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The molecular link between β- and γ-secretase activity on the amyloid β precursor protein

M. Tabaton[a,*] and E. Tamagno[b]

[a] Department of Neurosciences, Ophthalmology and Genetics, University of Genoa, Via De Toni 2, 16132, Genoa (Italy), Fax: +39010506938, e-mail: mtabaton@neurologia.unige.it
[b] Department of Experimental Medicine and Oncology, University of Torino (Italy)

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Abstract. Alzheimer's disease (AD) is characterized by an accumulation in the brain of amyloid β peptides (Aβ). The production of Aβ requires two sequential cleavages induced by β- and γ-secretases on the β-amyloid precursor protein (APP). Altered activity of these secretases is involved in the pathogenesis of AD. The expression and activity of β-secretase (BACE1) is augmented in the brain in late-onset sporadic AD. Mutant presenilin 1 (PS1), the major genetic defect of early-onset familial AD (FAD), alters the activity of γ-secretase, leading to increased production of Aβ42. Here we review the role of oxidative stress as a molecular link between the β- and the γ-secretase activities, and provide a mechanistic explanation of the pathogenesis of sporadic late-onset AD. We also discuss evidence for a role of the same mechanism in the pathogenesis of familial AD carrying PS1 mutations.

Keywords. Alzheimer's disease, BACE1, PS1, γ-secretase, oxidative stress, amyloid β.

Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder affecting the elderly population. The risk of AD increases dramatically in individuals above the age of 70, and it is predicted that the incidence of AD could increase a further threefold over the next 50 years. AD can be classified into two forms: sporadic AD, which accounts for the majority of cases, and a rare familial early-onset form (FAD), in which gene mutations have been identified. The pathological hallmarks of AD are intraneuronal neurofibrillary tangles (NFTs) composed of hyperphosphorylated tau protein and a deposition of amyloid β (Aβ) fibrils in the extracellular space. Central to the disease is an altered proteolytic processing of the Aβ precursor protein (APP), resulting in overproduction and aggregation of neurotoxic forms of Aβ. APP is a receptor-like type I glycoprotein, with a single membrane-spanning domain, a large extracellular N-terminus, and a shorter C-terminus. The amyloidogenic processing of APP involves two sequential cleavages operated by the β- and γ-secretases at the N- and C-termini of Aβ, respectively (Fig. 1a).

The β-secretase cleaves APP at the beginning of the sequence of Aβ, generating an extracellular soluble fragment, called sβAPP, and an intracellular C-terminal end, termed C99. The C99 is further cleaved within the membrane by the γ-secretase, with a broad specificity. The γ-secretase cleavage is a player in a novel physiological mechanism called Regulated Intra-membrane Proteolysis (RIP). The so-called ε-cleavage, occurring between Aβ residues 49 and 50, produces APP intracellular domain 50 (AICD50) [1], and this can translocate to the nucleus where it regulates gene expression, including the induction of apoptotic genes [2, 3]. The γ-cleavage produces Aβ fragments of different length, these being predominantly Aβ40 and Aβ42 with some Aβ38. A recent study identified an intracellular long Aβ species,

* Corresponding author.

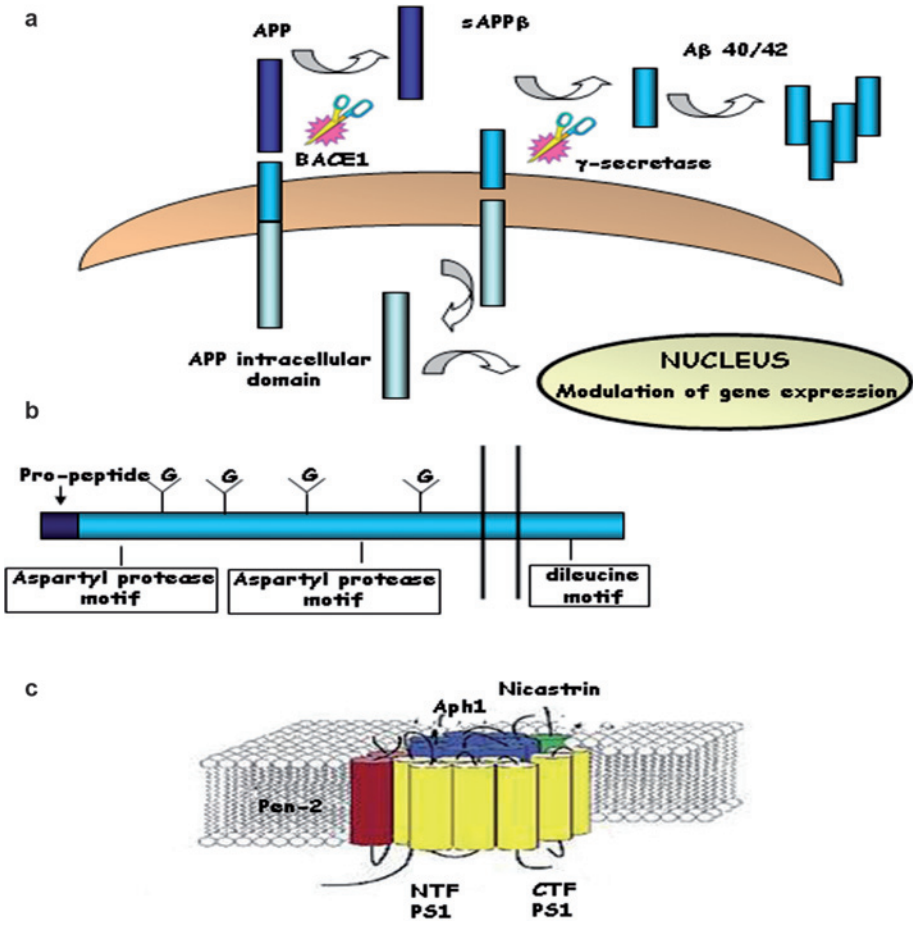

Figure 1. (*a*) Amyloidogenic processing of the amyloid precursor protein (APP). The amyloidogenic pathway involves BACE1 (β-site APP cleaving enzyme) activity, which releases βAPPs and produces an intracellular fragment that is cleaved by the γ-secretase complex. (*b*) The structure of BACE1. Sites for potential N-linked glycosylation are indicated (G). Aspartyl protease signature sequences are boxed, and their locations in the polypeptide are shown. The extreme C-terminus of the molecule is also enlarged to demonstrate the dileucine motif that has been implicated in the endosomal targeting of the protein. (*c*) The γ-secretase complex consists of four core members: presenilin, nicastrin, anterior pharynx-defective 1 (Aph1), and presenilin enhancer 2 (Pen2).

Aβ46, which results from a third cleavage, ζ-cleavage, located between the γ and ε cleavage sites [4]. The finding that γ-secretase inhibitors, such as DAPT and compound E, inhibit the secretion of Aβ40/42, while leading to an accumulation of Aβ46, leads one to question whether Aβ40/42 and Aβ46 are produced by the same or by different enzymes [5]. Moreover, the role of ε- and ζ-cleavage in the formation of Aβ and the relationship between the three cleavage types remain elusive.

The central role of Aβ in the pathogenesis of AD is supported by several lines of evidence. Aggregates of Aβ are neurotoxic and initiate a series of events, including the hyperphosphorylation of tau, which results in neuronal dysfunction and death [6–8]. In addition, all genes bearing mutations that increase the susceptibility to AD, including the genes for APP and presenilins (PS) 1 and 2, and, also, a specific form of apolipoprotein E (apo E) gene, facilitate the accumulation of Aβ42, influencing its production, aggregation, and clearance [9–11]. The cause of modified APP processing and Aβ42 accumulation in sporadic cases of AD is unclear, but could include a series of aging-related events, such as oxidative stress, impaired energy metabolism, and perturbed calcium homeostasis [12–14].

The β-secretase

BACE1 is a 501 amino acid aspartyl protease, with a signal peptide (1–21), a pro-domain (22–45) and a single trans-membrane domain (461–477) [15] (Fig. 1b). The 23-amino acid pro-domain is removed by furin or a furin-like activity enzyme [16, 17], and BACE1 is phosphorylated, N-glycosylated, and palmitoylated [18–20] during its maturation. The enzyme contains a di-leucine motif (LL) which is crucial for regulating the internalization of the protein to the endosomal compartment and also to lysosomes where is degraded [21–23]. The active site involves Asp93 and Asp289 and is on the luminal/extracellular side of the membrane and comprises the β-secretase cleavage site of APP. Thus, BACE1 and APP follow similar trafficking routes and co-localise within endosomes, this providing for optimal BACE1 activity due to a requirement for acidic pH [24, 25]. BACE1 exhibits all the known properties of the β-secretase [26]. BACE1

is known to be the sole β-secretase since Aβ is not synthesized in BACE1 knockout mice [27]. The expression of BACE1 is confined mainly to neurons. Its activity may lead to the release of substrates from neuronal membranes, thereby mediating paracrine signalling to neighbouring cells, that is, functioning in neuron-neuron or neuron-glia interactions. Recently, Willem et al. [28] showed that BACE1 favours peripheral nerve myelination induced by Schwann cells. High levels of BACE1 are expressed when peripheral nerves become myelinated. Moreover, deficiency of BACE1 resulted in the accumulation of unprocessed neuregulin 1, an axonally expressed factor required for glial cell development and myelination.

The γ-secretase complex

PS forms part of a tetrameric complex, including nicastrin, Aph1 (anterior pharynx-defective phenotype) and Pen2 (presenilin enhancer), that acts as the γ-secretase (Fig. 1c). Presenilin (PS) provides the active core of the γ-secretase complex. Two mammalian homologs, PS1 and PS2, are found. PSs are aspartyl proteases whose topology is characterized by seven- to eight-membrane spanning domains, with a sixth and seventh domain, containing aspartate residues (Asp257 and Asp385), being essential for the catalytic activity of the protease. The stabilization of PS is accompanied by a proteolytic maturation cleavage produced by an unknown 'presenilase' [29]. The resulting N-terminal fragment and C-terminal fragment each contribute one aspartyl residue to the catalytic site. PSs regulate the activity of the γ-secretase and determine the generation of Aβ peptides [30, 31]. Levels of Aβ are dramatically decreased in animal models lacking the expression of PS1 [32] but not of PS2 [33, 34], suggesting that among the two presenilins PS1 regulates the activity of γ-secretase and hence Aβ formation. Nicastrin (Nct) is a glycosylated integral membrane protein that binds both the N-terminal and the C-terminal of PS. Nct is synthesized as a precursor protein that requires PS to leave the endoplasmic reticulum to reach the cell surface. In PS-deficient cells, the Nct precursor accumulates in the endoplasmic reticulum. Conversely, the suppression of Nct by small interfering RNA (siRNA) results in decreased steady-state levels of PS, indicating that it is one of the stabilizing factors of fragments of PS [35]. Aph1 and Pen2 have been isolated as part of the γ-secretase complex in two separate genetic screens in *Caenorhabditis elegans* [36, 37]. Aph1 is a multimembrane spanning protein that is required for the correct subcellular transport of Aph1/Nct to the cell surface [38], while Pen2 is small protein and with a hairpin-like structure and that initiates endoproteolysis of PS [39].

BACE1 and the pathogenesis of sporadic AD

A serie of reports suggest that the activity of BACE1 is upregulated in sporadic late-onset AD brains [40–42]. The cause of altered APP processing and Aβ42 accumulation in sporadic AD remains unclear. Oxidative stress is potentially involved in the pathogenesis, since it is an age-dependent phenomenon, and aging is a major risk factor in AD [43]. Oxidative stress and Aβ production are reciprocally linked to each other because Aβ aggregates have been shown to induce oxidative stress both *in vivo* and *in vitro* [44–47]; in addition oxidative stress increases the production of Aβ [48–50]. We have recently proposed that a sequence of events might link oxidative stress, stress-activated protein kinases, BACE1 induction and apoptotic cell death, through an overproduction of Aβ (Fig. 2). Initially, we showed that oxidant agents and 4-hydroxynonenal (HNE), a product of lipid peroxidation, significantly increase the expression, protein levels, and activity of BACE1 in NT_2 neurons, without affecting the levels of full-length APP [51, 52]. These events are followed by a significant increase of intracellular Aβ peptides, as well as by morphological signs of apoptotic cell death. Finally, it was observed that the upregulation of BACE1 is modulated by c-jun-N-terminal kinase (JNK) and $p38^{MAPK}$, also known as stress-activated protein kinases (SAPKs) [49]. These findings suggest that the overproduction of Aβ, dependent on the upregulation of BACE1 induced by oxidative stress, contributes to the pathogenesis of the common, sporadic, late-onset form of AD, a major risk factor for which is aging.

Presenilin gene mutations and the pathogenesis of FAD

Mutations in PS1 and PS2 genes account for the majority of early-onset FAD cases. So far, more than 150 FAD-induced mutations in PS1 have been identified, and approximately 10 mutations have been found in the homologous gene PS2. Study of the effect of PS1 mutations is crucial for understanding the pathogenesis of AD. Mutations in PS genes certainly cause an increase in the ratio of Aβ42/Aβ40 species, as determined in mutant cells and transgenic mice [53–56]. The Aβ42 peptide has increased aggregation properties and is believed to trigger a pathogenic cascade leading to neurodegeneration in AD [57].

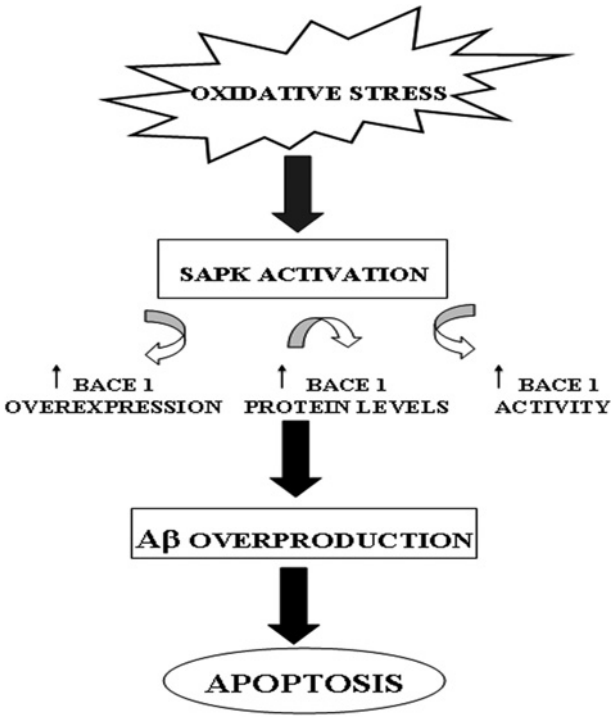

Figure 2. A sequence of the pathogenetic steps of sporadic AD linking oxidative stress, stress-activated protein kinases, BACE1 induction, and apoptotic cell death through overproduction of Aβ.

Indeed, Aβ42, although generated by neurons, at a 10-fold lower rate than Aβ40, is the main component of soluble and insoluble Aβ that accumulates in the AD brain [58]. Alternatively, PS mutations can cause FAD-inducing oxidative stress. In support of this hypothesis, Schuessel et al. [59] demonstrated the presence of oxidative stress in the brain of PS1 M146L transgenic mice, as assessed by increased HNE levels. Interestingly, the effect of the FAD mutation is dependent on the natural aging process, as only aged mice display increased ROS levels and oxidative damage. In addition, mitochondrial dysfunction and increased levels of HNE-modified protein were also detected in a different PS1 mutant transgenic mouse [60, 61]. FAD PS1 mutations result in heterogeneous clinical and pathological phenotypes that cannot simply depend on the extent of Aβ42/40 ratio. Assini et al. [62] described a novel R278K PS1 mutation in a kindred that showed a marked phenotypic variability, characterized by the presence in three of the affected members of paraparesis plus dementia, dementia only or pure paraparesis lasting 12 years. The coexistence of paraparesis and dementia was shown in subjects carrying different deletions of PS1 exon 9 and mutations [63–66]. The data indicate that a phenotype with paraparesis does not completely depend on the rate of the Aβ42/Aβ40 ratio. We were able to show a correlation between the molecular pattern of the Aβ species and the disease phenotype, which may reflect the type and degree of Aβ neurotoxicity. We also performed a comparative study of the association of soluble Aβ species, seen in normal brain aging, with sporadic AD [67]. The results showed the species of Aβ present in physiologically aging brains differed from that of brains with sporadic AD: the full-length Aβ1–42 was the predominant Aβ species in aging brain, while in AD the N-terminally truncated pyroglutamate 3–42 Aβ peptide was the most prevalent. Moreover, the Aβ species associated with AD forms oligomers more quickly, is more neurotoxic, and produces more severe membrane damage than the Aβ species associated with normal aging brain. Another correlation between the FAD phenotype and the pattern of Aβ species has been provided in a case study of an early onset AD brain bearing a PS1 S170F mutation. The disease presented cerebellar syndrome and myoclonus in the third decade followed later by rapidly progressive dementia. A histopathological examination showed a novel pattern of Aβ deposition that was widespread, abundant, and was heavy in the cerebellar cortex, where diffuse amyloid plaques were associated with a severe loss of the Purkinje cell arborization. The PS1 S170F mutation induced a threefold increase of both Aβ40 and Aβ42, and over 90% of the accumulating Aβ species was were N-truncated forms [68]. The N-terminally truncated Aβ peptides are derived from β-secretase cleavage [69]; therefore, the latter finding strongly suggests that β-secretase cleavage is also altered in PS1 mutations. It is tempting to speculate that the combination of different Aβ species, determined by genetic and epigenetic factors, leads to variable conformations of soluble Aβ aggregates that exhibit a large spectrum of toxicity.

The link between the β- and the γ-secretase

The functional interaction between β- and γ-secretase remain unclear. Some observations raise the possibility that PS1 influences the intracellular trafficking and maturation of BACE1 through the APP amyloidogenic pathway [70]. APP, PS1, and BACE1 are transported in the same membrane vesicles along axons, *via* the direct binding of APP to the kinesin light-chain subunit of kinesin-I, a microtubule motor protein. Furthermore, it has been shown that the levels of mature BACE1 are increased in human neuroblastoma cells stably expressing wild type PS1, as compared to native cells. Conversely, the maturation of BACE1 is impaired as a result of a stable expression of a dominant-negative mutant PS1 [71]. Recently, Minopoli and co-workers [72] demonstrated a corre-

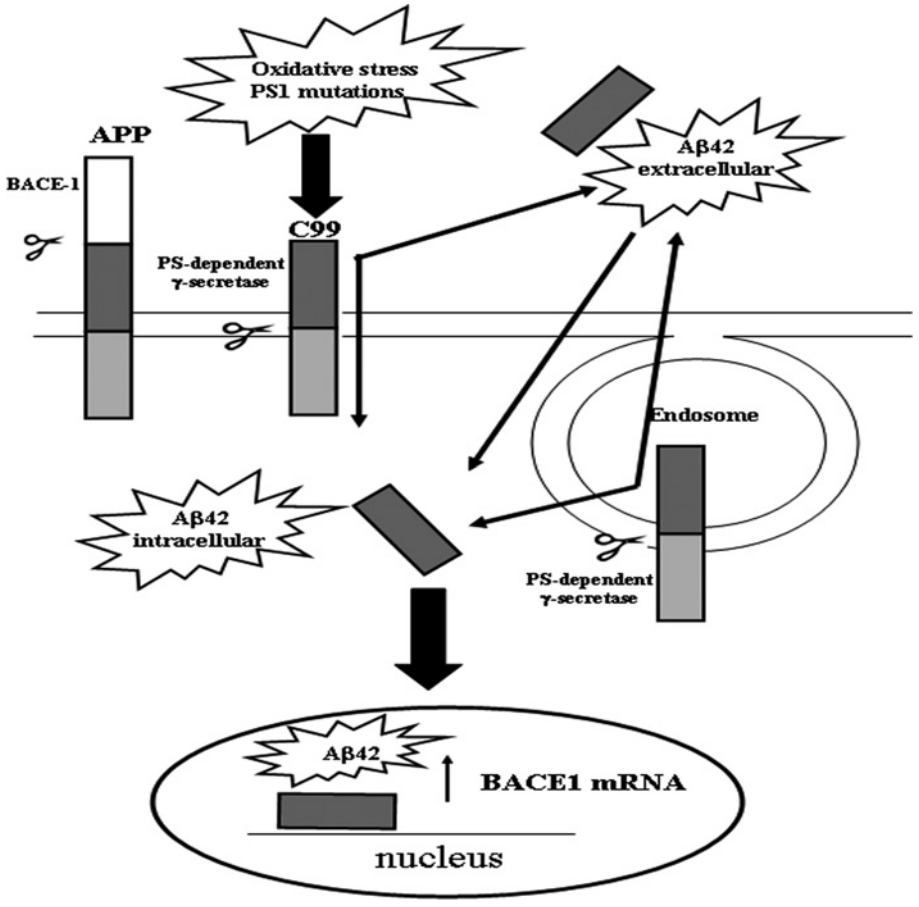

Figure 3. Pathogenetic hypothesis in which the increase in the γ-secretase cleavage of APP mediated by oxidative stress (sporadic AD) or by PS1 mutations (FAD) fosters the expression and activity of BACE1 through the release of Aβ peptides which act as signalling molecules.

lation between the induction of oxidative stress and an increase of γ-secretase cleavage. The pre-treatment of embryonic fibroblasts with hydrogen peroxide or etoposide, which is toxic due to HNE production, are able to drastically increase γ-secretase activity. Given that oxidative stress can mediate both γ-secretase and BACE1 activities, we would predict that oxidative stress is the molecular link between β- and γ-secretase, and that as a consequence the activities of the two endo-proteases are also linked. We have also found that oxidative stress increases PS1 expression and γ-secretase activity. In parallel, we observed that the increased expression of BACE1 is regulated by γ-secretase activity [E. Tamagno et al., unpublished].

These findings provide a mechanistic explanation for the role of oxidative stress in sporadic late-onset AD. Aβ induces oxidative stress and HNE production, which in turn increase the activities of the β- and γ-secretases; this then further enhances Aβ production. Hence, anti-oxidant agents have the potential of preventing AD by suppressing BACE1 and γ-secretase activities.

The molecular link between β- and γ-secretases may also be involved in the pathogenesis of FAD carrying PS1 mutations. The overproduction of N-terminally truncated Aβ peptides, which depends on β-secretase cleavage [69], are more abundant, relative to the full-length Aβ species, in the brains of FAD carrying PS1 gene mutations as compared to those with sporadic AD [73]. This indicates that mutant PS1 could affect the β-secretase activity. This hypothesis is supported by our results showing that PS1 mutations increase the expression and activity of BACE1. The activation of BACE1 requires γ-secretase cleavage of APP and is proportional to the amount of secreted Aβ42 [R. Borghi et al., unpublished].

Together, these findings suggest that an increase in the γ-secretase cleavage of APP mediated by oxidative stress (sporadic AD), or by PS1 mutations (FAD), fosters BACE1 expression and activity. Given that the expression of BACE1 appears to be mediated by γ-secretase cleavage, the substrate of which is APP, we investigated whether the presence of APP or APP derivatives might be essential in determining the expression of BACE1. Preliminary data indeed show that the activation of BACE1, mediated by oxidative stress or PS1 mutations, depends on the presence of APP. We recently proposed a model in which γ-secretase might influence the expression and activity

of BACE1 through the production of Aβ, the APP derivative resulting from γ-secretase cleavage, acting as a signalling molecule [74]. In this model, exogenously applied Aβ42 exerts a biphasic neurotoxic action, depending initially on the soluble form of Aβ and then mediated by aggregated fibrillar Aβ polymers. The soluble form of Aβ exerts a higher neurotoxicity than its fibrillar form because it causes apoptotic and necrotic cell death. The fibrillar form, being less cytotoxic, would activate BACE1 expression and activity, fostering amyloidogenic processing of APP that results in a further accumulation of Aβ. Our data suggest that there may be a positive feedback loop between the γ-secretase and the β-secretase cleavage of APP, mediated by the release of the Aβ peptides, that then act as signalling molecules. It would now be of interest to investigate whether the regulation of BACE1 operated by Aβ depends on its transcriptional activity. It is likely that Aβ42 promotes BACE1 transcription indirectly *via* the activation of intracellular signalling pathways, resulting in turning on transcription factors that elevate BACE1 transcription (Fig. 3).

Previous studies have proposed different physiological functions for Aβ [75, 76]. A particularly convincing finding came from Kamenetz et al. [77], who found that Aβ could be a player in a negative feedback loop that regulates synaptic activity. It appears likely that a more detailed knowledge of the genes activated by Aβ peptides may be a requirement to understand the precise series of events that cause dysfunction and degeneration of neurons in AD.

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