Molecular changes during the development of Alzheimer's disease
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Summary

Alzheimer’s disease (AD) is the most common form of dementia contributing to up to 70% of all dementia cases. It is characterized by deposition of amyloid β (Aβ) plaques, neurofibrillary tangles formed by hyperphosphorylated tau, gliosis, progressive neurodegeneration and synaptic dysfunction. AD progression is neuropathologically classified by the six Braak stages which are defined by the distribution of neurofibrillary tangles in the brain (Braak and Braak, 1991). While the familial, early onset form of AD is known to be caused by several mutations in genes encoding presenilin 1, presenilin 2 or amyloid β precursor protein, the underlying mechanism leading to the development of sporadic, non-inherited forms of AD is still not known. In this thesis we focused on the early molecular changes in AD since a better understanding of these initial changes may lead to the development of new therapeutic strategies aimed at preventing 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 dysfunctional insulin signaling, cerebrovascular changes and dysfunctions of mitochondria associated membranes and 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. Together these events have an influence on many other prominent, early occurring pathological features of AD including increased oxidative stress and free radical formation, DNA damage, disturbed energy metabolism and synaptic and neuronal degeneration.

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 (chapter 2). In the prefrontal cortex, senile plaques and neurofibrillary changes start to appear around Braak stage III, allowing for the detection of changes in gene expression before, during and after the onset of Alzheimer’s disease neuropathology. We detected two distinct patterns of tightly co-regulated gene groups: i) genes that were down-regulated in Braak stage II and upregulated from Braak stage III onwards and ii) genes that were upregulated in Braak stage II, but downregulated in later Braak stages. Most interestingly, we discovered a concerted increase in the expression of a set of genes involved in synaptic activity and plasticity in Braak stage II, just before the onset of the first clinical and neuropathological symptoms of AD. This increase was paralleled by an increase in intraneuronal Aβ levels. The functional implications of the increase of synaptic activity and plasticity for the development of AD remained, however, unclear: the increase in synaptic activity and plasticity may represent an endogenous compensatory mechanism that initially counteracts the detrimental effects of increased Aβ levels on synapse function, and this compensation may be lost in later stages of AD when cognitive decline becomes apparent. Alternatively, it is possible that the increase of synaptic activity and plasticity precedes the dysregulation of Aβ levels and contributes to a pathological buildup of Aβ, thereby causing synaptic dysfunction and later cognitive decline.

In chapter 2 and 3 we describe the protein localization and expression of 10 genes (RER1, SST, PSEN2, EGR1, ZNT3, BDNF, SPON2, C4B, LMOD1 and FOXJ1) by immunohistochemistry and Western blotting. These genes showed a clear dysregulation in their mRNA expression profile on the microarray and are mostly involved in Aβ generation or breakdown. We found that half of the studied genes showed protein expression changes similar to their mRNA changes at different Braak stages (RER1, PSEN2, ZNT3, C4B, EGR1, SST). The protein expression of the other genes did not resemble their mRNA profiles. This shows that changes in mRNA expression do not always directly predict changes in protein expression in the human cortex. One should be aware of these limitations when using mRNA levels as direct predictors for functionally relevant protein changes between samples of healthy and diseased subjects.

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 the functional characterizations of gene expression and protein changes observed in sporadic AD with a focus on synaptic changes. Using microarrays, we studied the genome-wide gene expression profiles in the frontal cortex of APPswe/PS1dE9 mice at the age of 2, 3, 6, 9, 12, and 15-18 months to investigate transcriptional changes that are associated with Aβ plaque formation and buildup. The gene expression profiles in the frontal cortex showed a pattern of elevated expression of genes with a function in the immune system as response to Aβ plaque buildup. We did not detect changes in genes involved in synaptic transmission or plasticity. A direct comparison of regulated gene transcripts showed that only 3 genes, plexin domain containing 2, complement component 4b, and Slc14a1 were significantly upregulated in both the mouse and human brain. These findings indicate that the cortex of the APPswe/PS1dE9 mouse is not a good model system for functional studies of synaptic activity and plasticity genes altered in sporadic AD.
The concerted changes in the expression of genes suggest an underlying regulatory mechanism that directs gene expression during the progression of sporadic AD. In chapter 5 we applied biocomputational analyses and cell culture studies to investigate transcription factors as regulatory mechanisms for concerted transcriptional changes in synaptic activity and plasticity genes. Employing a transcription factor binding site overrepresentation 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 stage II. As these transcription factors showed expression profiles very similar to the genes encoding proteins involved in synaptic activity and plasticity, 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. We show that an overexpression of Egr1, 2 and 4 individually induced the expression of up to 29% of the genes, while combinatorial expression of Egrs and Mef2c, specifically the combinations Egr1/4, Egr2/3, Egr1/3/4 and Mef2c/Egr1/2/3, induced more than 39% of the target genes regulated in early AD. This indicates that these transcription factors can indeed modulate the expression of synaptic activity and plasticity genes that are altered 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 show that Egr1, Egr2, Egr4 and Mef2c overexpression rendered these cells sensitive to Aβ associated neurotoxicity since extracellular Aβ induced death in primary neurons overexpressing these transcription factors. Overexpression of APP-CT100, the β-secretase product of APP and precursor to Aβ, increases production of intracellular Aβ. We found that overexpression of APP-CT100 decreased neurite length in primary neurons. These results form the basis for future experiments to investigate the effects of Egr transcription factors in combination with APP-CT100 overexpression on synaptic plasticity.

Taken together, we discovered that

**A set of synaptic activity and plasticity genes is increased in expression at early stages (Braak stage II) of AD.**
This finding highlights the existence of broad and concerted molecular changes in the brain of AD patients that occur prior to the occurrence of Aβ plaques and cognitive deficits.

**Aβ elicits an immune response in the APPswe/PS1dE9 mouse, but no changes in genes directly involved in synaptic function.**
This finding shows that this transgenic mouse does not replicate changes in synaptic activity and plasticity gene expression that occur in AD.

**The transcription factors Egr1 to 4 and Mef2c direct the concerted upregulation of a set of synaptic activity and plasticity genes that are also upregulated in the human brain during the early stages of AD**
This finding shows that changes in transcription factors may be causally responsible for synaptic alterations in AD.

**Overexpression of Egr1 to 4 and/or Mef2c renders neurons sensitive to Aβ neurotoxicity**
This finding shows that Egr1 to 4 and Mef2c might be directly involved in facilitating Aβ mediated neurotoxicity.