TRANSDIFFERENTIATION OF PANCREATIC ALPHA TO BETA CELLS USING EPI-CRISPR DIRECTED DNA METHYLATION

Marija Sinadinović[1], Jelena Arambašić Jovanović[1], Anja Tolić[1], Miloš Đorđević[1], Mirjana Mihailović[1], Nevena Grdović[1], Aleksandra Uskoković[1], Jovana Rajić[1], Goran Poznanović[1], Svetlana Dinić[1], Tomasz P. Jurkowski[2], Melita Vidaković[1]

[1]Institute for Biological Research "Siniša Stanković", University of Belgrade, Belgrade, Serbia; [2]Institute for Biochemistry, University of Stuttgart, Stuttgart, Germany.

Introduction: Since diabetes is characterized by impaired ability of pancreatic beta-cells to respond and/or produce insulin, new approaches for renewal and replacement of deficient beta-cells are indispensable. The aim of this study is direct pancreatic alpha- to beta-cells transdifferentiation by using a new synthetic epigenetic tool, Epi-CRISPR system. Using Epi-CRISPR system we aim to introduce targeted DNA methylation and subsequent repression of genes responsible for maintaining alpha-cell identity.
Methods: AlphaTC1-6 cells (a-cells) were transiently transfected with dCas9-Dnmt3a-Dnmt3L constructs and one or four different vectors containing guide RNA components for specific targeting the promoter region of aristaless-related homeobox gene (*Arx*). The success of a-cells transdifferentiation into insulin-producing cells was evaluated by measuring *Arx* and insulin mRNA level, amount of secreted insulin and by immunostaining of insulin/glucagon in the cells.
Results: We observed *Arx* transcriptional repression in a-cell transfected with Epi-CRISPR construct that targets the *Arx* gene promoter inducing subsequent methylation. At fifth day post-transfection the expression of *Arx* was decreased in a-cells followed by consequent increase in insulin (mRNA and protein level). At the same time, the glucagon levels remained unchanged. At twelfth day post-transfection the transfected cells start to lose glucagon while still secreting insulin.
Conclusion: This study is near to confirm Epi-CRISPR system functionality and to verify the concept of cell transdifferentiation through silencing of genes responsible for maintaining cell phenotype. The obtained results will be valuable for later Epi-CRISPRs use in mouse *in vivo* models of diabetes and eventually as a future therapy for diabetes attenuation in humans.

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