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 Leprt[D] (SDT fatty) rat

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

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.

Figure 3: Single peak analysis of kidney from SDT fatty rat at 17 weeks of age. a: optic image of section before IMS, b: image of single peak m/z 1866, medulla, c: glomeruli and pelvis d: cortico-medullar junction, e: cortex.

Figure 4: 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.

Figure 5: 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.