ABSTRACT

Introduction: Colorectal cancer (CRC) is one of the most frequent malignancies and affects approximately 5% of worldwide population. More than 75% of CRC cases represent sporadic forms. Susceptibility to nonhereditary CRC is significantly influenced by polymorphisms and mutations in low-penetrance genes. Variations in biotransformation and DNA repair genes may result in acumulation of toxins and DNA damage in cells leading to the development of cancer. Furthermore, different gene expression profiles of membrane transporters affecting the accumulation of anticancer drugs in tumour cells, e.g. ABC drug transporters, may largely influence inter-individual variability in drug response and chemotherapy outcome.

The aim of this study was to evaluate the role of genetic and lifestyle factors in the risk of onset and progression of colorectal cancer. This study followed selected genetic alterations in xenobiotic-metabolizing enzymes (CYP1B1, GSTM1, GSTT1, GSTP1, NQO1 and EPHX1) and genes involved in response to DNA damage (*CHEK2* and *NBN*), as potential CRC susceptibility factors. Another aim of this study was to investigate expression profile of all human ABC transporter genes to follow their prognostic and predictive potential in colorectal carcinoma.

Materials and methods: The polymorphisms and other genetic alterations were detected using real-time PCR, allele-specific PCR, RFLP-PCR and DNA sequencing methods in DNA samples extracted from peripheral blood lymphocytes of patients. The frequency of polymorphisms was evaluated and their importance was assessed with regard to the available epidemiological data. Gene expression was determined by qRT-PCR in paired samples of tumor and distant unaffected mucosa tissues. Stability of 23 reference genes was assessed and 6 reference genes were then used for normalization. Results were evaluated by REST2009 and SPSS programs.

Results: Carriers of variant Ser allele in codon 453 of *CYP1B1* (rs1800440) were at a significantly lower risk of CRC compared to carriers of the wild-type allele (adjusted odds ratio; aOR=0,68; CI=0,51-0,89; p=0,006). The combination of polymorphisms in codons 453 and 432 (rs1056836) of *CYP1B1* further increased the protective effect (aOR=0,53; CI=0,34-0,83; p=0,005). The *GSTM1-null* genotype was associated with a moderately elevated CRC risk (aOR=1,30; CI=1,01-1,68; p=0,044). Combined *GSTM1-null* and *GSTT1-null* genotype was associated with a significantly higher CRC risk compared to the presence of both full-length genes (aOR=1,58; CI=1,01-2,47; p=0,044). The increased risk of CRC was also associated with mutations in FHA domain of *CHEK2* gene (OR=2,3; 95% CI=1,3–4,0; p=0,003), and with the most frequent I157T mutation (OR=2,0; 95% CI=1,1–3,6; p=0,03). The results of the study suggest that the I157T and other alterations in its proximity predispose to sporadic but not to familial CRC in the Czech population. The rest of the observed polymorphisms had not any significant effect on CRC risk. By the evaluation of the expression levels of all 49 members of the human ABC transporters we found that the majority of the studied ABCs were down-regulated or unchanged between tumours and control tissues. *ABCA12*, *ABCA13*, *ABCB6*,

ABCC1, *ABCC2* and *ABCE1* were upregulated in tumours versus control tissues. Transcript levels of *ABCA12*, *ABCC7* and *ABCC8* increased in direction from colon to rectum. Additionally, transcript levels of *ABCB9*, *ABCB11*, *ABCG5* and *ABCG8* followed the reverse significant trend. The transcript level of *ABCC10* in tumours correlated with the grade (p=0,01). Therefore, the gene *ABCC10* appears to be a potential prognostic factor of CRC. Transcript levels of *ABCC6*, *ABCC11*, *ABCF1* and *ABCF2* were significantly lower in non-responders to palliative chemotherapy in comparison with responders. The disease-free interval of patients treated by adjuvant chemotherapy was significantly shorter in patients with low transcript levels of *ABCA7*, *ABCA13*, *ABCB4*, *ABCC11* and *ABCD4*. Therefore *ABCC11* may be a promising candidate marker for a validation study on 5-FU therapy outcome.

Conclusions: These results provide further evidence that interaction between metabolic and DNA repair gene variants contributes to colorectal carcinogenesis. This study gives a comprehensive view of the expression profiles of all known ABC transpoters in patients with colorectal cancer. The results, if validated by an independent study, may contribute to the establishment of markers of optimized anticancer therapy and improve balance between therapy efficiency and toxicity profiles.