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The ubiquitin-proteasome system in Alzheimer's disease

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Abstract

Accumulation of proteins is a recurring event in many neurodegenerative diseases, including Alzheimer's disease (AD). Evidence has suggested that protein accumulation may result from a dysfunction in the ubiquitin proteasome system (UPS). Indeed, there is clear genetic and biochemical evidence of an involvement of the ubiquitin proteasome system in AD. This review summarizes the data supporting an involvement of the UPS in the pathogenesis of AD, focusing on the data showing the relationship between Aβ and tau, the two hallmark lesions of AD, and the UPS.

Introduction

Over 24 million people worldwide suffer from some form of dementia with 4.6 million new diagnoses made every year and it is estimated that by 2040, 80 million people will be demented [1]. Alzheimer's disease (AD) is the most common neurodegenerative disorder and is responsible for approximately 60% of dementia cases [1]. Short-term memory loss and visual-spatial confusion are one of the earliest clinical manifestations in AD. As the disease progresses, memory loss becomes more severe and patients are unable to recognize familiar objects or persons. Eventually, decline in other cognitive domains manifests, including deficits in attention, language and spatial orientation and patients will not be able to maintain personal independence [2–5].

Pathologically, the AD brain is characterized by prominent atrophy and by a profound loss of neurons and synapses, which is restricted to specific brain regions critical for learning and memory, including the temporal and parietal lobes, the frontal cortex and the cingulate gyrus. In addition to neuronal loss and atrophy, the AD brain has two main lesions, extracellular amyloid plaques and intraneuronal neurofibrillary tangles (NFTs) [6]. Amyloid plaques are mainly formed by a small peptide called amyloid-β (Aβ) [7, 8], which can also accumulate intraneuronally [9], whereas NFTs are formed by hyperphosphorylated tau [10–13].

Aβ is generated by the sequential cleavage of a larger precursor, the amyloid precursor protein

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(APP), which is encoded by a gene located on chromosome 21 [14]. APP can be processed by a non-amyloidogenic pathway or an amyloidogenic pathway. In the non-amyloidogenic pathways, which is the most common, APP is cleaved by the α-secretase enzymes, which cut APP in the middle of the Aβ sequence, therefore precluding the formation of Aβ [15]. In the non-amyloidogenic pathway APP is cleaved by BACE1 [16–18], at the beginning of the Aβ sequence, thus liberating βAPP and a small carboxiterminal fragment, C99. Subsequently, C99 is further cleaved by the γ-secretase complex, formed by PS1 or PS2, Aph1, Pen2 and nicastrin generating Aβ40 and Aβ42 [19–23]. Aβ42 is more amyloidogenic form of Aβ and is the major species that accumulates in the AD brain [24]. Aβ can aggregate to form multimeric complexes of different molecular weights, ranging from low molecular weight oligomers to high molecular weight, highly organized fibrils. Although Aβ fibrils are the major component of the extracellular plaque deposits, recent evidence has elucidated the role of Aβ oligomers in the pathogenesis of AD [25–27].

NFTs are composed of tau, a microtubule-binding protein with several cellular functions, including regulation of cytoskeletal structure and function [28, 29]. Six different tau isoforms have been identified in the adult human brain, which differ by the presence of three or four microtubule binding domains at the C-terminal, represented by 18 amino acid repeat sequences that are tubulin binding sites [30]. At the N-terminal, tau is characterized by the presence or absence of one or two 29 amino-acid inserts. All six isoforms are generated by the alternative splicing of a single gene product [13, 31, 32]. Notably, the ratio of three to four repeats is equal in the adult brain; however, only the four-repeat isoforms are present in the fetal brain [33, 34]. This differential expression likely reflects the more plastic status of the foetal brain where tau is normally more phosphorylated than in the adult brain. The microtubule-binding properties of tau are mainly regulated by post-translational modifications, including phosphorylation at specific serine/threonine sites, glycation, ubiquitylation, sumoylation, nitration, proteolyis and glycosylation [35]. Importantly, there is direct evidence that tau phosphorylation inversely regulates its ability to bind to microtubules [36]. The phosphorylation state of tau is controlled by the activity of several kinases and phosphatases [37–39]. In AD and other tauopathies, tau is abnormally hyperphosphorylated therefore there is an increase in total levels of unbound tau that aggregates to form straight and paired helical filaments that form NFTs [36, 40]. Although evidence showed a correlation between NFTs and the memory decline in AD [41, 42], recent findings have dissociated NFTs with cognition and have indicated that more soluble forms of tau may be more toxic for the cell [43–46]. Although both views are not necessarily mutually exclusive, further studies are needed to elucidate the relationship between different forms of tau and cognitive impairments.

Biology of the UPS

The accumulation of Aβ and tau makes AD a proteins-misfolding disease, or proteopathy, and suggests that alterations in protein quality control mechanisms may be directly or indirectly involved in the disease pathogenesis [47–50]. This review will focus on evidence linking Aβ and tau pathology to the UPS.

Protein clearance by the UPS occurs in two sequential steps, a tagging reaction and a subsequent degradation of the tagged proteins by the proteasome system.

The tagging reaction

Ubiquitin is a small, highly conserved peptide present in all eukaryotic cells that is conjugated to the proteins that needs to be targeted to the proteasome [51]. This process occurs in three steps. First an ubiquitin monomer is activated in an ATP-dependent reaction by the ubiquitin-activating enzyme (E1). Subsequently ubiquitin is transferred to an ubiquitin-conjugating enzyme (E2). In the final step, ubiquitin is transferred to the target protein *via* an ubiquitin ligase (E3). The E3 ligase binds both the target protein and the complex E2-ubiquitin and facilitates the formation of a covalent bond between the ubiquitin monomer from the E2 enzyme and the target protein. Activated ubiquitin molecules are sequentially added to the first ubiquitin proteins to form a polyubiquitin chain [52, 53]. Proteins tagged with chains of four or more ubiquitins are recognized by the 26S proteasome for degradation [52–54]. It is the E3 ligase that confers specificity to the process by selectively

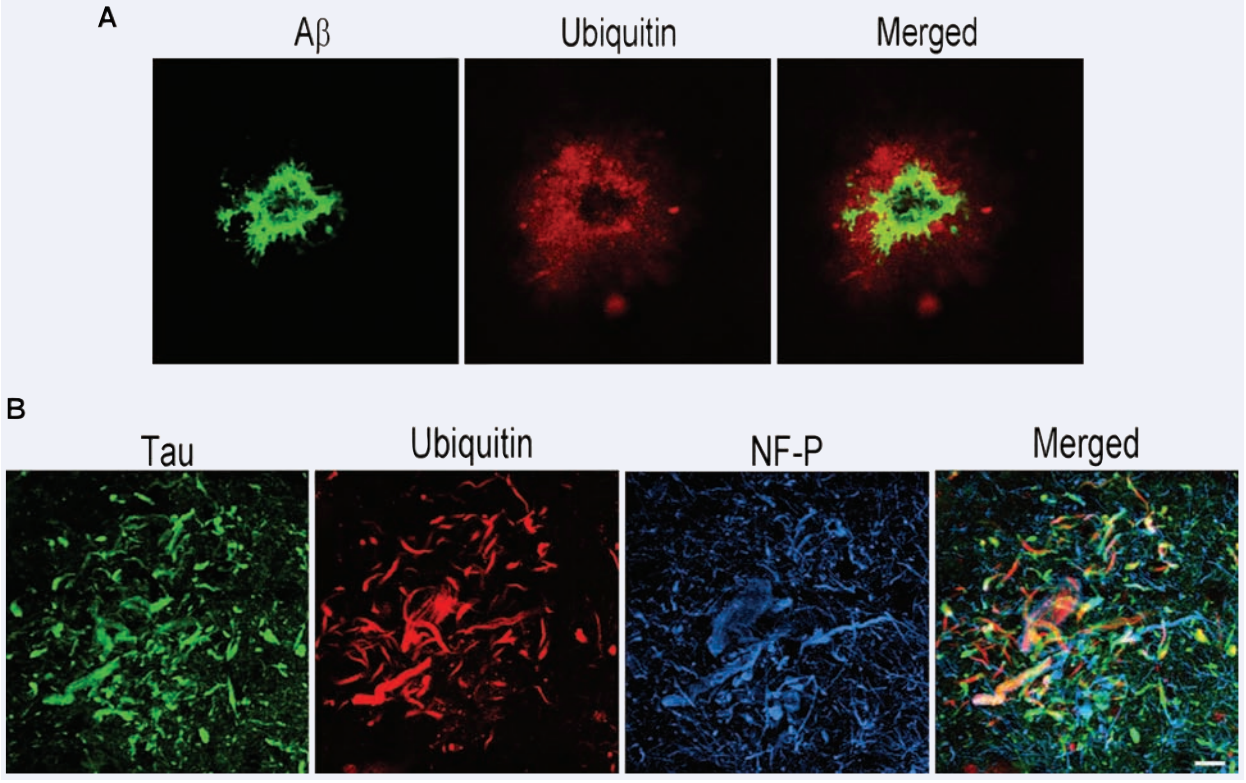

Fig. 1 Early evidence of proteasomal dysfunction in AD demonstrated the presence of ubiquitin-positive structures in AD brains. Representative microphotographs showing that ubiquitinated proteins are associated with amyloid plaques (**A**) and neurofibrillary tangles (**B**) in AD brains.

binding to a protein target. Ubiquitin monomers are liberated after proteasome degradation or are actively removed by the ubiquitin carboxyl-terminal hydrolases [55].

Structure of the proteasome

The proteasome, known as 26S proteasome, is formed by three major subunits, a 20S catalytic core and two 19S regulatory caps. The catalytic core, known as 20S proteasome, has a cylindrical structure formed by four-stacked rings. It contains three distinct proteolytic activities, a tripsin-like activity, a chymotrypsin-like activity and a peptidylglutamyl-like activity [56–58]. At each end of the 20S proteasome is a 19S regulatory subunit, which is formed by two different subcomplexes: a base formed by 10 different proteins that binds to the 20S proteasome and a lid, formed by 9 different proteins that recognize and binds polyubiquitinated proteins. In addition to recognizing the substrates for the 20S proteasome, the regulatory caps facilitate the access of the target proteins into the 20S proteasome by unfolding the substrate and opening the catalytic channel [59].

The involvement of the UPS in AD pathogenesis

Growing evidence suggest that alterations in the UPS function may be involved in AD pathogenesis. This view is supported by evidence showing that in AD brains ubiquitin accumulates in both plaques and tangles (Fig. 1) [60–64]. It has also been shown that these structures contain ubiquitin-B mutant protein (UBB+1), a mutant ubiquitin carrying a 19-amino acid C-terminal extension generated by a transcriptional dinucleotide deletion [65]. Notably, UBB+1 has been

shown to block ubiquitin-dependent proteolysis in neuronal cells [66], to cause neuritic beading of mitochondria in associating with neuronal differentiation [67] and it has been suggested to be a mediator of Aβ-induced neurotoxicity [68].

The AD brain is also characterized by the accumulation of oxidized proteins [69, 70], which may further exacerbate the decrease in proteasome activity [71]. Particularly intriguing is the finding that the ubiquitin carboxy-terminal hydrolase L1 (UCH-L1), an enzyme that hydrolyses ubiquitin from poly ubiquitinated proteins to liberate ubiquitin monomers, is oxidized in AD and is down-regulated in the specific brain regions of early AD cases [72, 73]. Finally, changes in proteasome subunit composition have been reported in the AD brain [74]. Taken together, these data strongly argue that dysfunctional UPS function maybe involved in AD pathogenesis. This view is further strengthened by recent genetic evidence showing positive association between AD and several single-nucleotide polymorphisms in UBQLN1, which encodes for an ubiquitin-like protein called ubiquilin [75].

Direct evidence of altered proteasome activity in AD brains has been reported [76, 77]. In particular, Keller and colleagues, demonstrated a selective decrease in proteasome activity in specific brain regions of AD cases. Very intriguing was the finding that proteasome activity was decreased in brain regions, such as the hippocampus, that are more susceptible to the AD pathology, whereas other less susceptible brain regions, such as the cerebellum, exhibited no changes in proteasome activity between AD and controls [77].

The interaction between Aβ and the proteasome

Growing evidence supports an interaction between Aβ and the proteasome system. In particular, early *in vitro* work using biochemical and scanning transmission electron microscopy experiments showed that Aβ40 directly binds to the inside of the proteasome along the peptide channel and selectively inhibits the chymotrypsin-like activity of the 20S proteasome [78, 79]. More recent evidence shows that Aβ42 also impairs proteasome activity [80, 81]. In particular it has been shown that Aβ42 can inhibit proteasome function at the same extent as a known proteasome inhibitor [81], raising the possibility that Aβ may be an endogenous inhibitor of the proteasome. These studies provide strong *in vitro* evidence that Aβ impairs proteasome function. One important question was if different assembly states of Aβ interacted differentially with the proteasome (*e.g.* monomers *versus* oligomers). This is pivotal as in the last few years there has been a growing appreciation of the toxic capacities of Aβ oligomers [25–27]. For example, it has been shown that Aβ oligomers, but not monomers or fibrils, inhibit long-term potentiation *in vivo* [27]. To determine how different assembly states of Aβ affect proteasome activity, we used a cell-free proteasome activity assay and found that Aβ40 and Aβ42 oligomers significantly decrease the tripsin-like activity, the chymotrypsin-like activity and the peptidylglutamyl-like activity of the proteasome in a dose-dependent manner [82]. Particularly interesting is the finding that Aβ toxicity can be mediated by its interaction with the proteasome. Analysis of gene expression profile of rat primary cortical neurons incubated with aggregated Aβ further supported a link between Aβ and the UPS [68]. In this work, the authors identify an ubiquitin-conjugating enzyme, E2-25K/Hip2 as a mediator of Aβ neurotoxicity [68]. Along these lines, it has been shown that the Aβ-induced synaptic dysfunction can be rescued by increasing expression of UCH-L1 [83].

Work in transgenic animal models of AD also supports a relationship between Aβ accumulation and UPS function. Towards this end, Oh and colleagues showed an inverse relationship between Aβ accumulation and proteasome function in the brains of mice overexpressing APP, suggesting the possibility that Aβ42 accumulation may be responsible for an age-dependent decrease in proteasome function detected in the brains of these mice [81]. Similarly, there was ~50% reduction in proteasome activity in primary neurons isolated from APP transgenic mice compared to neurons isolated from wild-type mice [84]. Moreover, a near complete normalization in proteasome activity to wild-type levels was obtained when APP mutant neurons were treated with a γ-secretase inhibitor [84], strongly suggesting a direct involvement of Aβ in the reduction of proteasome function. Using a transgenic animal model (3×Tg-AD) that develops both plaques and tangles in an age-dependent manner [85], we showed that proteasome activity was significantly decreased in the brains of 6- and 9-month old 3×Tg-AD mice but not in the brains of 12-month old mice [82]. These age-dependent changes in proteasome activity in the

3×Tg-AD mice correlate with the levels of intraneuronal Aβ, which are higher in 6- and 9-month old mice compared to 12-month old mice [86, 87]. The proteasome deficits were rescued by Aβ immunotherapy [82], thus confirming the hypothesis that Aβ accumulation impairs proteasome function *in vivo*. Further studies will be needed to confirm this hypothesis; in particular it will be important to determine if proteasome function increases in APP KO mice or in wild-type mice after blocking Aβ production.

The accumulation of Aβ is dependent of the balance between Aβ production and degradation. It is well established that different proteases are involved in Aβ degradation [88–91]. There is also evidence suggesting that Aβ is degraded by the proteasome. Lopez Salon and colleagues showed that upon inhibition of the 26S proteasome *via* lactacystin, there was a 40% and 50% decrease in radio-labelled Aβ42 in astrocytes and neurons, respectively [80]. Consistent with these results, it has been shown that in a cell-free assay, the 20S proteasome degrades both Aβ40 and Aβ42 [82]. Moreover, we showed a striking increase in intracellular Aβ40 and Aβ42 in N2A cells treated with a proteasome inhibitor [82]. To determine if Aβ is degraded by the proteasome *in vivo*, we injected a proteasome inhibitor into the cerebral ventricle of 4-month old 3×Tg-AD mice and measured Aβ levels 72 hrs later. Consistent with the *in vitro* data, these experiments showed that proteasome inhibition resulted in a significant increase in intraneuronal Aβ levels [82]. Taken together these data suggest that in addition to being degraded by specific proteases (*e.g.* IDE, NEP and ECE), Aβ is also degraded by the proteasome. Considering the well established decrease in proteasome function during aging [92, 93], and the data reported above, it is tempting to speculate that the age-dependent proteasome dysfunction may participate to the accumulation of Aβ in AD brains. Further supporting this idea, it has been shown that both PS1 and PS2 are degraded by the proteasome [94], thus a decrease in proteasome activity would likely increase γ-secretase activity and Aβ production.

A major unresolved question is how Aβ physically interacts with the proteasome. Proteasomes are found in the plasma and nucleus but are also associated with plasma and internal membranes [95]. In addition, a study using immuno-EM showed that the 20S subunit of the proteasome was also present in the outer membranes and inner vesicle of the multivesicular bodies [84]. Considering that Aβ is produced in the membranes [9] where the presence of the proteasome has been reported, it is possible that Aβ-proteasome interaction may occur there and not in the cytoplasm. At this point, this is just a possibility and further studies are necessary to clarify where Aβ and the proteasome interact.

The interaction between the UPS and tau

The degradation systems responsible for tau catabolism, a 'natively unfolded' protein, are not completely clear. It has been reported that tau can be cleaved by several proteases including calpains, caspases, cathepsins and thrombin. There is also growing evidence suggesting an involvement of the UPS in tau turnover. Towards this end, Keck *et al*. showed that the 20S proteasome co-precipitated with tau aggregates. Most notably, they showed that the amount of tau aggregates pulled down with an antibody to the 20S proteasome was higher in samples with low proteasome activity, suggesting an inhibitory interaction between tau aggregates and proteasome activity [76]. To further support this view, they showed, *in vitro*, that tau aggregates isolated from human AD brains can inhibit the proteasome, whereas non-aggregated tau isolated from AD brains or normal tau isolated from control brains was not able to do so [76]. These data show that different aggregation states of tau can dictate tau turnover *via* the proteasome.

There is also evidence that tau can be degraded by the proteasome. It has been shown that proteasome inhibition in cell culture inhibits tau degradation [82, 96]. Similar results were obtained by another group showing that inhibitors against the trypsin-like and glutamyl-like activities almost completely blocked tau degradation [97]. More directly, these authors also showed that tau was degraded after incubation with the 20S proteasome *in vitro* [97]. Taken together these studies provide strong experimental evidence for the involvement of the UPS in tau turnover.

Particularly interesting are the findings highlighting the role of ubiquitination in tau turnover, especially in light of the data showing that alteration in the ubiquitin-dependent proteasomal degradation may be involved in neurodegeneration [98]. To this end, it has been shown that tau co-immunoprecipitates with the carboxy terminus of heat shock protein70-interacting

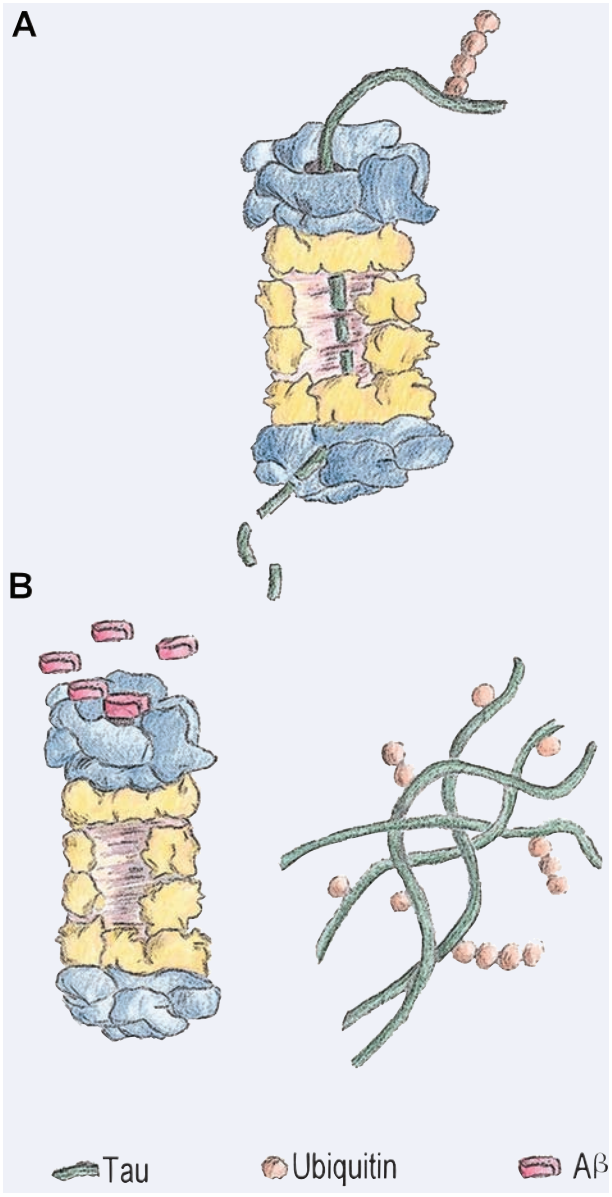

Fig. 2 Schematic representation of a possible scenario by which Aβ can mediate tau accumulation *via* the proteasome. During normal conditions, ubiquitinated tau is targeted to the proteasome for turnover (**A**). Aβ deposit can inhibit the proteasome impairing its normal function. As a consequence, tau cannot be degraded by the proteasome and accumulates into NFT (**B**).

protein (CHIP), an E3 ubiquitin ligase that ubiquitinates tau for degradation by the proteasome [99, 100]. These data were strongly supported by a recent work published by Dickey and colleagues showing that soluble phosphorylated tau accumulate in the brains of CHIP knockout mice [101]. Taken together, these data clearly indicate how proteasome activity is necessary for tau turnover but aggregated tau inhibits the proteasome. Normally a substrate to be bound by an E3 ligase, must undergo post-translational modification such as phosphorylation or oxidation. It remains to be established which post-translational modification have to occur in tau for it to be bound by CHIP. One hypothesis is that during tau pathogenesis, the CHIP-binding site on tau is unavailable, thus tau cannot be targeted to the proteasome. This hypothesis is consistent with data indicating that 'normal' tau and soluble tau that have not undergone major structural changes are degraded by the proteasome, whereas hyperphosphorylated and aggregated tau is resistant to proteasome degradation [76].

The UPS as a link between Aβ and tau interaction

Evidence from human genetic and transgenic animal models strongly supports a primary role of Aβ in AD pathogenesis. Particularly, the amyloid cascade hypothesis stipulates that Aβ is the upstream trigger of all cases of AD [102]. A major implication for this hypothesis is that Aβ accumulation is upstream of tau. Recent works in transgenic animals have supported such hierarchical interaction [43, 87, 103–107]; however, the molecular mechanisms underlying this link are just starting to get unveiled. To better understand the mechanism by which Aβ and tau are linked, we injected anti-Aβ antibodies into the brains of 3×Tg-AD mice and show that a week after the injection, there was a marked decrease in the Aβ deposits [103]. Most notably, we showed that Aβ clearance led to a significant reduction in early tau pathology but not late aggregated tau deposits. The mechanism underlying the tau clearance *via* an anti-Aβ antibody is mediated by the proteasome as concomitant injection of an anti-Aβ antibody with a proteasome inhibitor led to a reduction of Aβ deposits but no changes in tau pathology were detected [103]. These data indicate that the accumulation of Aβ may impair proteasome function thus facilitating tau accumulation. However, once Aβ is

cleared, normal proteasome function is reestablished and early tau deposits can be removed. In contrast, even if proteasome function is restored after removal of Aβ, aggregated tau cannot be removed by the proteasome [103]. This view is consistent with data showing that aggregated tau is not degraded by the proteasome and actually inhibits it [76].

Further supporting a role for the proteasome in the Aβ and tau interaction is the data showing an impairment of proteasome activity in the 3×Tg-AD mice that correlates with an increase in Aβ oligomers [82]. Remarkably, accumulation of Aβ and tau was found after direct inhibition of proteasome activity in the 3×Tg-AD mice [82]. Taken together, these data strongly suggest that the proteasome is a molecular link between Aβ and tau pathology (Fig. 2). Further studies will need to elucidate how Aβ-dependent proteasome inhibition can lead to tau accumulation. Considering the clear role of CHIP in tau removal, it is tempting to speculate that Aβ accumulation may alter CHIP function thus leading to the accumulation of tau.

Conclusions

The data reviewed here provide evidence that proteasome dysfunction may be involved in AD pathogenesis. It is tempting to speculate that the age-dependent decrease in proteasome activity may lead to the accumulation of both Aβ and tau. Additionally, once Aβ and tau aggregate, they can further decrease proteasome activity creating a vicious circle leading to more Aβ and tau accumulation. While the age-dependent decrease in proteasome activity seems to be a normal aging process, only a proportion of people accumulate Aβ and tau, thus other unknown mechanism may be involved in this vicious circle. A better understanding of these mechanisms may facilitate the identification of new pathways that may decrease and/or prevent the age-dependent proteasome dysfunction thus breaking the above-mentioned vicious circle.

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