



THE UNIVERSITY
OF BRITISH COLUMBIA

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University of British Columbia
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REVIEW

of the foreign scientific supervisor for the research work of Alzhanuly Bakhytzhan «**Development of a cell therapy approach for diabetes by engineering tunable insulin production in β -cells**» for the degree of Philosophy Doctor (Ph.D.) in the specialty 6D060700 – «Biology»

The dissertation of PhD candidate Bakhytzhan Alzhanuly is devoted to assessing feasibility of modulating insulin transcription in ordinary human cells and then in embryonic stem cells with utilizing CRISPR/Cas9 technology. Having proved the initial goal, he next performed in vitro differentiation of the CRISPR-edited stem cells into insulin-producing pancreatic β -cells while simultaneously assessing effectiveness of the CRISPR construct throughout the differentiation process.

Diabetes is one of the heaviest medical problems in society. Among the known types of the disease, the acute and most difficult to manage is type 1 diabetes, which is characterized by absence of the hormone insulin in the patient's body caused by autoimmune destruction of β -cells. To date, the only available means of maintaining patient's life is insulin injection, while the most potential therapy is the transplantation of healthy insulin-producing cells. However, the widespread shortage of donors greatly limits the possibilities. Therefore, seeking solutions to obtain new cells suitable for transplantation has become an extremely important medical need.

While completing his dissertation Bakhytzhan has designed several guide RNAs (gRNAs) for directing CRISPR construct to the promoter of the insulin gene and then a new vector has been created based on lentivirus to deliver selected gRNAs to target cells. Plasmids with inactive nuclease dCas9 and transcription activator VP64 and transcription repressor KRAB, dCas9-VP64 and dCas9-KRAB, respectively, were obtained and validated. A stable HEK 293 cell line expressing proteins of these plasmids was produced. Then the effectiveness of the developed CRISPR complex (dCas9 nuclease, gRNA, transcription regulator) in HEK 293 cells was tested by transducing the previously obtained dCas9-VP64 and dCas9-KRAB HEK 293 cells. As a result, he demonstrated that the developed genetic approach is able to activate and further reduce the expression of insulin in these cells.

He next moved on to the H1 line of human embryonic stem cells. The CRISPR-dCas9 plasmids and the lentiviral vector were introduced into the H1 stem cells, validated, and in vitro directed differentiation into insulin-synthesizing pancreatic β -cells was performed using protocols available at the time of the research. As results of the differentiation, the final cell line expressing key markers of natural β -cells such as NKX 6.1, MAFA and insulin was obtained. The resulting cells showed significantly increased insulin expression compared to the expression of somatostatin and glucagon.

Thus, the candidate proposed and then experimentally showed the possibility of modulating insulin transcription by CRISPR-Cas9 technology in human HEK 293 cells as well as in the H1 embryonic stem cells. It has also been shown that the genetically modified H1 cells can be differentiated to pancreatic β -cells, without losing expression of key genes of the CRISPR construct. I firmly believe the obtained results of Bakhytzhan holds a great potential in developing a new cellular therapy approach against type I diabetes.

Being a promising early-career scientist Bakhytzhan practices a professional attitude in conducting scientific work. He treats tasks with full dedication and constantly acts as an initiator in optimizing the workflow and an active participant in scientific discussion. It was a pleasure working with him along while he was visiting us and since then we have been in close contact. I hope for more collaboration with him in the future.

Therefore, having said the abovementioned, I believe that Bakhytzhan himself and his research work deserve the degree of Philosophy Doctor (Ph.D.) in the specialty 6D060700 – «Biology».

Sincerely,



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