Review

MOLECULAR METABOLISM

Interrogating islets in health and disease with single-cell technologies

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ABSTRACT

Background: Blood glucose levels are tightly controlled by the coordinated actions of hormone-producing endocrine cells that reside in pancreatic islets. Islet cell malfunction underlies diabetes development and progression. Due to the cellular heterogeneity within islets, it has been challenging to uncover how specific islet cells contribute to glucose homeostasis and diabetes pathogenesis. Recent advances in single-cell technologies and computational methods have opened up new avenues to resolve islet heterogeneity and study islet cell states in health and disease.

Scope of review: In the past year, a multitude of studies have been published that used single-cell approaches to interrogate the transcriptome and proteome of the different islet cell types. Here, we summarize the conclusions of these studies, as well as discuss the technologies used and the challenges faced with computational analysis of single-cell data from islet studies.

Major conclusions: By analyzing single islet cells from rodents and humans at different ages and disease states, the studies reviewed here have provided new insight into endocrine cell function and facilitated a high resolution molecular characterization of poorly understood processes, including regeneration, maturation, and diabetes pathogenesis. Gene expression programs and pathways identified in these studies pave the way for the discovery of new targets and approaches to prevent, monitor, and treat diabetes.

Keywords Pancreatic islet; Endocrine cell; Single-cell; Heterogeneity; Type 2 diabetes; RNA-seq

1. INTRODUCTION

The islets of Langerhans in the pancreas contain five different endocrine cell types, namely beta, alpha, delta, gamma (also called PP cells), and epsilon cells, which each produce a different hormone. These hormones are secreted in response to metabolic cues and together orchestrate the maintenance of blood glucose homeostasis. Pancreatic hormones do not function in isolation, but influence each other's release through endocrine, paracrine, and autocrine feedback mechanisms [1,2]. It is well established that individual cells of a given islet cell type are heterogeneous in nature and that this heterogeneity forms an important basis for islet behavior [3]. Islet cell heterogeneity has been most extensively studied for beta cells, which play a critical role in the pathogenesis of diabetes. While it is well known that damage or loss of beta cells causes diabetes, how other endocrine cell types contribute to disease pathogenesis is not fully understood. Moreover, it is still largely unclear if different cellular states and subpopulations within islet cell types contribute to diabetes pathogenesis.

First observations that beta cells are heterogeneous and differ in regard to insulin secretion were made more than 30 years ago. Salomon and Meda developed methods to visualize insulin release from individual beta cells and reported substantial differences between individual cells [4]. The idea of functionally relevant heterogeneity among beta cells was further bolstered by studies showing that beta cell subpopulations exhibit different sensitivity to glucose [5,6] and change dynamically in response to glucose exposure [7–11]. It has been further suggested that the functional state of individual beta cells affects their fragility, as differences in insulin expression, glucose responsiveness, and oxidative state between beta cells have been associated with susceptibility to oxidative and cytokine-induced damage [12–14]. In the 1990s, the use of microscopy techniques and the development of fluorescent dyes greatly expanded the research of islet function with a strong visual impact at the single-cell level. Calcium imaging provided crucial information on the calcium influx pattern in response to glucose in different islet cell types [15–17]. Measurement of cytosolic calcium in individual beta cells further revealed heterogeneity of calcium oscillations in response to different secretagogues among individual beta cells [18]. One shortcoming of these early studies was that they were conducted in dispersed islet cells and therefore lacked spatial resolution and presence of functionally relevant cues in the intact islet. Recent studies, using optical interrogation of intact islets in tissue slices have overcome these limitations and have convincingly demonstrated functional differences

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between individual beta cells in rodent and human islets [19–21]. In addition to insulin secretion, heterogeneity among beta cells also exists for other features. For example, beta cells differ with regard to their proliferative activity [22,23] and expression of senescence markers, such as p16Ink4a [24,25].

Until recently, most of our knowledge about how islet cells change their molecular features in response to different physiological and pathological conditions was obtained by studying whole islets. As these studies detect global patterns, they represent an average dominated by the most abundant cell types and thus mask contributions from more rare cell types and subpopulations. Fluorescence-activated cell sorting (FACS)-enrichment has been utilized to study alpha, beta, and delta cell populations. In mice, this has been accomplished by genetically tagging endocrine cell populations and isolating these cells based on a fluorescent reporter [26–29]. This strategy, however, cannot be applied to human cells, and sorting strategies for the less abundant gamma and epsilon cells are not available. In humans, other methods, such as cell sorting based on surface markers or intracellular proteins, have been used to obtain cell type-specific transcriptomes of islet cells [30–36]. But even with sorted cells, population-based profiling of islet cell types masks the variation across individual cells, thus limiting insight into different cell states or subpopulations. Recently, cell sorting strategies have been developed that can separate beta cell subpopulations in both mice and humans based on expression of specific marker genes [23,31]. Molecular analysis of these subpopulations has revealed differences with regard to proliferative capacity and responsiveness to nutrient cues. Grompe and colleagues identified distinct beta cell populations in humans that exhibit differences in basal and stimulated insulin secretion, as well as gene expression profiles [31]. The relative abundance of these subpopulations was found to be significantly altered in islets from donors with type 2 diabetes (T2D). It may be that the different subtypes influence pathogenesis through differences in susceptibility to metabolic stress, proliferative capacity, or maturation state.

Over the past decades, we have amassed a wealth of knowledge about islet function in health and disease, employing electrophysiological, microscopy, genetic, and population-based gene expression profiling approaches. More recently, an abundance of different single-cell technologies has been developed that allows even higher-dimensional analyses of isolated single cells [37–39]. These new techniques have been applied to islet cells, as demonstrated by multiple papers published on this topic in the last year, and represent a breakthrough in islet biology and diabetes research. In this review, we summarize what we have learned from studying islets at the single-cell level using new single-cell technologies to investigate islet cell function, physiology, and pathogenesis (Figure 1). Moreover, we highlight current challenges encountered when analyzing the high-dimensional data obtained using new single-cell technologies, as well as discuss how these new technologies can be utilized in the future to further interrogate islets.

2. NEW SINGLE-CELL METHODOLOGIES TO STUDY ISLET CELL PHYSIOLOGY AND FUNCTION

Just in the past year, multiple studies using new single-cell technologies have been published with the goal to understand how specific pancreatic cell types contribute to glucose homeostasis and diabetes pathogenesis (Table 1). These studies analyzed different tissues, including whole pancreas and isolated islets from mouse and human in healthy and diabetic conditions. The studies varied greatly in regard to the experimental technology used to generate single-cell expression data as well as applied methods for data analysis. To capture individual cells from pancreatic tissue, flow cytometry [40–44] and microfluidic methods [45–49] were utilized. While the most common technology used to study the molecular profile of individual pancreatic cells has been RNA-seq [41–49], proteomic approaches including imaging mass spectrometry (IMS) and mass cytometry have also been applied [40,50]. Recent advancements in computational methods and the development of new algorithms to reconstruct and investigate molecular processes has aided in reducing these high-dimensional data to an interpretable form. By revealing the cellular heterogeneity found in the pancreas, studies using single-cell technologies have advanced our understanding of cell function and cell communication in the pancreas. The technologies enabled the study of less abundant islet cell types and revealed previously unknown cellular states and subpopulations of endocrine cells. Together, these studies have uncovered new functions for islet cells and allowed a high-resolution molecular characterization of poorly understood processes, including islet cell regeneration, maturation, and T2D pathogenesis.

2.1. Adult islet cell function

Single-cell profiling allows the interrogation of less abundant endocrine cell types, which have remained elusive in studies of both whole islets and sorted islet cells. Multiple studies from this past year that have uncovered the transcriptional signatures of individual human delta, gamma, and epsilon cells from human islets suggest important and novel roles for each islet cell type in sensing and integrating specific systemic cues to govern islet function [42,43,47]. These novel insights are based on the observations that receptors for cell signaling pathways are specifically enriched in individual islet cell types (Figure 1). Compared to other islet cell types, delta cells, for example, highly express receptors for leptin (*LEPR*) and ghrelin (*GHSR*) [42,43,47], suggesting that pancreatic responses to these appetite-regulating hormones are mediated by these cells. Indeed, work by Huising and colleagues has recently demonstrated that ghrelin selectively activates delta cells and promotes somatostatin release from pancreatic islets [27]. Delta cells also exhibit high expression of receptors for specific neurotransmitters (dopamine; *DRD2*) and growth factors (*ERBB4*) [43,47], while gamma cells selectively express receptors for acetylcholine and serotonin [42,47]. Epsilon cells, which make up less than 1% of endocrine cells, also uniquely express various receptors for neurotransmitters, endorphins, prostaglandins, and glycoproteins [43]. Thus, single-cell analysis has uncovered novel roles for these rare islet cell types as integrators of systemic cues and metabolic signals in the islet.

While much has been uncovered studying transcriptomes of the less abundant endocrine cell types, novel insight gained by single-cell RNA-seq analysis into the function of beta and alpha cells has been more limited. Genes displaying high expression in beta or alpha cells overlapped largely with those found in previous transcriptome studies employing cell sorting methods to isolate beta or alpha cells from mouse and human islets [43,47–49]. While the observation that beta cells express genes associated with glucose sensing, uptake, and metabolism confirmed prior studies, the single-cell analysis revealed a previously not fully appreciated heterogeneity in gene expression among individual beta cells. Previous studies provided evidence for beta cell heterogeneity both at a functional and gene expression level [21,23,31,51]; however, the extent of this heterogeneity could only be fully resolved by single-cell analysis. Using computational methods, such as principal component analysis (PCA) and t-Distributed Stochastic Neighbor Embedding (tSNE) to visualize groups of cells with similar transcriptional profiles, recent single-cell studies have

