Targeted Aβ proteomics – A tool to study the pathogenesis of Alzheimer's disease

AKADEMISK AVHANDLING

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Avhandlingen baseras på följande arbeten:

I. **Portelius, E**; Westman-Brinkmalm, A; Zetterberg, H; Blennow, K. Determination of β-amyloid peptide signatures in cerebrospinal fluid using immunoprecipitation-mass spectrometry. Journal of proteome research. 5, 1010-1016, 2006

II. **Portelius, E**; Zetterberg, H; Andreasson, U; Brinkmalm, G; Andreasen, N; Wallin, A; Westman-Brinkmalm, A; Blennow, K. An Alzheimer's disease-specific β-amyloid fragment signature in cerebrospinal fluid. Neuroscience letters. 409, 215-219, 2006

III. **Portelius, E**; Tran, A; Andreasson, U; Persson, R; Brinkmalm, G; Zetterberg, H; Blennow, K; Westman-Brinkmalm, A. Characterization of amyloid β peptides in cerebrospinal fluid by an automated immunoprecipitation procedure followed by mass spectrometry. Journal of proteome research. 6, 4433-4439, 2007

IV. **Portelius, E**; Price, E; Brinkmalm, G; Stiteler, M; Olsson, M; Persson, R; Westman-Brinkmalm, A; Zetterberg, H; Simon, AJ; Blennow, K. A novel pathway for amyloid precursor protein processing. Neurobiology of aging. In press, 2009

V. **Portelius, E**; Zhang, B; Gustavsson, M; Brinkmalm, G; Westman-Brinkmalm, A; Zetterberg, H; Lee, V; Trojanowski, J; Blennow, K. Effects of γ-secretase inhibition on the amyloid β isoform pattern in a mouse model of Alzheimer's disease. Submitted

VI. **Portelius, E**; Andreasson, U; Ringman, JM; Buerger, K; Daborg, J; Buchhave, P; Hansson, O; Harmsen, A; Gustavsson, M; Hanse, E; Galasko, D; Hampel, H; Blennow, K; Zetterberg, H. Distinct cerebrospinal fluid amyloid β peptide signatures in sporadic and *PSEN1* A431E-associated familial Alzheimer's disease. Submitted

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ABSTRACT

Alzheimer's disease (AD) is an age-related progressive neurodegenerative disorder of the central nervous system. Diagnosis and monitoring of sporadic AD has long depended on clinical examination of individuals with end-stage disease. The accumulation of amyloid-β (Aβ) peptides in specific brain regions is believed to represent the earliest event in the pathogenesis of the disease and there is developing consensus for the use of cerebrospinal fluid (CSF) Aβ as a core biomarker for the mild cognitive impairment stage of AD.

Aβ has been the subject of extensive research aimed at identifying markers for the disrupted balance between the production and clearance of the peptide. Many studies on Aβ in plasma, cell media, and CSF have been based on immunoassays such as enzyme-linked immunosorbent assays where specific antibodies are used to discriminate between for example the 40- and 42-amino acid long Aβ peptides (Aβ1-40 and Aβ1-42, respectively). The aim of this thesis was to develop a targeted Aβ proteomic approach using immunoprecipitation (IP) and mass spectrometry (MS).

To study Aβ in CSF, a highly specific IP was developed and combined with MS. Using various Aβ specific antibodies with different epitopes, more than 20 Aβ isoforms have so far been identified and verified. Furthermore, a relative abundance pattern including Aβ1-16, and Aβ1-42 in CSF, distinguished sporadic AD patients from non-demented control subjects with a high degree of accuracy in two independent studies.

The IP-MS method was automated and further optimized which improved the speed of sample preparation and thus sample capacity. By adding isotopically labelled internal standards, variations in the IP and the MS desorption/ionization processes were diminished. This increased the possibility of using the method in AD diagnostics and of estimating the concentration of the Aβ isoforms present in CSF.

To address from which processing pathways the shorter isoforms arise, for example Aβ1-15/16/17, a cell model accurately reflecting the Aβ isoform pattern in CSF was developed. The optimized and automated IP-MS method was used to determine changes in the Aβ isoform pattern induced by α-, β-, and γ-secretase inhibitor treatment. All isoforms longer than and including Aβ1-17 were γ-secretase dependent, whereas shorter isoforms were γ-secretase independent. These shorter isoforms, including Aβ1-15, were reduced by treatment with α- and β-secretase inhibitors, suggesting the existence of a third and previously unknown APP processing pathway.

The described APP processing pathway was further investigated by exploring the effects of γ-secretase inhibition on the Aβ isoform pattern in brain and CSF from transgenic mice. As in the cell model, all fragments longer than and including Aβ1-17 decreased upon γ-secretase inhibition, whereas the shorter isoforms, e.g. Aβ1-15, increased. These data, together with the cell model data, strongly suggest that Aβ1-15 and Aβ1-16 may be generated through a third metabolic pathway by concerted β- and α-secretase cleavage of APP.

Key words: Alzheimer's disease, cerebrospinal fluid, immunoprecipitation; mass spectrometry, β-amyloid

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