SNAPSHOT ON POTENTIAL INVOLVEMENT OF ENZYME SECRETASE IN ALZHEIMER’S DISEASE

Erica Sequeira and N. Saraswathy*

Shobhaben Pratapbhai Patel School of Pharmacy and Technology Management,
SVKM’S NMIMS, Mumbai, India.

ABSTRACT
Alzheimer’s disease is the most common form of dementia. It is a progressive and irreversible neurodegenerative disorder and characterized clinically by progressive loss of memory, cognition, reasoning, judgment and emotional stability. The brains of people with Alzheimer’s disease have two abnormal structures such as the senile plaques containing amyloid β and the neurofibrillary tangles containing Tau, which are the hallmarks of the disease. Over time, this damage spreads to other areas of the brain, such as the grey matter (responsible for processing thoughts) and the hippocampus (responsible for memory). This results in decline in the neuronal mass and cognitive functions. The actual pathogenesis of Alzheimer’s disease is thought to begin many years before the diagnosis of Alzheimer’s disease. Thus such a long "preclinical" phase of Alzheimer’s disease would provide a critical opportunity for therapeutic intervention and prevent the disease in future. These amyloid plaques are formed due to the proteolytic cleavage of amyloid precursor protein (APP) by β-secretase and γ-secretase. This article reviews on the secretases, that are responsible for the pathogenesis of Alzheimer’s disease, molecular explanation of the possible neuropathogenesis, the cleavage products generated by different processing patterns and the potential role of those cleavage products.

Keywords: Alzheimer’s disease, amyloid, neurofibrillary tangles, APP, γ-secretase, α-secretase, β-secretase, oxidative stress.
INTRODUCTION
Alzheimer's disease is a degenerative central nervous system disorder which is characterized with extensive loss of specific neuronal cells. Both the environmental and genetic factors contribute to the risk of developing AD, which increases with age [1]. With the current aging population, Alzheimer’s disease is developing as a social burden for the health care system, national economy in addition to the emotional burden for the immediate family members. Prevalence of Alzheimer’s disease is 0.8% in individuals aged 65 - 69 years and upwards of 28.5% in persons aged 90 years and older [2]. Individuals with Alzheimer’s exhibit neuronal degeneration and characteristic structures such as amyloid plaques and neurofibrillary tangles, the latter consists largely of hyperphosphorylated twisted filaments of the microtubule-associated protein tau. The Aβ peptides are produced as a result of excessive processing of the amyloid precursor protein (APP), which is the trans-membrane protein found in neurons and other cells [3]. Amyloid plaques are composed primarily of 40 and 42 amino acid peptides called Aβ40 and Aβ42, respectively which are derived from amyloid precursor protein (APP) by sequential proteolysis catalyzed by several enzymes [3], predominantly due to the β secretase. In particular ageing, head injury/trauma/stroke, genetics such as Apo E4/4 allele carriers, cardiovascular disease and type 2 diabetes, all predispose an individual towards onset of Alzheimer’s disease [4]. There are two forms of the disease, a genetics based early onset familial Alzheimer’s disease which is caused due to the autosomal dominant mutations in either APP[5,6] or the presenilin genes and a more prevalent age-dependent form called sporadic Alzheimer’s’ disease.

AMYLOID PRECURSOR PROTEIN (APP) AND ITS FUNCTION
The APP gene is located on chromosome 21 in humans with three major isoforms arising from alternative splicing fashion [7] which are APP695, APP751 and APP770. APP751 and APP770 are expressed in most tissues and contain a 56 amino acid Kunitz Protease Inhibitor (KPI) domain within their extracellular regions. APP695 is predominantly is expressed in neurons and lacks the KPI domain [8, 9]. There are reports showing that the protein and mRNA levels of KPI-containing APP isoforms are elevated in AD brain [10], this occurs due to a prolonged activation of extrasynaptic NMDA receptor in neurons which can shift the expression from APP695 to KPI-containing APP isoforms, and is also associated with increased production of Aβ [11]. Thus, a dysregulation in the splicing of APP RNA contributes to disease pathogenesis. APP plays a role in neurite outgrowth and synaptogenesis, neuronal protein trafficking along the axon, transmembrane signal
transduction, cell adhesion, calcium metabolism. [12] Thus the net effect of APP depends on the proportion of APP metabolites produced and their combinations [13]. Full-length APP is a type I transmembrane protein and is synthesized in the endoplasmic reticulum (ER) and then transported through the Golgi apparatus to the trans-Golgi-network (TGN) where the highest concentration of APP is found in neurons at steady state [14,15]. During the transport of APP, it was found that APP interacts with kinesin-I and functions as a kinesin-I membrane receptor to mediate axonal transport of β-secretase (BACE1) and PS1 [16,17].

However, another study failed to verify the interaction between APP and kinesin-I and the co-transport of BACE1 and PS1 with APP [18]. A study showed that increased doses of APP markedly decreased retrograde transport of nerve growth factor and resulted in degeneration of forebrain cholinergic neurons in a mouse model of Down's Syndrome [19]. APP was also found to interact with high-affinity choline transporter (CHT) and that, APP deficiency affected CHT endocytosis [20]. Hence most of the studies suggest that APP plays some role in regulating protein trafficking. Thus the Alzheimer disease occurs due to the inappropriate cleaving of the APP due to the two membrane bound secretases, namely α and β secretase which results in the accumulation of Aβ in the senile plaques, this triggers the pathophysiological changes and cause a decline in the cognitive abilities and memory loss.

VARIous SECRETASES LINKED WITH ALZHEIMER’S DISEASE

α-Secretase : One of the most important enzyme, α-secretase is responsible for cleaving the Amyloid Precursor Protein(APP) [21-23]. The activity of this enzyme is mediated by a series of membrane bound proteases which are members of the ADAM (a disintegrin and metalloprotease) family [24]. The α-secretases cleave APP within the Aβ sequence itself and thus generating a soluble APPsα ectodomain and a membrane-bound carboxy-terminal fragment [24], the carboxy-terminal fragment is degraded in lysosomes [25, 26] or may be further processed by the γ-secretase [27]. This results in the formation of a series of short hydrophobic peptides including Aβ17–40 and Aβ17–42, which are together are termed as p3 fragments [28]. Processing of APP by α-secretase is considered to be protective for AD Patients because the enzymes cleave within the Aβ sequence, thereby preventing the production of Aβ [24]. sAPPα (Soluble alpha APP) plays an important role in neuronal plasticity/survival and is protective against excitotoxicity [29,30]. sAPPα also regulates neural stem cell proliferation and is important for early brain development [31,32]. It inhibits
the stress-induced CDK5 activation and participate in various neuroprotective reagent-mediated excitoprotection [33-36].

One interesting feature observed is that the expression of sAPPα alone is able to rescue the abnormalities of APP deficient mice [37], implying that most of APP’s physiological function is mediated by sAPPα. The early in vitro studies have also demonstrated that sAPPα protects cultured neurons against oxygen-glucose deprivation and excitotoxicity by inhibiting calcium currents and increasing potassium currents and thus stabilizing the resting membrane potential [38,39], also promotes neurite outgrowth, synaptogenesis and cell adhesion[30,40]. According to a study it was observed that α secretase mRNA in the hippocampus was down regulated and its activity decreased after chronic brain hypoperfusion [41]. It was also observed that α secretase decreased in animals after ischemic brain injury too [42]. This subsequently causes an increased cleavage of the amyloid precursor protein in an amyloidogenic pathway, resulting in the formation of the β- amyloid peptide. The biologically relevant site for these actions was located to the carboxy-terminus of sAPPα, spanning the region from just amino-terminal to the β-secretase cleavage site to the carboxy-terminal end with a heparin binding motif at the carboxy-terminus being the most important [39] thus the amino terminal end of sAPPα was not required for these effects.

The in vivo studies reported that sAPPα, when administered intracerebroventricularly resulted in the enhancement of learning and memory in mice and rats [43]. However the neuroprotective action of α-secretases via the shedding of soluble APPsα ectodomain will stand true only if p3 fragment produced by α-secretase activity is not pathogenic, and if increasing α-secretase activity actually lowers Aβ production[24]. Increasing α-secretase activity, increases the production of APPsα, and has been reported to be neuroprotective and growth promoting[44], but the consequences of chronically upregulating α-secretase-mediated cleavage of other substrates remains unknown[24]. Interestingly, transgenic approaches that over express the metalloprotease ADAM10 in mice resulted in increased APPsα generation and are protective against amyloidosis in the human APPV717I transgenic mouse model [45, 46].Thus α-secretase activity is upregulated when ADAM10 is over expressed. α-secretase activity is also upregulated in response to the signaling peptide PACAP[47].

Another finding is that the manipulation of ADAM17 can alter α-cleavage of APP and Aβ generation. The regulated α-cleavage which is abolished in ADAM17-deficient cells,
suggests that ADAM17 is likely the α-secretase responsible for regulated APP cleavage [48]. Additionally, an ADAM17 inhibitor prevented the regulated α-secretase activity in human neurons [49], thus it has a therapeutic significance.

However, the current evidence suggests that the identified α-secretases demonstrate a high degree of redundancy, and which α-secretases are responsible for APP cleavage in neurons and other brain cells is still unclear [50-54]. Until this problem is overcome, optimizing the development of therapeutic compounds that directly activate the α-secretases would be difficult and risky [24]. Protein kinase C, mitogen-activated protein kinases, tyrosine kinases and calcium-mediated pathways are all known to be involved in regulating α-secretase activity, and developing the compounds that stimulate α-secretase via these pathways is safer and has a high efficacy [55]. Retinoic acid derivatives have been proposed to increase transcription of ADAM10 and thus could also be used to indirectly stimulate α-secretase-mediated cleavage of APP [56].

Thus stimulating one or more of the signal transduction pathways involved in the regulation of α-secretase activity might be an alternative and an indirect method of promoting α-secretase-mediated cleavage of APP.

**β-Secretase**: β-secretase enzyme is responsible for the formation of the amyloid plaques, thus inhibition of this enzyme would eliminate the harmful pathogenic steps which are involved in AD. In the year 1999, five different research groups had independently reported the molecular cloning of β-secretase and, consequently, this enzyme has been given various names, such as β-site APP cleaving enzyme (BACE), aspartyl protease 2 and membrane-associated aspartic protease 2 [57-61]. β-secretase (BACE1) is a type 1 transmembrane aspartic protease and its expression is increased in situations of cellular stress, such as during energy deprivation, hypoxia and ischemia [62,63], and an oxidative stress [64] has been shown to increase BACE1 expression in a γ-secretase dependent fashion. Soon after BACE1 was discovered, a homologous protein, BACE2, was identified. BACE1 and BACE2 share 64% amino acid sequence homology, raising the possibility that BACE2 is also a β-secretase [24]. Unlike BACE1, however, which is highly expressed in neurons of the brain, BACE2 is expressed at low levels in the brain and does not have the same cleavage specificity for APP that BACE1 does [24].
The subcellular localization of BACE1 is predominantly within the trans-Golgi network and endoplasmic reticulum [65]. Although BACE1 reaches the plasma membrane due to vesicle traffic, it is recycled quickly, and very little BACE1-mediated APP cleavage occurs at the plasma membrane; instead APP is cleaved by BACE mostly in endocytic vesicles. Its observed that within the secretory pathways of the endoplasmic reticulum and trans-Golgi network, APP and BACE1 are located in separate membrane microdomains, due to binding of APP to X11/Munc18 proteins [66]. Neuronal depolarization leads to Munc18 phosphorylation and causes APP to relocalize in BACE1-containing membrane microdomains [67]. Thus due to the depolarization which causes the relocalization there is an increase in BACE1-mediated APP cleavage and Aβ production [68]. Thus, increased neuronal depolarization increases APP cleavage by both α-secretase and BACE1 in a competitive fashion [68]. It will therefore be of interest to determine if neuronal activity can be normalized and improved by increasing one activity over the other [69]. BACE1 is located in cholesterol rich lipid rafts [70], but reduction of Aβ generation and improvement in cognitive function by lowering brain cholesterol levels in AD patients still remains to be seen [70-72].

Several evidence suggest that inflammation and excitotoxicity leading to increase in free radicals causes the activation of the nuclear factor-κB (NF-κB), which is responsible for the pathogenesis of AD [73,74]. Thus this can influence the amount of Aβ production to some extent, since BACE1 expression appears to be modulated by stress [75,76]. An increase of NF-κB expression is observed in the hippocampus and entorhinal cortex of AD patients, thus two cerebral areas altered in this pathology [77]. NF-κB is a dimeric transcription factor and the two pathways responsible for its activation are canonical and the alternative pathways [78]. It has been observed that there is an activation of canonical pathway [79-82] involved in a feedback controlled mechanism by which Aβ activates NF-κB, which, in turn, regulates the production of Aβ peptides [80-82], this is seen in several in vitro studies which have suggested that NF-κB could be activated by Aβ peptides in primary cultured neurons [79,80]. This observation is indirectly consistent with the fact that NF-κB has been observed in cells surrounding or within the amyloid plaques [79-82]. An inhibition of NF-κB activation reduces Aβ secretion in vitro [83-85], and it was suggested that this could occur by interfering with βAPP processing [85-90]. According to current data on experimental brain, ischemia is known to cause an over expression, production and activity of β-secretase [91-94].
App and β-Secretase 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 in endoplasmic reticulum and Golgi en route to the cell surface [95].

The subcellular compartments where β-secretase functions have acidic interior, near the optimal pH for its protease activity. Thus the Aβ-secretase inhibitor drug should ideally be able to penetrate these subcellular membranes so as to reach the sites of Aβ production.

In addition, since majority of Aβ in the body is produced in the brain, a β-secretase inhibitor drug must have the ability to penetrate the blood–brain barrier (BBB) [95]. Aβ-secretase inhibitor was conjugated to a carrier peptide for penetrating the BBB was shown to reach the brain and reduce Aβ in AD mice [96]. Due to the absence of a serious phenotype its studied that the elimination of processing for other β-secretase substrates is not a serious safety concern for the clinical application of β-secretase inhibitors, which 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 [97, 98]. Immunization of β-secretase reduced Aβ in the brain which improved the cognitive performance of AD mice [99]. Heterozygous β-secretase knock-out transgenic AD mice with 15% reduction of brain Aβ showed a dramatic reduction of amyloid plaques at old age [100]. Thus β is the potential target molecule that is used for the treatment of AD.

γ-Secretase: After α- and β-cleavage, the carboxyl terminal fragments (CTFs) of APP, known as αCTF and βCTF are further cleaved by γ-secretase so as to generate p83 and Aβ, respectively [13].

The p83 fragment is rapidly degraded and widely believed to possess no important function. γ-secretase-mediated cleavage is unique in that the cleavage takes place within the transmembrane domain, though the exact site can vary. γ-cleavage can yield both Aβ40, the majority species, and Aβ42, the more amyloidogenic species, as well as release the intracellular domain of APP (AICD) [13]. Through several genetic, pharmacological, protein and cell biology studies, it is now clear that γ-secretase is a multi-subunit aspartyl protease that cleaves APP and many other type 1 transmembrane proteins within their transmembrane domains [101, 102]. Presenilin 1 and presenilin 2 (PS1 and PS2) form the catalytic core of γ-secretase, and three accessory proteins, anterior pharynx-defective 1 (APH-1), nicastrin, and presenilin enhancer protein 2 (PEN2) are also a part of the γ-secretase complex [101, 103,
103], these three are responsible for the maturity and stability of γ complex. The γ-secretase complex seems to have a high degree of heterogeneity since humans have two presenilin genes and two APH1 isoforms—APH-1A, which can be alternatively spliced to long and short forms, and APH-1B—and six different functional γ-secretase complexes have been identified [105,106]. However rodents have an extra Aph1C gene, which probably arose due to the duplication of Aph1B [107,108]. Therefore, rodent γ-secretases might display a higher degree of heterogeneity than human γ-secretase. Thus the subunit of the γ-secretase complex which is heterogenic suggests that selective targeting of one particular subunit might be an effective alternative treatment strategy to nonselective γ-secretase inhibition [13]. Selective removal of APH-1B and APH-1C in a mouse model of AD has already been shown to decrease Aβ plaque formation and improve behavioral deficits, without adverse effects attributable to impaired Notch signaling being reported [109]. CD147 is a transmembrane glycoprotein and interacts with all the essential γ-secretase components. Interestingly, downregulation of CD147 increases Aβ production but its overexpression has no effect on Aβ generation [110]. Strong evidence suggests that the γ-secretase complex is located primarily in the ER, Golgi/TGN, endocytic and intermediate compartments, most of which (except the TGN) are not major subcellular localizations for APP [111,112]. In addition to cleaving APP CTFs, γ-secretase cleaves a series of functionally important other transmembrane proteins also, including Notch [113], cadherin [114], tyrosinase [115], ErbB4 [116], CD44 [117], etc.) [118].

The cleavage of various substrates appears to be dependent on the subcellular compartment; APP is mainly cleaved in the TGN. Thus a disturbance in the localization of the γ-secretase complex may play some role in abnormal Aβ generation and Alzheimer’s disease pathogenesis. Current data shows that the microglial cells and astrocytes exhibit an overexpression of presenilin and nicastrin after brain injury [119]. The protein products of the genes on chromosomes 14 and 1 are presenilin 1 and presenilin 2 respectively. According to the first study of presenilin 1 mRNA overexpression in the gerbil ischemic hippocampus, was performed by Tanimukai et al [120]. It was observed that post ischemia there was a selective induction of presenilin 1 gene in neurons of CA3 area and dentate gyrus.

In another research the expression of presenilin1 mRNA was observed in the rat ischemic hippocampus, cortex, cerebellum and striatum and there was an increase in the level of presenilin mRNA in the hippocampus and cortex [121].
But their expression in cerebellum and striatum displayed no significant increase which correlates very well with areas unaffected by Alzheimer’s disease. There is a correlation between the triggered oxidative stress and the increased \( \gamma \)-secretase cleavage of amyloid precursor protein [122,123].

Oxidative stress can influence the \( \beta \)-secretase and \( \gamma \)-secretase activities[123,124,125,126]. Another data have exhibited that a full length presenilin interacts with the immature \( \beta \)-secretase activity via direct interaction and facilitates the trafficking of \( \beta \)-secretase to different compartments of cells [127]. Thus such studies provide a molecular explanation for the role of the oxidative stress in sporadic Alzheimer’s disease pathogenesis.

**CONCLUSION**

Several studies have been conducted in characterizing the molecules involved in APP processing and the functions of the APP cleavage products. Thus overproduction and accumulation of A\( \beta \) in the brain are key pathogenic events in Alzheimer’s disease progression which occurs due to the dysregulation of the secretases which otherwise have physiological roles in the body. Therefore a basic understanding of the biochemistry of the key players in this complex disease is the most important, since it provides the framework for developing drugs to alleviate the severe pathology that these molecules cause.

Ischemic oxidative stress causes an over expression of \( \beta \)-secretase and \( \gamma \)-secretases thereby increasing the \( \beta \) amyloid peptide which results in Alzheimer’s disease. Thus more research in future is possible to prevent the oxidative stress induced formation of \( \beta \) amyloid peptide, which can directly alleviate the disease.

**REFERENCES**


125. Tabaton M, Tamagno E. The molecular link between \( \beta \)- and \( \gamma \)-secretase activity on the amyloid \( \beta \) precursor protein. Cell Mol Life Sci, 2007;64:2211–2218
