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Candidate biomarkers for synaptic pathology: neurogranin, neuroligins and neurexins in neurodegenerative disorders

Akademisk avhandling

Som för avläggande av medicine doktorsexamen vid Sahlgrenska akademin, Göteborgs universitet, kommer att offentligen försvaras i **R-aulan**, Hus R-SU/Mölndals sjukhus, fredagen den **4 Juni** 2021, klockan **9:00**

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Avhandlingen baseras på följande delarbeten

I. Becker B, Nazir F H, Brinkmalm G, **Camporesi E**, Kvartsberg H, Portelius E, Bostrom M, Kalm M, Hoglund K, Olsson M, Zetterberg H, and Blennow K. *Alzheimer-associated cerebrospinal fluid fragments of neurogranin are generated by Calpain-1 and prolyl endopeptidase*. Molecular Neurodegeneration, 2018. **13**(1): p. 13-47.

II. Nazir F H, **Camporesi E**, Brinkmalm G, Lashley T, Toomey C E, Kvartsberg H, Zetterberg H, Blennow K, and Becker B. *Molecular forms of neurogranin in cerebrospinal fluid.* Journal of Neurochemistry, 2020. **00**(1): p.1-18

III. **Camporesi E**, Tammaryn L, Johan G, Lantero-Rodriguez J, Hansson O, Zetterberg H, Blennow K, and Becker B. *Neuroligin-1 in brain and CSF of neurodegenerative disorders: investigation for synaptic biomarkers.* Acta Neuropathologica Communications, 2021. **9**(1): p. 9-19

IV. **Camporesi E**, Johanna N, Vrillon A, Cognat E, Hourregue C, Zetterberg H, Blennow K, Becker B, Brinkmalm A, Paquet C, and Brinkmalm G. *Quantification of the trans-synaptic partners neuroligin-neurexin in CSF of neurodegenerative diseases by parallel reaction monitoring mass spectrometry*. Manuscript

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Candidate biomarkers for synaptic pathology: neurogranin, neuroligins and neurexins in neurodegenerative disorders

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Abstract

Synapses are small units through which neurons communicate in the brain. They represent the site of memory formation and cognitive abilities and are thus primarily affected by neurodegenerative diseases such as Alzheimer's disease (AD), the leading cause of dementia in the elderly population. Despite great research efforts, there is no definitive cure to date. Moreover, AD diagnosis is challenged by comorbidities and the long prodromal asymptomatic disease stage that conceal early pathological changes in the brain. These pathological changes affect synapse structure and function and cause the cognitive decline observed in AD and other neurodegenerative diseases. Furthermore, synaptic degeneration has been described as an early event in AD and as a better correlate of the degree of cognitive decline or disease severity than *e.g.*, amyloid-β deposits, a major pathological hallmarks of AD. For these reasons, synaptic proteins are considered candidate biomarkers for the detection of early pathological changes in AD and to track cognitive decline. The study of synaptic proteins is further important to understand pathological events affecting synapses and to discover novel biomarkers to detect such changes.

In this thesis, we investigated the postsynaptic proteins neurogranin (Ng) and neuroligins (Nlgn) in brain tissue and cerebrospinal fluid (CSF), focusing on AD. These initial investigations were expanded to include the presynaptic binding partners of Nlgns, the neurexins (NRXN), to gain an overview of changes of this class of synaptic adhesion proteins in neurodegenerative diseases. The aim was to identify changes in concentration and fragmentation patterns of these proteins during AD and to evaluate the usefulness of NRXNs and Nlgns as biomarkers for synaptic pathology in neurodegenerative diseases, primarily AD.

The investigation into proteolytic processing of Ng led to the identification of two enzymes that can cleave the protein, probably in sequence, generating the C-terminal Ng fragments previously described to appear in increased levels in the CSF of AD patients. Additionally, CSF was found to contain full-length Ng of different molecular forms and new N-terminal fragments. These studies revealed the complexity of Ng's processing during AD and open up for further investigations of the potential utility of these new species as biomarkers for synaptic pathology in AD. The study of the transmembrane proteins Nlgn and NRXN were directed toward their soluble extracellular fragments, which are cleaved off and released in response to synaptic activity. Initial investigations for Nlgn1 showed that its fragment levels were decreased in different brain regions in AD. However, the greatest differences were found in frontal grey matter of a group of primary tauopathies, showing marked reduction, results that encourage further investigations of the protein in those diseases. The simultaneous quantification of Nlgns and NRXNs with a targeted parallel reaction monitoring mass spectrometry method in CSF revealed no changes in the levels of the targeted protein fragments along the disease continuum, suggesting that these proteins do not reflect synaptic dysfunction during AD.

In conclusion, the research conducted in this thesis has increased our understanding of the role of the processing of the synaptic proteins Ng, Nlgns and NRXNs and provides the groundwork for future investigations of those proteins in AD and other neurodegenerative diseases. Furthermore, they will inform the development of new tools to monitor synaptic dysfunction and increase the interpretability of those proteins as biomarkers in neurodegenerative diseases.

Keywords: synaptic dysfunction, Alzheimer's disease, biomarkers, neurogranin, neurexins, neuroligins, cerebrospinal fluid, brain tissue

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