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- Title: Study of the role of RNA-based technologies for improving β-cell mass in type 1 diabetes

Summary

The current challenge of research teams working on diabetes cell therapy is to find new sources of cells that meet several requirements: 1) simple isolation protocols from native tissue; 2) capacity for in vitro expansion and/or availability in large numbers; 3) significant β-cell differentiation potential or insulin-secreting capacity; 4) preservation of insulin secreting capacity after transplantation.

In our investigations, we developed a system that allows expansion of human pancreatic duct cells to clinically useful levels. The proliferating cells showed evidence of partial epithelial-mesenchymal transition and were driven in vitro towards β-like cells. After reprogramming with cocktails of small molecules, human duct-derived cells (HDDCs) acquired a broad array of β-cell-specific genes and, in these cell populations, up to 3.1% of the cells stained for insulin. The differentiated HDDCs were able to secrete significant amounts insulin in the culture media. The current research aimed at developing new technologies for direct reprogramming of our HDDCs into β cells, based on the overexpression of defined transcription factors.

Three transcription factors (i.e. PDX1, NGN3 and MAFA) are essential for normal pancreas development and their overexpression, either separate or combined, can lead to differentiation of many cell types into β cells. However, current techniques for overexpression of these factors invariably depend on viral vectors, impeding their clinical use. Very recently it has been demonstrated that the use of synthetic modified mRNAs could allow human cells to reprogram to pluripotency or to somatic lineages. This technique is very appealing because it completely eliminates the risk of genomic integration and insertional mutagenesis inherent to all DNA-based methodologies and thus, if efficient for differentiation procedures, it has a high potential for clinical application.

In collaboration with Dr. A. Vetere at the Broad Institute of MIT and Harvard (Boston), we generated synthetic modified mRNAs for PDX1, NGN3, and MAFA to differentiate our HDDCs into β cells. We observed that MAFA smRNA was sufficient and efficient to drive HDDCs toward a β-cell lineage. After 7 daily MAFA smRNA transfections, HDDCs acquired the expression of β-cell hallmark genes and immunostaining assays showed insulin protein expression in up to 40% of HDDC populations (called β-HDDCs) transfected with MAFA. β-HDDCs also acquired the expression of typical β-cell proteins, including PDX1, synaptophysin and GLUT2.

Functional assays showed that β-HDDCs released significant amounts of human insulin in the media respectively in basal and stimulated conditions using high glucose, KCl and IBMX. Consistent with the insulin secretion analysis, increased levels of intracellular calcium concentrations were observed by monitoring cytoplasmic influx. These data suggest that β-HDDCs secreted insulin in low glucose conditions and significantly responded to increased levels of glucose stimulation. Transplantation of β-HDDCs into diabetic SCID-beige mice confirmed their functional glucose-responsive insulin secretion and their capacity to mitigate hyperglycemia. Our data describe a new, reliable, and fast procedure in adult human pancreatic cells to generate clinically relevant amounts of new β cells with potential to reverse diabetes.
Original paper:

Review paper:
Corritore E, Lee YS, Sokal EM, Lysy PA.
β-cell replacement sources for type 1 diabetes: a focus on pancreatic ductal cells.