In-situ Reduction Coupled with Imaging Mass Spectrometry as a Novel Method for T1D Biomarker Identification in nPOD Tissue Samples

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Purpose: There is an unmet medical need to identify novel biomarkers of type 1 diabetes (T1D). In T1D insulin producing cells are the selective target for inflammatory autoimmune destruction. Our long-term goal is to characterize differentially expressed proteins in human pancreatic tissues using matrix-assisted laser desorption ionization mass spectrometry imaging (MALDI-MSI). The ability to track relative insulin expression during MALDI-MSI allows for verification of β-cell status and novel pathways leading to beta cell damage though in-situ MS-MS has been used in the identification of molecules directly from tissues, there are technical limits on the identification of biomolecules with molecular masses ≥ 4000 Da. We have used in-situ reduction of disulphide bonds in the proteins of pancreatic tissue sections to generate optimum sized components that are amenable to direct MS/MS. Presently, there is no known cure for T1D and diabetics endure the invasive conventional treatment of insulin replacement therapy. Here, we apply MALDI-IMS after on- tissue reduction to mine the proteome of the pancreatic islet of Langerhans as a means of identifying key players involved in insulitis.

Methods: Human Diabetic and non-diabetic pancreatic section donated from Network for Pancreatic Organ Donors with Diabetes nPOD were placed in OCT and snap frozen in 2-methylbutane submerged in liquid nitrogen. Tissue slices were cut 7µm thick in a -20°C cryostat and mounted on glass slide for hematoxylin and eosin staining. A sequential slice was cut and mounded onto an indium coated mass spectrometry slide for MALDI-IMS. The tissue was then washed and fixed in ethanol and left to dry in a desiccator until it was homogeneously sprayed with matrix in an automated sprayer (Bruker Daltonics). The sprayed tissue was subjected to MALDI-IMS. Using a pre-clinical model in which a target gene was deleted in the nod mouse, this tool was able to detect unique proteins of interest that could distinguish between the diabetic NOD and protected non-diabetic NOD-Alox15null.mice. In the human tissue samples, we have used in-situ reduction of disulphide bonds in the proteins of pancreatic tissue sections to generate optimum sized components (≥ 4000 Da) that are amenable to direct MS/MS.

Summary of Results: Mass spectrometry data showed that we can clearly differentiate between the pancreases of a NOD versus NOD-Alox15null using IMS to identify the islets and Langerhans and potential novel biomarkers that are specific to each group. In human tissue, we succeeded in the identification of mature insulin, a 5.8 kDa multidomain protein comprised of smaller A and B chains. Reduction generated the individual chains and MS/MS analysis effectively identified the B chain with an m/z of 3430.664. The MALDI-MSI image of the 5812.85 insulin peak before reduction and the 3430.664 peak after reduction both co-localized with the healthy pancreatic islets.

Conclusions: This study showed that insulin, a relatively large molecule can be easily identified in human pancreatic sections via MALDI-IMS. This approach is now being used to determine differential protein expression between a human diabetic and non-diabetic pancreas. We expect to identify candidates to be useful in the identification of therapeutic molecular targets in β-cells.