Abstract

Charles University in Prague
Faculty of Pharmacy in Hradec Králové
Department of Biochemical Sciences

Candidate: Bc. Barbora Hanousková
Supervisor: Ing. Petra Matoušková, Ph.D
Title of diploma thesis: **Construction of plasmids for human GPx7 gene reporter assay**

Glutathione peroxidases (GPx) catalyze the reduction of H_2O_2 and organic hydroperoxides to H_2O or corresponding alcohols. GPx remove compounds dangerous for the cell and protect cells against oxidative stress. Until now, in human genome in total 8 GPx coding genes were nidentified.

Thesis deals with GPx7, a monomeric enzyme with antioxidant effect and that participates in protein folding. Lower expression of GPx7 was observed in breast cancer, Barrett´s dysplasia and esophageal cancer. GPx7 might be a classic tumor marker and its inactivation can lead to cancer development.

Possibly, microRNAs (miRNAs) cause a low expression of the GPx7 gene. MiRNAs are small non-coding single stranded RNA molecules that are significantly involved in regulation of gene expression. Their main function is inhibition of gene expression at post-transcriptional level, thus prevention translation of mRNA into protein. It is caused by specific binding miRNA to binding sites in 3´UTR region of mRNA.

The aim of this thesis is the construction of plasmids with ligated 3´UTR region of GPx7 containing the miRNA binding sites and their mutated counterparts. Specific vector pmiR-GLO was used for cloning, suitable for subsequent luciferase assay. These plasmids will be used for further experiments.