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# Mass Spectrometric In-Depth Proteome Analysis of the Kidneys from Rat Model of Diabetic Nephropathy

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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 have tried to generate in depth proteomic analysis of DN using diabetic animal model, a new inbred rat strain, Spontaneously Diabetic Torii (SDT) fatty rats, which develop hyperglycemia with obesity after 17 weeks of age in comparison with normal control Sprague Dawley (SD) rats of the same age. In this study, we applied matrix-assisted laser desorption/ionization (MALDI) imaging mass spectrometry (IMS) for the study of pathological aspects of DN, especially generating in depth proteome analysis by integrating MALDI-IMS and shotgun analysis (timsTOF Pro system).

## 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**

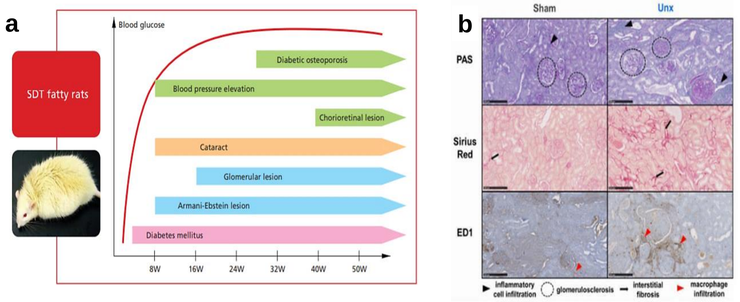



**Figure.1 a:** Time course of diabetic complications of SDT fatty rats [2].
**b:** Histological changes in kidney after 10weeks of treatment, one side kidney removed and salt loading.

### ▸ Histopathological examination of the kidney

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

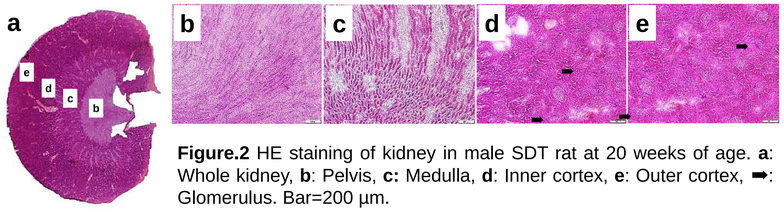


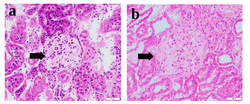

**Figure.2** HE staining of kidney in male SDT rat at 20 weeks of age. **a**: Whole kidney, **b**: Pelvis, **c:** Medulla, **d**: Inner cortex, **e**: Outer cortex, ➡: Glomerulus. Bar=200 µm.



| Groups / Pathological findings | Weeks of age 8 | | | 16 | | | 24 | | | 32 | | | 40 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | − | + | +++ | − | + | +++ | − | + | +++ | − | + | +++ | − | + | +++ |
| SDT-fa/fa rat | | | | | | | | | | | | | | | |
| Glomeruli | | | | | | | | | | | | | | | |
| glomerulosclerosis, diffuse | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 1 | 4 | 0 |
| Renal Tubule | | | | | | | | | | | | | | | |
| Armanni-Ebstein change | 5 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 2 | 3 | 0 | 0 | 2 | 3 |
| tubular dilation, diffuse | 5 | 0 | 0 | 0 | 0 | 2 | 3 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 5 |
| hyaline cast, diffuse | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 2 | 3 | 0 |
| SDT-+/+ rat | | | | | | | | | | | | | | | |
| Glomeruli | | | | | | | | | | | | | | | |
| glomerulosclerosis, diffuse | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 |
| Renal Tubule | | | | | | | | | | | | | | | |
| Armanni-Ebstein change | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 |
| tubular dilation, diffuse | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 |
| hyaline cast, diffuse | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 |
| SD rat | | | | | | | | | | | | | | | |
| Glomeruli | | | | | | | | | | | | | | | |
| glomerulosclerosis, diffuse | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 |
| Renal Tubule | | | | | | | | | | | | | | | |
| Armanni-Ebstein change | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 |
| tubular dilation, diffuse | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 |
| hyaline cast, diffuse | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 |

Each of the findings was assigned one of four qualitative grades: normal (-), slight (+), moderate (++), or severe (+++) abnormalities. Each value in the Table (e.g., 0, 3, 2, 3, 4, 5) is the number of rats with the pathology.

Histopathological findings in kidneys from female SDT-fa/fa. SDT −+/+, and SD rats




HE stain of kidney in a: SD and b: SDT fatty rat. ➡: Glomerulus

### ▸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

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

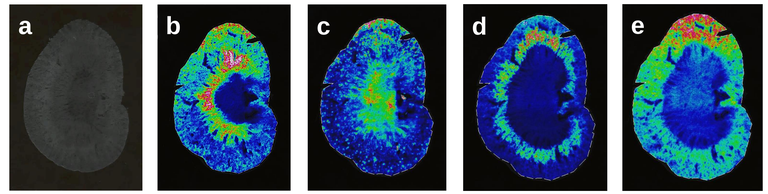



**Figure.3** Single peak analysis of kidneys from SDT fatty rats at 17 weeks of age. **a:** optic image of section before IMS **b:** image of single peak m/z 1886, medulla, **c:** glomeruli and pelvis **d:** cortico-medullar junction**, e:**cortex.

### ▸Visualization of glomeruli with MALDI-IMS

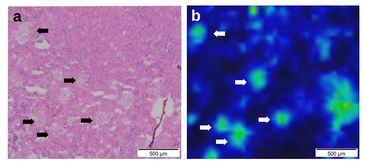



**Figure.5** Analyte in the glomeruli of SDT fatty rat. **a:** HE staining of renal cortex from SDT fatty rat. **b:** IMS of renal cortex from the same SDT fatty rat with a. Black arrows indicate glomeruli at identical area from serial section of the same kidney, HE staining. White arrows indicate distribution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

### ▸Renal Segmentation of diabetic changes with MALDI-IMS

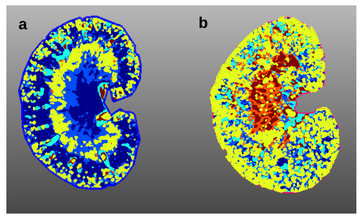
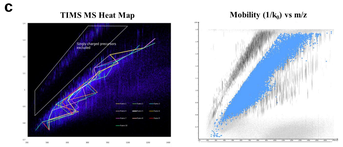



**Figure.6** Segmentation map of SD and SDT fatty rat. **a:** Segmentation map from SD rat. **b:** : Segmentation map from SDT fatty rat. C. Results of shot-gun analysis with Tims TOF-Pro.




## Conclusions

Visualizing structure and function of kidneys of SD and 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.