Trends in **Molecular Biology** • Special issue

Abstract Book

CoMBoS2

2nd Congress of Molecular Biologist of Serbia

ISBN-978-86-82679-15-8

Belgrade • 2023

CoMBoS2 – the Second Congress of Molecular Biologists of Serbia, Abstract Book – Trends in Molecular Biology, Special issue
06-08 October 2023, Belgrade, Serbia
Online Edition
https://www.imgge.bg.ac.rs/lat/o-nama/kapacitet-i-oprema/istrazivacka-delatnost
https://indico.bio.bg.ac.rs/e/CoMBoS2

IMPRESSUM

PUBLISHER:
Institute of Molecular Genetics and Genetic Engineering (IMGGE), University of Belgrade

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Institute of Molecular Genetics and Genetic Engineering (IMGGE),
University of Belgrade
Belgrade, 2023
ISBN 978-86-7078-173-3

CHANGES IN UP AND DOWN REGULATED GENE EXPRESSION AFTER TRANSIENT SUPPRESSION OF *ARX* GENE IN PANCREATIC ALPHATC1-6 CELL LINE

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Introduction: The Aristaless-related homeobox (*Arx*) gene plays a key role in the development and maintaining pancreatic alpha cell phenotype, and as such represents an excellent target for alpha cell identity change towards insulin-producing cells. Therefore, this cell switch and increase in beta cell mass could be of potential use in diabetes management.

Methods: On the fifth day after transient transfection with dCas9-Dnmt3a3L-KRAB construct and four gRNAs targeting *Arx* promoter, αTC1-6 were sorted to collect GFP-positive (transfected) cells (EpiC). The mRNA-seq libraries were pooled in equimolar amounts and sequenced in a single end-setting on the Illumina NextSeq 500 High output machine with 75 bases long reads. KEGG pathway overrepresentation analysis was performed using an application on all significantly up- or downregulated genes using default settings.

Results: Directed induction of DNA methylation on the *Arx* gene promoter reduces its expression and causes the up-regulation of 357 genes, while 266 genes were down-regulated in EpiC compared to Mock transfected cells. The KEGG pathways analysis of biological processes confirmed several biological pathways associated with genes differentially expressed in EpiC vs. Mock transfected cells at the 5th post-transfection day (pval ≤ 0.05). As the most significant, up-regulated pathways we found Type II diabetes, Insulin secretion, Longevity regulation pathways. As significant, down-regulated pathways pop-up Fatty acid metabolism and PPAR signaling pathway.

Conclusion: Reduction of *Arx* mRNA level is sufficient to initiate the transdifferentiation process of alpha cells into insulin-producing cells by triggering several biological pathways tight related to insulin secretion and function.

Key words: CRISPR/dCas9; alpha cells; methylation; Arx gene; epigenetic editing

Acknowledgements: This study was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Agreement no. 451-03-47/2023-01/200007) and by research grant from AstraZeneca within the European Foundation for the Study of Diabetes (EFSD): European Diabetes Research Programme in Cellular Plasticity Underlying the Pathophysiology of Type 2 Diabetes.