Review

MOLECULAR METABOLISM

Systematic single-cell analysis provides new insights into heterogeneity and plasticity of the pancreas

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ABSTRACT

Background: Diabetes mellitus is characterized by loss or dysfunction of insulin-producing β-cells in the pancreas, resulting in failure of blood glucose regulation and devastating secondary complications. Thus, β-cells are currently the prime target for cell-replacement and regenerative therapy. Triggering endogenous repair is a promising strategy to restore β-cell mass and normoglycemia in diabetic patients. Potential strategies include targeting specific β-cell subpopulations to increase proliferation or maturation. Alternatively, transdifferentiation of pancreatic islet cells (e.g. α- or δ-cells), extra-islet cells (acinar and ductal cells), hepatocytes, or intestinal cells into insulin-producing cells might improve glycemic control. To this end, it is crucial to systematically characterize and unravel the transcriptional program of all pancreatic cell types at the molecular level in homeostasis and disease. Furthermore, it is necessary to better determine the underlying mechanisms of β-cell maturation, maintenance, and dysfunction in diabetes, to identify and molecularly profile endocrine subpopulations with regenerative potential, and to translate the findings from mice to man. Recent approaches in single-cell biology started to illuminate heterogeneity and plasticity in the pancreas that might be targeted for β-cell regeneration in diabetic patients.

Scope of review: This review discusses recent literature on single-cell analysis including single-cell RNA sequencing, single-cell mass cytometry, and flow cytometry of pancreatic cell types in the context of mechanisms of endogenous β-cell regeneration. We discuss new findings on the regulation of postnatal β-cell proliferation and maturation. We highlight how single-cell analysis recapitulates described principles of functional β-cell heterogeneity in animal models and adds new knowledge on the extent of β-cell heterogeneity in humans as well as its role in homeostasis and disease. Furthermore, we summarize the findings on cell subpopulations with regenerative potential that might enable the formation of new β-cells in diseased state. Finally, we review new data on the transcriptional program and function of rare pancreatic cell types and their implication in diabetes.

Major conclusion: Novel, single-cell technologies offer high molecular resolution of cellular heterogeneity within the pancreas and provide information on processes and factors that govern β-cell homeostasis, proliferation, and maturation. Eventually, these technologies might lead to the characterization of cells with regenerative potential and unravel disease-associated changes in gene expression to identify cellular and molecular targets for therapy.

Keywords β-Cell heterogeneity; Single-cell analysis; Diabetes; Regeneration; Endocrine cells; Transdifferentiation; Dedifferentiation; Maturation; Subpopulations

1. INTRODUCTION

Diabetes mellitus is a complex and multifactorial disease characterized by progressive loss or dysfunction of the insulin-producing β-cells in the pancreas. This results in chronic hyperglycemia and systemic metabolic complications and, in the long-term, causes multi-organ damage including nephropathy, retinopathy, and enteropathy. Today, over 382 million people worldwide have been diagnosed with diabetes and the number is expected to rise to 592 million by 2035 [1]. Type 1 diabetes (T1D) is an autoimmune disorder caused by destruction of β-cells through cytotoxic T-cells. Unlike in T1D, onset of the more prevalent type 2 diabetes (T2D) is usually in adulthood and is often consequence of genetic predisposition, obesity and lack of physical exercise. T2D is triggered by insulin resistance of the peripheral tissues, which is concomitant with β-cell mass expansion, β-cell exhaustion, and gradual loss of functional β-cell mass through β-cell dedifferentiation and/or β-cell death [2,3]. Thus, the common feature of both pathologies is loss of functional β-cells. Despite its high prevalence and increasing impact on global health, diabetes is still incurable and our knowledge of the underlying pathomechanisms is far

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from complete. Current treatments succeed in reducing symptoms; however, they fail to alleviate long-term complications and require lifelong compliance from patients. Therefore, intensive efforts in the field of diabetes research are put into the development of novel therapeutic strategies to stop the progression of the disease and restore functional β-cell mass.

Human islet transplantation from cadaveric donors has been successfully established as a therapeutic treatment for a subset of patients with "brittle" T1D that do not respond to standard conventional and intensive insulin therapies and suffer from kidney failure [4,5]. However, donor shortage and risks associated with life-long immunosuppression demand the development of alternative therapies. Two main strategies are currently extensively explored to replace lost and/or dysfunctional β-cells: i) *in vitro* differentiation of β-cells from stem cells and ii) endogenous β-cell regeneration. The former holds great promise for cell-replacement therapy and tissue engineering. In the past years, major advances have enabled the generation of monohormonal and glucose-responsive β-like cells from human embryonic stem cells and patient-derived induced pluripotent stem cells [6–8]. Importantly, these cells were able to secrete insulin and restored normoglycemia in diabetic mice [9]. Still, prior to application in humans, the differentiation efficiency and functionality of *in vitro* generated β-like cells needs to be improved. In this regard, the field would benefit greatly from a better understanding of the postnatal β-cell maturation process and the identification of biomarkers that label the different maturation stages and functional glucose-responsive β-cells. In addition, their immune-protection as well as safety must be guaranteed as not fully differentiated stem cells might have teratoma-initiating potential.

Stimulating regeneration of insulin-producing cells from cells residing within the adult pancreas or even in other metabolically active organs, such as the liver or gut (not discussed in this review), is an appealing approach that could bypass the aforementioned hurdles. The main routes pursued to restore functional β-cell mass *in situ* include boosting the replication of remaining β-cells, maturation of immature (dedifferentiated) β-cell subpopulations, mobilization of putative precursors present in the adult pancreas and reprogramming of other cell types into insulin-producing β-like cells (Figure 1) [10]. Important in this respect is the existence of β-cell subpopulations that differ in their glucose responsiveness, proliferative activity, maturation state, or susceptibility to metabolic deregulation in animal models [11]. Moreover, adult exocrine and other endocrine cell types showed the ability to reprogram and produce insulin under certain conditions [12]. Further characterization of these candidate sources for the generation of new insulin-producing cells as well as the identification of biomarkers and therapeutic targets requires detailed dissection of the cellular heterogeneity within the pancreas and their underlying molecular mechanisms. To this end, single-cell studies might be paradigm changing. Single-cell technologies allow for simultaneously measuring the expression of tens to thousands of genes (e.g. single-cell RNA sequencing) or proteins (e.g. single-cell mass cytometry, flow cytometry) in individual cells with high-throughput and precision. Clustering of cells as per their expression profiles allows for unbiased detection and characterization of cell types and states including rare or unanticipated subpopulations that are masked in bulk analyses (Figure 2). By pooling many cells with partially correlated measurements, one can derive rich molecular profiles without prior knowledge of defining criteria and screen for subtype specific marker genes even if only a limited number of transcripts or proteins per cell are captured [13,14]. In addition, single-cell measurements provide an accurate temporal resolution of continuous processes, such as differentiation or reprogramming, as cells of all present (transient and stable) stages are

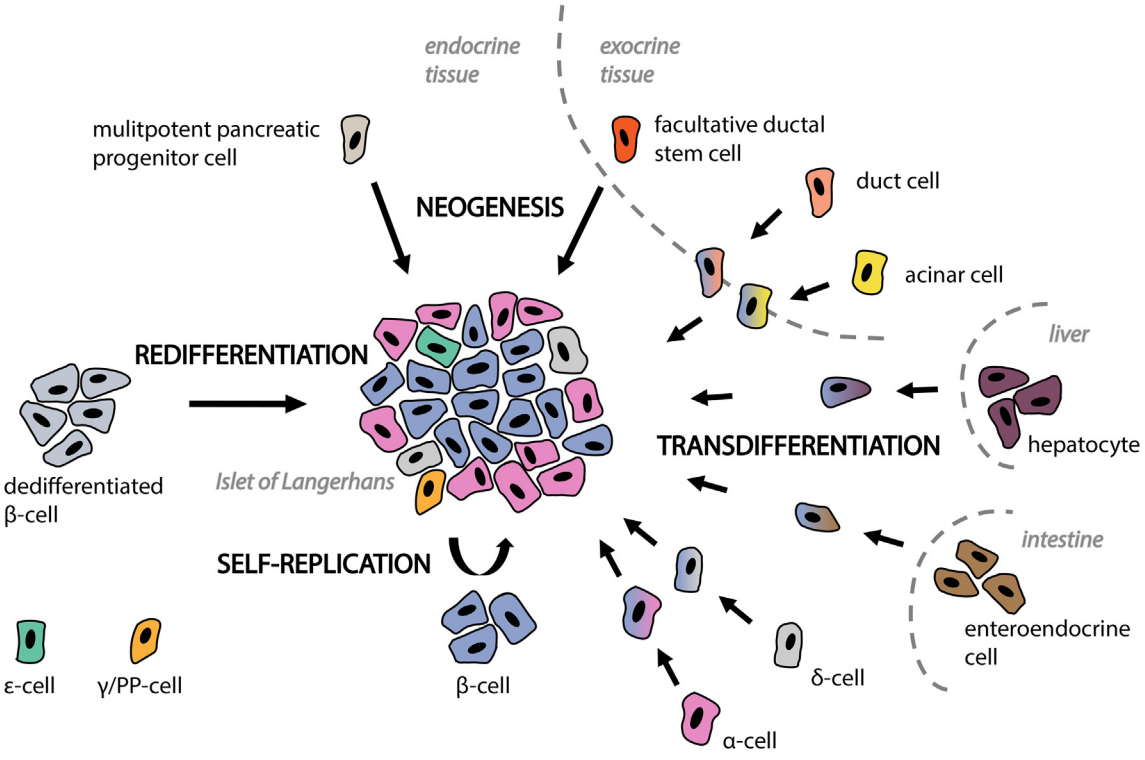

Figure 1: Main routes to restore functional β-cell mass *in situ*. Schematic summarizing the possible ways of β-cell regeneration that are discussed in the text.

