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In Situ proteomics of Kidney from Type 2 Diabetes Mellitus (T2DM) Rat using MALDI-Imaging Mass Spectrometry

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Overview

MALDI-IMS, Proteome, Diabetes, Kidney, Biomarker

Introduction

Diabetic nephropathy (DN) is the main cause of dialysis treatment. Conventional diagnostic criteria of DN using albuminuria and serum creatinine to estimate GFR (eGFR) as well as proteinuria are still insufficient because cases in which kidney function declines without significant increase in proteinuria. By contrast, changes in the morphology of the kidney have been observed at an early stage of the diabetes. Clinical observations along with histological changes in living renal tissues has been demanded. Here we focus on the minimal changes of diabetic kidney at early stages of the disease progression at proteomic level on animal model.

Methods

▸ Animals

Kidneys of Spontaneously Diabetic Torii (SDT) fatty rats without hemi-section at 17 weeks of age were sacrificed and resected kidneys were snap-frozen in liquid nitrogen.

Spontaneously Diabetic Torii Lepr^{fa} (SDT fatty) rat

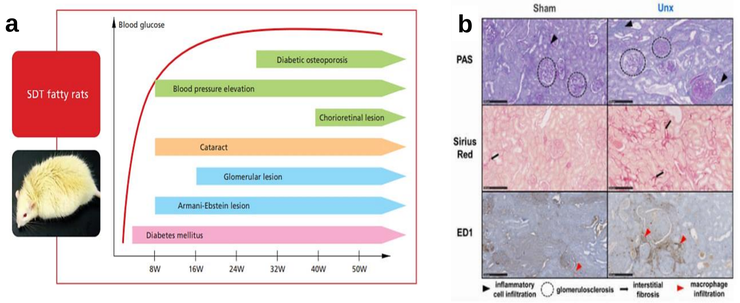


Figure.1 a: Time course of diabetic complications of SDT fatty rats [2].
b: Histological changes in kidney after 10weeks of treatment, one side kidney removed and salt loading.

▸ Histopathological examination of the kidney

10 µm sections from kidneys of SDT fatty rats after IMS were subjected to standard hematoxylin/eosin (HE) staining.

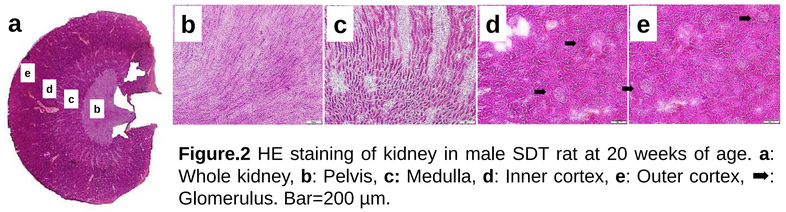


Figure.2 HE staining of kidney in male SDT rat at 20 weeks of age. **a**: Whole kidney, **b**: Pelvis, **c:** Medulla, **d**: Inner cortex, **e**: Outer cortex, ➡: Glomerulus. Bar=200 µm.

▸MALDI imaging mass spectrometry (IMS)

Sinapic Acid (SA) and α-cyano-4-hydroxycinnamic acid (CHCA) 10 mg/ml in 50 % and 70 % Acetic Acid (0.1 % and 1 % TFA) was uniformly deposited on the slide by using TM-Sprayer (HTX Imaging). On tissue digestion with trypsin was performed with TM-Sprayer. Then extracted peptides and proteins, and measured by using rapifleX (Bruker Daltonics) with a spatial resolution of 50 µm. Ions were detected in mass range of m/z 2,000-25,000 and 800-5,000.

▸Shotgun proteomics with the tims TOF Pro

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

▸Data analysis

Obtained mass spectra as well as annotated proteins and peptides were visualized with flexImaging and SCiLS Lab 2018 software. About 2,000 proteins were successfully annotated with Proteinscape 4.0, and database was Swiss-prot.

Results

▸Single peak analysis and protein distributions

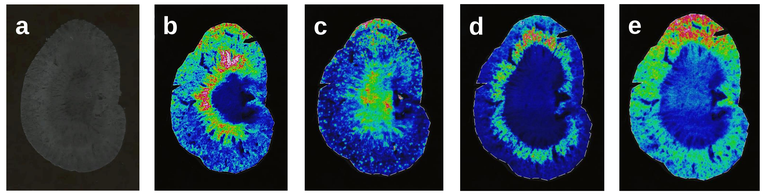
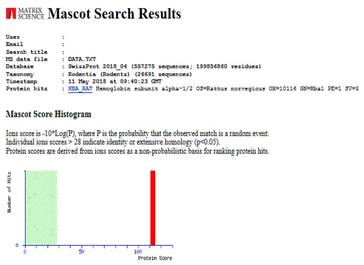


Figure.3 Single peak analysis of kidneys from SDT fatty rats at 17 weeks of age. **a:** optic image of section before IMS **b:** image of single peak m/z 1886, medulla, **c:** glomeruli and pelvis **d:** cortico-medullar junction**, e:**cortex.

▸MS/MS Ion search


MATRIX SCIENCE **Mascot Search Results**

User :
Email :
Search title :
MS data file : DATA.TXT
Database : SwissProt 2018_04 (557275 sequences; 199856860 residues)
Taxonomy : Rodentia (Rodents) (26691 sequences)
Timestamp : 11 May 2018 at 09:40:23 GMT
Protein hits : HBA_RAT Hemoglobin subunit alpha-1/2 OS=Rattus norvegicus OX=10116 GN=Hba1 PE=1 SV=2

Mascot Score Histogram

Ions score is -10*Log(P), where P is the probability that the observed match is a random event.
Individual ions scores > 28 indicate identity or extensive homology (p<0.05).
Protein scores are derived from ions scores as a non-probabilistic basis for ranking protein hits.

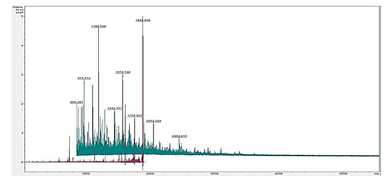


Figure.4 MS/MS ion search of single peak. After MS/MS analysis of m/z 1886, it was identified as hemoglobin subunit alpha.

▸Visualization of glomeruli with MALDI-IMS

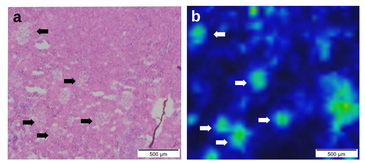


Figure.5 Analyte in the glomeruli of SDT fatty rat. **a:** HE staining of renal cortex from SDT fatty rat. **b:** IMS of renal cortex from the same SDT fatty rat with a. Black arrows indicate glomeruli at identical area from serial section of the same kidney, HE staining. White arrows indicate distribution of single spectra shown in dotted square area in Figure 3c. Bar＝500 mm.

Conclusions

Successfully visualizing structure and function of kidneys of SDT fatty rat with MALDI-IMS at proteomic level.
High speed MALDI-IMS combined with shotgun proteomics with tims TOF Pro is now ongoing for further detecting early diabetic marker protein and peptide.