TP 173

Exploring human brain proteome with Alzheimer's disease (AD) with MALDI Imaging Mass Spectrometry in combination with shotgun proteomics

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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- 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 MALDI-imaging mass spectrometry (MALDI-IMS) in combination with shotgun proteomics on human autopsy brain tissues to obtain a comprehensive mapping.

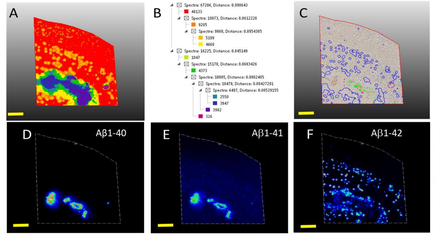


Figure.1 Visualization of Aβ in the human brain

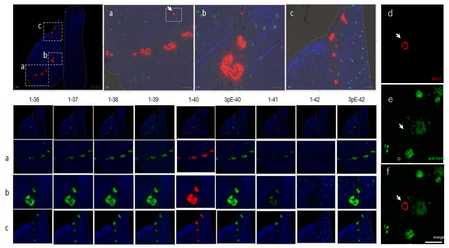


Figure.2 IMS for frozen AD/CAA brain sections at higher resolution

MALDI-IMS with rapifleX MALDI Tissuetyper demonstrated the detailed distributions of both Aβx-40 and Aβx-42 (x = 2, 4, 5, 6, 7, 8, 9, and 11pE) in AD accompanied with moderate CAA brain. Furthermore, MALDI-IMS with timsTOF flex detected shorter Aβ peptide, including Aβ1-29, Aβ10-40 and Aβx-42 (x = 3, 3p). As the next step, we have challenged to integrate in depth AD brain proteome with MALDI-IMS and a shotgun proteomics using intact and on tissue digestion technology. For those shotgun proteomics data, we have compared epitope preference of peptide sequences from identified proteins in human brains. A new protocol that combines MALDI-IMS and shotgun proteomics is useful for elucidating the pathology of AD brains.

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. 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 5 AD patients and 5 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	78	6	1
5	M	83	5	1
6	M	84	1	0
7	M	78	1	0
8	M	70	1	0
9	M	73	1	0
10	M	81	1	0

Table.1 Clinical and pathological data of AD / CAA cases and control

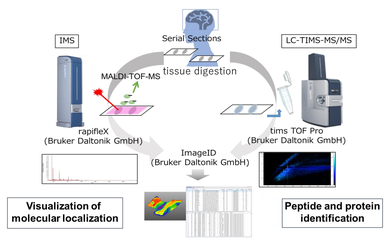
MALDI Imaging

【Non digestion IMS】
- Matrix :sinapinic acid (SA)
- Spatial resolution :50-70 μm
- Mass range :*m/z* 200～5000
- Mode :positive linear mode
- Measurement :tims TOF fleX
- Statistical analysis :SciLS Lab 2016a.

【Digested IMS】
- Matrix : α-cyano-4-hydroxycinnamic acid (CHCA)
- Spatial resolution :50 μm
- Mass range :*m/z* 200～4000
- Mode :positive reflector mode
- Measurement :rapifleX
- Image acquisition :Flex Imaging
- Statistical analysis :SciLS Lab 2016a

Shotgun proteomics

By using timsTOF Pro with nanoElute(Bruker Daltonics) shotgun proteomics was performed with the same tissue sample. Column used was 25 cm x 75 μm 1.6 μm C18 column. Number of MS/MS ramps was 10PASEF scan.



Data analysis

Obtained mass spectra imaging were visualized with flexImaging 5.0, SCiLS Lab 2019b software.
About 2,000 proteins were successfully annotated with Proteinscape 4.0, and database was Swiss-prot.

Results and Discussions

【Non digestion IMS】

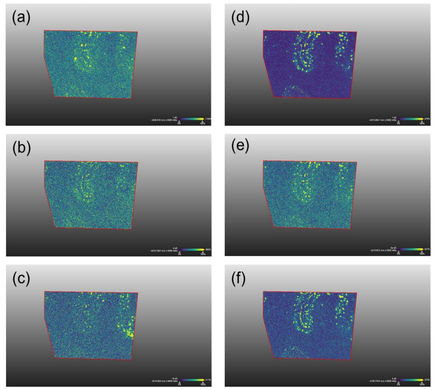
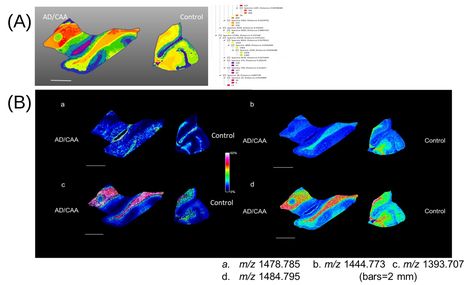


Figure.3 Visualization of the difference in N-terminal truncation of Aβ.
a)Aβ1-40 (x=1), *m/z* 4330.818, b)Aβ2-40 (x=2), *m/z*4215.730
c)Aβ10-40 (x=10), *m/z* 3315.822, d)Aβ1-42 (x=1), *m/z* 4515.054
e)Aβ3p-42 (x=3p), *m/z* 4310.873, f)Aβ4-42 (x=4), *m/z* 4199.774
The difference in distribution especially with Aβx-42 was clarified.

【Digested IMS】



a. m/z 1478.785 *b. m/z* 1444.773 *c. m/z* 1393.707
d. m/z 1484.795 (bars=2 mm)

Figure.4 Visualization of the digested proteins
We acquired segmentation map of AD/CAA and control brains (A).
This clustering analysis identifies plaque-like and vessel-like structures in the occipital cortex.
MALDI-IMS images suggest various peptides annotated (B).

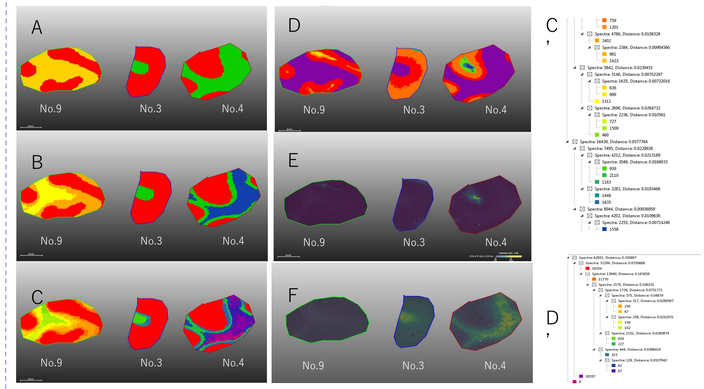


Figure.5 Segmentation map with digested peptides / proteins, obtained by MALDI-IMS of a frozen brain section.
A~D. Segmentation map obtained from a putative image analysis of digested peptides and proteins through MALDI-IMS. C' and D'. The clusters and substructures and their relations are shown as nodes. E. Distinct peptide localization patterns resembling leptomeningeal vascular structures. F. Distinct peptide localization patterns specific to AD (No.3) and AD/CAA (No.4) not in control case (No.9). Those are calculated and extracted by ROC analysis based on segmentation data set.

Summary

1) **AD/CAA by visualizing Aβ with undigested native peptide imaging.**
2) **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.**
3) **As a next step, we have succeeded in visualizing and identifying proteome of human brains with on tissue digestion method in combination with shotgun proteomics.**
4) **Current strategy enables us to elucidate AD/CAA pathology in leptomeningeal paces as well as brain parenchyma through *in depth* proteomics.**

References

Ikegawa M, Nirasawa T, Kakuda N, et al., Visualization of Amyloid beta Deposits in Human Brain with Matrix-assisted Laser Desorption / Ionization Imaging Mass Spectrometry. ***J. Vis. Exp.*** 145, e57645, doi:10.3791/57645. (2019)
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).