Distribution of cross-linking enzymes, their activity and their substrates in Alzheimer's disease brain

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Research question and background

Alzheimer's disease (AD) is characterized by accumulation of amyloid-beta protein (Aβ) in senile plaques (SPs) and cerebral amyloid angiopathy (CAA). Aβ interacts with itself resulting in Aβ multimers, oligomers, protofibrils and mature fibrils, as found in SPs and CAA. However, it is unclear what triggers this Aβ aggregation process. A possible cause for this aggregation-cascade is an enzyme-induced conformational change of Aβ resulting in so-called 'seed' to which more Aβ protein could bind. Enzymes that interact with Aβ and change its conformation or bioactivity via post-translational modifications might therefore play a crucial role in the initiation of the aggregation process. Transglutaminases (TG) are calcium-dependent enzymes that catalyze several reactions, amongst which the formation of molecular cross-links. TG2 or tissue transglutaminase (tTG) is linked to neurodegenerative diseases, and presence of tTG and its activity in SPs and CAA in AD have been reported recently by our group (Wilhelmus et al. Brain Pathol 2009). Another cross-linking enzyme is lysyl oxidase (LOX), a copper-dependent extracellular matrix (ECM) amine oxidase. Altough LOX is linked to the central nervous system, information on the role of LOX in the pathogenesis of AD hallmarks is very limited.

AIM: studying the association of cross-linking enzymes, in particular tTG and LOX, their activity and their substrates with the pathological hallmarks of AD, using immunohistochemisty, biochemical assays and specific enzyme-activity assays on snap-frozen brain tissue.

Methods and tissues used

Immunohistochemistry and specific enzyme-activity assays on snap-frozen cryo sections of AD cases with substantial (CAA), Aβ Braak stage C, Neurofibrillary tangles (NFT) Braakstage 5 or 6, as well as control cases with Aβ Braak stage O, NFT Braakstage 0 or 1 was performed.

Results and conclusion

We found presence of tTG in early stage CAA, where it colocalised with the deposited Aβ. In contrast, in later stages of CAA, both tTG and its cross-links do not colocalise with the deposited Aβ anymore, but are present in an abluminal and luminal halo enclosing the Aβ deposition. Furthermore, tTG substrates and important ECM components colocalise with tTG and its cross-links in the halos that enclose Aβ deposition in CAA. Together our data suggest that tTG plays a unique role in CAA development and progression. Initially, tTG levels are elevated in early stages of CAA, and may even precede Aβ deposition, whereas in later stages tTG is likely to be involved in alteration and/or remodelling of the ECM in CAA.

In addition, we demonstrated association of LOX with CAA and both classic and diffuse SPs in AD cases. In addition, we found LOX staining in reactive astrocytes associated with these pathological lesions. Based on our findings, we suggest that LOX is involved in the formation and progression of both CAA and SPs in AD.