Heterogeneity of β-cells

Loss of β-cell mass and function leads to the pathogenesis of type 1 and type 2 diabetes.

β-Cell neogenesis from endogenous progenitor pools is expected as a potential cell source for the treatment of diabetes. However, the existence of β-cell progenitors was challenged by Dor et al., that self-replication of pre-existing β-cells is the major mechanism for β-cell regeneration. Our knowledge of pancreatic β-cells would lead to a better understanding and manipulation of the maturation or proliferation of β-cells for regenerative medicine. Recently, Bader et al. showed that β-cells can be divided into two populations, a proliferation-competent population and mature β-cells, marked by the expression of Fltp (also known as Flattop and Cflap126), a Wnt/planar cell polarity (PCP) effector. Fltp is expressed not only in β-cells, but also in other pancreatic endocrine cells. The authors generated an Fltp reporter mouse line, and combined with another marker gene, NK6 homeobox 1, they sorted Fltp-positive and Fltp-negative β-cells (Figure 1). Fltp-positive β-cells showed higher cell-cycle inhibitor p27 expression, and lower 5-ethynyl-2’-deoxyuridine incorporation, suggesting that these cells are less proliferative than Fltp-negative β-cells. The authors carried out gene expression profile analysis, and found that Fltp-lineage positive cells are enriched with genes that are important for mature β-cell function, mitochondrial function, and receptors and signaling. In contrast, the Fltp-lineage negative population is enriched with genes associated with G-protein coupled receptor, Wnt/β-catenin and mitogen-activated protein kinase signaling transductions. Biochemical analyses, such as mitochondrial gene expression, ultrastructural observations, and measurements of the oxygen consumption rate of sorted Fltp-positive and Fltp-negative cells showed that Fltp-positive cells represent mature β-cell subpopulation, whereas Fltp-negative cells consisted of immature and proliferative cell population. Transplantation of the Fltp-lineage negative cells into pregnant mice showed that these cells are capable of undergoing compensatory proliferation. Whereas when transplanted into a high-fat diet mouse model, the Fltp-lineage positive cells became hypertrophic, and showed an increased in islet volume. These results further show that the seemingly different phenotypes are derived from different β-cell populations. Furthermore, Fltp-lineage negative proliferation-competent β-cells could differentiate into Fltp-lineage positive mature β-cells. This result implies that immature proliferative β-cells are expandable, and would be a target for therapeutic strategies for treatments of diabetes.

What is the function of Wnt/PCP in the pancreas? Fltp was identified as a Wnt/PCP gene, which is transcriptionally activated during PCP acquisition in ciliated tissues. The expression of Fltp in the pancreas increases during β-cell maturation and islet formation. Knockout of Fltp cells did not show impaired development, proliferation or maturation of β-cells. No glucose tolerance or insulin sensitivity phenotype was observed. However, glucose-stimulated insulin secretion (GSIS) was significantly decreased in isolated islets from Fltp knockout mice. In humans, the FLTP ortholog (CFAP126, also known as CIorf192) is associated with metabolic traits. The expression of CFAP126 was significantly downregulated in human islets from prediabetic individuals, and further downregulated in type 2 diabetic individuals. Taken together, their results suggest that Wnt/PCP effector FLTP regulates GSIS, and distinguishes proliferative and mature β-cells. How does FLTP function to affect GSIS? Fltp is a non-canonical Wnt/PCP effector. The canonical Wnt
pathway and non-canonical Wnt pathway have been shown to take part in development, morphogenesis, and tissue polarity and cell differentiation. In particular, Wnt/PCP signaling controls cell polarization, which regulates the orientation of cells, and thus drives cell movements and determines the function of cells in diverse tissues. The authors tested if the morphology of β-cells had some relationship with their function. The authors noticed that apical-basal polarity and the expression of Wnt/PCP genes were increased in Fltp-positive β-cells, and that in 3-D pseudo-islet cultures, proteins associated with β-cell maturation, such as MafA, NK6 homeobox 1 and urocortin3, were induced. Treatment with the Wnt/PCP ligand, Wnt5A, during re-aggregation of islet cells and pseudo-islet of Min6 insulinoma cells, β-cell maturation markers were significantly upregulated. This result shows an interesting implication that 3-D architecture upregulates and Wnt/PCP signaling activates β-cell maturation, and increases GSIS. In contrast, treatment of a human β-cell line, EndoC-bH1b cells, with a canonical ligand, WNT3A, but not with WNT4 or WNT5A, increased proliferation.

Are there any other molecular markers to distinguish the heterogeneity of β-cells? Heterogeneity of β-cells is also reported by other groups. Recently, Dorrell et al.\(^4\) reported that human islet endocrine cells can be subdivided into four subpopulations and can be distinguished by differential expression of two antigens, ST8 alpha-N-acetylgalactosaminide-2,8-sialyltransferase and CD9, both are surface antigens. Dorrell et al.\(^4\) sorted out human endocrine β-cell sub-populations according to the expression of these two antigens, and studied their gene expression profiles. These subpopulations are present in normal adult islets, and have diverse gene expression profiles, and distinct basal and GSIS. The β-cell distribution is markedly altered in type 2 diabetes, therefore raising the possibility that the β-cell subpopulations defined by these two antigens might be medically relevant. Unlike Bader et al.\(^2\), there are no lineage tracing data of these subpopulations, and therefore it remains to be determined whether conversion from one population to another occurs.

It has been under debate whether β-cell regeneration from the stem cell pool occurs. Plasticity of the endocrine pancreas remains, so that transdifferentiation was reported to occur under certain conditions. Recently, dedifferentiation of β-cells has been reported\(^5\). β-Cells are not homogeneous, as we expected. Our better knowledge of the characteristics of β-cells would enable us to target β-cells for appropriate manipulation to expand mature β-cells for regenerative medicine to cure diabetes.

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**REFERENCES**

1. Dor Y, Brown J, Martinez Ol, et al. Adult pancreatic beta-cells are formed by self-duplication rather than stem-cell differentiation. Nature 2004; 429: 41–46.
2. Bader E, Migliorini A, Gegg M, et al. Identification of proliferative and mature β-cells in the islet of Langerhans. Nature 2016; 535: 430–434.
3. Lange A, Gegg M, Bertscher I, et al. Fltp T2AiCre: a new knock-in mouse line for conditional gene targeting in distinct mono- and multiciliated tissues. Differentiation 2012; 83: S105–S113.
4. Dorrell C, Schug J, Canaday PS, et al. Human islets contain four distinct subtypes of β cells. Nat Commun 2016; 7: 11756.
5. Talchai C, Xuan S, Lin HV, et al. Pancreatic β Cell Dedifferentiation as a Mechanism of Diabetic β Cell Failure. Cell 2012; 150: 1223–1234.

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