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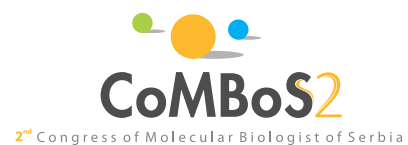
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APPLICATION OF CRISPR/CAS9 TECHNOLOGY FOR *IN VITRO* DISEASE MODELLING IN GLYCOGEN STORAGE DISEASE TYPE IB

Marina Parezanovic,¹ Marina Andjelkovic,¹ Nina Stevanovic,¹ Kristel Klaassen,¹ Vesna Spasovski,¹ Milena Ugrin,¹ Jovana Komazec,¹ Sara Stankovic,¹ Nikola Jovic,¹ Sonja Pavlovic,¹ Maja Stojiljkovic,¹ Anita Skakic¹

¹*Institute of molecular genetics and genetic engineering, University of Belgrade, Belgrade, Serbia*

Introduction: Glycogen storage disease type Ib (GSD-Ib) is an autosomal recessive disorder characterized by fasting hypoglycemia and the accumulation of glycogen in the liver, kidneys and intestinal mucosa. Recent studies revealed that chronic endoplasmic reticulum (ER) stress and increased apoptosis play a role in the progression of disease manifestations. Although dietary control is commonly utilized to manage hypoglycemia, there is still a lack of effective pharmacological therapy. Therefore, the establishment of proper model system is essential for testing novel treatment approaches.

Methods: To create GSD-Ib *in vitro* model system, CRISPR/Cas9-knockout (KO) method was used to introduce a deletion in *SLC37A4* gene in the FlpInHEK293 cells. Characterization of CRISPR/Cas9-KO model system was performed using Sanger sequencing, RT-qPCR and Western Blot. Additionally, the expression analysis of ER stress and apoptotic markers was performed.

Results: Sanger sequencing confirmed the presence of c.14_100del in *SLC37A4* gene. The expression level of *SLC37A4* was decreased to 26.8% in the *SLC37A4*^{-/-} cell line compared to the *SLC37A4* wild-type along with Western blot analysis, which confirmed reduced target protein level in *SLC37A4*^{-/-} clones. Furthermore, ER stress (*ATF4*, *DDIT3*, *HSPA5*, *XBP1s*) and apoptotic (*BCL2*, *BAX*, *CASP3*, *CASP7*) markers expression levels were significantly altered in *SLC37A4*^{-/-} clones compared to wild-type, which proved that we created a suitable GSD-Ib *in vitro* model system.

Conclusion: Utilizing CRISPR/Cas9 technology, we established cellular GSD-Ib model system that mirrors increased ER stress and apoptosis. This model system could be used to facilitate a comprehensive understanding of disease mechanisms and enable testing of potential treatment effectiveness.

Key words: CRISPR/Cas9 knockout; Glycogen storage disease type Ib; *in vitro* disease modelling; endoplasmic reticulum stress; apoptosis

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