Göteborg, 2019

Tau fragments: role as biomarkers and in the pathogenesis of Alzheimer's disease and other tauopathies

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 11 october 2019, klockan 09:00

av Claudia Cicognola

Fakultetsopponent:
Marc I. Diamond, MD, Professor
UT Southwestern, USA

Avhandlingen baseras på följande delarbeten

I. **Cicognola C**, Brinkmalm G, Wahlgren J, Portelius E, Gobom J, Cullen NC, Hansson O, Parnetti L, Costantinescu R, Wildsmith K, Chen H, Beach TG, Lashley T, Zetterberg H, Blennow K, Höglund K. *Novel tau fragments in cerebrospinal fluid: relation to tangle pathology and cognitive decline in Alzheimer's disease.* Acta Neuropathologica, 2019 Feb;137(2):279-296.

II. Blennow K, Chen C, **Cicognola C**, Wildsmith K, Manser P, Sanabria Bohorquez S, Zhang Z, Xie B, Peng J, Hansson O, Kvartsberg H, Portelius E, Zetterberg H, Lashley T, Brinkmalm G, Kerchner G, Weimer R, Ye K, Höglund K. *Cerebrospinal fluid levels of a tau fragment ending at amino acid 368 correlate with tau PET: a candidate biomarker for tangle pathology in Alzheimer's disease.* Submitted to *Brain*.

III. Foiani MS, **Cicognola C**, Ermann N, Woollacott IOC, Heller C, Heslegrave AJ, Keshavan A, Paterson R, Ye K, Kornhuber J, Fox NC, Schott JM, Warren JD, Lewczuk P, Zetterberg H, Blennow K, Höglund K, Rohrer JD. *Searching for novel CSF biomarkers of tau pathology in frontotemporal dementia – an elusive quest*. Journal of Neurology, Neurosurgery and Psychiatry, 2019 Jul;90(7):740-746.

IV. **Cicognola C**, Satir T, Brinkmalm G, Matečko-Burmann I, Agholme L, Bergström P, Becker B, Zetterberg H, Blennow K, Höglund K. *Tauopathy-associated tau fragment ending at amino acid 224 is generated by calpain-2 cleavage*. Manuscript.

SAHLGRENSKA AKADEMIN

GÖTEBORGS UNIVERSITET

Göteborg, 2019

Tau fragments: role as biomarkers and in the pathogenesis of Alzheimer's disease and other tauopathies

Claudia Cicognola

Department of Psychiatry and Neurochemistry, Institute of Neuroscience and Physiology, Sahlgrenska Academy at the University of Gothenburg, Gothenburg, Sweden, 2019

Abstract

Tau protein is physiologically expressed in neurons, where it is involved in microtubule assembly and stability. Tau functions are rigorously regulated by a series of modifications, *e.g.* phosphorylation and dephosphorylation. When these mechanisms are dysregulated or other modifications occur, this leads to a group of diseases defined as "tauopathies", characterized by build-up of tau protein aggregates in neurons and glial cells (neurofibrillary tangles, astrocytic plaques, tufted astrocytes). Tauopathies include, among others, Alzheimer's disease (AD), frontotemporal dementia (FTD) and diseases characterized by frontotemporal lobar degeneration (FTLD) such as progressive supranuclear palsy (PSP) and corticobasal degeneration (CBD). Among the many post-translational modifications that tau can undergo, proteolytic processing is gaining increasing attention, as many studies have shown that cleavage of tau in brain is related to disease. It has also been consistently observed that tau in cerebrospinal fluid (CSF) consists of a series of fragments, with predominance of N-terminal and mid-region fragments compared to C-terminal ones. The aim of this thesis was to identify and quantify specific tau fragments in CSF with novel targeted immunoassays, and assess their potential as biomarkers for different tauopathies. We identified two major pools of tau consisting of species cleaved at either amino acid (aa) 123 or 224, reflecting different mechanisms of tau processing in AD. While cleavage generating tau N-123 is part of the physiological tau turnover, the generation of tau N-224 was shown to have clinical relevance, being related to AD; N-224 tau showed significantly higher concentrations in AD CSF compared to control and was related to worsening cognitive performance over time. Also, in the primary tauopathies PSP and CBD, N-224 tau did not correlate to total tau (t-tau) content, showing promise as a candidate biomarker for tauopathies other than AD. Based on previous reports of tau cleavage by asparagine endopeptidase (AEP) at aa 368, we also developed a new immunoassay targeting tau fragments cleaved C-terminally of aa 368 (tau 368). Our results demonstrate that, although tau 368 is measurable in CSF and overall increased in AD, only a small portion of the total content of CSF tau ends at 368. Instead, most of tau 368 is retained in tangles, as shown by the decrease in the tau 368/t-tau ratio over the course of disease and immunohistochemical staining of tangles. Of potential clinical relevance, we also showed a strong negative association of the CSF tau 368/t-tau ratio and uptake of the tau PET tracer [^{18}F]GTP1, supporting the hypothesis that the ratio reflects underlying tau pathology and entrapment of tau 368 in tangles. When applying the newly-developed immunoassays to CSF from a FTD cohort, we observed that none of the measures showed a significant difference between the likely FTLD-TDP-43 and likely FTLD-tau pathology groups. However, when normalised for t-tau, N-224 showed a significant difference between FTLD-tau and FTLD associated to TAR DNA-binding protein 43 (FTLD-TDP-43), suggesting that, although the novel measures do not have a superior diagnostic accuracy to the classic tau biomarkers, there are different patterns in fragment concentrations between the pathological groups, and different profiles for each tauopathy. Finally, since the N-224 fragment showed potential clinical relevance in the differential diagnosis of tauopathies, we aimed to identify the enzyme responsible for cleavage at aa 224. By using a fluorescence resonance energy transfer (FRET) peptide, containing a tau sequence which included aa 224, and high resolution mass spectrometry, we identified the enzyme responsible for cleavage as calpain-2. We confirmed the results in a gene knock-down SH-SY5Y cell model, where we measured a significant reduction in tau N-224 in the cell media after knock-down of the calpain-2 gene. These findings suggest that the calpain-2 pathway should be investigated as a possible target in the treatment of tauopathies.

Keywords: tau, fragments, Alzheimer's disease, tauopathy, cerebrospinal fluid

ISBN 978-91-7833-558-9 (PRINT)
ISBN 978-91-7833-559-6 (PDF)

http://hdl.handle.net/2077/60768