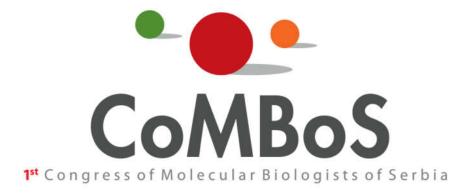
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THERAPEUTIC GENOME METHYLATION FOR CELL REPROGRAMMING EDITING: USE OF EPI-CRISPR-INDUCED TARGETED DNA

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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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