

Evaluation of the Endo-Lysosomal System and the Ubiquitin-Proteasome System in Neurodegenerative Diseases

Akademisk avhandling

Som för avläggande av medicine doktorexamen vid Sahlgrenska akademien, Göteborgs universitet, kommer att offentlig försvaras i R-aulan, Hus R, SU/Mölnads Sjukhus, Göteborgsvägen 31, Mölndal, fredagen den 16 mars 2018, klockan 09:00

av Simon Sjödin

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Avhandlingen baseras på följande delarbeten

- I. **Sjödin S**, Öhrfelt A, Brinkmalm G, Zetterberg H, Blennow K, and Brinkmalm A. Targeting LAMP2 in human cerebrospinal fluid with a combination of immunopurification and high resolution parallel reaction monitoring mass spectrometry. *Clinical Proteomics* 2016, 13:4.
- II. **Sjödin S**, Hansson O, Öhrfelt A, Brinkmalm G, Zetterberg H, Brinkmalm A, and Blennow K. Mass Spectrometric Analysis of Cerebrospinal Fluid Ubiquitin in Alzheimer's Disease and Parkinsonian Disorders. *Proteomics Clinical Applications* 2017, 11:11-12.
- III. **Sjödin S**, Brinkmalm G, Öhrfelt A, Parnetti L, Paciotti S, Hansson O, Hardy J, Blennow K, Zetterberg H, and Brinkmalm A. CSF Levels of Endo-Lysosomal Proteins and Ubiquitin in Alzheimer's and Parkinson's Disease. Manuscript.
- IV. **Sjödin S**, Woollacott I, Brinkmalm G, Foiani M, Heller C, Lashley T, Öhrfelt A, Blennow K, Brinkmalm A, Rohrer J, and Zetterberg H. Cerebrospinal Fluid Levels of Lysosomal Proteins and Ubiquitin in Clinical and Familial Subtypes of Frontotemporal Dementia. Manuscript.

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ABSTRACT

Neurodegeneration is the process of neuronal cell loss where the symptoms will reflect the regions affected. Neurodegenerative disorders including Alzheimer's disease (AD), Parkinson's disease (PD) and frontotemporal dementia (FTD) are all characterised by an accumulation of protein aggregates suggesting impaired production or turnover of these proteins. Hence, dysfunctional proteostasis is implicated in neurodegenerative disorders. In AD, there is a decreased turnover of endocytic and autophagic vesicles and an accumulation of endo-lysosomal proteins and ubiquitin in brain tissue. Lysosomal dysfunction has been indicated in PD by the link of disease risk and genetic alterations associated with lysosomal storage disorders as well as by decreased expression of lysosomal proteins in disease afflicted regions. Disease causing mutations and genetic risk factors in FTD suggest altered function of the autophagic and endo-lysosomal system to be involved in the pathogenesis.

The aim of this thesis was to examine dysfunctional proteostasis in neurodegenerative diseases by developing assays to monitor proteins from the autophagic and endo-lysosomal system and the ubiquitin-proteasome system in human cerebrospinal fluid (CSF). Proteins from the endo-lysosomal system and the ubiquitin-proteasome system have been identified and quantified in CSF using mass spectrometry (MS)-based proteomics. Principally, three methods have been developed; 1) lysosomal membrane protein LAMP2 was purified from CSF by immunoprecipitation followed by tryptic digestion and quantification by liquid chromatography (LC) and parallel reaction monitoring MS (PRM-MS); 2) full length ubiquitin was isolated from CSF by solid-phase extraction (SPE) followed by quantification by LC PRM-MS; and 3) finally, a panel of endo-lysosomal proteins, *e.g.*, LAMP2, and ubiquitin, were analysed using tryptic digestion, peptide isolation by SPE and quantification by LC PRM-MS. CSF samples from cohorts including subjects with AD, PD, clinical FTD subtypes and FTD mutation carriers, as well as controls, were analysed with the developed assays.

In AD the CSF levels of several endo-lysosomal proteins, including LAMP2, were elevated compared to controls. CSF ubiquitin was also found to be elevated in AD compared to controls. In contrast, CSF levels of endo-lysosomal proteins and ubiquitin in PD were found to be decreased. Investigation in clinical subtypes of FTD and mutation carriers showed limited alterations in the CSF levels of endo-lysosomal proteins, suggesting dysfunctional proteostasis not to be readily detected in CSF in FTD. Our results showing altered CSF levels of proteins involved in proteostasis in AD and PD might indicate pathological alterations in the autophagic and endo-lysosomal system and the ubiquitin-proteasome system. Although further studies are needed, CSF ubiquitin in AD and endo-lysosomal proteins and ubiquitin in PD might serve as potential biomarkers in these disorders.

Keywords: Alzheimer's disease, Parkinson's disease, frontotemporal dementia, dysfunctional proteostasis, cerebrospinal fluid, mass spectrometry

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