Abstract

Diabetes mellitus type I is severe autoimmune disease which is caused by destruction of insulin-producing β-cells in pancreas. Diabetic patients are dependent on external usage of insulin during their whole life. Nowadays the only treatment of diabetes type I is transplantation of entire pancreas or isolated Langerhans islets. Due to the fact that this kind of treatment is very demanding and limited availability of suitable donors, the researchers are intensively working on development of new alternative ways how to produce the insulin-producing cells.

One of the possible approaches on producing insulin-positive cells is transdifferentiation of pancreatic exocrine cells via transcription factors. In this diploma thesis, the transdifferentiation of exocrine cells AR42J was carried out with *in vitro* synthesized mRNA encoding transcription factors Pdx1, Ngn3 and MafA. The primary mRNA structure was optimized in order to prepare highly stable mRNA which is correctly translated into the protein. The main stabilizing elements in mRNA structure include 3' and 5' untranslated region derived from highly stable β-globin mRNA. In order to verify the function of synthetic mRNA the immunofluorescence staining of transcription factors has been investigated.

Synthetic mRNAs encoding transcription factors Pdx1, Ngn3 and MafA were utilized for transdifferentiation of exocrine cells into insulin-producing cells. After 10 days of repeated transfection with these three transcription factors the exocrine cells became insulin-positive. The level of expression was dependent on the composition of cultivation media. Transdifferentiation of exocrine cells using mRNA for transcription factors Pdx1, Ngn3 and MafA allowed us to derive insulin-positive cells with characteristic features of pancreatic β-cells. (In Czech)

Key words: pancreatic cells, protein expression, transfection, cell culture