●ThP221

Exploring human brain proteome of Alzheimer's disease (AD) using MALDI Imaging Mass Spectrometry in combination with lipidomics *in situ*

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【Introduction】

Neuropathology of Alzheimer's disease (AD) is characterized by the accumulation and aggregation of Amyloid β (Aβ) peptides into extracellular plaques of the brain. The Aβ peptides, composed of forty amino acids, are generated from amyloid precursor proteins (APP) by β-secretase and γ-secretases. Aβ is deposited not only in cerebral parenchyma but also in leptomeningeal and cerebral vessel walls, known as cerebral amyloid angiopathy (CAA). While a variety of Aβ peptides were identified, detailed production and distribution of individual Aβ peptides in pathological tissues of AD and CAA is not fully addressed. Here, we develop a novel protocol of MADLI-imaging mass spectrometry (MALDI-IMS) in combination with mapping on human autopsy brain tissues to obtain a comprehensive molecular mapping.

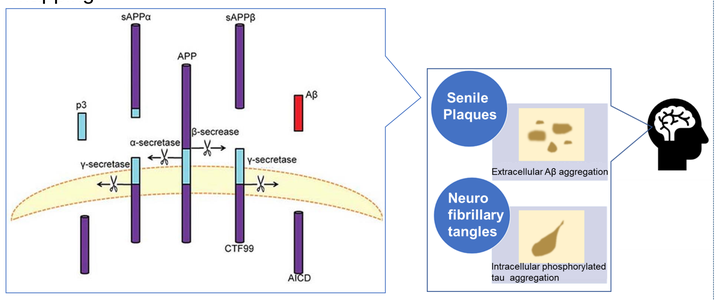

Figure 1. Pathogenic Aβ peptide generation by γ-secretase
The Aβ peptides, composed of forty amino acids, are generated from amyloid precursor proteins (APP) with following cleavage mediated by β- and γ-secretases. APP undergoes gradual cleavage by γ-secretase to produce Aβ of different lengths. In particular, Aβ42 is known to have high aggregability.

【Methods】

Subjects

Human cortical specimens for IMS and immunohistochemistry were obtained from those brains that were removed, processed and placed in -80˚C within 8h postmortem at the Brain bank at Tokyo Metropolitan Institute of Gerontology. Frozen tissue sections were cut on a cryostat at a 20 µm thickness onto ITO glass slides. For mass spectrometric measurements, tissue areas were defined using the fleximaging (Bruker Daltonik GmbH)For all brains registered at the brain bank we obtained written informed consents for their use for medical research from patients or patient's family. Each brain specimen was taken from occipital cortex of 3 AD patients and 3 controls. (table1)

case	gender	age at death	Braak Stage	CAA
1	M	83	5	0.5
2	M	88	5	1
3	M	84	5	2
4	M	70	1	0
5	M	73	1	0
6	M	81	1	0

Table1. Clinical and pathological data of AD/CAA cases and non pathological brains

MALDI Imaging

【Intact IMS】
- Matrix : 2,5-Dihydroxybenzoic Acid (DHB)
- Spatial resolution :50 µm
- Mass range :*m/z* 200～5000
- Mode :positive linear mode
- Measurement :tims TOF flex
- Statistical analysis :SciLS Lab 2022a.

【Lipid IMS】
- Matrix : 2,5-Dihydroxybenzoic Acid (DHB)
- Spatial resolution :50 µm
- Mass range :*m/z* 200～4000
- Mode :positive reflector mode
- Measurement :tims TOF fleX
- Statistical analysis :SciLS Lab 2022a

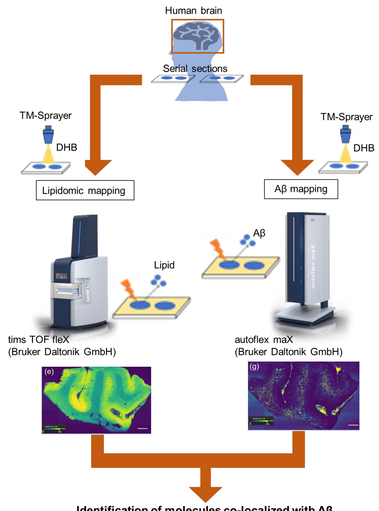

Figure2. An integrated workflow of MALDI-IMS

【Results and Discussions】

【Intact IMS】
By the current protocol, detected short Aβ peptide, including Aβ1-29, Aβ10-40 and Aβx-42 (x = 3, 3p). single ion images of the individual Aβ peptides observed with MALDI-IMS are almost assigned to Aβ species. The difference in distribution especially with Aβx-42 was clarified.

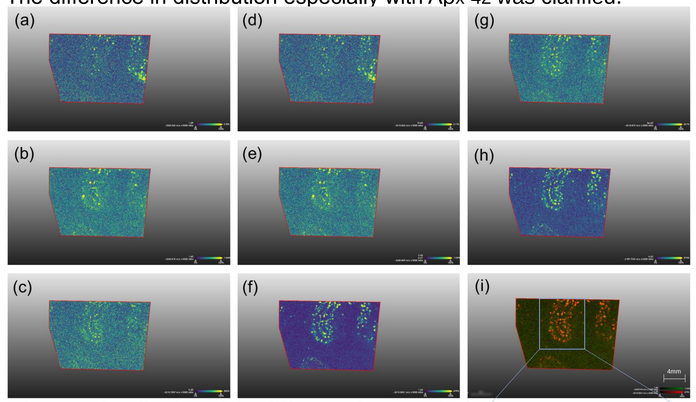

Figure3. Visualization of the difference in N-terminal truncation of Aβ
a) Aβ1-29, *m/z* 3320.523, b) Aβ1-40, *m/z* 4330.818 c) Aβ2-40, *m/z* 4215.730, d) Aβ10-40, *m/z* 3315.822 e) Aβ2-41, *m/z* 4328.889, f) Aβ1-42, *m/z* 4515.0541 g) Aβ3p-42 , *m/z* 4310.873, h) Aβ4-42, *m/z* 4199.7743 i) Merged image of Aβ1-40(green) and Aβ1-42(red)
j) immunohistochemistry for total Aβ (82E1), k) Hematoxylin-Eosin stain

【Lipid IMS】

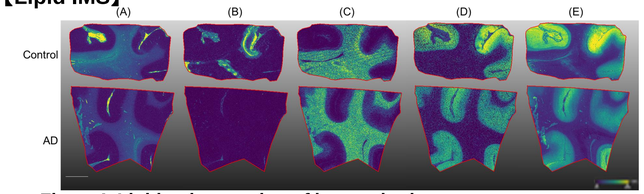

Figure4. Lipidomic mapping of human brain
(A) Sphingomyelin SM (34:1) $C_{39}H_{79}N_2O_6P$, *m/z* 703.572
(B) Monoglyceride MG (22:2) $C_{25}H_{46}O_4$, *m/z* 428.372
(C) Ceramides Cer (42:2) $C_{42}H_{81}NO_3$, *m/z* 648.629
(D) Phosphatidylethanolamine PE (40:6) $C_{45}H_{78}O_8P$, *m/z* 814.533
(E) Phosphatidylcholine PC (32:0) $C_{40}H_{80}NO_8P$, *m/z* 756.549, Bar = 8 mm

m/z 703 as sphingomyelin (34:1) is localized in blood vessels of both non-pathological brain and AD. *m/z* as ceramides, phosphatidylcholine and phosphatidylethanolamine are also detected same localization pattern in control and AD brain, which is unique to gray matter and white matter, respectively.

Furthermore, distribution of *m/z* 428 as monoglyceride (22:2) is characteristic of non-pathological brain (fig4 (B)).

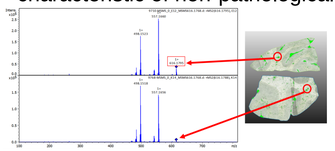

Figure5. MS/MS measurement of *m/z* 616.1 by tims TOF fleX
To identify molecule of *m/z* 616.1, we used tims TOF fleX. Area were defined at meningeal vessels of each sections.

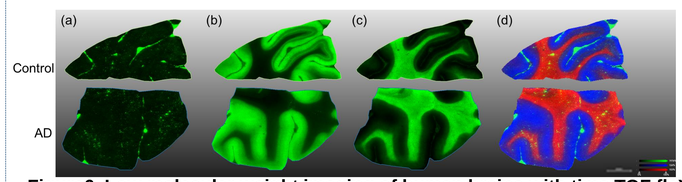

Figure6. Low molecular weight imaging of human brains with tims TOF fleX
(a)Image for *m/z* 616 derived from Heme B (b)Image of *m/z* 772 as phosphatidylcholine for grey matter (c) Image of *m/z* 851 as cerebroside for white matter (d)Merged image of cerebroside (red), phosphatidylcholine (blue) and Heme B (green), bar = 5mm

Heme B was widely distributed at white matter and meningeal vessels for both AD and non pathological brains. There have been few reports weighting on white matter pathology in terms of amyloid deposition in AD brains. However, here we confirmed that low molecular weight imaging of AD and non pathological human brains were clearly delineated with white matter area in Figure 6 (d).

【Summary】

- We have succeeded in visualizing not only the difference in the C-terminal truncation but also the difference in the N-terminal truncation of Aβ.
- We imaged lipids in the human brain and attempted to identify molecules that differ between AD and non-pathological brains.
- We have determined m/z 616 as heme with MS/MS analysis which is also detected in certain distribution manner. Heme B was widely distributed at white matter and meningeal vessels for both AD and non pathological brains. This implicated white matter pathology will give us a novel clue to understand AD pathology.
- Current strategy accelerates the diagnosis and the clarification of the pathogenesis of AD and will benefit unraveling molecular mechanisms underlying senile plaque and neurofibrillary tangles formation as well as neuronal loss in human brains.

【References】

Kakuda N, Miyasaka T, Iwasaki N, et al. Distinct deposition of amyloid-β species in brains with Alzheimer's disease pathology visualized with MALDI imaging mass spectrometry. ***Acta Neuropathologica Comm.*** 5: 73. (2017).