SHIMADZU

WP 082

Development of an IP-MALDI Method for Analyzing Endogenous Tau Fragments in Cerebrospinal Fluid

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1. Introduction

◆Tau, a microtubule-associated protein, has gained attention in dementia research. This protein, essential for neuronal structure and function, undergoes hyperphosphorylation and fragmentation in Alzheimer's disease (AD). These changes are thought to be involved in disease pathogenesis. The present study aimed to develop a highly sensitive Nano-LC/MALDI-MS method for analyzing endogenous tau fragments in cerebrospinal fluid (CSF), potentially providing insights into AD pathogenesis and aiding biomarker and therapeutic development.

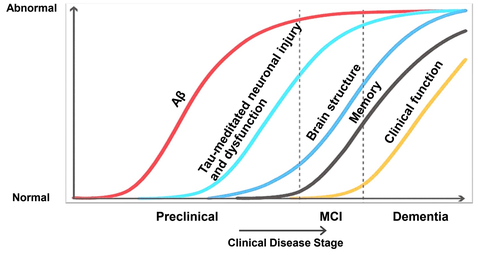

Fig. 1 AD pathological cascade model[1)]

Fig. 2 Schematic of tau protein (2N4R)

2. Methods

2-1. Materials

- CSF#1-18: Human cerebrospinal fluid (Access biologicals, ProteoGenex, PrecisionMed)

2-2. Immunoprecipitation

- Anti-Tau antibody: Anti-Tau, Mouse-Mono (HT7) (Thermo Fisher Scientific, MN1000)
- Anti-Tau antibody: Anti-Tau, Mouse-Mono (77G7) (Biolegend, 816702)
- Magnetic beads: Dynabeads M-270 Epoxy (Thermo Fisher Scientific, 14301)
- Surfactant: n-Dodecyl-β-D-maltoside (DDM) (Dojindo Molecular Technologies, D316)

2-3. Nano-flow liquid chromatography

- HPLC: LC-20AD nano, SIL-40C XSi (Shimadzu Corporation)
- Column: Column Probe, UK-C18 3 μm 150 x 0.075 mm (Imtakt)
- Mobile phase: A) 0.1% Trifluoroacetic acid, B) 0.1% Trifluoroacetic acid / Acetonitrile
- Flow rate: 200 nL/min
- Time program: B conc. 0%(0-35 min) - 40%(65 min) - 100%(65.01-72 min) - 0%(72.01- 100min)
- Injection volume: 2.0 μL

2-4. Mass spectrometry

- MS: MALDI-TOF/TOF-MS AXIMA Performance™, MALDI-QIT TOF-MS AXIMA Resonance™ (Shimadzu/Kratos)
- MALDI matrix: Recrystalllized 2,5 dihydroxybenzoic acid (LaserBio Labs)

◆Immunoprecipitation (IP) was carried out using monoclonal antibody HT7/77G7 to capture the Mid-domain/MTBR tau, respectively. The isolated and enriched tau fragments were subsequently analyzed by Nano-LC/MALDI-MS. Nano-LC eluent was spotted on a 150 μm-diameter DHB matrix by using a column-integrated spotting probe.

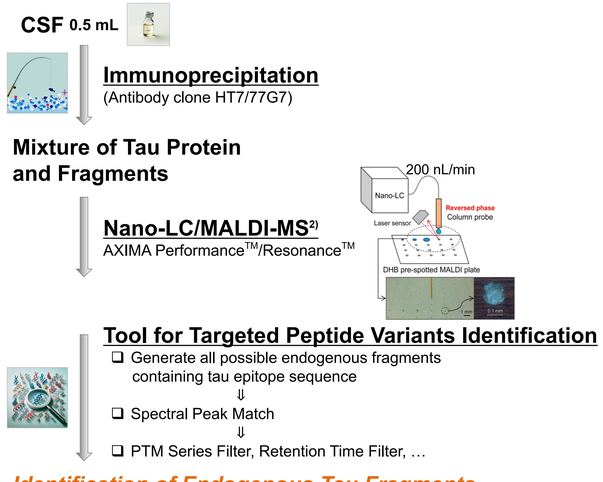

Fig. 3 Workflow of IP and Nano-LC/MALDI-MS method for tau fragment analysis in CSF

3. Results

3-1. Mid-domain tau analysis

◆The mid-domain targeted IP-MS analysis of 18 CSF samples revealed a diversity of endogenous tau fragments. Several of these fragments, including previously unreported ones, exhibited extensive phosphorylation. The abundance and phosphorylation patterns of all fragments varied significantly across the samples. This remarkable heterogeneity in tau fragments and their phosphorylation states likely reflects the complex processing of tau and the progressive pathophysiology of tauopathies. Further studies are crucial to elucidate the clinical significance of these fragments and their potential role in understanding tau-related neurodegenerative diseases.

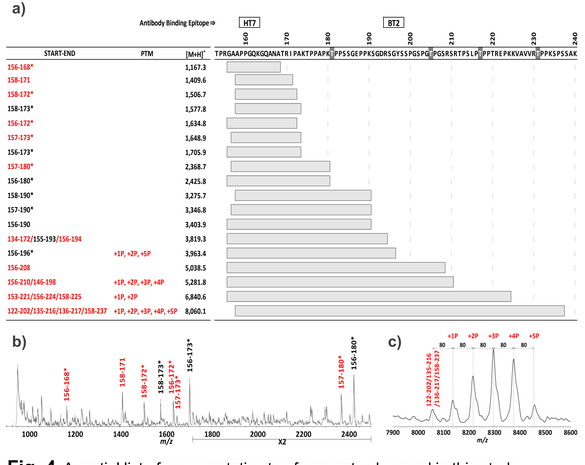

Fig. 4 A partial list of representative tau fragments observed in this study
(a) The +1P, +2P, etc., indicate the number of phosphorylations observed, without specifying the exact sites of phosphorylation, in addition to the unmodified form. Unreported tau fragments are indicated in red. * indicates tau fragments identified by MS/MS analysis. [M+H]+ represents the calculated average mass of unmodified form. The schematic shows only candidate tau fragments beginning with 156 or 158, if there is more than one candidate.
(b, c) Representative mass spectra showing the tau fragments around m/z 1,000-2,400 and m/z 8,200.

3-2. MTBR tau analysis

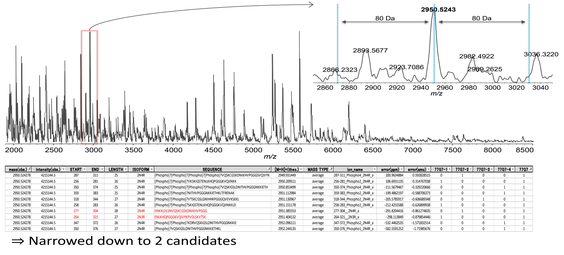

Fig. 5 Typical mass spectrum shows peaks potentially corresponding to the MTBR-tau fragments in CSF.

◆We have observed several MS peaks, potentially representing endogenous tau fragments derived from the MTBR targeted by IP-MS analysis. We are currently utilizing the tool developed in this study to generate and filter candidate sequences with post-translational modifications, working towards identifying them.

4. Conclusion

◆For the identification of endogenous tau fragments, including phosphorylated species, in CSF samples, a highly sensitive immunoprecipitation and Nano-LC/MALDI-MS method coupled with a tool for targeted peptide variants identification is being developed.

◆We have observed dozens of unreported endogenous tau fragments, including multiple phosphorylated forms in CSF. These fragments could potentially be used as diagnostic biomarkers for tau-related neurodegenerative diseases.

Reference

1) Jack, Clifford R Jr *et al.* The Lancet. Neurology vol. 12,2 (2013): 207-16.
2) Hioki, Yusaku *et al.* Analytical chemistry vol. 86,5 (2014): 2549-58.
3) Cicognola, Claudia *et al.* Acta neuropathologica vol. 137,2 (2019): 279-296.