Let's not forget: Peptidases in Alzheimer’s disease

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SUMMARY AND CONCLUSIONS
SUMMARY

In this thesis, we investigated the role of peptidases in Alzheimer’s disease (AD). AD is characterized by extracellular aggregates consisting of amyloid-β (Aβ) peptides and accumulation of these peptides starts decades before the onset of the first symptoms. We described which peptidases degrade the Aβ peptide and how their levels and activity change during the progression of AD. We are particularly interested in changes that occur in the presymptomatic stages of AD. Understanding of the processes involved in these stages may allow for therapeutic intervention before the disease becomes manifest. Furthermore, we investigated whether changes in peptidase activity in presymptomatic stages of AD can be used to diagnose the disease.

Production and secretion of the Aβ peptide can be affected by neuronal activity. In early stages of AD, neurons are hyperactive, but the role of neuronal hyperactivity is not known. In chapter 2, we discuss the two-faced nature of neuronal hyperactivity; neuronal hyperactivity can serve as a protective mechanism to deal with early pathological changes in the brains of patients, but may also be a pathogenic contributor to AD. Specific molecular mechanisms by which neuronal (hyper)activity affects production and degradation of the Aβ peptide are also reviewed.

In chapter 3, we examined changes in peptidase activity in the brains of presymptomatic AD patients and AD mouse models. We analyzed degradation rates of a fluorogenic Aβ40 peptide (qAβ40) that becomes fluorescent upon degradation, which enables detection of early changes in Aβ clearance during progression of AD. The insulin-degrading enzyme (IDE) was identified as the main peptidase degrading the monomeric qAβ40, but not oligomeric forms of qAβ40. Degradation rates of qAβ40 were measured in post mortem hippocampal tissue of AD patients, and we showed for the first time that qAβ40 degradation is already decreased in the presymptomatic stages of the disease. This decrease was correlated with reduced protein levels of IDE. We also provide evidence that two commonly used AD mouse models do not mimic the alterations in Aβ clearance as observed in human AD. qAβ40 degradation was not diminished during AD development and IDE activity and levels were not decreased in those mouse models. Therefore we state that these mouse models are not suitable to study early changes in Aβ degradation in human AD.

In chapter 4, we investigate whether measuring qAβ40 degradation can serve as a diagnostic and prognostic tool for early AD. In cerebrospinal fluid (CSF) but not in blood plasma of AD patients, a decrease in qAβ40 degradation could be detected already in the presymptomatic stages. This decrease was coincided with decreased IDE levels. However, in ante mortem CSF obtained by lumbar puncture, qAβ40 degradation did not correlate with clinical diagnosis and did not differentiate
stable mild cognitive impaired (MCI) subjects from MCI subjects that converted to AD within 2 years. In conclusion, qAβ40 degradation was not proven to be a diagnostic or prognostic marker by itself, but a combination of the qAβ40 degradation assay and other biomarkers such as Aβ42 levels, may increase diagnostic accuracy. Chapter 5 describes a filed patent for the application of quenched Aβ40 peptide degradation as a diagnostic and prognostic tool in AD. In chapter 6, we describe in detail the method that uses quenched fluorescent peptides to study peptide degradation in living cells and cell lysates. The use of peptidase inhibitors can help to elucidate the role of different peptidases in degradation of specific peptides.

In chapter 7, we study gene expression changes before, during and after the development of AD in an AD mouse model with the use of microarray technology. Gene expression was analyzed in the prefrontal cortex, which is already affected early during disease progression, and especially expression of genes involved in the immune response and activation of glia cells was increased. This is in contrast to gene expression changes in the prefrontal cortex of AD patients. Here, expression was increased of genes involved in synaptic activity and plasticity. In conclusion, the AD mouse models may be used to study immune responses during AD development, but are not representative for progression of AD in patients.

In chapter 8, we describe a proteomic screen that was performed by stable isotope labeling by amino acids in cell culture (SILAC) to identify (in)direct proteomic targets of tripeptidyl peptidase II (TPP2). TPP2 is involved in many cellular processes, including antigen presentation, cancer, cell proliferation and DNA repair. We investigated proteins that alter their expression upon TPP2 inhibition by butabindide or B6 (a newly developed inhibitor) or TPP2 knock down. Levels of phosphorylated ERK1 and ERK2 appeared to be reduced in the nucleus and downstream pathways were inhibited. We suggest that TPP2 affects the cellular processes mentioned above by regulating ERK1 and ERK2 phosphorylation. In addition, we showed for the first time that diminished TPP2 activity resulted in stronger synaptic contacts and increased levels of the amyloid precursor protein (APP) and that TPP2 may therefore be involved in neurological disorders such as AD.

In chapter 9, we investigate the effect of TPP2 activity on levels of APP. By using a pulse-chase assay in which newly synthesized APP was radiolabeled, we showed accumulation of O-glycosylated APP in the Golgi complex upon knock down of TPP2. After inhibition of TPP2 in SH-SYSY cells and organotypic hippocampal mouse brain slices, higher levels of C-terminal APP fragments were detected that resulted from α-secretase cleavage (CTF-α), suggesting increased processing of APP via the non-amyloidogenic pathway. Therefore, TPP2 seems to regulate APP levels and processing by regulating its cellular trafficking and we suggest a possible role for TPP2 in AD.
CONCLUSIONS

In this thesis we show that in presymptomatic stages of AD Aβ degradation is decreased. We suggest that especially the diminished levels of IDE contribute to less efficient Aβ degradation in these stages and that it may initiate Aβ accumulation, plaque formation and the progression to AD. We also showed that measuring Aβ degradation rates in ante mortem CSF may become a diagnostic tool, but only in combination with other biomarkers. Furthermore, TPP2 activity affects APP levels, processing and trafficking and we suggest a role for TPP2 in AD. This thesis contributes to a better understanding of the role of peptidases in neurodegenerative disease, and we show that these peptidases are important players in the initiation and progression of AD.