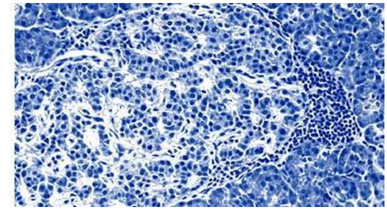
nPOD
Network for Pancreatic Organ Donors with Diabetes



miRNA Signatures in Human Pancreatic Slices during Glucose Stimulation

AUTHORS

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PURPOSE

Through a working group effort nPOD has successfully established that it is possible to conduct studies of live pancreas slices from organ donors, in which the cytoarchitecture of the organ is preserved and islets are not subjected to the stress of isolation. In Miami, we have conducted functional assessment of pancreatic cell function from slices obtained from 12 nPOD donors (with and without T1D). Insulin and glucagon secretion were assessed upon glucose and KCl stimulation. miRNAs are small noncoding RNA molecules known to regulate gene expression and are emerging as novel biomarkers for many diseases. Our laboratory has been investigating serum levels of miRNAs as biomarkers of islet autoimmunity and/or progression to T1D. To investigate how glucose stimulation impacts miRNA expression, we measured miRNA levels in the perifusates of human pancreas slices and correlated levels with insulin and glucagon release upon glucose stimulation.

METHODS

Human pancreas slices were obtained from 3 nPOD donors, without T1D; 1 donor was male (6482) and 2 were females (6261 and 6462), and they were 11, 14, and 13 years old, respectively. Slices were produced at the nPOD laboratory in Gainesville and shipped overnight to Miami. Upon arrival, slices were allowed to rest for two hours and then assayed for insulin and glucagon secretion following stimulation with glucose 3 mM for 10 min, glucose 16.7 mM for 20 min, glucose 1mM for 30 min, KCl 25mM for 5 min and glucose 3 mM for 10 min. Insulin and glucagon secretion were measured for each point via ELISA. Four time points per donor were selected for each donor, and point selection was based on the insulin and glucagon secretion data from each donor. The baseline point corresponded

to a glucose level of 3 mM, then we selected two samples corresponding to the peaks in the GSIS (glucose stimulate insulin secretion) curve upon stimulation with 16.7 mM glucose, and the 4th sample was taken at 1 mM glucose representing the shift between insulin and glucagon secretion. Frozen perifusate samples were collected and later miRNAs were assayed using the EdgeSeq platform from HTG Molecular. This RNAseq based assay allows for the detection and quantification of over 2,083 miRNAs without prior miRNA extraction in a 15 µL volume. The samples were assayed individually and then grouped for statistical analyses using the DESeq2 Statistical Pipeline (version 1.14.1) from HTG Molecular Diagnostics, Inc. The DESeq2 package provides methods for estimating and testing differential expression using negative-binomial generalized linear models. Differential expression outputs are the mean value of each probe for each group after normalization, the log2-transformed average expression of each probe across all groups after normalization, the estimated fold change between the two groups (transformed from log-fold change). Significance is defined for $p<0.05$ after correction for false discovery rate using the Benjamini and Hochberg method.

SUMMARY OF RESULTS

We analyzed data from 12 samples from 3 nPOD donors. All 2,083 miRNAs were detected in all samples tested. Levels measured were within the detection limits for other sample types such as serum. To identify miRNAs showing changes in levels of expression during the stimulation, we compared miRNA levels as follows: 3 mM vs 16.7 mM Glucose; 3 mM vs 1 mM Glucose; 16.7 mM vs 1 mM Glucose. Of the 2,083 miRNAs tested, 139 (6.67%) showed changes in levels of expression that reached statistical significance: 1) levels of 124 miRNAs varied in response to a shift from low to high glucose and in parallel with the peaks of insulin secretion; 2) levels of 15 miRNAs changed from high to low glucose and mirrored the glucagon secretion. Among these miRNAs, 8 miRNAs (miR-29a-3p, miR-103a-3p, miR-140-5p, miR-181a-5p, miR-24-3p, miR-25-3p, miR-26b-5p and miR-27b-3p) have been linked to T1D in more than one published study and in our experimental setting seem to be related to insulin secretion. miR-197-3p and miR-30c-5p were also previously linked to T1D and seem to be related to glucagon secretion. Some microRNAs exhibited a parallel response pattern for both glucagon and insulin secretion. However, some of them exhibit an unparallel pattern of secretion which may indicate different mechanisms of release.

CONCLUSIONS

Our pilot studies using human pancreas slice perifusates show that it is possible to measure miRNAs during GSIS. We indeed observed significant level changes in a subset of miRNAs which reflect glucose stimulation. While these results were obtained from whole pancreas tissue, the response to stimulation suggests that the changes in miRNA levels most likely reflect changes in islet function during the response to glucose. Thus, these results help define the relationship of certain miRNAs with beta cell function. In addition, several of the miRNAs reproducibly associated with T1D in published studies exhibited significant changes during GSIS, further validating their relationship to islet cell responses and to T1D. Further studies should provide insight into alterations of miRNA responses during GSIS in nPOD donors with T1D.