Pancreas consists of the exocrine and endocrine compartment. The exocrine compartment is made of acinar cells (the most common cells in the pancreas) and ducts secrete and transport pancreatic juice to the intestine to aid digestion. The Langerhans's islets comprise the endocrine compartment - are relatively rare (only 5% of the pancreas) clusters of five different endocrine cell types that secrete hormones into the blood. One type of endocrine cells is a beta-cell that secretes hormone insulin to regulate blood glucose levels. Any abnormalities in the cellular composition and pancreatic cells' functional capacity lead to different diseases, with diabetes being the most common. Last year more than 415 million people worldwide were affected by diabetes, and the number of new cases is increasing annually. Though there are some general treatments available for diabetes, there is no cure. Diabetes often leads to devastating secondary diseases or complications impairing life quality and span, or even premature death. Therefore, diabetes is a significant health and socioeconomic burden. Proof-of-principle studies show that be providing new beta cells, patients become euglycemic. We do not have enough donor islets for all diabetes patients. Thus, there is an urgency to better understand how beta cells develop to create robust source of human, functional beta cells for research and clinical needs.

Over the last decade, we have studied signals that allow the generating beta cells from human pluripotent stem cells (hPSC). We choose PSCs as a starting point since they can proliferate almost indefinitely in vitro and make any cell type on our body. To make human beta cells from PSCs, cells must transverse through multiple stages, much like during development. We and others developed reliable ways of making early pancreatic progenitors. However, the ultimate step, derivation of mature beta cells that can read glucose levels and secrete adequate insulin levels, is still not efficient enough for regenerative medicine's broad needs. The success at the last step of pancreatic differentiation is hindered by the gap of knowledge about the signals that regulate beta-cell differentiation and maturation.

Here we propose to study how cells surrounding the developing beta cells, like mesenchyme (M) and endothelium (E), impact the endocrine islet differentiation. We postulate that different cells, forming together pancreas, communicate with each other and regulate their development. This pancreatic microenvironment is absent in commonly used in vitro beta-cell differentiation protocols. Therefore, we will combine the organ and stage-specific mesenchyme and endothelium with pancreatic progenitors to increase to make more and better functioning beta cells. We preliminary identified candidates- niche-derived growth factors- that induce insulin expression in pancreatic progenitors. We will further investigate the impact of these candidate factors on beta cell differentiation and maturation. We will also look into molecular mechanism be to uncover which signaling pathways changed by the M-E derived factors that regulate beta cell derivation. Here, we propose to use advanced biochemistry, pancreatic differentiation combined with precise gene editing, and single-cell RNA-sequencing to study mechanisms regulating insulin cell induction from hPSCs. Finally, we will test whether beta cells derived in the presence of M-E cells or their secreted factors, can adequately function and secrete insulin, specifically when glucose levels are elevated.

Five types of pancreatic endocrine cells work together to regulate blood glucose levels. Most research efforts focus on generating beta cells. Recently protocols to generate glucagon-expressing alpha cells have been developed. Interestingly, no specific signals are known that regulate other endocrine cells in vitro derivation. As preliminary data, we uncovered the new growth factors derived from M-E cells that induce somatostatin expressing delta cells from human pancreatic progenitors. We will extend these preliminary data. Furthermore, using cutting-through molecular biology techniques, for instance, single-cell RNA-sequencing, we will determine the molecular mechanism regulating delta-cell induction from human PSC-derived endocrine progenitors.

Our overreaching goal is to establish how pancreatic niche, defined as mesenchyme and endothelial cells surrounding pancreatic progenitors, contribute to beta and other islet cell development. In the long term, this study might facilitate the development of a cell-therapy for people with diabetes.