# Understanding of human insulin maturation for cell and gene therapy in diabetes

The pancreas consists of the exocrine and endocrine compartment. Exocrine compartment 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 endocrine compartment is made up of islets of Langerhans - relatively rare (only 5% of the pancreas) clusters of 5 different endocrine cell types that secrete hormones into the blood. One type of endocrine cells is a beta cell that secretes hormone insulin and regulates glucose levels in the blood. Any abnormalities in the proper cellular composition and the functional capacity of pancreatic cells 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. Thus, diabetes often leads to many devastating secondary diseases or complications impairing life quality, span, or even premature death. Therefore, diabetes is a significant health and socio-economic burden and brings urgency to a better understanding of how pancreatic insulin-secreting beta cells function to develop regenerative medicine-based therapies and preventive strategies for diabetes and other pancreatic diseases.

Over the last decade, we have studied signals that allow the generation of human beta cells from pluripotent stem cells. We developed reliable ways of making human beta cells for the broad needs of regenerative medicine, including studies on beta cell development, physiology, and pathophysiology. Here we are proposing to study how to make these *in vitro*-derived human beta cells more efficient in insulin secretion. Insulin secretion has to be precisely regulated to prevent too low or too high blood glucose levels. Therefore, insulin secretion is a multistep process with many regulatory mechanisms. Here, we propose to use advanced biochemistry, structural biology, and human stem cell pancreatic differentiation combined with precise gene editing to study mechanisms regulating insulin maturation. Insulin is first synthesized as a precursor that undergoes several modifications to yield active mature insulin. This maturation process involves proinsulin folding, in which many cellular factors are involved. The abnormalities in insulin folding lead to decreased insulin secretion or even beta cell death, resulting in diabetes.

Interestingly, folding mechanisms are similar between proinsulin and a closely related insulin-like growth factor 2 (IGF-2), which is also secreted by pancreatic cells. Yet, pro-insulin folding had to evolve a unique mechanism to address highly dynamic needs for insulin secretion in response to changing blood glucose levels. Here we prose to study similarities and cellular factors common to proinsulin and IGF-2 folding and ones that are insulin specific. To further investigate the role of these proinsulin specific factors in proinsulin folding and insulin secretion, we will abolish their expression in human beta cells using state-of-the-art genetic engineering techniques. Finally, we will test whether inactivation of pro-insulin specific folding elements can impair the ability of human beta cells implanted into diabetic mice to maintain proper glucose levels.

Our overreaching goal is to elucidate molecular mechanisms of proinsulin folding in beta cells, identify cellular factors critical in the process, and finally make attempts to use them as targets for diabetes treatment. In the long term, this study might facilitate the development of a cell and gene therapy for people with diabetes.