In depth proteomics of the kidneys from autoimmune type I diabetes rat model through MALDI - Imaging Mass Spectrometry

Introduction

Diabetic nephropathy (DN) is one of the serious chronic complications of type 1 diabetes (TID). Although microalbuminuria has been used as a primary marker of DN in early stage, it is sometimes difficult to make an early diagnosis of DN simply relied on the current criteria. Unraveling pathogenesis of DN and finding earlier markers of DN is extremely important.

Microvascular lesions in the renal glomeruli is a typical hallmark of the DN. Aberrant homeostasis in blood glucose tolerance may cause systemic damages to vascular systems including glomerulus and arteriole. In addition, several lines of evidence show hyperglycemia is not a single factor of diabetes – induced microvascular complication.

Here we adopt matrix-assisted laser desorption/ionization (MALDI) mass imaging mass spectrometry (IMS) approach to study early proteomic tissue marker of the kidneys from KDP rat, which has been well established T1DM model in comparison with KND rat as normal control.

Methods

HiMALDI-IMS

10 μm cryosections were cut and transferred to Indium-Tin-Oxide (ITO) coated glass slides. Trypsin and α-Cyano-4-hydroxycinnamic acid (HCAC) was uniformly deposited on the slide using the TM-SprayTM (HTX Imaging) device and measured using flexImaging (Bruker Daltonik GmbH) with a spatial resolution of 50 μm in linear mode. Ions were detected in a mass range of m/z 800 to 3000.

LC-MS/MS

For the serial section of the tissues exactly prepared with the same protocol for MALDI-IMS, proteins and peptides were extracted to be analyzed with timsTOF Pro (Bruker Daltonik GmbH) with nanoElute (Bruker Daltonik GmbH). Obtained mass spectra from LC-MS/MS were identified peptides and proteins in the tissue with ProteinScape (Bruker Daltonik GmbH). By Image ID analysis to integrate these data visualized peptides and proteins in the tissue.

Results and Discussion

Histopathological examination in the kidney

Kidneys from KDP rats at 7 weeks of age were dissected and snap-frozen in liquid nitrogen. Normal KND rat kidneys were obtained at 7 weeks of age as control. Sacrifice time was not identical.

Animals

Fresh frozen kidneys from KND and KDP rats were cut into 6 μm to 10 μm sections. The sections were mounted on glass slides and subjected to standard Hematoxylin-Eosin (H&E) staining, Periodic acid-Schiff (PAS) staining and Immunohistochemistry (IHC).

Summary

- Kidneys from T1D animal model. KDP rat at 7 weeks of age were successfully applied to MALDI-IMS and a shotgun proteomics.
- We have identified both morphological and functional proteins from kidneys of KND and KDP rats at 7 weeks of age through shotgun proteomics.
- By Protein ID analysis, we have succeeded in visualization of the identified proteins, such as Moein with MALDI-IMS, which can be a traceable marker for DN development in the future study.

References