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

Yumiko Toyama[1], Hongsun Park[2]; Ryo Kajita[3]; Nobuto Kakuda[1]; Tomohiro Miyasaka[1]; Takashi Nirasawa[3]; Shigeo Murayama[4]; Nobuyuki Nukina[2]; Yasuo Ihara[2]; Masaya Ikegawa[1]
[1]Doshisha University, Kyotanabe, Japan
[2]Graduate School of Brain Science, Doshisha University, Kyotanabe, Japan
[3]Bruker Japan K.K., Yokohama, Japan;
[4]The Brain Bank for Aging Research, Tokyo Metropolitan Geriatric Hospital and Institute of Gerontology, Japan

同志社大学 Doshisha University

BRUKER

Introduction

Alzheimer's disease (AD) is a debilitating, progressive, unremitting chronic degenerative illness of the brain. Neuropathology of AD is characterized by the accumulation and aggregation of Amyloid β (Aβ) peptides into extracellular senile plaques (SP) of the brain and intra-neuronal neurofibrillary tangles (NFT) of hyper-phosphorylated tau protein, both of which comprise highly insoluble, densely packed filaments. The Aβ peptides are generated from amyloid precursor proteins (APP) by β- and γ-secretases. Aβ deposited not only in cerebral parenchyma but also in leptomeningeal and cerebral vessel walls. This has been known as cerebral amyloid angiopathy (CAA).

In advance study, MALDI-imaging mass spectrometry (MALDI-IMS) successfully clarifies Aβ distributions in brains with AD and CAA by ourselves. Here, we modify and upgrade protocol for MALDI-IMS on autopsied brain tissues to obtain in depth protein mapping. For this purpose, we integrate IMS data with shotgun proteomics. As precise proteome identification of pathological depositions accelerates the diagnosis and the clarification of AD, our strategy will benefit unravelling pathogenesis of common neurodegenerative diseases.

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.

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

Frozen tissue sections were cut on a cryostat (CM1950, Leica Microsystems, Wetzlar, Germany) at a 10 mm thickness onto indium-tin-oxide–coated glass slides (Bruker Daltonics, Bremen, Germany). Before matrix coating, treated with a formic acid. For mass spectrometric measurements, tissue areas were defined using the FlexControl 3.8 and FlexImaging 5.0 software packages (both Bruker Daltonics). Spectra were acquired using the rapifleX MALDI Tissuetyper (Bruker Daltonics) in positive linear mode, whereas ions were detected in a mass range of m/z 2,000 to 20,000 with spatial resolution of 20, 50 and 100 mm. A ready-made protein standard was used for spectra calibration (Bruker Daltonics). Visualization and statistical analysis were used FlexImaging and SciLS Lab 2016a (SCiLS, Bremen, Germany).

Immunohistochemistry

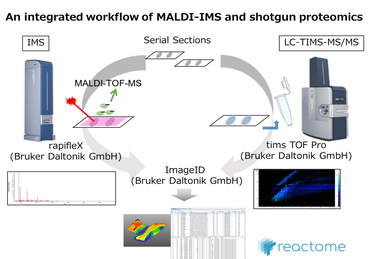
Fresh frozen sections were post-fixed by 4% paraformaldehyde (PFA) in phosphate buffered saline (PBS). After rinses with PBS-T, bound antibodies were visualized with secondary antibodies conjugated with Alexa dyes (life technology). The specimens were analyzed by confocal-laser-scanning-microscope (LSM700; Carl Zeiss Inc.)

Shotgun proteomics with the tims TOF Pro

By using tims TOF Pro with nanoElute（Bruker Daltonics）shotogun 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

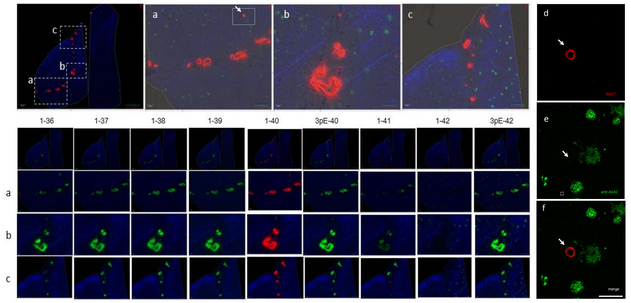


Figure.1 IMS for frozen AD/CAA brain sections (No.3) at higher resolution. Various C-terminal and N-terminal truncated and modified Aβ peptides in AD accompanying severe CAA (NO. 3). Aβ1-36 to Aβ1-41 are preferentially deposited in leptomeningeal blood vessels, while Aβ1-42 and Aβ1-43 are deposited in the cerebral parenchyma as senile plaques. d~f: Brain sections of the occipital cortex from AD brains were immunostained and focused on arteriole and cerebral parenchyma (arrowhead in a) using antibodies against Aβ40 (**d**: BA27) or Aβ42 (**e**: anti-Aβ42 polyclonal) and merged view (**f**). Resolution = 20 mm.

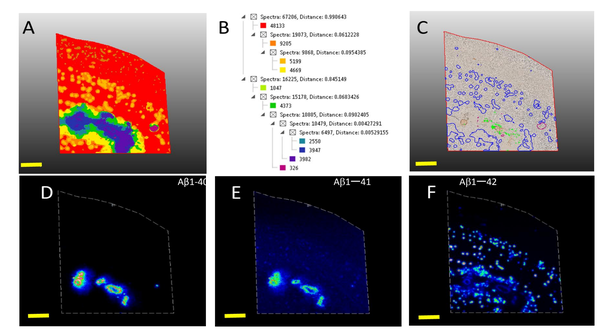


Figure.2 Segmentation map with native peptides / proteins, obtained by MALDI-IMS of a frozen AD/CAA brain section, reveals putative senile plaque, large vessel structures, and small parenchymal arterioles. A Segmentation map obtained from a putative image analysis of MALDI-IMS data. B. Bisecting k-means based clustering analysis identified plaque-like and vessel-like structures in the occipital cortex. The clusters and substructures and their relations are shown as nodes (e.g. , 1-0-0). C. Distinct Ab peptide localization patterns resembling plaques (blue), subarachnoid space (green), and arteriole (red) structures. D. Ab 1-40, E. Ab 1-41, and F. Ab 1-42.

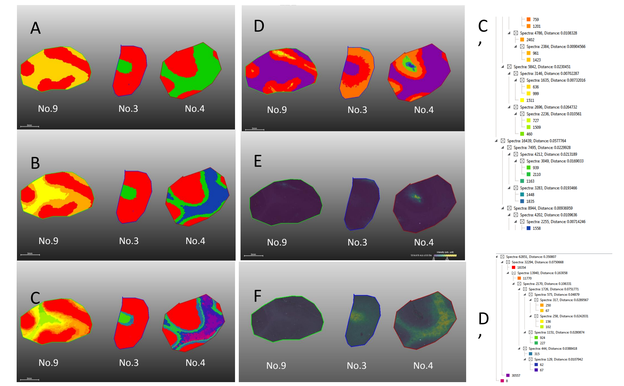


Figure.3 Segmentation map with digested peptides / proteins, obtained by MALDI-IMS of a frozen brain section. A~D. Segmentation map obtained from a putative image analysis of digested peptides and proteins through MALDI-IMS. C' and D'. The clusters and substructures and their relations are shown as nodes. 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) MALDI-IMS is a powerful strategy in deciphering pathogenesis of AD/CAA by visualizing amyloid beta proteoform with undigested native peptide imaging.
2) 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.
3) Current strategy enables us to elucidate AD/CAA pathology in leptomeningeal spaces as well as brain parenchyma through *in depth* proteomics.

References

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