Gene Expression Profiling of Islet Cell Subtypes

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Pancreatic endocrine cells are co-located into clusters called the islets of Langerhans that are comprised of glucagon producing alpha cells, insulin secreting beta cells, somatostatin generating delta cells, and other cell types. Type 1 diabetes results from an autoimmune process in which autoreactive T cells destroy the insulin producing beta cells, requiring the patient to inject insulin to regulate their blood glucose levels. Thus far, attempts to cure diabetes via islet transplantation have been limited by insufficient donor supply, inconsistent isolated islet quality, continued autoimmunity, alloimmune rejection, and limited beta cell regeneration. Diabetes research has focused on preventing the autoimmune response, promoting stem cell to beta cell differentiation, and defining the factors that influence beta cell proliferation. Islet research, in turn, has been limited to whole islet studies since, isolating the islet cell subtypes has not been possible. Using a method recently developed for mouse islet cells (Pechhold et al. Nat Biotechnol. 2009 Nov; 27(11):1038-42), that uses intracellular hormone staining and flow cytometry, we are able to sort human islets into populations uniquely expressing glucagon, insulin, or somatostatin. Further, we have developed a human gene array to measure candidate gene expression using a quantitative nucleic protection assay (qNPA). This technique uses 50 base oligomers that specifically recognize RNA from each gene of interest, overcoming limitations caused by the harsh conditions required for intracellular staining. We report gene expression analysis for specific hormones and transcription factors expressed in each islet cell population. We are further modifying this technique to study nonhuman primate islets, and investigate the specific proteome and miRNA profiles for individual islet cell populations. The goal of these studies is to characterize the genetic differences between the islet cell populations and understand which factors control beta cell regeneration and proliferation.

Pancreatic Islet Architecture & Cell Purification

The adult human islet is comprised of alpha, beta, and delta cells. The cells are dispersed in a heterogeneous manner throughout the islet.

Gene Expression Profiling of Islet Cell Subtypes

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Abstract

Pancreatic endocrine cells are co-located into clusters called the islets of Langerhans that are comprised of glucagon producing alpha cells, insulin secreting beta cells, somatostatin generating delta cells, and other cell types. Type 1 diabetes results from an autoimmune process in which autoreactive T cells destroy the insulin producing beta cells, requiring the patient to inject insulin to regulate their blood glucose levels. Thus far, attempts to cure diabetes via islet transplantation have been limited by insufficient donor supply, inconsistent isolated islet quality, continued autoimmunity, alloimmune rejection, and limited beta cell regeneration. Diabetes research has focused on preventing the autoimmune response, promoting stem cell to beta cell differentiation, and defining the factors that influence beta cell proliferation. Islet research, in turn, has been limited to whole islet studies since, isolating the islet cell subtypes has not been possible. Using a method recently developed for mouse islet cells (Pechhold et al. Nat Biotechnol. 2009 Nov; 27(11):1038-42), that uses intracellular hormone staining and flow cytometry, we are able to sort human islets into populations uniquely expressing glucagon, insulin, or somatostatin. Further, we have developed a human gene array to measure candidate gene expression using a quantitative nucleic protection assay (qNPA). This technique uses 50 base oligomers that specifically recognize RNA from each gene of interest, overcoming limitations caused by the harsh conditions required for intracellular staining. We report gene expression analysis for specific hormones and transcription factors expressed in each islet cell population. We are further modifying this technique to study nonhuman primate islets, and investigate the specific proteome and miRNA profiles for individual islet cell populations. The goal of these studies is to characterize the genetic differences between the islet cell populations and understand which factors control beta cell regeneration and proliferation.

Sorted Islets Gene Expression Profiles

Figure 2: Sorting Alpha, Beta, and Delta Cells. The gating hierarchy (A) and strategy (B-E): viable cells are selected by gating out debris (low fsc) and VRC nonspecific fluorophore binding (VRC is pre-incubated with PE and high PE labeling is gated out B-C). Each hormone expressing cell population is then gated (insulin = APC (D); glucagon = Pacific Blue (E); somatostatin = 488 (E)), doublets are excluded per the FSC-Height vs. FSC-Area plot (F-H) and a final sort gate is generated by plotting fluorophore of interest versus the remaining nonspecific fluorophore (I-K). Note the insulin (red) cells have the highest side scatter, due to their granular nature (L). Following sorting, a small amount of sorted insulin cells were reanalyzed to determine if the cells have been properly sorted and are only insulin positive. Cells only appear in the initial insulin gate, within the singlet gate, and within the sort gate. All other debris and non-insulin expressing cells have been excluded. These sorted cells provide the mRNA that will be used in the qNPA arrays to measure gene expression within each islet cell subtype.

Conclusions

We have shown that we can purify adult human islets into individual cellular populations. This is the first step in understanding the genetic and environmental components that regulate increased beta cell proliferation and beta cell mass. In the absence of full-length miRNA for RT-PCR or next generation sequencing, the qNPA technique provides candidate gene expression profiles for these cells.

Sorted Islets Gene Expression Profiles

Figure 4: qNPA Results. Three human donor samples were sorted and purified alpha and beta cell populations (as well as a population that did not stain for insulin, glucagon, or somatostatin) were assessed for their gene expression profiles. Hi10-10 = male, age 48, BMI 34.4; HI11-2 = female, age 55, BMI 31.6; HI11-6 = unknown gender, age 66, BMI 34, diagnosed T2DM. Insulin and glucagon were highly expressed in beta and alpha cells, respectively, while glucagon mRNA is also detected in purified beta cells.

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