Final Update for Dr. Li’s project: **A Safe and Cost-Effective Stem Cell Approach for Treating Diabetes**

Human pluripotent stem cell (hPSC)-derived pancreatic β cells are an attractive cell source for treating diabetes. However, current derivation methods are still inefficient and often generate mixed cell populations. The differentiation protocols are also highly dependent on individual cell lines and exhibited high variability when applied in other cell lines. These issues represent major barriers in applying hPSC-derived β cells for cell replacement therapy.

To address these issues, we first optimized the steps from hPSCs to pancreatic progenitors (PP) and devised a strategy to efficiently cluster hPSC-derived PP cells into 3D structures. These PP 3D clusters were then directly subjected to differentiation with well-established protocols. However, only about 15% of the cells became genuine β cells. Upon detailed analysis, we found that PP 3D clusters tend to lose their identity prematurely. To stabilize the PP 3D clusters, we performed a systematic screen and identified a combination of 10 chemicals that not only retains the pancreatic progenitors in 3D clusters but also enhances their potentiality towards β cells. This additional step of treating with these 10 chemicals a major advancement for efficient generation of β cells from hPSCs.

To further increase β cell yield, we attempted to optimize the step-wise procedure for differentiating 10 chemical cocktail treated PPs into endocrine progenitors (EP), then into immature β (iβ) cells, and finally functional β (Fβ) cells. We initially used the classical method, which employed markers specific to intermediate stage cells as the readout of the screening. But we found that the cells cultured in the resulting conditions were not efficient in generating mature β cells. Thus, we designed a more stringent screen that instead used late-stage markers (NKX6.1+/INS+ for this case) as the readout, termed “late-stage readout strategy”. Using this strategy, we were able to screen novel conditions for all these three steps, and the implementation of these novel chemical combinations resulted in an unprecedented high efficiency of generating β cells from different sources of hPSCs. The derived β cells were functional and able to reverse hyperglycemia in mice within two weeks. Our protocol provides a robust platform for studying the basic biology of human β cells and developing hPSC-derived β cells for cell replacement therapy.

All this work has been submitted for publication and is currently under revision. Once it is accepted, we will communicate it to the DRC. The DRC has been acknowledged as supporting this work in the manuscript.