ACTIVATING INSULIN EXPRESSION IN H1 STEM CELLS

Alzhanuly B.[1,2], Sharipov K.[1,3]

[1] M.A.Aitkhozhin Institute of Molecular Biology and Biochemistry, 86 Dosmukhamedov Str., Almaty, 050012, Kazakhstan;
[2] Al-Farabi Kazakh National University, 71 Al-Farabi St., 050040, Almaty, Kazakhstan
[3] Kazakh National Medical University named after S.D.Asfendiyarov, 94 Tole bi Str., 050000, Almaty, Kazakhstan

Corresponding author: bakhytzhan.alzhanuly@gmail.com

Background. CRISPR/Cas9 technology has equipped researchers around the globe with broad possibilities for benefit of science. Type 1 diabetes is a disease, which develops as a result of destruction of the insulin-secreting β-cells of pancreas by the body's immune system. To date the only way of managing the disease is external insulin injection, and there is no functional cure yet. In this regard, there is an opportunity for a potential cell therapy which is leveraging human embryonic stem cells (ESCs) with CRISPR-edited insulin gene (*INS*) expression. Overall, The ESCs are now under full scale research as an inexhaustible supply of β-cells for future transplantation into the patients with diabetes type 1. In this work, we studied the feasibility of modulating insulin transcription in an ESC line using CRISPR/Cas9 technology.

Methods. The *INS* in the H1 line of ESCs was targeted with a lentivirus-expressed guide RNAs-bound CRISPR-dCas9 complex. A deactivated Cas9 nuclease (dCas9) linked to a synthetic VP64 transcription activation factor was used for the activation. Guide RNAs, to target insulin promoter, were separately packaged into a lentivirus and the viral product was used to infect previously obtained dCas9-VP64 H1 cells. Quantitative PCR was used to identify fold increase in *INS* mRNA expression. Insulin itself as a protein was detected using Hoechst staining.

Results. Nearly 900x (compared to controls) insulin expression was observed in the CRISPR- edited H1 stem cells. The Hoechst staining confirmed the activated insulin transcription by successfully detecting the hormone as an expressed protein.

Conclusion. Despite regulation of insulin gene is complicated in nature, the study showed feasibility of targeting and activating insulin expression in H 1 line of embryonic stem cells using CRISPR/dCas9 approach. Moreover, detecting the activated protein product was possible with Hoechst staining. The results provide a path to designing a potential approach for obtaining a new line of stem cells-derived insulin-producing cells for future therapy options against type 1 diabetes.

Acknowledgment. This research has been funded by the Science Committee of the Ministry of Science and Higher Education of the Republic of Kazakhstan (Grant No. AP25795747).

Keywords: *diabetes mellitus, type I diabetes, insulin, CRISPR, H1 stem cells*