Shaping the future: The importance of morphological changes in delamination and cell fate determination of pancreatic endocrine progenitors

Pancreas is an organ consisting of exocrine and endocrine compartments. While exocrine part secretes enzymes to aid digestion, the endocrine cells collectively regulate blood glucose homeostasis. β-cells (beta) are especially important since they produce insulin in response to changing blood glucose levels, to keep it on a healthy level. In diabetes β-cells are either lost or dysfunctional which leads to major health problems. Directed differentiation of hPSCs (human pluripotent stem cells) provides an attractive platform for regenerative medicine to study diabetic mechanisms and treatments. It allows studying developmental mechanisms in the human context, otherwise hindered by the scarcity and ethical ambiguity of human fetal tissues available for research. Decade of developing and refining human pancreatic differentiation protocols resulted in not only robust and reliable generation of different types of pancreatic progenitors from hPSCs, but also in the development of 3D differentiation protocols allowing investigation of the morphological events. Ultimately, hPSC-derived β-cells could one day serve as a cellular therapy for diabetic patients. However, despite the enormous progress, application at clinics and broad research is hindered by an incomplete understanding of signals that control β-cell generation. For this purpose, it is crucial to understand pancreatic developmental processes, which can be translated to the generation of human β-cells *in vitro*.

This project aims to discover new signals regulating the delamination process and cell fate determination of pancreatic endocrine progenitors (EPs), which are the inevitable stage for endocrine cell formation. **Here, we propose that those processes are interconnected, namely morphological changes and mechanistic signals during EP delamination influence cell specification.** We focus on AMOTL2 (Angiomotin-Like 2), which we found to be upregulated in the newly identified EP delaminating subpopulation in murine, specifically at e16.5 (embryonic day 16.5) but not e14.5 (embryonic day 14.5). E16.5 EPs were shown to be more prone to form β-cells, a most-wanted type of cells in diabetes therapy. **We further hypothesize that AMOTL2 is a critical regulator of the Hippo pathway downstream effector YAP, which is known to be involved in both EP detachment and endocrine commitment in pancreatic settings. Also, we suggest that AMOTL2 regulates cell size and shape in hPSCs, also in YAP-dependent manner.**

Here, we generated AMOTL2 gene knockout in hPSCs and noticed altered cell and colony morphology, and overconfluency, which we suggest, is stemming from increased cell size. We will use 3-dimensional (3D) hPSC differentiation to gain an insight into morphological changes of AMOTL2-deficient cells and their significance for human pancreatic development. We will test the hypothesis in the following aims:

1) **Shape-shifters: AMOTL2 role in cell and colony morphology in hPSCs and early pancreatic differentiation** where we will confirm the reason behind overconfluency – is it increase in cell divisions, in cell death, or in cell size. We will also assess whether cell size and shape are dependent on the density of cells in the culture. Further, we will investigate the mechanism underlying those changes. Our prime candidate is the Hippo pathway but we will also check if any other pathways might be involved.
2) **Shaping the future: How cell morphology influences pancreatic differentiation and cell fate determination processes** where we will focus on EPs, cells without which there are no β-cells. We will check how many EPs are forming and if they form at the appropriate time. We will again check the cell division and death rates, and cell size, since they may differ in different kinds of cells. Then, we will focus on the process of delamination and we will look for the mechanism involved. Next, we will check if AMOTL2 knockout EPs can generate β-cells and if those cells are capable to secrete insulin in response to changing glucose levels, which is characteristic of normal β-cells. We will examine it first *in vitro*, in cell culture, and then *in vivo* by transplanting our cultured β-cells into diabetic mice to see if they are able to restore control over blood glucose levels.