Molecular dissection of renal amyloidosis with MALDI - Imaging Mass Spectrometry and shotgun proteomics on paraffin embedded biopsy tissue section

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Introduction

The amyloidoses are a group of disorders in which soluble proteins aggregate and deposit extracellularly in tissues as insoluble fibrils. The kidney is one of the most frequent sites of amyloid deposition. Current diagnosis relies on histopathological examination, commercially available antibodies lack specificity and sometimes failed to diagnose properly.

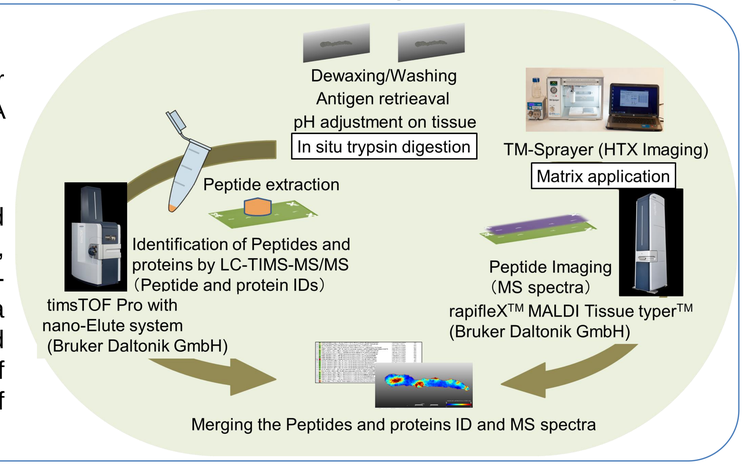
In this study, we aimed to identify and to subtype amyloid proteins with high accuracy in paraffin-embedded tissue sections fixed in formalin-acetic acid-alcohol (FAA), using MALDI-IMS and shotgun proteomics. We established an in depth tissue proteomics at single nephron level.

Methods

Tissue samples were transferred from Necker-Enfants malads Hospital. Four sample groups of immunoglobulin light chain (AL)-κ, AL-λ, serum amyloid A (SAA) and non-pathological control were analyzed.

【MALDI-IMS and shotgun protemics】

Tissue sections were mounted onto ITO slide glasses. Pretreatment was carried out as dewaxing, antigen retrieval using AR Buffer (pH = 10 mM Citrate Buffer), on-tissue digestion with trypsin and deposition of MALDI matrix CHCA using TM-Sprayer. MALDI-IMS was done by using rapifleX MALDI Tissue typer with a spatial resolution of 50 μm and 20 μm. Serial tissue sections were applied shotgun proteomics using TOF Pro with nanoElute system. The number of MS/MS lamps was 10 PASEF scan (1PASEF=12 MS/MS). Statistic analysis of mass spectra was performed with SCiLS Lab 2019 software.



Results

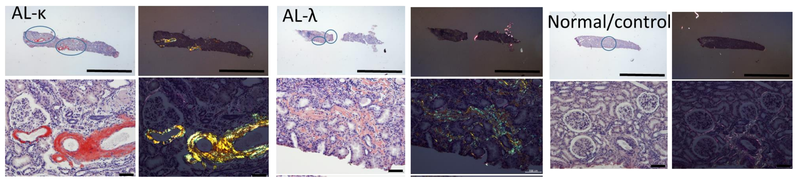


Fig.1. Histochemistry. DFS stain highlights glomeruli and segmental capillary wall of amyloid deposits. Upon polarization stained material shows characteristic apple-green birefringence, confirming the presence of amyloid. (left: bright-field observation, right: polarizing observation, Bars; 2500 μm, 200 μm)

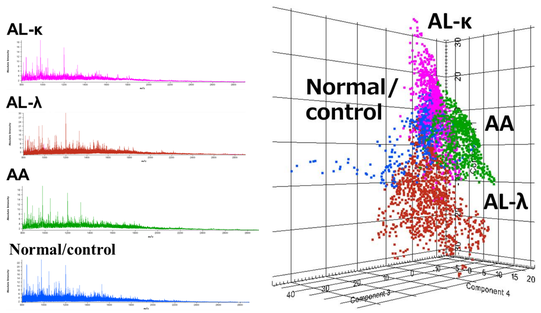


Fig.2. Typing by Principal Component Analysis (PCA)

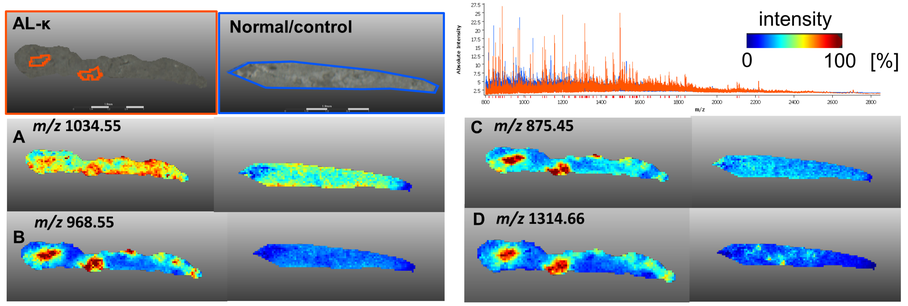


Fig.3. MS ion images of each peptide were shown with SCiLS Lab software. A to D are part of those showing a significant difference in the comparison between the DFS staining positive site of AL-κ type amyloid nephropathy(■) and the whole non-amyloidosis kidney(■) by Receiver Operating Characteristic (ROC) analysis. Mass range: *m/z* 800-3000, Spatial resolution: **50** μm

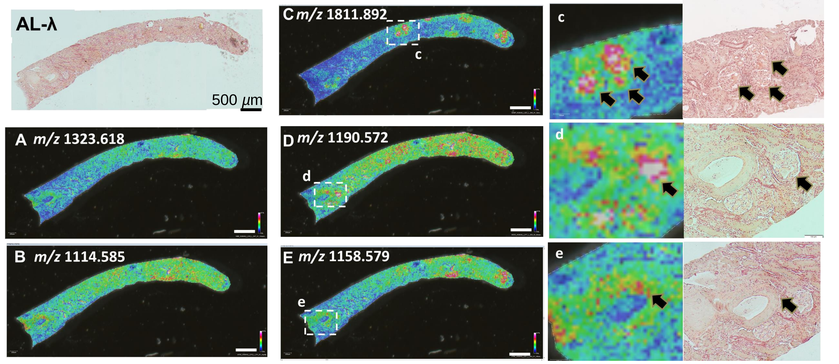


Fig.4. MS ion images of each peptide were shown with imageID software. We visualized deposited proteins such as Apolipoprotein E (B), Serum amyloid P component (C), Vitronectin (E), as well as uncharacterized proteins, Vimentin (A), Moesin (D), were identified in AL-λ type amyloidosis. Mass range; *m/z* 800-3000, Spatial resolution; **20** μm

Conclusions & Perspectives

- **MALDI-IMS with shotgun analysis enabled single nephron proteomics on formalin fixed renal biopsy samples.**
- **We were able to discover yet uncharacterized proteins co-localized with amyloid deposits from renal biopsy samples, useful in elucidating renal amyloidogenesis.**

References

1. R. Casadonte *et al.*, Imaging mass spectrometry analysis of renal amyloidosis biopsies reveals protein co-localization with amyloid deposits. *Analytical and Bioanalytical Chemistry*, 5323-5331 (2015) .
2. T. Nakanishi *et al.*, Topologies of amyloidogenic proteins in Congo red positive sliced sections of formalin-fixed paraffin embedded tissues by MALDI-MS imaging coupled with on-tissue tryptic digestion. *Clin Biochem.* **46**(15), 1595-1600 (2013) .