Spatial Mapping of Lipids and Elements by Mass Microscopy and Integration with LA-ICP-MS in the Diabetic Mouse Pancreata

Koji Okuda[1]; Ei-ichi Matsuo[1], Jeffrey Dahl[2]; Toshiya Matsubara[2]; Shinichi Yamaguchi[1]; Jannine I. Gamayot[3]; Shiuhwei Chen[3]; Christine M. Kusminski[3]; Philipp. E. Scherer[3]; Ruth Gordillo[3]

1. Shimadzu Corporation, Kyoto, Japan; 2. Shimadzu Scientific Instruments, Columbia, MD; 3. University of Texas Southwestern Medical Center, Dallas, TX,

UT Southwestern Medical Center

SHIMADZU Excellence in Science

Introduction.

Pancreas endocrine function critically relies on the islets of Langerhans that secrete insulin and glucagon as well as other hormones. Previous pancreas LC-MS/MS lipidomics studies required islets isolation by a lengthy process involving tissue digestion and manual selection, potentially compromising metabolite basal levels [1]. MALDI-TOF imaging allows for in situ global assessment of lipids in pancreata cryosections. Our methodology was applied to a mouse model of diabetes, the adipocyte-specific-doxycycline-inducible mitochondrial ferritin (FtMT) overexpression, which displays massive beta-cell hyperplasia as its most striking phenotype [2].

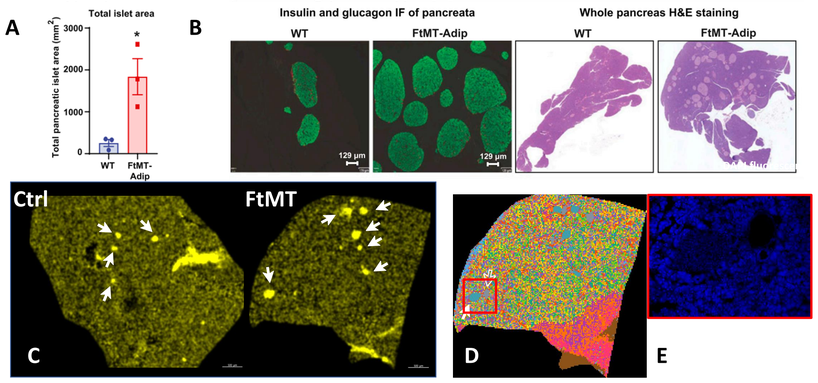

Figure 1. **A)** Total pancreatic islet area (mm^2) [2]. **B)** Insulin IF staining of pancreata and (left) and H&E staining of whole pancreas from WT and FtMT-Adip mice [2]. **C)** Mouse pancreas false color mass spectrometry imaging (25 µm spatial resolution) of SM 34:1;O_2 m/z 741.527 $[M+K]^+$. Transgenic mouse pancreas displays considerably larger size islets. **D)** Unsupervised spatial segmentation calculation of MSI data (15 clusters) allows for differentiations of islets and blood vessel regions. **E)** DAPI fluorescence staining of red marked area in **D.**

Methods and Instrumentation.

Frozen mouse pancreata was cryosectioned into 10 µm-thick sections. Tissue micro-sections were mounted on In_2O_3-SnO_2 (Millipore-Sigma, Burlington, MA). Automated matrix deposition system was achieved with an iMLayer™ (Shimadzu Corporation, Japan). 2-mercaptobenzothiazole (MBT, positive ion mode) and 1,5-diaminonapthalene (DAN, negative ion mode) were used as MALDI matrix reagents. Mass spectrometry imaging (MSI) analysis was performed on an iMScope™ QT mass microscope (Shimadzu Corporation). Spatial elemental analysis was performed on an imageBIO266 (Elemental Scientific Lasers, USA) coupled to an ICPMS-2030 (Shimadzu Corporation). Imaging data processing was performed using IMAGEREVEAL™ MS software package (Shimadzu Corporation).

References. 1. Ye et al. J clin Invest 2018, 128(3):1178-1189 2. Kusminski et al. Diabetes 2020 69(3):313-330 3. MetaboAnalyst 5.0 www.metaboanalyst.ca

Acknowledgements. Shimadzu Corporation (JP) and Shimadzu Scientific Instruments (USA) for the collaborative efforts in mass spectrometry technology resources and useful discussion. Dr. José A. Fernández at the University of the Basque Country for useful discussion.

Results.

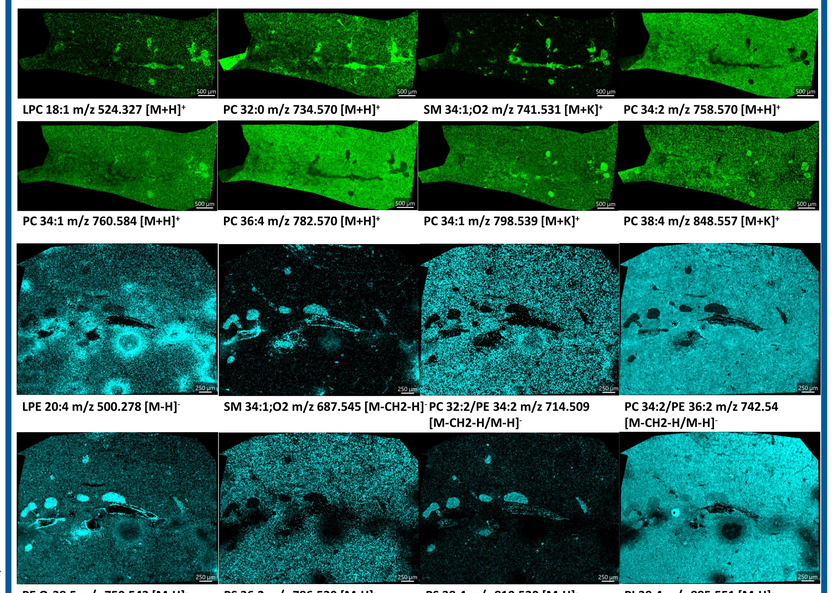

Figure 2. Top panel (green) positive ion mode analysis of wild-type (WT) pancreas cryosection at 6 µm spatial resolution. Bottom panel (cyan) negative ion mode analysis of WT pancreas cryosection at 5 µm spatial resolution.

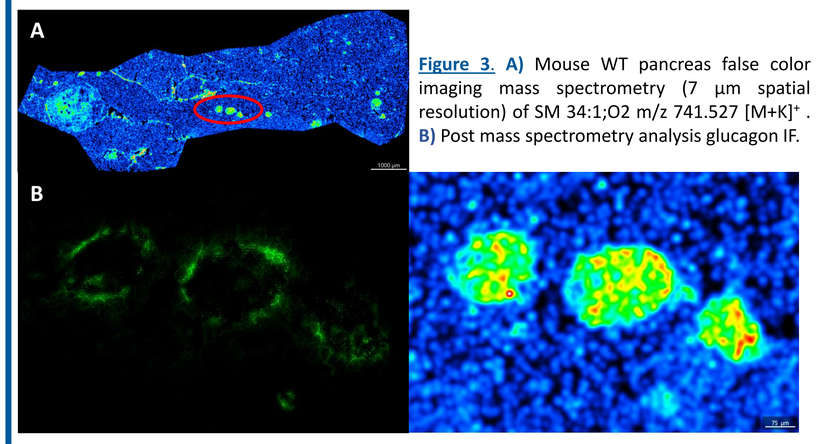

Figure 3. **A)** Mouse WT pancreas false color imaging mass spectrometry (7 µm spatial resolution) of SM 34:1;O2 m/z 741.527 $[M+K]^+$. **B)** Post mass spectrometry analysis glucagon IF.

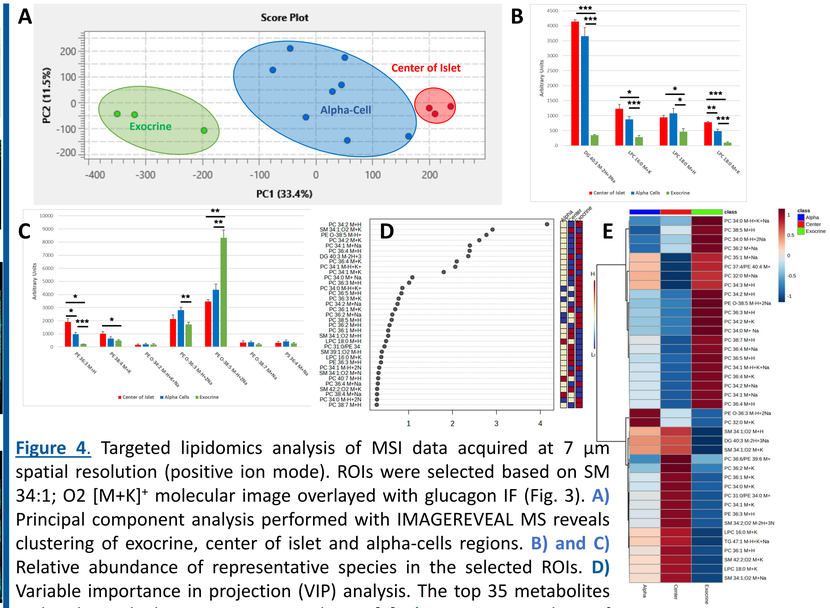

Figure 4. Targeted lipidomics analysis of MSI data acquired at 7 µm spatial resolution (positive ion mode). ROIs were selected based on SM 34:1; O2 $[M+K]^+$ molecular image overlayed with glucagon IF (Fig. 3). **A)** Principal component analysis performed with IMAGEREVEAL MS reveals clustering of exocrine, center of islet and alpha-cells regions. **B) and C)** Relative abundance of representative species in the selected ROIs. **D)** Variable importance in projection (VIP) analysis. The top 35 metabolites with relative higher VIP score are shown [3] **E)** Heat map analysis of pancreata MSI lipidomics only. The top 40 most relevant features are shown [3].

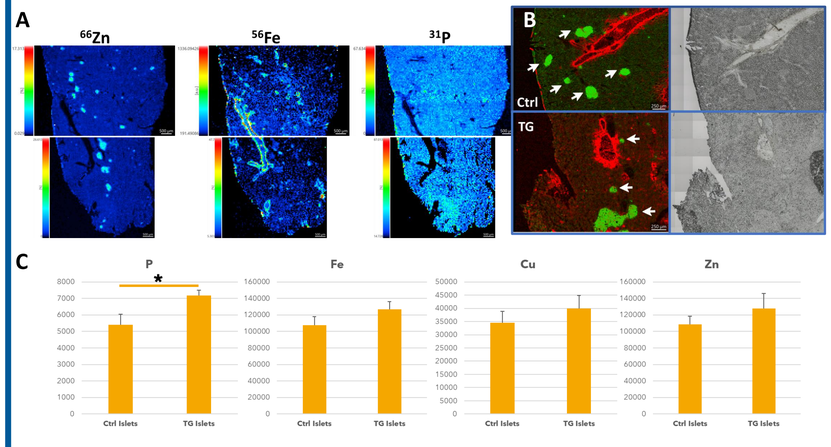

Figure 5. **A)** LA-ICP-MS imaging analysis of mouse pancreas at 50 µm spatial resolution. Zn accumulates in the islets of Langerhans, Fe in blood vessels, while P is ubiquitous. **B)** Overlay of elemental images of Zn (green) and Fe (red) in control and FtMT overexpressing pancreas (analysis at 5µm spatial resolution). TG mice cryosection displays islets hyperplasia. High resolution optical images are also displayed. **C)** Elements relative abundance in islets and exocrine regions.