● WP381

Dissecting Cerebral Amyloid Angiopathy (CAA) and Alzheimer's Disease (AD) brains with X-ray Phase-contrast microtomography combined with MALDI-Mass Spectrometry Imaging

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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. 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. So far, we have succeeded in obtaining comprehensive Aβ mapping with our standard protocol of Matrix-assisted laser desorption/ionization-based mass spectrometry imaging (MALDI-MSI) (Kakuda et al., 2017; Toyama et al., 2024). As the next step, we will reconstruct MSI data set obtained with two-dimension (2D) into three-dimensional (3D) modality by integrating MALDI-MSI with synchrotron radiation-based X-ray phase-contrast microtomography. Through our current protocol, we will answer if amyloid beta metabolism in CAA and AD will be influenced by retarded glymphatic flow and its molecular basis with multi-omics study will be discussed.

【Methods】

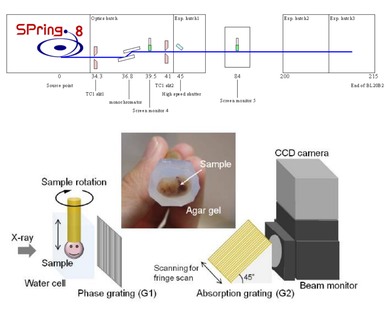

Figure 1. Spring-8: X-ray Phase Contrast Tomography, Harima, Japan.

Human samples were obtained with informed consent by the Brain Bank at the Tokyo Metropolitan Institute of Gerontology and Doshisha University. Cortical specimens from AD / CAA and Control patients were obtained and were transferred to the laboratory at room temperature in formalin fixed status. Size of sample block were 11~20 × 23~35 × 5 mm. The microtomographic analyses were performed at the BL20B2 beamline of the synchrotron radiation facility SPring-8. X-ray microtomography data were analyzed by Amira software. After obtaining data at the Spring-8, agarose gels were removed with heating procedures and were embedded into paraffin block by preserving the exact orientation of the brain samples.

MALDI Mass Spectrometry Imaging

Matrix : 2,5-Dihydroxybenzoic Acid (DHB) Spatial resolution:50 μm; Mass range: m/z 200～5000 for Ab and m/z 500～2000 for lipids; Mode: positive mode, MSI Measurement: tims TOF flex for Aβ and rapiFlex for lipids, Statistical analysis :SciLS Lab 2022a.

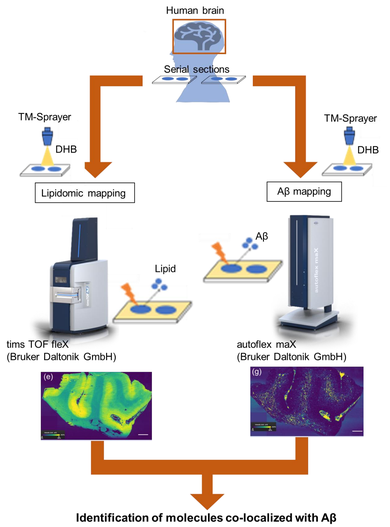

Figure 2. An integrated workflow of MALDI-MSI and X-ray Phase Contrast Tomography

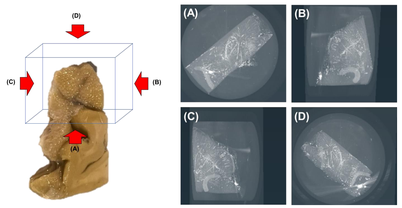

Figure 3. X-ray phase-contrast microtomography. Arrows in left panel (A), (B), (C), and (D) represent orientation of 3D phase contrast CT images. Right panel is an example of merged images. This view can be observed in a 3D mode.

Immunohistochemsitry of amyloid-β in neuritic plaques and vessel walls.

Anti-amyloid-β immunostaining (left: clone 82E1 and right: 11A1) of a postmortem section of the frontal lobe from CAA patient (left) reveals co-existing neuritic plaques (right, top) and cerebral amyloid angiopathy (CAA; right, bottom) in leptomeningeal and cortical vessels.

【Results and Discussions】

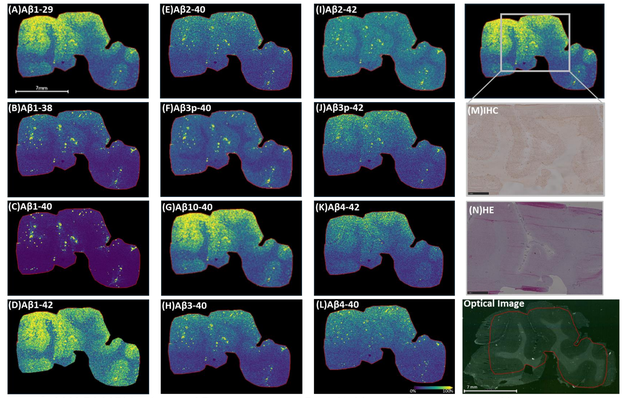

Figure 4. MALDI-MSI for frozen AD brain section (Case1). Various C-terminal and N- terminal truncated Aβ peptides in AD occipital lobe cortex. From A) to O): A) Aβ 1-29, B) 1-38, C) 1-40, D) 1-42, E) 2-40, F) 3p-40, G) 10-40, H) 3-40, I) 2-42, J) 3p-42, K) 4- 42, L) 4-40, M) Immunohistochemistry of anti Aβ antibody (82E1). N) HE staining, and O) Optic image of occipital lobe from AD Case 1. Scale bar indicates 7 mm. From Toyama et al. 2024.

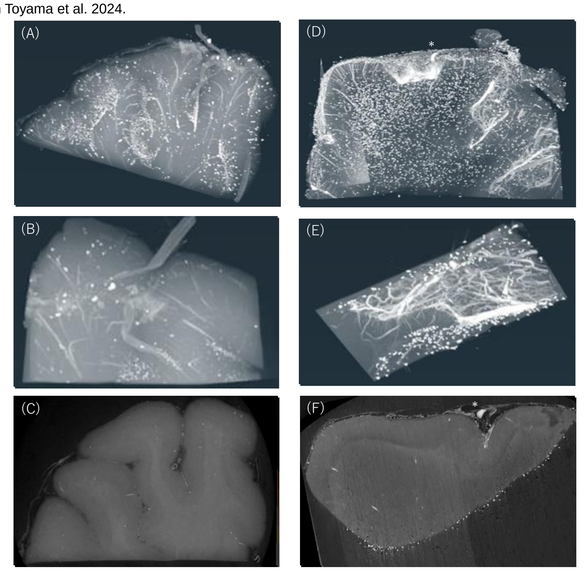

Figure 5. Phase reconstructed slices of Control (A, B, C) and CAA (D, E, F) brain sample obtained at higher resolution through X-ray phase contrast CT. The region of interest outlined in A is shown in B. White asterisk (*) indicates the identical region of high CT value in 3D (D) and 2D (F) mode from CAA brain.

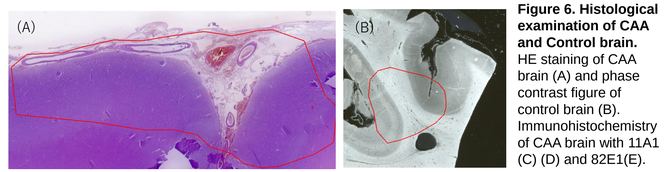

Figure 6. Histological examination of CAA and Control brain. HE staining of CAA brain (A) and phase contrast figure of control brain (B). Immunohistochemistry of CAA brain with 11A1 (C) (D) and 82E1(E).

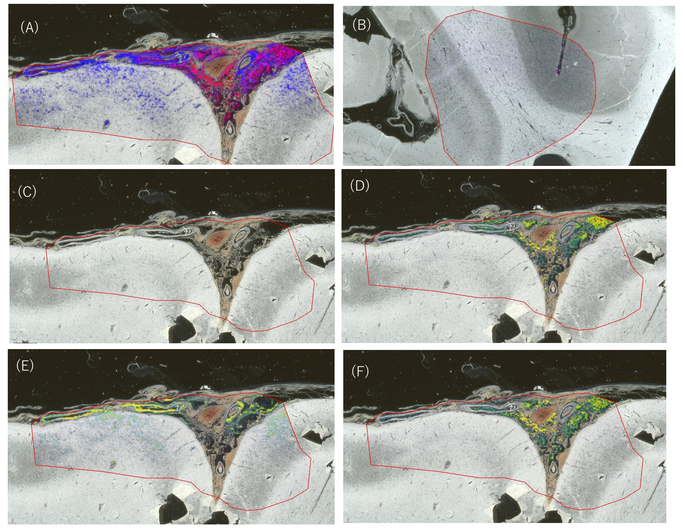

Figure 7. MALDI-MSI for FFPE samples from CAA and Control brains. Brain samples were re-embedded after X-ray phase contrast CT scanning at Spring-8. (A) Single mass imaging for three compounds (D to F) were merged in (A) CAA brain and (B) Control brain. (C) Phase contrast figure for CAA. (D) to (F) represent three different compounds. Leptomeningeal vessels from arteries and veins were histologically differentiated.

【References】

Kakuda N, Miyasaka T, Iwasaki N, Nirasawa T, Wada-Kakuda S, Takahashi-Fujigasaki J, Murayama S, Ihara Y, Ikegawa M. Distinct deposition of amyloid-β species in brains with Alzheimer's disease pathology visualized with MALDI imaging mass spectrometry. ***Acta Neuropathol Commun.*** 2017 Oct 16;5(1):73.

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