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Mini Review

Secretases as therapeutic targets for Alzheimer's disease

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ABSTRACT

Accumulation of amyloid-β (Aβ) is widely accepted as the key instigator of Alzheimer's disease (AD). The proposed mechanism is that accumulation of Aβ results in inflammatory responses, oxidative damages, neurofibrillary tangles and, subsequently, neuronal/synaptic dysfunction and neuronal loss. Given the critical role of Aβ in the disease process, the proteases that produce this peptide are obvious targets. The goal would be to develop drugs that can inhibit the activity of these targets. Protease inhibitors have proved very effective for treating other disorders such as AIDS and hypertension. Mutations in APP (amyloid-β precursor protein), which flanks the Aβ sequence, cause early-onset familial AD, and evidence has pointed to the APP-to-Aβ conversion as a possible therapeutic target. Therapies aimed at modifying Aβ-related processes aim higher up the cascade and are therefore more likely to be able to alter the progression of the disease. However, it is not yet fully known whether the increases in Aβ levels are merely a result of earlier events that were already causing the disease.

1. Alzheimer's disease (AD)

Alzheimer's disease (AD) is a progressive, irreversible neurodegenerative disease of the brain [1]. AD is the most common cause of dementia in aging populations. It is characterized by a loss of short-term memory and deterioration in behavior and intellectual performance. In advanced stages of the disease, all memory and mental function may be lost [2]. Almost 5 million individuals in the United States currently have undergone these changes. The U.S. Alzheimer's Association estimates that by 2025, over 22 million people worldwide will be afflicted [3]. The onset of AD is not clear. It is difficult to distinguish clinically between mild, or early AD, and normal aging. The disease is divided into two major classes, early and late onset, with 60 or 65 years of age generally as the dividing point. Early-onset AD is rare and affects only 0.02% of those aged 30–59. In its early stages, AD often goes unrecognized or is misdiagnosed, because most of the early symptoms of AD are similar to the consequences of aging. There is no single test that accurately diagnoses AD. Hence, doctors use a variety of assessments and laboratory measurements to make a diagnosis (Table 1) [4,5]. A definitive diagnosis of Alzheimer's disease is possible only by examining brain tissue after death. The two hallmark pathological lesions of the disease are neuritic plaques and neurofibrillary tangles in the cerebral cortex and limbic system [6,7].

2. Aβ plaque and PHF tau tangle

Amyloid is a protein that is normally found throughout the body. β-Amyloid (Aβ), a 37–43 amino acid amyloid, is derived from stepwise proteolytic cleavage of the amyloid-β precursor protein (APP), a large, type I membrane protein, by β-secretase (BACE) and γ-secretase [8]. Although Aβ42 accounts for only about 10% of total Aβ secreted from cells (~90% is the 40 amino acid variant Aβ40), hydrophobic and highly insoluble Aβ42 is the major component of amyloid plaques. The progressive accumulation of the Aβ42 peptide is a key feature of AD [9]. Amyloid plaque deposits are found outside nerve cells (neurons).

Intracellular paired helical filaments (PHF) represent the second of the two major AD hallmarks [10]. The PHF tangles are located inside neurons and their branching projections such as axons and dendrites. Tangles are composed of microtubule-associated (tau) proteins. Much of the tau protein in tangles presents as highly insoluble filaments. Analysis of PHF tangles using antibodies specific for various phosphorylated tau proteins revealed that tau in tangles is hyperphosphorylated by several kinases such as cyclin-dependent kinase 5 (cdk5) [11].

Aggregates of Aβ outside a neuron can initiate a cascade of events that include the alteration of tau proteins inside the cell. In particular, the Aβ aggregates can ultimately change the cellular activity of enzymes such as kinases [12,13]. The affected kinases then add too many phosphates to tau, changing the proteins' chemical properties and causing them to form insoluble filaments. Mutations in the tau gene itself can also generate tau filaments and

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Table 1
Diagnosis of Alzheimer's disease.

Laboratory tests	
Urine tests	Measuring the level of neural thread protein (NTP) or isoprostane in urine (e.g. Nymox's AlzheimAlert)
Blood tests	Examine the blood count, thyroid and live function, and levels of glucose and other blood-based indicators of illness
CSF markers	Detection of total-tau, phospho-tau, Aβ42 in CSF
Genotyping	Single nucleotide polymorphisms (SNPs) of AD-related genes (e.g. ApoE E4 allele, cholesterol-related genes)
Brain-imaging scan	
PET scans	Use chemical markers (FDDNP, Pittsburgh compound B) which bind to plaques or large amount of amyloid deposits
MRI scans	Useful for identifying AD after or before clinical symptoms appear
CT scans	Produce two-dimensional brain images to rule out brain tumors or blood clots in the brain as the reason for symptoms

cause other types of neurodegenerative diseases besides AD [14,15]. Thus, the formation of tau filaments is apparently a more general event leading to neuronal death, whereas amyloid plaques are the specific initiator in AD.

3. Amyloid cascade hypothesis

The two key features of Alzheimer's disease, amyloid plaques and PHF tau tangles, were first discovered by the German neurologist Alois Alzheimer 100 years ago. The observation of these anomalies begin the modern era of research on neurodegenerative disease. Are the plaques and tangles responsible for the degeneration of neurons, or are they merely markers of where neuronal death has already occured? Over the past 30 years, genetic and molecular biological studies have provided accumulating evidence supporting the hypothesis that the production of Aβ peptides and the formation of cortical amyloid plaques cause the onset and progression of AD. Researchers discovered connections between AD and the genes that regulate the production of Aβ [16]. The evidence came from studies of families at especially high risk of getting AD. Members of these families carry rare genetic mutations that predestine them for the disease at a relatively young age [17]. The first genetic mutations causing AD were discovered in the APP gene [18,19]. Most of the mutations cluster at or very near the sites within APP that are normally cleaved by protease called α-, β-, and γ-secretases. Mutations in two related genes dubbed presenilin 1 and 2 also cause very early and aggressive forms of Alzheimer's disease [20]. These mutations increase the formation of either Aβ in general or a particular type of Aβ that is highly prone to forming deposits by favoring proteolytic processing of APP by β- or γ-secretase [21,22]. Aggregates of Aβ outside a neuron can initiate a cascade of events that include alteration of tau proteins inside the cell (Fig. 1). Overproduction of Aβ leads to neuronal and synaptic loss and, hence, cognitive decline. Although scientists still do not understand exactly how the soluble assemblies and insoluble filaments of Aβ disrupt and kill neurons, the amyloid hypothesis has become the focus of much AD research [23]. These findings have led to the development of a large variety of different pharmacological and immunological approaches aimed at lowering brain levels of Aβ [24,25].

4. Aβ production: APP processing

Aβ is a short peptide and a normal product of APP metabolism. APP is a single transmembrane protein that is cotranslationally translocated into the endoplasmic reticulum and then posttranslationally modified by glycosylation through secretory pathways [25]. Both during and after trafficking, it generates several small peptides by a variety of proteolytic cleavages. The first occurs 12 amino acids N-terminal to the transmembrane domain by α-secretase. This processing releases the large soluble ectodomain fragment (α-APPs) into the lumen and/or extracellular space and leaves an 83-residue C-terminal fragment (CTF; C83) in the membrane. Alternatively, some APP proteins can be cleaved at 16-residues N-terminal to the β-cleavage site, generating a slightly smaller ectodomain derivative (β-APPs) and retaining a 99-residue CTF (C99) in the membrane [26]. Subsequently, C83 and C99 are processed by γ-secretase activity, producing P3 and amyloid-β,

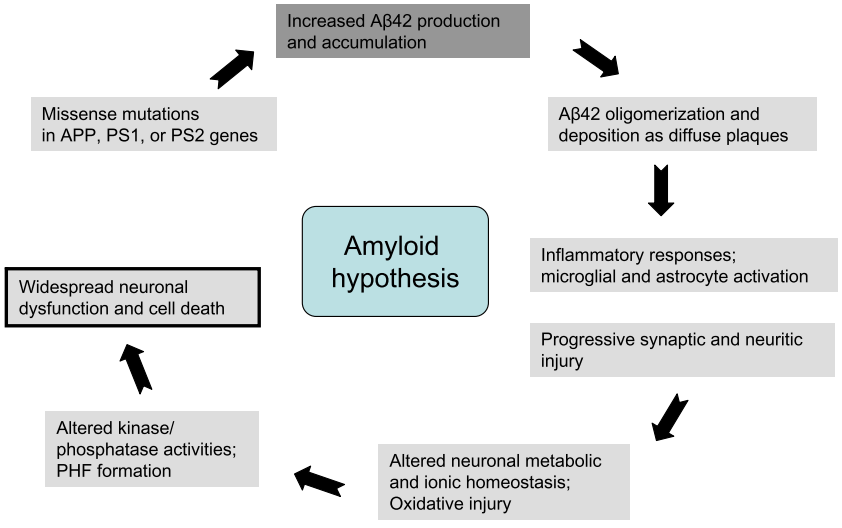

Fig. 1. Amyloid hypothesis: a hypothetical sequence of the pathogenetic steps for familial forms of Alzheimer's disease. Mutations of APP or presenilins elevate the Aβ1–42/1–40 ratio. Aβ oligomers induce inflammatory responses, oxidative damage, neurofibrillary tangles and subsequently, synaptic and neuritic injury and neuronal loss.

respectively. The sites of cleavage of the C83 and C99 fragments by γ-secretase are located in the middle of the transmembrane domain [27].

The γ-secretase generates a number of isoforms of 39–43 amino acid residues in length. Aβ40 is the most abundant form of Aβ synthesized (80–90%). Although Aβ42 represents only about 10% of secreted Aβ, this longer and more hydrophobic variant is abundantly present in the amyloid plaques observed post mortem in AD patients [28]. An understanding of the production of Aβ42 in particular is essential for elucidating the molecular mechanism of AD pathogenesis and may also lead to the development of new chemotherapeutic agents in AD.

5. γ-Secretase complex in AD

γ-Secretase is an unusual protease that apparently cleaves within the transmembrane helix of numerous type I glycoproteins including APP and Notch. Despite several pieces of evidence indicating that membrane-anchored proteases known as secretases or sheddases are involved in APP metabolism and Notch signaling, the identity of γ-secretase was not clear for many years. Unlike other secretases such as α-secretase and β-secretase, γ-secretase have peculiar intramembrane proteolysis activity—they can achieve the unusual feat of using water to cut the protein inside the water-hating (hydrophobic) environment of the cellular membrane [29]. Over the last several years, molecular identification of the γ-secretase complex has shown that four different members are essential for this enzymatic activity: presenilin 1 (PS1) [30], anterior pharynx-defective (APH-1) [31], presenilin enhancer 2 (PEN-2) [31] and nicastrin (NCT) [32]. Assembly of the four components of γ-secretase leads to PS autoproteolysis into two fragments that contribute an aspartate to the active site. Much evidence suggests that PS or γ-secretase is also involved in the regulation of cellular Ca^{2+} homeostasis and Ca^{2+}-mediated cell death [33,34].

NCT undergoes complex N-glycosylation during its maturation within the ER and Golgi compartments [35]. The extracellular DAP domain of NCT is essential for substrate recognition and processing [36]. NCT is also specifically required for transport of the γ-secretase complex to the cell surface [37]. APH-1 and PEN-2 act as cofactors in the active γ-secretase complex [31]. APH-1 has scaffolding roles in the initial assembly of γ-secretase and in the enzymatic function of the final complex [38]. PEN-2 is incorporated at the final step of γ-secretase complex assembly and may assist in the endoproteolysis of PS [39]. Six variants of γ-secretase exist based upon different combinations of the two PS proteins and the three different Aph-1 proteins [40].

γ-Secretase is essential for the proteolysis of many molecules besides APP. For example, it has important roles in normal cellular physiology and pathophysiology (Table 2) [41]. After cleavage by γ-secretase, the active cytoplasmic domains of some substrates translocate to the nucleus where they serve to regulate gene expression [42]. Because of the role of γ-secretase in Aβ formation, γ-secretase has emerged as a promising molecular target for the treatment of AD. In recent years, γ-secretase inhibitors have been designed and synthesized by several groups. However, most of these inhibitors are not specific for γ-secretase cleavage of APP, and equally inhibit the processing of other γ-secretase substrates. Not surprisingly, then, inhibition of γ-secretase activity has been associated with serious adverse effects in animal models. Therefore, modulation of APP processing without interfering with other signaling is an important therapeutic goal in the development of γ-secretase regulators [43]. In this context, it is important to understand γ-secretase-mediated proteolysis in order to clarify this enzyme's role as a therapeutic target in AD (and possibly in other diseases in which γ-secretase is involved) [44].

6. β-Secretase (BACE-1) in AD

β-Secretase is a novel membrane-bound protein with homology to the pepsin family of aspartyl proteases (BACE-1: β-site APP cleaving enzyme 1) [45–47]. BACE-1 fulfills most of the requirements expected for a candidate β-secretase. It is highly expressed in brain and is colocalized with CTFs and Aβ. Oxidative stress-induced BACE-1 expression is regulated by γ-secretase activity, providing evidence of a feed-forward pathogenic mechanism of AD in which oxidative stress increases Aβ production, which, in turn, enhances oxidative stress [48,49]. Genetic studies suggest that blocking β-secretase's activity eliminates Aβ formation in brain without any apparent negative consequences [50,51]. β-Secretase inhibitors were designed primarily to prevent the formation of Aβ primarily and deposition of amyloid fibrils in the brain. The three-dimensional structure of β-secretase has been reported [23]. This provides useful information for computer-based drug design of potential inhibitors. Compound screening and medicinal chemistry are being pursued vigorously to identify potent small-molecule inhibitors that can fit the large active site of β-secretase and still penetrate the blood brain barrier (BBB). Two transition-state analog inhibitors modeled on the β-secretase cleavage site of the Swedish mutation have been reported to have relatively low IC_{50} concentrations: P10–P4 'StatVal', $IC_{50} \sim 30$ nM; OM99-2, $IC_{50} \sim 1.6$ nM [47,52].

7. α-Secretase in AD

In non-amyloidogenic pathways, α-secretase cleaves the amyloid precursor protein (APP) within the sequence of Aβ peptides. α-Secretase cleavage precludes β-secretase and γ-secretase cleavage, and releases an N-terminal extracellular domain known as

Table 2
γ-Secretase substrates.

Protein	Product	Potential function of cleavage product	Protein	Product	Potential function of cleavage product
APP	AICD	Transcriptional activity	ApoER2	ND	Regulation of signaling
Notch	NICD	Transcriptional activity	Delta	DIICD	Regulation of cell proliferation
APLP1/2	ALID	Transcriptional activity	E-cadherin	Ecad/CTF2	Regulation of cell adhesion
ErbB4	B4ICD, E4ICD, 4ICD	Transcriptional activity	Ephrin-B1	ND	Regulation of actin polymerization
CD44	CD44ICD	Transcriptional regulation	Ephrin-B2	Ephrin-B2/CTF2	Phosphorylation of Src
DCC	DCCICD	Transcriptional activity	GluR3	ND	Modulation of receptor activity
γ-Protocadherin	γ-ICD or Pcdhg-CTF2	Transcriptional activity	HLA-A2	HLA-A2ICD	Degradation of HLA-A2
LRP1	LRP1ICD	Transcriptional regulation	N-cadherin	Ncad/CTF2	Regulation of cell adhesion
Jagged	JICD	Transcriptional activity	Nectin 1α	NEICD	Regulation of cell adhesion
SCNB2	β2-ICD	Regulation of cell adhesion	TYRP1/TYRP2	ND	Pigment regulation
Syndecan 3	SICD	Control of localization of associated proteins	$p75^{NTR}$	P75ICD	Activation of Rho

Abbreviations: ICD, intracellular domain; ND, not detected.

α-APPs with neurotrophic and neuroprotective properties. α-Secretases are members of the ADAM (a disintegrin and metalloprotease domain) family, which are expressed on the surface of cells and anchored in the cell membrane [53,54]. ADAM10 has been identified as possessing α-secretase activity. Shunting APP towards the α-secretase pathway lowers Aβ levels in the brain. An increase in α-secretase activity is an attractive strategy for the treatment of AD and may be achieved by selectively modulating key signaling pathways.

8. Screening strategies for secretase inhibitors

Why are there no effective drugs for AD? The reason is that the underlying etiology of AD is not understood at the level which is required for drug discovery. During the past decade, several advances have been achieved for treatment of AD (Table 3). Several candidate therapies are undergoing clinical trials and have yielded some promising preliminary results. Inhibition of the production of insoluble Aβ is a widely pursed strategy for the treatment of AD. Several simple assays have developed and appied in highthroughput screening of candidate β- and γ-secretase inhibitors for preventing Aβ production.

8.1. FRET

Fluorescence resonance energy transfer (FRET) occurs between two fluorophores if they are within close proximity (~10 nm) of each other. During fluorescence emission, some of the donor's excitation energy is non-radiatively transferred to the acceptor fluorophore, which leads to a characteristic shortening in the donor fluorophore lifetime. After labeling the two epitopes of interest with donor and acceptor fluorophores, the presence of a second lifetime indicates that a proportion of the donor-labeled epitopes is in close proximity to the acceptor-labeled epitopes. Biochemical assays for BACE-1 inhibitor screening have been reported in the fluorescence resonance energy transfer (FRET) format [55]. However, the reported and commercial BACE-1 FRET substrates suffer from relatively poor solubility, due in part to the hydrophobicity of their fluorophores.

8.2. C99–GVP

γ-Secretase reporter assays using the APP C99–GVP constructs have been reported [56]. The GVP construct consists of the Gal 4 DNA-binding/VP16 transactivation (GVP) domain fused to the C99 region of APP, which serves as a substrate for γ-secretase *in vivo*. After transmembrane processing of C99, the AICD fragment containing a GVP moiety is translocated into the nucleus via nuclear localization signals in the GVP moiety. In the nucleus, GVP specifically binds to and transactivates the UAS promoter of the luciferase reporter gene [57]. Also, the C99–GVP construct harbors the native APP cytoplasmic tail, ensuring correct intracellular targeting by the sorting signals contained within the tail. Specificity and sensitivity were confirmed by the addition of selective γ-secretase inhibitors. This assay offers several advantages for detection of AICD which can be quantified because the presence of the GVP domain reduces cytosolic degradation of the AICD fragment. This assay has the possibility of being useful in highthroughput screening of candidate γ-secretase inhibitors for APP.

8.3. C99–Tet on (C99–rtTA)

To detect γ-secretase activity in living cells, researchers developed a C99–rtTA chimeric assay system by incorporating the cDNA encoding the C99 domain of APP into an rtTA (reverse tetracycline-controlled transactivator) [58]. rtTA protein has a TRE (tetracycline-responsive element) binding region and a VP16 activation domain. Cleavage of the chimeric protein C99–rtTA liberates the C-terminal region, including the rtTA, which is translocated to the nucleus and, in the presence of doxycycline, activates the expression of GFP from pTRE-GFP. Using this modified assay system, it is possible to monitor γ-secretase activity in living cells in real time, and signal monitoring is simple under a fluorescence microscope or in a plate spectrofluorometer.

8.4. AP–APP for BACE (SEAP–APP)

Chimeric constructs that possess alkaline phosphatase (AP) fused to APP are widely used for studying APP processing and BACE activity [59]. SEAP–APP is a very sensitive indicator of BACE cleavage. After co-transfection of the SEAP–APP construct with a ß-gal reporter construct and target, SEAP activity was measured in the medium and normalized to ß-gal activity. Because wild-type APP is a rather poor substrate for BACE-1, substrates were developed based on the sequence of the Swedish mutant of APP, an FAD mutation that enhances Aβ production. It enabled development of a sensitive biochemical assay in which the BACE-1 concentration can be as low as 100 pM. Moreover, this assay and substrate are applicable for counterscreening BACE-2 and cathepsin D.

9. Perspectives

During the past decade, researchers have made tremendous progress toward understanding the molecular events of AD that trigger the illness, and they are now exploring a variety of strategies for slowing or halting these destructive processes.

Inhibition of Aβ production could be therapeutic in the early clinical phases of AD, particularly in patients with minimal cognitive impairment. Several small-molecule inhibitors for β- or γ-secretase that can cross the blood–brain barrier have been discovered. In the case of γ-secretase inhibitors, these could be designed to decrease Aβ production by some 30–40% or so, hopefully without interfering in a quantitatively meaningful way with Notch processing. An alternative and attractive approach would be to use small molecules to bind Aβ monomers and prevent their assembly into cytotoxic oligomers. For example, an

Table 3
Therapeutic strategies for Alzheimer's disease.

- Inhibit β-, γ-secretase to prevent production of amyloidogenic Aβ
- Targeting Aβ oligomers to prevent the oligomerization of Aβ or enhance its clearance from the cerebral cortex:
  - Immunotherapy: active or passive Aβ immunization
  - Selective dagradation of Aβ oligomers: IDE, plasmin, cathepsin B, neprilysin
  - Destabilization of Aβ oligomers: Alzhemed
- Anti-inflammatory strategy
- Modulating cholesterol homeostasis; cholesterol-lowering drugs
- Chelate metal ions Cu^{2+} amd Zn^{2+} which support Aβ aggregation
- Broad amyloid-based strategy; prevent synaptotoxic and neurodegenerative effects

anti-aggregation compound blocked amyloid fibril formation. An advantage of the anti-oligomerization strategy is that one would be targeting a purely pathological event in the disease, rather than interfering with normal metabolic reactions such as those of β- and γ-secretases. Because the success of any one of these strategies cannot be predicted, two or more approaches might ultimately be combined for greater success.

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References

[1] D.M. Walsh, D.J. Selkoe, Deciphering the molecular basis of memory failure in Alzheimer's disease, Neuron 44 (2004) 181–193.
[2] M.P. Mattson, Pathways towards and away from Alzheimer's disease, Nature 430 (2004) 631–639.
[3] R. Scatena, G.E. Martorana, P. Bottoni, et al., An update on pharmacological approaches to neurodegenerative diseases, Expert Opin. Investig. Drugs 16 (2007) 59–72.
[4] J.L. Cummings, Alzheimer's disease, N. Engl. J. Med. 351 (2004) 56–67.
[5] P.M. Doraiswamy, G.L. Xiong, Pharmacological strategies for the prevention of Alzheimer's disease, Expert Opin. Pharmacother. 7 (2006) 1–10.
[6] F.M. LaFerla, S. Oddo, Alzheimer's disease: A[beta], tau and synaptic dysfunction, Trends Mol. Med. 11 (2005) 170–176.
[7] G.K. Gouras, C.G. Almeida, R.H. Takahashi, Intraneuronal A[beta] accumulation and origin of plaques in Alzheimer's disease, Neurobiol. Aging 26 (2005) 1235–1244.
[8] D.J. Selkoe, Alzheimer's disease: genes, proteins, and therapy, Physiol. Rev. 81 (2001) 741–766.
[9] M.E. Figueiredo-Pereira, S. Efthimiopoulos, N. Tezapsidis, et al., Distinct secretases, a cysteine protease and a serine protease, generate the C termini of amyloid beta-proteins Abeta1–40 and Abeta1–42, respectively, J. Neurochem. 72 (1999) 1417–1422.
[10] M. Goedert, C.M. Wischik, R.A. Crowther, et al., Cloning and sequencing of the cDNA encoding a core protein of the paired helical filament of Alzheimer disease: identification as the microtubule-associated protein tau, Proc. Natl. Acad. Sci. USA 85 (1988) 4051–4055.
[11] G. Michel, M. Mercken, M. Murayama, et al., Characterization of tau phosphorylation in glycogen synthase kinase-3beta and cyclin dependent kinase-5 activator (p23) transfected cells, Biochim. Biophys. Acta 1380 (1998) 177–182.
[12] F. Kuperstein, N. Reiss, N. Koudinova, et al., Biphasic modulation of protein kinase C and enhanced cell toxicity by amyloid beta peptide and anoxia in neuronal cultures, J. Neurochem. 76 (2001) 758–767.
[13] F. Kuperstein, E. Yavin, ERK activation and nuclear translocation in amyloid-beta peptide- and iron-stressed neuronal cell cultures, Eur. J. Neurosci. 16 (2002) 44–54.
[14] M. Hong, V. Zhukareva, V. Vogelsberg-Ragaglia, et al., Mutation-specific functional impairments in distinct tau isoforms of hereditary FTDP-17, Science 282 (1998) 1914–1917.
[15] C. Mailliot, T. Bussiere, M. Hamdane, et al., Pathological tau phenotypes. The weight of mutations, polymorphisms, and differential neuronal vulnerabilities, Ann. N. Y. Acad. Sci. 920 (2000) 107–114.
[16] J. Hardy, D.J. Selkoe, The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics, Science 297 (2002) 353–356.
[17] R. Sherrington, E.I. Rogaev, Y. Liang, et al., Cloning of a gene bearing missense mutations in early-onset familial Alzheimer's disease, Nature 375 (1995) 754–760.
[18] C. Haass, C.A. Lemere, A. Capell, et al., The Swedish mutation causes early-onset Alzheimer's disease by beta-secretase cleavage within the secretory pathway, Nat. Med. 1 (1995) 1291–1296.
[19] D. Scheuner, C. Eckman, M. Jensen, et al., Secreted amyloid beta-protein similar to that in the senile plaques of Alzheimer's disease is increased in vivo by the presenilin 1 and 2 and APP mutations linked to familial Alzheimer's disease, Nat. Med. 2 (1996) 864–870.
[20] J. Perez-Tur, S. Froelich, G. Prihar, et al., A mutation in Alzheimer's disease destroying a splice acceptor site in the presenilin-1 gene, Neuroreport 7 (1995) 297–301.
[21] D.R. Borchelt, G. Thinakaran, C.B. Eckman, et al., Familial Alzheimer's disease-linked presenilin 1 variants elevate Abeta1–42/1–40 ratio in vitro and in vivo, Neuron 17 (1996) 1005–1013.
[22] K. Duff, C. Eckman, C. Zehr, et al., Increased amyloid-beta42(43) in brains of mice expressing mutant presenilin 1, Nature 383 (1996) 710–713.
[23] D.J. Selkoe, D. Schenk, Alzheimer's disease: molecular understanding predicts amyloid-based therapeutics, Annu. Rev. Pharmacol. Toxicol. 43 (2003) 545–584.
[24] D. Schenk, Amyloid-beta immunotherapy for Alzheimer's disease: the end of the beginning, Nat. Rev. Neurosci. 3 (2002) 824–828.
[25] P.S. Aisen, S. Gauthier, B. Vellas, et al., Alzhemed: a potential treatment for Alzheimer's disease, Curr. Alzheimer Res. 4 (2007) 473–478.
[26] L. Marlow, M. Cain, M.A. Pappolla, et al., Beta-secretase processing of the Alzheimer's amyloid protein precursor (APP), J. Mol. Neurosci. 20 (2003) 233–239.
[27] C. Yu, S.H. Kim, T. Ikeuchi, et al., Characterization of a presenilin-mediated amyloid precursor protein carboxyl-terminal fragment gamma. Evidence for distinct mechanisms involved in gamma-secretase processing of the APP and Notch1 transmembrane domains, J. Biol. Chem. 276 (2001) 43756–43760.
[28] J.T. Jarrett, E.P. Berger, P.T. Lansbury Jr., The carboxy terminus of the beta amyloid protein is critical for the seeding of amyloid formation: implications for the pathogenesis of Alzheimer's disease, Biochemistry 32 (1993) 4693–4697.
[29] M.S. Wolfe, D.J. Selkoe, Biochemistry: intramembrane proteases—mixing oil and water, Science 296 (2002) 2156–2157.
[30] A. Herreman, L. Serneels, W. Annaert, et al., Total inactivation of gamma-secretase activity in presenilin-deficient embryonic stem cells, Nat. Cell Biol. 2 (2000) 461–462.
[31] R. Francis, G. McGrath, J. Zhang, et al., Aph-1 and pen-2 are required for Notch pathway signaling, gamma-secretase cleavage of betaAPP, and presenilin protein accumulation, Dev. Cell 3 (2002) 85–97.
[32] G. Yu, M. Nishimura, S. Arawaka, et al., Nicastrin modulates presenilin-mediated notch/glp-1 signal transduction and betaAPP processing, Nature 407 (2000) 48–54.
[33] Y.H. Choi, A.R. Gwon, H.Y. Jeong, et al., Contribution of gamma-secretase to calcium-mediated cell death, Neurosci. Lett. 469 (2010) 425–428.
[34] D.G. Jo, J.W. Chang, H.S. Hong, et al., Contribution of presenilin/gamma-secretase to calsenilin-mediated apoptosis, Biochem. Biophys. Res. Commun. 305 (2003) 62–66.
[35] D.S. Yang, A. Tandon, F. Chen, et al., Mature glycosylation and trafficking of nicastrin modulate its binding to presenilins, J. Biol. Chem. 277 (2002) 28135–28142.
[36] A. Capell, C. Kaether, D. Edbauer, et al., Nicastrin interacts with gamma-secretase complex components via the N-terminal part of its transmembrane domain, J. Biol. Chem. 278 (2003) 52519–52523.
[37] K. Shirotani, D. Edbauer, A. Capell, et al., Gamma-secretase activity is associated with a conformational change of nicastrin, J. Biol. Chem. 278 (2003) 16474–16477.
[38] W.J. Luo, H. Wang, H. Li, et al., PEN-2 and APH-1 coordinately regulate proteolytic processing of presenilin 1, J. Biol. Chem. 278 (2003) 7850–7854.
[39] N. Takasugi, T. Tomita, I. Hayashi, et al., The role of presenilin cofactors in the gamma-secretase complex, Nature 422 (2003) 438–441.
[40] M.S. Wolfe, The gamma-secretase complex: membrane-embedded proteolytic ensemble, Biochemistry 45 (2006) 7931–7939.
[41] A.L. Parks, D. Curtis, Presenilin diversifies its portfolio, Trends Genet. 23 (2007) 140–150.
[42] H.N. Woo, J.S. Park, A.R. Gwon, et al., Alzheimer's disease and Notch signaling, Biochem. Biophys. Res. Commun. 390 (2009) 1093–1097.
[43] A. Petit, F. Bihel, C. Alves da Costa, et al., New protease inhibitors prevent gamma-secretase-mediated production of Abeta40/42 without affecting Notch cleavage, Nat. Cell Biol. 3 (2001) 507–511.
[44] T.V. Arumugam, S.L. Chan, D.G. Jo, et al., Gamma secretase-mediated Notch signaling worsens brain damage and functional outcome in ischemic stroke, Nat. Med. 12 (2006) 621–623.
[45] I. Hussain, D. Powell, D.R. Howlett, et al., Identification of a novel aspartic protease (Asp 2) as beta-secretase, Mol. Cell. Neurosci. 14 (1999) 419–427.
[46] R. Vassar, B.D. Bennett, S. Babu-Khan, et al., Beta-secretase cleavage of Alzheimer's amyloid precursor protein by the transmembrane aspartic protease BACE, Science 286 (1999) 735–741.
[47] S. Sinha, J.P. Anderson, R. Barbour, et al., Purification and cloning of amyloid precursor protein beta-secretase from human brain, Nature 402 (1999) 537–540.
[48] D.G. Jo, T.V. Arumugam, H.N. Woo, et al., Evidence that gamma-secretase mediates oxidative stress-induced beta-secretase expression in Alzheimer's disease, Neurobiol. Aging 31 (2010) 917–925.
[49] E. Tamagno, M. Guglielmotto, M. Aragno, et al., Oxidative stress activates a positive feedback between the gamma- and beta-secretase cleavages of the beta-amyloid precursor protein, J. Neurochem. 104 (2008) 683–695.
[50] Y. Luo, B. Bolon, S. Kahn, et al., Mice deficient in BACE1, the Alzheimer's beta-secretase, have normal phenotype and abolished beta-amyloid generation, Nat. Neurosci. 4 (2001) 231–232.
[51] S.L. Roberds, J. Anderson, G. Basi, et al., BACE knockout mice are healthy despite lacking the primary beta-secretase activity in brain: implications for Alzheimer's disease therapeutics, Hum. Mol. Genet. 10 (2001) 1317–1324.
[52] V. John, Human beta-secretase (BACE) and BACE inhibitors: progress report, Curr. Top. Med. Chem. 6 (2006) 569–578.
[53] M. Asai, C. Hattori, B. Szabo, et al., Putative function of ADAM9, ADAM10, and ADAM17 as APP alpha-secretase, Biochem. Biophys. Res. Commun. 301 (2003) 231–235.

[54] J.D. Buxbaum, K.N. Liu, Y. Luo, et al., Evidence that tumor necrosis factor alpha converting enzyme is involved in regulated alpha-secretase cleavage of the Alzheimer amyloid protein precursor, J. Biol. Chem. 273 (1998) 27765–27767.
[55] B.L. Pietrak, M.C. Crouthamel, K. Tugusheva, et al., Biochemical and cell-based assays for characterization of BACE-1 inhibitors, Anal. Biochem. 342 (2005) 144–151.
[56] H. Laudon, P.M. Mathews, H. Karlstrom, et al., Co-expressed presenilin 1 NTF and CTF form functional gamma-secretase complexes in cells devoid of full-length protein, J. Neurochem. 89 (2004) 44–53.
[57] H. Karlstrom, A. Bergman, U. Lendahl, et al., A sensitive and quantitative assay for measuring cleavage of presenilin substrates, J. Biol. Chem. 277 (2002) 6763–6766.
[58] D.G. Jo, J. Jang, B.J. Kim, et al., Overexpression of calsenilin enhances gamma-secretase activity, Neurosci. Lett. 378 (2005) 59–64.
[59] S. Efthimiopoulos, K.M. Felsenstein, K. Sambamurti, et al., Study of the phorbol ester effect on Alzheimer amyloid precursor processing: sequence requirements and involvement of a cholera toxin sensitive protein, J. Neurosci. Res. 38 (1994) 81–90.