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"Characterization of conformational changes in the catalytic core of γ-Secretase: Implications for Alzheimer's disease pathology and normal aging"

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SUMMARY

Alzheimer's disease (AD) is characterized by neuronal loss, deposition of senile plaques composed of amyloid-β (Aβ) peptide, and accumulation of tau-containing neurofibrillary tangles. Numerous biochemical and genetic studies point to Aβ as an essential trigger for the disease, even though neuropathological features such as neurofibrillary tangles, synaptic loss and neuronal loss show better correlation with dementia progression and severity of the disease. Aβ peptides of various lengths are generated after sequential cleavage of the amyloid precursor protein (APP) by β- and γ-secretases. The catalytic subunit of the γ-secretase enzyme complex is a transmembrane protein named Presenilin 1 (PS1). Topological studies suggest that PS1 adopts an intra-membranous ring-like structure, with N- (NT) and C-termini (CT) in close proximity, which is critical for its function. More than 160 mutations have been identified that spread through the entire sequence of the PS1 molecule, and are associated with early-onset familial Alzheimer's Disease (fAD). We have previously shown that fAD-linked mutations in PS1 lead to a consistent change in PS1 conformation, alter APP positioning within the membrane and affect PS1/γ-secretase alignment with the APP substrate.
By employing Förster-resonance energy transfer (FRET)-based imaging our laboratory has previously determined that a close PS1 NT-CT proximity correlates with predominant cleavage of APP at ≥Aβ42 position, whereas an "open" PS1 conformation is associated with predominant cleavage at ≤Aβ40, resulting in an increase or decrease of the Aβ42/40 ratio, respectively, thereby establishing PS1 conformation as a reliable readout for the Aβ42/40 ratio in vitro and in vivo. Longer and more aggregation-prone Aβ species, such as Aβ42, are implicated in neurotoxicity linked to AD. Strong evidence, that the ratio of the Aβ42 to Aβ40 peptides, rather than absolute Aβ levels, plays a pivotal role in neurodegeneration has been presented recently.
In brain tissue of sAD patients, when compared to age-matched normal controls, an increased Aβ42/40 ratio was observed. Furthermore, a small but significant increase in the Aβ42/40 ratio was shown to occur during normal aging in the wildtype mouse brain. This suggests that changes in PS1 conformation similar to those caused by fAD mutations may occur in sAD and- in a similar matter but to a lesser extend- also during normal aging. To be able to reduce generation of the toxic Aβ≥42 species, it is crucial to identify factors that are upstream and that can modulate the precision of APP cleavage by PS1/γ-secretase. There is extensive literature supporting a role for oxidative damage in the pathogenesis of AD and oxidative stress is a well-known age-related factor. An interdependent link between Aβ and oxidative stress is well established; Aβ has been shown to induce oxidative stress and pro-oxidants, in turn, increase Aβ production.

In the current study we show that pathogenic conformational changes in endogenous wildtype PS1, similar to those found with mutant PS1, occur in the brain during normal aging and are observed in sporadic AD. By employing the FLIM assay in brain tissue sections we present evidence for a significant increase of the proportion of neurons with "closed" PS1 conformation in sAD cases, compared to cognitively normal age-matched controls. We also show that PS1 conformational changes are more pronounced in neurons close to Aβ plaques.
Using a mouse model of AD we found that these changes accelerate in an age-dependent manner and, importantly, precede Aβ-deposition in the brain. This pathogenic change in PS1 seems to precede plaque deposition in the Tg2576 mouse model of AD. Importantly, we found that the observed phenomenon also occurs in wildtype mice as they age. Furthermore, we provide evidence that PS1 conformational shift is critically influenced by stress factors that are unique to the microenvironment of Aβ-plaques, and that oxidative stress alters PS1 conformation *in vitro*. The results of this study provide important information about the timeline of pathogenic changes in PS1 conformation during aging. Finally, we provide a molecular mechanism by which oxidative stress may trigger Aβ accumulation and toxicity in aging neurons and in sporadic AD brain.