ABSTRACT

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Title of diploma thesis: Establishment of screening methods to find new regulators of the activity of phosphoglycolate phosphatase

This work deals with the siRNA-based genomic screening for the modification of phosphoglycolate phosphatase (PGP) activity. 235 proteins were affected by transient transfection of siRNAs *in vitro*. Each siRNA was used in duplicates and the control was carried out by two control siRNAs. After downregulation of protein by siRNA PGP activity was evaluated, whether any modifications of PGP activity have occurred. PGP was the main research target.

The main goal of this study before the screening was to set up a method, to create a reliable protocol. The whole study was 96 plate well. It was necessary to find the right conditions to measure PGP activity in two cell types (HEK AD 293 and Hep G2). Subsequently, optimal conditions were set up to influence expression of the protein. The method was optimalized using PGP siRNAs and 2 types of transfection reagents were tested. During our study the following methods were used: PGP activity assay, Bicinchoninic acid assay, and western blotting.

After the protocol has been drawn up, screening was performed to influence protein expression by siRNA. Due to interest, hepatic cells (Hep G2) were used for screening. Used siRNAs affect the proteins interfered with AMPK signalling, fatty acid and glucose metabolism. After incubation of siRNA PGP activity was evaluated in cell lysates. Research has shown several candidates that can affect PGP. Because of ongoing research, these candidates are not specified in this work.