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 autoimmune, 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 acid 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.

Figure 1: Islet Cell Purification Flowchart. We dissociate human islets into a single cell suspension, fix and permeabilize the cells with PFA and saponin, respectively, stain for intracellular hormones using insulin, glucagon, and somatostatin antibodies that are either pre-labeled or are recognized by secondary antibodies conjugated to fluorescent probes. The dissociated and stained islets are sorted into individual cell types using fluorescence-activated cell sorting technology.

Figure 2: Sorting Alpha, Beta, and Delta Cells. The gating hierarchy (A-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)). doubles are excluded per the FSC-Height v. 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.

Figure 3: qNPA Setup. Two part preparation: (A = sample preparation) fixed and sorted cells are incubated with gene specific 50-mer probes, S1 nuclease digests unbound probes and mRNA. The remaining gene specific probes are transferred to the qNPA plate. (B = plate preparation) each 96-well has a 4x4 grid map printed on it. Each spot within the well corresponds to a specific gene and is programmed with linkers that recognize one of the sixteen respective spots. The sample qNPA probe is added and binds to the programming linker. The detection linker hybrids to the probe and the detection probe binds to it. After incubation with HRP substrate, photons are measured as an indicator of signal intensity.

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.

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 mRNA for RT-PCR or next generation sequencing, the qNPA technique provides candidate gene expression profiles for these cells.