
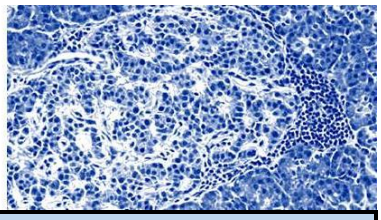

Comprehensive Proteomics Analysis of Human Islets Reveals GDF15 as a Protective Factor in Response to Proinflammatory Cytokines

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PURPOSE

Type 1 diabetes (T1D) is characterized by the autoimmune mediated destruction of pancreatic β-cells. The inflammatory process is mediated by autoreactive cytotoxic T-cells (CD8) and macrophages, as well as by pro-inflammatory cytokines secreted by these immune cells. In this study we sought to identify protein signatures that reflect the health of islet cells when exposed to a cocktail of pro-inflammatory cytokines (IL-1β, IFN-γ) and may serve as biomarkers and/or protective agents for β-cell stress/death during the development of T1D.

METHODS

Human pancreatic islets obtained from 10 cadaveric donors were treated with or without pro-inflammatory cytokines (IL-1β and IFN-γ) for 48 h, then their proteins were digested with trypsin and the resulting peptides barcoded with chemical tags before analysis using 2D liquid chromatography coupled with tandem mass spectrometry. The results were validated by performing an independent proteomic analysis of islets from 2 additional donors, cultured and treated with cytokines in an independent laboratory. We subsequently investigated the protective role one of these proteins identified in this screen by studying its molecular and functional role in prevention of β-cell stress/death in a mouse model of T1D, and also tested for the presence/absence of this protein in nPOD pancreas tissue sections from control, T1D, and T2D donors.

SUMMARY OF RESULTS

Proteomics analysis led to the identification of 11,325 proteins, of which 387 were significantly and differentially expressed in islets exposed to pro-inflammatory cytokines. Evaluating each protein for adequate power indicated that 87.7% of proteins had a power of greater than 0.8 to detect a fold-change of 1.5 for sets 1 and 2, respectively. (Figure S1).

The power analysis confirmed that the size of the present study is appropriate to investigate even small changes in protein abundances in response to the cytokine treatment. Informatics analysis revealed activation of multiple pathways related to immune response, type 1 diabetes, cell death and cytokine signaling. Additionally, of the 387 significantly differentially expressed proteins, 207 were also detected in the independent proteomic analysis and 182 (88%) validated (i.e. same direction of change). Of interest growth/differentiation factor 15/GDF15 was found to be consistently downregulated upon cytokine treatment, a phenomenon that appears to be regulated at the post-transcriptional level, as identified by polyribosomal profile analysis. Pretreatment of MIN6, EndoC-BH1 cells and human islets with recombinant GDF15 protected cells from cytokine mediated β-cell death, and its levels were noted to be downregulated in islets harboring insulitis from NOD mice. Similarly, GDF15 was noted to be reduced in islets from nPOD donors with T1D compared to controls, but not in donors with T2D. Treatment of prediabetic NOD mice with recombinant GDF15 showed a significant decrease in insulitis and β-cell oxidative stress, with subsequent decrease in the incidence of T1D.

CONCLUSIONS

We present a unique resource for the identification of human islet proteins regulated by pro-inflammatory cytokines. By mining this new dataset, and integrating it with available RNA sequencing data, we detected an imbalance between pro- and anti-apoptotic proteins modulated by IL-1β + IFN-γ in β cells. This imbalance includes a post-transcriptional down-regulation of GDF15, presently shown to act as an anti-apoptotic protein. This finding provides a proof of concept for the utility of the present resource. Additional extension and mining of the human islet proteome has the potential to indicate novel avenues for the therapy of diabetes.