SRP Diabetes Postdoctoral Fellowship Program 2023

Project title: *Integrating transcriptomics and chemical biology for induction of β-cells*

Project outline

Background: Diabetes features a reduction in the number of functional β-cells, a key pathologic event that causes or exacerbates the dysregulation of blood glucose levels (*1*). Regenerating insulin-producing β-cells might prove a better treatment for diabetes, which is at present controlled but not reversed by insulin injections. There is currently no efficient stimulator of β-cell regeneration in higher model organisms and humans, but prominent β-cell regeneration occurs in zebrafish (*2*). Here, we will take an integrative approach—combining bioinformatics with chemical and functional studies in zebrafish and human cells—to identify a specific subpopulation of ductal cells in the pancreas that can be induced to form β-cells. By converting single-cell RNA-seq data from zebrafish into the human orthologs, we perform integrated analyses of mixed zebrafish and human ductal cells to deduce which human cells might have progenitor potential. We then leverage a novel technology we recently developed for knocking-in genes that allows us to generate Cre-expressing zebrafish lines (*3*), and use them to lineage-trace specific progenitor populations, as well as isolate the ductal progenitor cells and their differentiated descendants to delineate the mechanisms of β-cell neogenesis by single-cell RNA-seq. We will then test the identified pathways on human organoids derived from ductal cells to determine whether their effects on β-cell neogenesis are conserved across species, as we previously done in another project (*4*).

Objectives: Our overarching goal is to identify clinically viable targets that can affect β-cell neogenesis and form the basis of a road to the reversal of diabetes. We will pursue our goal through the following three independent, but interconnected objectives:

Objective 1: Through comparative analysis of single-cell transcriptomics, we propose a progenitor population in the pancreatic duct

Objective 2: Explore pancreatic progenitors and their features in zebrafish

Objective 3: Transitioning from zebrafish to human organoids

Methodology & Work plan:

Objective 1: Through comparative analysis of single-cell transcriptomics, we propose a progenitor population in the pancreatic duct. By converting single-cell RNA-seq data from zebrafish into the human orthologs, we can perform integrated analysis of mixed zebrafish and human ductal cells. Since we can deduce which cluster of zebrafish ductal cells has progenitor potential (based on the location where newly formed endocrine cells appear in vivo), we can infer which cell cluster might have progenitor potential in the human duct. Markers of the corresponding human pancreatic duct cluster with inferred progenitor potential will be examined in sections of human pancreata and organoids.

Moreover, cellular signaling between different pancreatic cell types will be identified using several bioinformatics tools, which examine interactions in >100 signaling pathways. The identified pathways will be perturbed, using mutagenesis or small molecules know to target the pathways, and assessed for involvement in progenitor maintenance or differentiation of progenitors to β-cells in zebrafish.

Objective 2: Explore pancreatic progenitors and their features in zebrafish. To explore the features of the identified progenitor population(s), we will determine the cellular transformation during differentiation to β-cells. To do so, we perform single-cell RNA-seq of lineage-traced ductal progenitor cells and their differentiated descendants. Thus, we delineate the mechanisms of β-cell neogenesis by identifying pseudotime trajectories, transitional cellular states, regulon and differentiation signatures. Critical genes/pathways at branch points

will be perturbed, using mutagenesis or small molecules know to target the pathways, and assessed for involvement in progenitor maintenance or differentiation of progenitors to β-cells in zebrafish.

Objective 3: Transitioning from zebrafish to human organoids. By determining whether the effects of hits from Objectives 1 & 2 are conserved across species we will test them on human organoids derived from ductal cells, i.e. by assessment of insulin (and other β-cell specific markers) by qPCR, immunohistochemistry and ELISA.

Using single-cell RNA-seq we will delineate the mechanisms of β-cell neogenesis, by a similar pipeline as used in Objective 2 for zebrafish. We will perform single-cell RNA-seq of the human organoids to gain mechanistic insight into β-cell neogenesis after induction by hits identified through Objectives 1 & 2.

Relevance of project for diabetes: **(i)** Leveraging integrated single-cell RNA-seq analysis of pancreatic ductal cells from different species to identify clusters of progenitors and markers thereof, as well as cellular interactions, are innovative ways of targeting diabetes. **(ii)** Using the newly identified progenitor cells to identify genes and small molecules that induce differentiation to β-cells in zebrafish and human organoids will bring us closer to the development of a novel class of drugs that can increase β-cell mass — as an alternative approach to reverse diabetes.

References

1. G. C. Weir, S. Bonner-Weir, Islet beta cell mass in diabetes and how it relates to function, birth, and death. *Annals of the New York Academy of Sciences* **1281**, 92-105 (2013).
2. O. Andersson *et al.*, Adenosine Signaling Promotes Regeneration of Pancreatic beta Cells In Vivo. *Cell Metab* **15**, 885-894 (2012).
3. J. Mi, O. Andersson, Efficient knock-in method enabling lineage tracing in zebrafish. *bioRxiv* **doi.org/10.1101/2022.07.15.500272**, (2022).
4. C. Karampelias *et al.*, MNK2 deficiency potentiates beta-cell regeneration via translational regulation. *Nat Chem Biol*, (2022).

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