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THERAPEUTIC GENOME METHYLATION FOR CELL REPROGRAMMING EDITING: USE OF EPI-CRISPR-INDUCED TARGETED DNA

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Introduction and aim: Diabetes is the perfect candidate for cell replacement therapy since it is caused by either an absolute (type 1 diabetes) or relative (type 2 diabetes) defect of insulin-producing pancreatic beta cells (b-cells). We focused on applying a novel synthetic epigenetic tool (Epi-CRISPRs) for a straightforward, one-step transdifferentiation of mouse pancreatic alpha (a-cells) to b-cell by targeted DNA methylation and suppression of genes essential for maintaining pancreatic cell identity (homeobox Arx gene (*Arx*)).
Methods: The a-cells were transiently transfected with four different Epi-CRISPR constructs and co-transfected with a single guided RNA (gRNA) or with a mix of different gRNAs all targeting different promoter regions of *Arx*. After 5, 8 and 12 days post-transfection, DNA and RNA were isolated and the cells were immunostained. The transdifferentiated cells were analysed for key features of bona fide cells, using qPCR to assess *Arx* expression, and immunostaining of insulin/glucagon and ELISA for measuring secreted insulin.
Results: We succeeded to transiently transfect a-cells with Epi-CRISPR constructs and 275 gRNA/mix gRNA. The suppression of *Arx* in a-cells was confirmed on days 5 and 8 post-transfection. The reduction of glucagon synthesis and beginning of insulin production in transfected a-cell was confirmed and visualised by immunostaining. Whether DNA methylation-mediated suppression of *Arx* in a-cells lead to their transdifferentiation to insulin-producing cells, will be confirmed by bisulfite sequencing.
Conclusion: We are on the right course of developing a clear-cut technology capable of providing a perfect delivery system for increasing the number of insulin-producing cells *in vitro*.

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