Scope and outline

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The primary aim of this thesis is to gain a better understanding of the molecular mechanisms that occur during the development and progression of Alzheimer’s disease (AD), the most prevalent form of dementia. AD progression is neuropathologically classified by the six Braak stages which are defined by the amount and localization of neurofibrillary tangles in the brain. Neurofibrillary tangles form, next to amyloid beta (Aβ) plaques, gliosis and neuronal loss, a major hallmark of AD. Despite extensive research, the etiology, especially of the sporadic form of the disease, still remains poorly understood. Understanding the molecular events that occur early in the disease process might reveal possibilities for the development of future therapeutic strategies against AD that could be applied early in the disease process and prevent its progression. We therefore focus on the early molecular changes in AD since these initial changes may be the cause of the severe cognitive decline in later stages of the disease.

In chapter 1 we review the latest emerging evidence on causal factors for sporadic AD. This review identified insulin dysfunction, pathological cerebrovascular changes, dysfunction of mitochondria-associated membranes and/or synaptic changes as potential disease-initiating factors. They are likely to interact with each other and initiate and facilitate the development of AD pathology by accelerating the production and deposition of Aβ.

In order to directly investigate the molecular changes that occur during the development and progression of sporadic AD, we generated genome wide gene expression profiles of the human prefrontal cortex at all Braak stages using microarrays (see chapter 2). We discovered a concerted increase in the expression of a set of genes involved in synaptic activity and plasticity. This increase occurs early in Braak stage II, just before the onset of the first clinical and neuropathological symptoms of AD and is paralleled by an increase in intraneuronal Aβ levels. These data form the basis for chapters 3 and 5, where we study protein changes during the progression of AD (chapter 3) and investigate the functional relationship between synaptic activity and plasticity and Aβ levels (chapter 5).

In chapter 3 we describe the protein localization and expression of 10 genes (RER1, SST, PSEN2, EGR1, ZNT3, BDNF, SPON2, C4B, LMOD1 and FOXJ1) using immunohistochemistry and Western blotting. These genes showed a clear dysregulation in their mRNA expression profiles on the microarray and are mostly involved in Aβ generation or breakdown. We show that half of the studied genes showed protein expression changes similar to their mRNA changes at different Braak stages indicating that changes in mRNA expression do not always predict changes in protein expression in the human cortex.

Transgenic mouse models are commonly used to investigate AD. In chapter 4 we studied whether the APPswe/PS1dE9 mouse model would be a suitable model for functional characterizations of the gene expression and protein changes
observed in AD. Using microarrays, we generated gene expression profiles of the frontal cortex of APPswe/PS1dE9 mice before, during and after the development of plaque pathology. Our particular focus was whether synaptic changes observed in the human AD brain are replicated in this AD mouse model. The results indicate that this mouse model for AD mimics only the inflammatory changes observed in late stage AD patients while the global gene expression changes in these mice are very distinct from those observed in the human brain.

In chapter 5 we applied bio-computational analyses and cell culture studies to investigate the changes in synaptic plasticity and activity genes in AD. The concerted changes in the expression of these genes suggest an underlying regulatory mechanism that directs gene expression during the progression of AD. Employing a transcription factor binding site analysis, we identified a significant enrichment of early growth response 1 to 4 (Egr1 to 4) and myocyte enhancer factor-2 c (Mef2c) transcription factor binding sites in the promoters of the set of genes involved in enhanced synaptic activity and plasticity in early Braak stages. As these transcription factors showed expression profiles very similar to the ones of synaptic activity and plasticity genes, we investigated whether Egr/Mef2c transcription factors are direct modulators of the increase in expression of synaptic activity and plasticity genes in early stages of AD. Furthermore, we investigated whether overexpression of Egr and Mef2c transcription factors alters the effects of Aβ on primary neurons. We demonstrate that Egr and Mef2c transcription factors directed the regulation of synaptic activity and plasticity genes that are upregulated in early stages of the disease (Braak II). Moreover, we show that Egr/Mef2c transcription factors rendered neurons in culture more vulnerable to toxic effects of Aβ.

We discuss the results obtained in the preceding chapters in chapter 6 and we focus on two main questions: 1. how to use human microarray data for further studies on the molecular mechanisms involved in AD? and 2. how toxic is synthetic, extracellular Aβ for cultured cells?. We also discuss the limitations of the work presented in this thesis and we propose future in vivo and electrophysiological experiments to answer questions that arose during the studies presented here.
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