Laser Microdissection Coupled Nano-Proteomic Characterization of Functional Dysregulation in Pancreatic Islets of Pre-Type 1 Diabetic Patients

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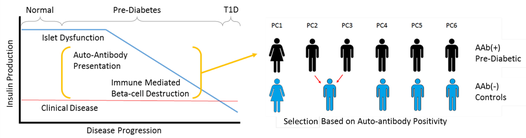
Overview

- The etiology and early pathogenesis of type 1 diabetes leading to clinical disease are still unknown[1].
- We hypothesize that dysfunctional insulin producing beta-cells may participate in disease pathogenesis by provoking immune response.
- Deep proteomic profiling of pre-symptomatic autoantibody positive (AAb+) patients compared to age/sex matched controls shows dysregulation of important cellular processes including translation, endoplasmic reticulum stress, and exocytosis.

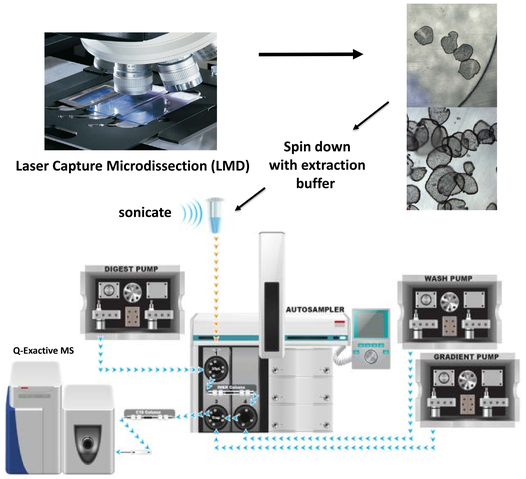
Introduction

- There are many known genetic risk factors that increase the likelihood that one could develop T1D. However, even individuals with the strongest risk factors may never go on to develop the disease. Only a 50% disease concurrence exists among identical twins.
- What drives one at-risk individual to develop the disease when another at-risk individual does not?
- Prior to onset of clinical T1D, the majority of early-stage patients experience a period of non-symptomatic immune attack against insulin producing beta cells within the Islet of Langerhans. The initiating provocation is unknown.
- Autoantibodies against beta-cell associated proteins are present and some islets experience an immune mediated state of inflammation known as insulitis.
- During this non-symptomatic phase beta-cells are still able to produce sufficient insulin to maintain glucose homeostasis despite declining beta cell mass.
- The ability to study beta-cells under immune attack, before they are eliminated in the disease state, is a rare an special opportunity.
- Herein we use a highly sensitive ultra-low starting material LC-MS/MS method to identify significant protein differences between healthy and pre-disease patients.

Methods



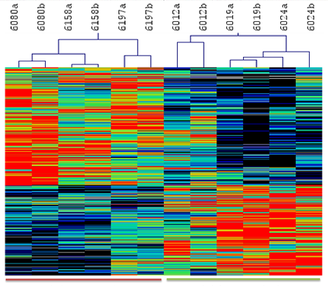
- AAb+ and age/sex matched control samples were obtained from Network for Pancreatic Organ Donors with Diabetes (nPOD), a project funded by the Juvenile Diabetes Research Foundation (JDRF).
- Laser capture microdissection of islets from 10 µm sections of pancreata were pooled to yield 2-3 islet equivalents (IEQs), about 0.5 µg protein.
- Samples were processed using an automated inline ultra-small scale bottom-up proteomics platform called the simplified nanoproteomics platform (SNaPP)[2].
- Protein identification and quantification was completed with MaxQuant software[3] with MBR and peptide level FDR<1%.
- The final three-by-three comparison included only doubly AAb+ patients with the most commonly detected AAb types.
- Statistical analyses were complete using Inferno, MeV, David, Ingenuity Pathway Analysis, and Skyline software.



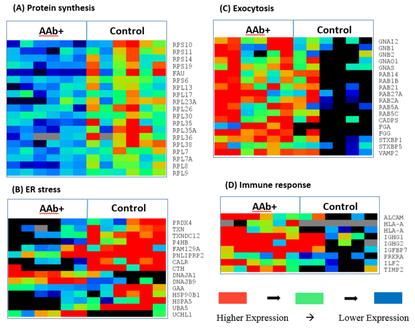
Results

Pre-symptomatic AAb+ individuals demonstrate similar patterns of Islet dysfunction involving endoplasmic reticulum stress, translational deficiencies, and increased exocytotic demands

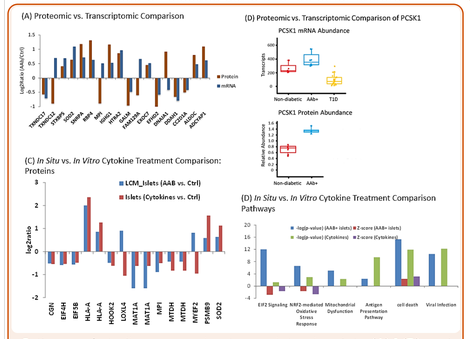
nPOD Case ID	Case Type	Age	Sex	Race	nPOD Case ID	AAb Type	Age	Sex	Race
6012	non-diabetes	68	Female	Caucasian	6080	GADA+ mIAA+	69.2	Female	Caucasian
6019	non-diabetes	42	Male	Caucasian	6158	GADA+ mIAA+	40	Male	Caucasian
6024	non-diabetes	21	Male	Caucasian	6197	GADA+, IA-2A+	22	Male	African American



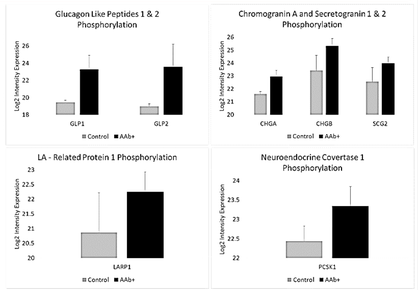
Hierarchal clustering of patients shows strongest correlation based on disease state over all other factors.



(A) Deficiencies in proteins involved in translation, (B) Loss of ER responsive stress proteins and protein chaperones, (C) Increased proteins involved in exocytosis and secretion, (D) Increase of immune responsive proteins.



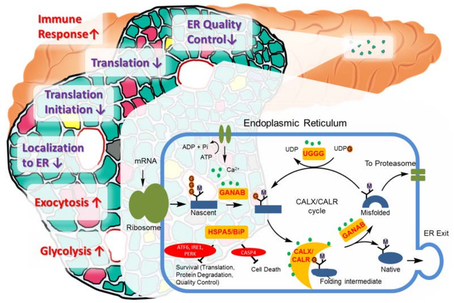
Orthogonal studies comparing transcriptomics data (A)&(B) and in vitro study evaluating effects of cytokine treatment (C)&(D).



Significant phosphorylated post translationally modified proteins identified in the study. Of special note: GLP1 and GLP2 which are produced exclusively by the alpha cells within the islet, indicated potential islet wide dysregulation.

Conclusions

- Non-symptomatic AAb+ individuals share similar patterns of cellular dysfunction involving protein synthesis, ER stress response, protein processing, and exocytosis.
- This state of dysregulation could create an error prone environment to produce improperly translated and processed proteins which are immunogenic thereby provoking the immune response that ultimately leads to the destruction of the insulin producing beta cells.
- ER dysfunction appears to play a central role in the most highly impacted cellular processes of AAb+ individuals.
- Novel post translational modifications identified in this study indicate islet wide dysfunction, not just with beta cells.



Acknowledgements

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Career Opportunities: For potential openings in Integrative Omics Group at PNNL please visit http://omics.pnl.gov/careers

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