



Trends in **Molecular Biology** • Special issue

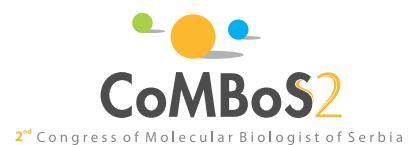
Abstract Book

CoMBoS²

2nd Congress of Molecular Biologist of Serbia

Belgrade • 2023

ISBN-978-86-82679-15-8



**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**

FOR THE PUBLISHER:

Dr. Sonja **Pavlović**

EDITOR:

Dr. Zorana **Dobrijević**

EDITORIAL REVIEW BOARD:

Prof. Dr. Silvana **Andrić**

Dr. Valentina **Ćirković**

Dr. Ivica **Dimkić**

Prof. Dr. Branko **Jovčić**

Prof. Dr. Gordana **Matić**

Ass. Prof. Dr. Milena **Milutinović**

Dr. Aleksandra **Stanković**

Dr. Nemanja **Stanisavljević**

Dr. Maja **Stoiljković**

EDITOR IN CHIEF:

Prof. Dr. Dušanka **Savić-Pavićević**

DESIGN:

Ivan **Strahinić**

All rights reserved

Institute of Molecular Genetics and Genetic Engineering (IMGGE),

University of Belgrade

Belgrade, 2023

ISBN 978-86-7078-173-3

© Copyright 2023 by Institute of Molecular Genetics and Genetic Engineering (IMGGE), University of Belgrade
Belgrade • 2023

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

Marija Đorđević,¹ Clarissa Feuerstein-Akgoz,² Jelena Arambašić Jovanović,¹ Anja Tolić,¹ Jovana Rajić,¹ Ana Sarić,¹ Nevena Grdović,¹ Svetlana Dinić,¹ Aleksandra Uskoković,¹ Mirjana Mihailović,¹ Clarissa Gerhauser,² Tomasz Jurkowski,³ Melita Vidaković¹

¹*Institute for Biological Research "Siniša Stanković" - National Institute of Republic of Serbia, University of Belgrade, Belgrade, Serbia;* ²*German Cancer Research Center (DKFZ), Heidelberg, Germany;* ³*School of Biosciences, Cardiff University, Cardiff, Wales, United Kingdom*

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 ($p\text{-val} \leq 0.05$). 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.