From the Transcriptome to Electrophysiology: Searching for the Underlying Cause of Diabetes

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Cells within the islet of Langerhans are heterogeneous. Camunas-Soler et al. (2020) implement a patch-seq technique to collect both transcriptomic and electrophysiological data from the same cell. By doing so, they discover new genes that correlate with functional heterogeneity and find that shifts in these correlations indicate β cell compensation in type 2 diabetes.

Insulin secreting β cells have long been known to be heterogeneous, in terms of morphology and function (Pipeleers, 1992; Salomon and Meda, 1986). To understand the molecular mechanisms driving this heterogeneity, there has been a surge in single-cell sequencing and other high-throughput approaches (Segerstolpe et al., 2016; Wang and Kaestner, 2019). There have also been interesting discoveries in terms of functional heterogeneity (Benninger and Hodson, 2018). However, a major gap to date has been reconciling the functional heterogeneity present within the islet with the rich array of single-cell data that indicates a host of diverse sub-populations. Camunas-Soler et al. (2020) implement a "patch-seq" approach to bridge this gap and collect both molecular information via single-cell RNA sequencing (scRNA-seq), and functional information via recording patch clamp on the same cell, from islets of healthy individuals and those with type 2 diabetes (T2D) (Figure 1).

Patch clamp is a long-established technique to interrogate the electrophysiological properties of a cell, including ionic currents, membrane potential, exocytosis, and cell size. In patch-seq, the patch pipette is supplemented by an additional pipette to extract the cell contents for scRNAseq analysis. Patch-seq was first developed for determining electrophysiological, transcriptomic, and morphologic profiling of individual neurons (Cadwell et al., 2016). Adopting this approach, the authors manually performed patch clamp of 1,369 human islet cells (including α, β, δ, and other endocrine cells). This is an impressive feat in and of itself given that patch clamp is known to be a slow but accurate method to profile cell function. Following cell content extraction for scRNAseq analysis, the team compiled a dataset permitting the visualization and analysis of how heterogeneity in gene expression correlates with heterogeneity in ionic currents and exocytosis in islet endocrine cells. They also examined how this differs between islets from healthy subjects and those with T2D. While generating an impressive dataset that will be a valuable resource for islet biology researchers, the authors themselves describe several important findings.

By correlating gene expression with islet function on a cell-by-cell basis, the authors discovered already known genes but also a number of novel genes not previously linked to β cell function such as cell adhesion molecules. The authors also validate via knockdown that OGDHL, FAM159B, TSPAN1, and RGS9 regulate β cell function. Further, RBP4, which has previously been shown via scRNAseq analysis to mark a sub-population of β cells, was shown here to also mark a β cell sub-population with reduced function.

Demonstrating the robustness of their dataset Camunas-Soler et al. (2020) developed a model in which the electrophysiology of the β cell could be predicted from a subset of gene expression signatures. The authors selected a network of genes with significant correlation to more than one aspect of electrophysiology (that is, Ca^{2+} or Na^{+} channel current or exocytosis). The authors used this gene set to generate a model that effectively predicted both ionic currents as well as exocytosis in a separate test set of data. This approach may prove useful to analyze other scRNAseq datasets to predict the function of different population clusters, without the need to patch another 1,000 cells—to the relief of many labs! However, whether the genes analyzed are driving ionic currents and exocytosis, or whether they simply correlate with increased function, remains to be determined.

Reduced β cell exocytosis is known to occur in T2D, and this was demonstrated by the authors. Although one may assume genes discovered to correlate with exocytosis would be decreased in β cells from individuals with T2D, the authors surprisingly observed the converse: genes that correlate with exocytosis in non-diabetic subjects were upregulated in β cells from donors with T2D but negatively correlate with exocytosis. The authors demonstrated that distinct molecular pathways are linked to reduced exocytosis in healthy subjects (often metabolic pathways) and subjects with T2D (often immune response and cell cycle pathways). This transcriptional shift was also observed in subjects with obesity and thus strongly hints at an underlying mechanism of islet compensation under metabolic stress. Indeed, one gene that positively correlates with function in healthy subjects but negatively correlates with function in subjects with T2D is the transcription factor ETV1: knockdown experiments supported its shift to a negative role in T2D. However, a key unresolved question—what is underlying insufficient compensation in T2D—may be gained upon further analysis of data from individuals with obesity and T2D.

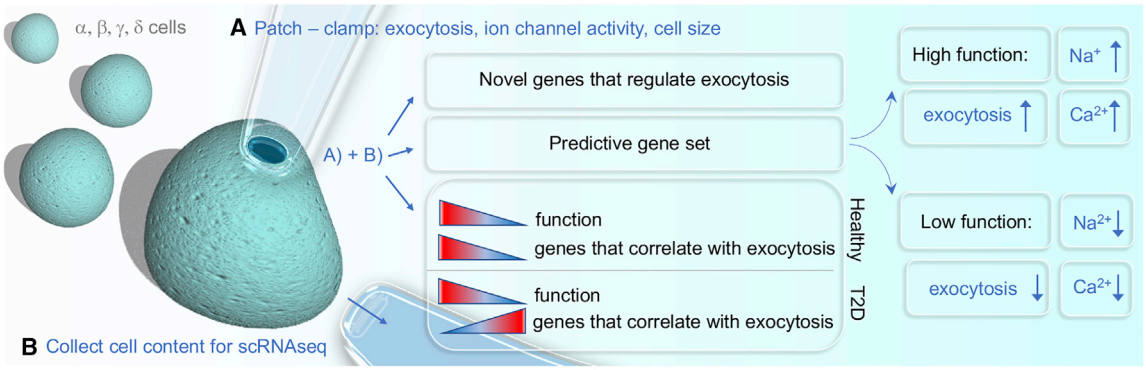

Figure 1. Patch-Seq Measurements in Islet Endocrine Cells
Patch-seq combines patch clamp recordings of cell electrophysiological properties with cell content collection for scRNAseq analysis. Applying this to islet endocrine cells, the authors identify novel genetic regulators of human β cell function under healthy conditions and in T2D.

Finally, the authors performed patch-seq on "bio-banked" frozen samples, including those of subjects with T1D. They demonstrated that β cells, from individuals with T1D, although infrequent, show relatively normal gene expression signatures, albeit with an increase in genes related to immune activation. Interestingly RBP4 expression decreased, hinting at a loss of the β cell sub-population with reduced function. In contrast, α cells showed more extensive transcriptional and functional changes. These findings support recent studies demonstrating significant α cell dysfunction in T1D (Brissova et al., 2018) and the utility of frozen bio-banked samples.

Overall this study provides a trove of data, unprecedented in detail, revealing novel genes that regulate β cell function, and novel gene signature changes and mechanisms underlying endocrine cell dysfunction in diabetes. One obvious caveat is that these studies were performed in dissociated islet cells. We know from many studies that extensive cross talk between endocrine cells within the islet is critical for their function and likely gene expression. For example, crosstalk between β cells regulates their electrical activity, whereas interactions between α, δ, and β cells regulate exocytosis. Thus, whether the link between transcriptional and functional heterogeneity in healthy and diabetic cases holds within the intact islet remains to be determined. However, there is no barrier to performing these studies in intact pancreas tissue. Such studies will also reveal how the islet microenvironment, including neurons, blood vessels, and resident immune cells, impacts β cell molecular identity and function. Another open question is whether the authors found evidence for previously identified functional sub-populations, such as highly functional sub-populations or those with signatures of low maturity.

In conclusion, Camunas-Soler et al. (2020) implemented a powerful combination of the patch-seq technique, network analysis, and machine learning to link functional and molecular β cell heterogeneity. They identified genetic drivers of β cell function, suggested new compensatory trends that occur in T2D, and demonstrated the ability to measure function and the transcriptome in cryo-preserved T1D samples.

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