ABSTRACT

Diabetes mellitus is a severe metabolic disease associated either with loss or functional impairment of insulin-producing β-cells. A major goal in diabetes research is to develop effective ways of pancreatic β-cells restoration. One of the possible approaches is the stimulation of β-cell proliferation.

Transcription factor c-Myc is one of the promising targets for stimulation of β-cell proliferation. However, the adenovirus-mediated long-term overexpression of this oncoprotein is associated with risk of cellular dedifferentiation, apoptosis and development of cancer. Therefore, the applicability of an alternative approach for the c-Myc ectopic expression, based on the in-vitro transcribed synthetic mRNA, was verified in this thesis. Use of in vitro transcribed c-Myc-mRNA allows achieving short-term overexpression of transcription factor c-Myc in host cells. High level of c-Myc expression by the β-cells, was detected following the transfection with c-Myc-mRNA. However, within the 48 hours the expression level of c-Myc protein declined to the basic level. The physiological degradation of c-Myc-mRNA by the host cells is a key factor that reduces the risk of cancer development.

Transfection of rat islet cells with c-Myc-mRNA resulted in a significantly increased β-cell proliferation. Under the physiological conditions, the proliferation rate of adult rat β-cells achieves 3 %, whereas c-Myc-mRNA induced β-cells proliferation ranged from 35 to 70 % depending on the concentration of applied synthetic mRNA. Furthermore, addition of CHIR99021, GSK3β inhibitor, increased the number of proliferating β-cells by additional 5-13 %.

In addition it was revealed that synthetic mRNA based overexpression of c-Myc led to the increased expression of cell cycle regulators (cyclin and cyclin-dependent kinases) in pancreatic islets cells. Furthermore, the expression of genes encoding pancreatic hormones, genes that regulate insulin secretion and glucose metabolism and genes encoding certain transcription factors has also increased in islet cells transfected with c-Myc-mRNA. (In Czech)

Key words:

pancreatic β-cells, proliferation, c-Myc, *in vitro* transcription