

Increasing the interpretability of Alzheimer-related biomarkers: cell- and cerebrospinal fluid-based studies with focus on neurogranin

Akademisk avhandling

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av Faisal Hayat Nazir

Fakultetsopponent:

Henrik Ahlenius, Associate Professor
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Avhandlingen baseras på följande delarbeten

- I. Bergström P, Agholme L, **Nazir FH**, Satir TM, Toombs J, Wellington H, Strandberg J, Bontell TO, Kvartsberg H, Holmström M, Boreström C, Simonsson S, Kunath T, Lindahl A, Blennow K, Hanse E, Portelius E, Wray S, Zetterberg H. *Amyloid precursor protein expression and processing are differentially regulated during cortical neuron differentiation*. Sci Rep. 2016 Jul 7;6:29200.
- II. **Nazir FH**, Becker B, Brinkmalm A, Höglund K, Sandelius Å, Bergström P, Satir TM, Öhrfelt A, Blennow K, Agholme L, Zetterberg H. *Expression and secretion of synaptic proteins during stem cell differentiation to cortical neurons*. Neurochem Int. 2018 Dec;121:38-49.
- III. Becker B, **Nazir FH**, Brinkmalm G, Camporesi E, Kvartsberg H, Portelius E, Boström M, Kalm M, Höglund K, Olsson M, Zetterberg H, Blennow K. *Alzheimer-associated cerebrospinal fluid fragments of neurogranin are generated by calpain-1 and prolyl endopeptidase*. Mol Neurodegener. 2018 Aug 29;13(1):47.
- IV. **Nazir FH**, Camporesi E, Brinkmalm G, Zetterberg H, Blennow K, Becker B. *Molecular forms of neurogranin in cerebrospinal fluid*. Manuscript.

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Increasing the interpretability of Alzheimer-related biomarkers: cell- and cerebrospinal fluid-based studies with focus on neurogranin

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Abstract

Biomarkers for Alzheimer's disease (AD) is a growing field of research. A particularly vibrant field during recent years has been biomarkers for synaptic dysfunction. Sensitive assays for a synaptic protein called neurogranin (NRGN) have produced very interesting results when applied on cerebrospinal fluid (CSF) from AD patients and there are several other biomarker candidates that are thought to reflect different aspects of AD pathophysiology. The aim of this thesis was to investigate the expression and secretion of selected Alzheimer-associated biomarkers in a newly developed model of stem cell-derived cortical neurons that recapitulate the *in vivo* time frames of cortical development. For NRGN, we further investigated the processing and detection of its various molecular forms in CSF.

First, human induced pluripotent stem cell (hiPSC)-derived cortical neurons were used to determine the expression and processing of one of the core AD biomarkers, amyloid precursor protein (APP)-derived amyloid beta (A β). Our findings suggested that APP was expressed throughout the differentiation, but its processing shifted during neuronal stages. The AD-associated amyloidogenic pathway was activated in mature cortical neurons. Although amyloid and tau pathology are the defining neuropathological lesions, synaptic dysfunction and degeneration are thought to be the earliest events in AD. Thus, secreted synaptic proteins in CSF during neurodegeneration could serve as potential AD biomarkers; a notion that has been supported by several studies on changes in concentration of NRGN in CSF in AD during recent years. To learn more about this biomarker, its expression and secretion were investigated in hiPSC-derived cortical neurons. We also examined three additional markers, namely synaptotagmin-1, SNAP-25 and GAP-43. NRGN, synaptotagmin-1 and SNAP-25 expression peaked in mature neurons, while GAP-43 expression was highest in immature cortical neurons and its secretion peaked in mature cortical neurons. The increased expression of synaptic proteins coincided with neurite network formation, which suggests that secretion of these proteins to the extracellular space reflects synapse maturity.

For one of the synaptic proteins, NRGN, C-terminal peptides have been detected at increased levels in CSF from AD patients. Nonetheless, the enzyme(s) that generate these peptides were not known. Here, we identified calpain 1 (CALP1) and prolyl endopeptidase (PREP) as enzymes that cleave NRGN and its fragments. The fragments generated through cleavage by human CALP1 and PREP may suggest an increase in the activation and/or expression of these enzymes in AD. Further, CSF analysis revealed the presence of several molecular forms of NRGN that may represent NRGN fragments, monomers and oligomeric forms, or complexes of NRGN with yet unidentified binding partners. Furthermore, we determined that the ratio of C-terminal fragments to total-NRGN was about 50% in a CSF pool.

Taken together, the results of this thesis show that a human-derived neuronal model can teach us a great deal on biomarker processing and secretion into biofluids, which may increase the interpretability of the biomarker results and tell us more about the underlying disease processes, which they may reflect.

Keywords: Alzheimer's disease, biomarker, neurogranin, APP, human iPSCs, CSF

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