Abstract

Several malignant diseases, such as colorectal, pancreatic, breast or ovarial cancers, are primarily treated with cytostatics 5-fluorouracil (5-FU). 5-FU undergoes biotransformation in human body and arising metabolites induce the damage and subsequent apoptosis in the target cells.

The main aim of this diploma Thesis was the determination of methylation in promoter regions of 14 candidate genes participating on 5-FU biotransformation: *TK1, PPAT, RRM1, RRM2, UCK2, UCK1, UMPS, TYMP, UPP1, UPP 2 SLC29A1, UPB1, DPYS and DPYD.* We hypothesize that the methylation in promoter regions regulates mRNA transcription of the above candidate genes. We have conducted appropriate analyses in 128 colorectal cancer patients, for whom both tumor and nonmalignant adjacent tissues were available. Sample processing and analysis involved DNA isolation, bisulfite conversion of unmethylated cytosines to corresponding uracils, methylation-specific analysis of melting curves with high resolution for theproper methylation analysis and gel electrophoresis to separate PCR products.

For the majority of the studied genes (*TK1, PPAT, RRM1, RRM2, UCK2, UCK1, UMPS, TYMP, UPP1, SLC29A1 and DPYD*) we did not detect any aberrant methylation in promoter regions. In genes *DPYS, UPB1* and *UPP2* we recorded various degree of promoter methylation. Statistically significant differences ($P < 0.05$) in methylation by comparing tumor tissue and adjacent mucosa were observed in testing set for genes *UPB1 and DPYS* and for *DPYS* in validation set I.

In collaboration with Natl. Inst. Public Health we were comparing expressions of genes with elevated levels of promoter methylation. However, hypermethylation of promoter regions in genes involved in 5-FU biotransformation did not affect mRNA transcription.

We have also analysed studied parameters as prognostic markers in relation to the 5-FU therapy. In general, overall survival was not different in relation to hypo- or hypermethylation of *DPYS, UPB1* and *UPP2* promoter regions. However, patients treated with 5-FU with simulateneous hypo- or hypermethylation in *DPYS, UPB1* and *UPP2* exhibit better prognosis than those treated with other cytostatics.

Key words: 5-Fluorouracil, mechanisms of epigenetics, DNA methyaltion, bisulfite conversion, methylation specific high resolution melting.