The role of protein kinases in Alzheimer's disease

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General Summary

Recent studies suggest that the pathogenesis of Alzheimer's disease (AD) might be a result of neuroinflammation in response to stress, which is followed by alterations in neuronal physiology and is accompanied by synaptic loss, neuronal dysfunction and subsequent neuronal death. AD is characterised by a long pre-clinical phase (20-30 years), during which significant brain pathology manifests itself. Neuroinflammation and synaptic failure are considered early events in the course of AD and are hence attractive targets for the prevention and pharmacological intervention to slow down or halter disease progression. Substantial evidence implicates aberrant protein kinase signalling in AD. An increase in kinase activity results in hyper-phosphorylation of the tau protein and the formation of toxic aggregates (tangles) in neuronal cells, while the extracellular amyloid beta protein (Aβ) is phosphorylated and forms extracellular insoluble deposits (plaques).

In **Chapter 1** the result of literature data mining of the NCBI PubMed database is presented using each of the 523 known protein kinases in combination with the search term 'Alzheimer's disease'. A list of protein kinases implicated in either 'neuroinflammation' and/or 'synaptic changes' in AD was generated.

To shine light onto early events in AD pathogenesis and the role of protein kinases, protein kinase profiling of 100 hippocampal post mortem brain tissue lysates of patients with AD and non-demented controls (Braak stages O-VI) was performed using a peptide-based microarray platform. The results show an overall decrease of protein kinase activity, which correlates well with disease progression. Protein kinase activity already decreases at pre-clinical stages of AD pathology (Braak I-III). Bioinformatics analysis (STRING) in combination with pathway analysis allowed the identification of the Ephrin-receptor A1 (EphA1) kinase, a risk gene for AD, and sarcoma tyrosine kinase (Src), which is involved in memory formation. In addition, protein kinases that have not previously been associated with AD were identified such as protein tyrosine kinase 6 (PTK6/BRK), feline sarcoma oncogene kinase (FES) and fyn-associated tyrosine kinase (FRK) (**Chapter 2**).

Even though protein kinase CK2 (former casein kinase II) is one of the best-studied kinases, its involvement in AD is far from clear. We find increased levels of CK2 in the hippocampus and temporal cortex of AD patients compared to non-demented controls. CK2 immunoreactivity is specifically increased in astrocytes that are associated with amyloid deposits, one of the hallmarks of AD. The function of CK2 was investigated using human U373 astrocytoma cells and human primary adult (HPA) astrocytes. Stimulation with IL-1β or TNF-α results in the secretion of pro-inflammatory cytokines MCP-1 and IL-6. The CK2 inhibitor CX-4945 shows a dose-dependent reduction of IL-1β or TNF-α secretion. These results suggest that CK2 might be considered a potential drug target for the modulation of neuroinflammation in AD (**Chapter 3**).

Activation of signalling pathways through chronic neuroinflammation is thought to lead to the activation of tau kinases, tau dysfunction followed by the dysfunction of synapses. The aberrant signalling at synapses and the subsequent loss of neurons occur early in AD and correlate with the cognitive decline observed in patients with mild cognitive impairment. The Eph receptor A4 (EphA4) kinase that we identified (**Chapter 2**) had been linked to memory loss in a transgenic mouse model for AD.

This inspired an investigation into the localization of EphA4 in human hippocampal tissue of AD and non-demented control cases. We found that the total amount of EphA4 is the same in AD compared to controls. In contrast, the localization of EphA4 immunoreactivity is altered as EphA4 is found in plaque-like structures in AD cases and co-localizes with neuritic plaques, one of the hallmarks of AD pathology. This re-distribution is already apparent at early stages (Braak stage II) (**Chapter 4**).

In order to test whether the observed re-localization affects the kinase activity, EphA4 kinase activity in human hippocampal brain tissue lysates derived from AD and non-demented control cases was compared. A new kinase immunodepletion assay (KID) was developed and used in combination with kinase activity profiling. Employing KID made it possible to selectively deplete EphA4. Interestingly, protein kinase activity of EphA4 remains unchanged in AD as compared to controls, revealing that the re-distribution of EphA4 happens independently of its kinase activity (**Chapter 5**).

The need for biomarkers that reflect early stages of AD pathology led us to explore the potential of using protein kinase activity profiling as a clinical biomarker for AD. We show that serine/threonine protein kinase activity is present in clinical CSF and that the activity is significantly lower in AD compared to controls (p-value < .05). Kinases that are differentially active in CSF include members of the CaMK family and the AGC kinase group (**Chapter 6**).

In **Part V, Chapter 7** the findings of this thesis are discussed. A few examples of protein kinases involved in early processes in AD pathophysiology such as neuroinflammation and synaptic changes are presented. In addition, the therapeutic potential of protein kinases as well as kinase inhibitors and activators currently in clinical tests are mentioned.