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Patch-seq shows the heterogeneity of pancreatic islet cells

Dysfunction of pancreatic islets plays an important role in the onset and progression of type 2 diabetes. The change of gene expression in pancreatic islets, which is affected by genetic factors, environmental factors, or both, impairs insulin or glucagon secretion and results in the abnormal regulation of glucose metabolism. The expression of genes in pancreatic islets has generally been analyzed in isolated islets using immunoblotting or quantitative real-time polymerase chain reaction. However, these methods are unable to overcome the problem of heterogeneity in pancreatic islets. Pancreatic islets consist of various cells (α , β , γ and pancreatic polypeptide) that secrete diverse hormones. The heterogeneity of pancreatic islets makes it difficult to evaluate the data from isolated islets accurately. Recently, single-cell ribonucleic acid (RNA) sequencing (scRNA-seq) analysis has been widely used to examine global gene expression in single cells. This methodology showed the existence of heterogeneous gene expression, even in the same type of cell. In addition, there are various pancreatic β -cells that show different phenotypes in a single islet. scRNA-seq analysis can be used to resolve these problems associated with examining gene expression in cells with distinct phenotypes. However, without electrophysiological analysis, it is impossible to determine whether the change of gene expression is the cause or result of hormone secretion, as gene expression is changed by its signal feedback.

In 2016, Patch-seq was established by Cadwell *et al.*¹ and Fuzik *et al.*² for the

analysis of electrophysiology, gene expression and morphology in neuronal cells. They promoted the classification of cell types by Patch-seq, which is the analysis of morphology and scRNA-seq followed by the study of firing patterns in neuronal cells. Recently, Camunas-Soler *et al.*³ used Patch-seq to analyze the functions and gene expression profiles of individual cells in pancreatic islets (Figure 1). Remarkably, they examined the association between the pathogenesis of type 2 diabetes and the phenotypes of pancreatic islets using 1,369 human islet cells, which showed more heterogeneity than rodent islet cells, from type 2 diabetes patients and non-diabetes (ND) controls. After they classified the isolated pancreatic tissues into α , β , γ , pancreatic polypeptide and acinar cells on the basis of the expression of key marker genes in each cell type, they examined exocytosis and ion channel currents. When evaluating the results of Patch-seq, it is important to confirm that the experimental techniques used did not affect the gene expression detected with scRNA-seq. Camunas-Soler *et al.*³ examined the effect of single-cell dispersion, patch clamp and other methods on gene expression, and they confirmed that these procedures had no effects on the results of scRNA-seq.

In this study, their examinations showed an association between the comprehensive gene expression profile and exocytosis in single pancreatic islet cells. In particular, insulin secretion from pancreatic β -cells has been considered to be unique in each cell, even if they reside within the same islet. Patch-seq enabled the authors to evaluate the expression of genes that regulate each phase of exocytosis (early, total and late). Furthermore, Patch-seq is expected to be useful in identifying unknown novel molecules, because it is a comprehensive analysis method. The

authors carried out small interfering RNA knockdown of molecules that were identified as exocytosis-associated factors by Patch-seq using islets from ND controls. This experiment showed the reduction of exocytosis after the knockdown of oxoglutarate dehydrogenase L, family with sequence similarity 159 member B, tetraspanin 1 and regulator of G-protein signaling 9. The specific roles of these molecules in insulin secretion remain obscure, but Patch-seq might shine a light on the novel mechanisms of hitherto unnoticed molecules.

In addition, Patch-seq might also enable the identification of molecules that can explain the heterogeneity of pancreatic β -cells. For instance, retinol-binding protein 4 has been reported to be heterogeneously expressed in pancreatic β -cells^{4,5}, but it has been considered to have no contribution to the functional heterogeneity of β -cells. However, in this study, Patch-seq showed that Na currents and exocytosis were reduced in retinol-binding protein 4-positive β -cells. Furthermore, the expression of molecules that affect insulin secretion, such as potassium inwardly-rectifying channel subfamily J member 8, adenosine triphosphate-binding cassette subfamily C member 9 and sodium voltage-gated channel alpha subunit 3, was found to be decreased in these cells. These findings might suggest the mechanisms underlying the heterogeneity of pancreatic β -cells.

Next, using pancreatic β -cells from type 2 diabetes patients, the authors investigated the associations between exocytosis and gene expression in each cell. Unexpectedly, molecules that were positively associated with exocytosis in pancreatic β -cells of ND controls were increased in the β -cells of type 2 diabetes patients. This phenomenon seems to be contradictory, but perhaps it might be a compensatory reaction

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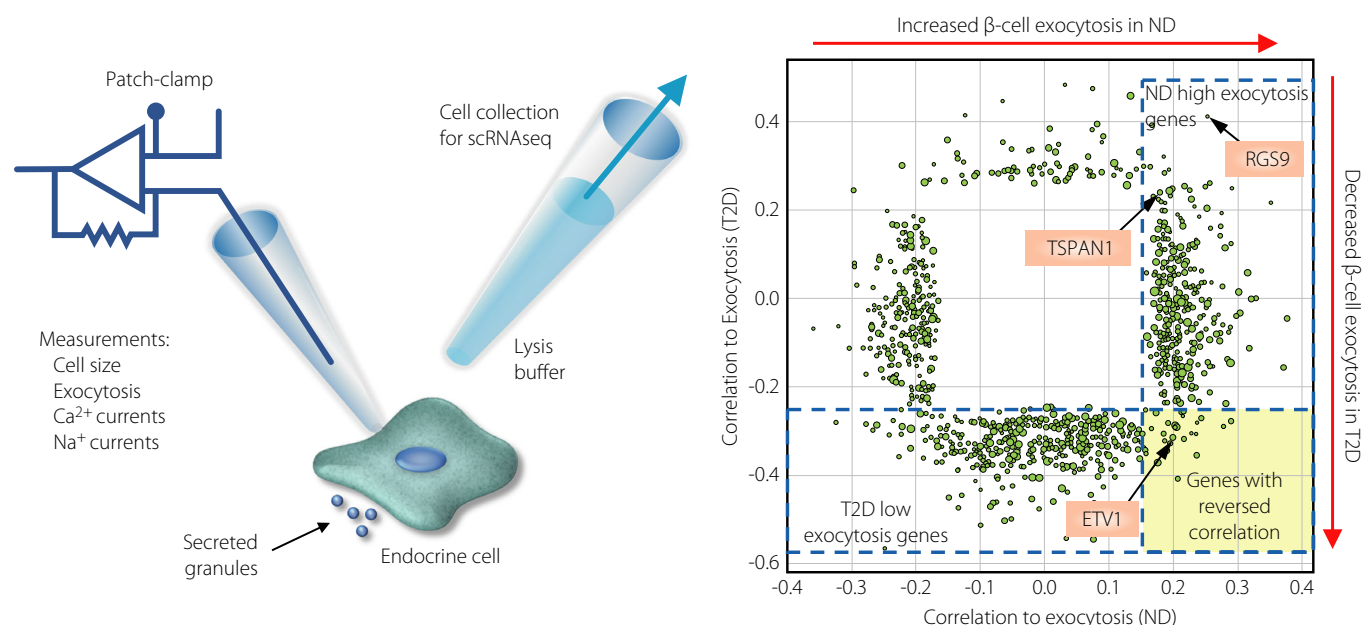


Figure 1 | Patch-seq measurements in pancreatic endocrine cells. Patch-seq was established as a two-step process: (i) electrophysiological measurements using whole-cell patch-clamp; and (ii) collection of cellular content using a larger secondary pipette filled with lysis buffer. This analysis showed that genes positively correlated with exocytosis in non-diabetes (ND) controls were unexpectedly upregulated in β -cells from type 2 diabetes patients, but negatively correlated with exocytosis. Reproduced with permission by Camunas-Soler *et al.*, *Cell Metab*, 2020³ with some modifications. Copyright and all rights reserved. RNA, ribonucleic acid; T2D, type 2 diabetes.

according to insulin demand in type 2 diabetes patients. Finally, however, there was a reduction of exocytosis in the pancreatic β -cells of type 2 diabetes patients compared with those of ND controls. Interestingly, differences in the molecules associated with reduced exocytosis in pancreatic β -cells were found between type 2 diabetes patients and ND controls. For instance, although the transcription factor erythroblast transformation specific translocation variant 1 was highly expressed and associated with reduced exocytosis in the pancreatic β -cells of type 2 diabetes patients, it was found to be associated with increased exocytosis in the β -cells of ND controls (Figure 1).

Finally, the authors carried out Patch-seq analysis in the islets of patients with type 1 diabetes using cryopreserved samples. The pancreatic β -cells of type 1 diabetes patients showed no differences in terms of electrophysiological findings and gene expression of β -cell markers from

cryopreserved pancreatic β -cells of ND controls. In contrast, the β -cells of type 1 diabetes patients showed an increase in natural killer 6 homeobox 1 expression and a decrease in natural killer 2 homeobox 2 expression, concomitant with a reduction of Ca channel activity compared with the β -cells of ND controls. It is of great interest that these results suggest an abnormality of glucagon secretion in individual cells of type 1 diabetes patients.

In this article, Camunas-Soler *et al.*³ analyzed the functional and gene expression heterogeneity of human pancreatic endocrine tissue by using Patch-seq. The results obtained using single cells showed an association between exocytosis and gene expression in the pancreatic β -cells of type 2 diabetes patients, and these results might help to identify novel mechanisms in insulin secretion. In addition, this method was shown to be useful for the analysis of the pancreatic islets of type 1 diabetes patients. In the future, Patch-seq is expected to contribute to the

complete characterization of pancreatic islets in diabetes patients.

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DISCLOSURE

The author declares no conflict of interest.

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