Summary

Colorectal cancer is one of the most common malignant neoplasms in the world. The molecular mechanisms leading to colon cancerogenesis are complex and not yet fully understood. Disturbances in the TGFβ signaling pathway play a significant role in the pathogenesis of colorectal cancer. Signaling by transforming growth factor β is involved in the control of many important cellular processes, such as cell growth, differentiation, proliferation, and apoptosis. SMAD genes are closely related to the TGFβ pathway, being key mediators in this pathway. They are responsible for the regulation of the transcription of many genes, contributing to the control of excessive proliferation of gastrointestinal epithelial cells. The SMAD4 gene is a suppressor gene located on chromosome 18 in a region that is frequently lost in colorectal cancer. Inactivation of this gene may lead to the intensification of neoplastic changes in the large intestine.

One of the goals of this study was to evaluate the expression of the SMAD4 gene in patients with colorectal cancer and to relate the data obtained with the disease stage. The relative expression level of the SMAD4 gene was determined by real-time PCR. The obtained expression results were compared with the clinical and pathological parameters characterizing the studied group of patients. Based on the obtained data, no correlation was found between the level of SMAD4 gene expression and the parameters of the clinical advancement of the tumor. The lack of dependence may result from the small diversity within the studied group in relation to the assessed pathological parameters.

Another assumption of the study was the assessment of 3 single nucleotide polymorphisms within the SMAD3 and SMAD4 genes, which may play a significant role in the predisposition to the development of colorectal cancer or in the regulation of the severity of changes related to cancer progression. Genotyping was performed by the RFLP-PCR method with the use of specific restriction enzymes. Based on the obtained results, no significant correlation was found between the frequency of specific genotypes for the analyzed SNPs and the risk of developing colorectal cancer or the severity of the disease stage. Extending the presented studies to a larger study group could confirm the importance of the studied SNPs as potential markers of colorectal cancer.

The last stage of the work was to analyze the influence of the cytotoxic effect of 5-FU on cellular processes and on the expression level of the SMAD4

gene. Chemotherapy with 5-FU is used as an adjuvant treatment in many colorectal cancer patients. One of the main problems limiting the implementation of therapeutic procedures based on 5-FU in patients is the phenomenon of chemoresistance to the preparation used. The participation of inactivation of the SMAD4 gene in initiating this phenomenon in patients with colorectal cancer has been confirmed. The evaluation of the effect of fluorouracil on the expression level of the SMAD4 gene in the colorectal cancer cells of the CACO-2 line was performed using the Ral-time PCR technique. Assessment of 5-FU cytotoxicity was performed with the MTT test, and the effect of 5-FU on the induction of apoptosis and / or DNA damage was analyzed by cytometric tests. The results obtained from this part of the study showed that when 5-FU was used in higher concentrations and with longer exposure time, the expression of the SMAD4 gene significantly increases, which may translate into better effectiveness of 5-FU therapy.