Colorectal cancer is one of the most commonly diagnosed cancer and a leading cause of death from cancer. DNA repair defects have been associated with an individual susceptibility to cancer. Therefore, polymorphisms of DNA repair genes, including XRCC1 gene, are suspected to may increase the risk of colorectal cancer.

The aim of the study was to examine the association between Arg399Gln polymorphisms of XRCC1 gene and the occurrence of colorectal cancer. Research and understanding of the molecular basis of the formation of colorectal cancer will allow for typing of genetically loaded persons and qualifying them to a high-risk group.

Material and methods. In case-control study we genotyped 150 colorectal cancer patients and 170 healthy subjects from Polish population. Analysis was performed by PCR-restriction fragment length polymorphism (PCR-RFLP).

Results. We found that Gln/Gln genotype is associated with increased risk of colorectal cancer (OR 1.984; Cl 95% 1.070-3.677; p=0.029). We also found that Arg/Gln genotype is a risk factor for progression of tumor growth (OR 3.52; Cl 95% 1.157-10.707; p=0.023).

Conclusions. The current state of research suggests a link between Arg399Gln XRCCI polymorphism and increased risk of colorectal cancer. Therefore, we conclude that the Arg399Gln polymorphism of XRCCI gene may underlie at the molecular basis of the causes of colorectal cancer.

Key words: colorectal cancer, risk factors, gene polymorphism

Among malicious tumours, colorectal cancer is the second most common. The risk of developing colorectal cancer significantly increases with age and reaches its peak in the seventh decade of life. The standarized mortality rate per 100 000 is 12.4 for men and 8.7 for women. Early diagnosis is one of the most important therapy factors and significantly improves the survival rate of patients (the 5-year survival rate in Poland fluctuates around 30%, whereas in Western Europe the rate exceeds 50%). The causes of colorectal cancer have not yet been established. It is estimated that in 65-85% of cases the cancer is sporadic, the rest are hereditary and familial. Approximately 3% of colorectal cancers are caused by mutations of strongly predisposed genes and, independently, 10-30% of cases are familial. Despite the cause of most colorectal cancers being environmental factors, studies show that individual predispositions for developing this cancer may depend on genetic changes, including changes in genes involved in the process of DNA repair.

In mammalian cells there are four basic mechanisms of DNA repair: base-excision repair (BER), nucleotide-excision repair (NER),
mismatch repair (MMR) and double-strand breaks repair (DSB). Damage to DNA bases, caused by deamination, oxidation or alkylation, is repaired mainly by base-excision. Among the known polymorphisms of the DNA repair genes, the \textit{XRCC1} gene polymorphism has been repeatedly studied as potentially connected with susceptibility to the occurrence of various cancers. The product of the expression of \textit{XRCC1} is a protein involved in the DNA repair process in the BER mechanism. Because the polymorphisms of \textit{XRCC1} gene may affect the level of DNA repair, leading to carcinogenesis, it is important to establish the molecular causes of the carcinogenic process. Genetic tests enable the diagnosis of individuals with increased risk of developing cancer and helps to put them into a group which can be placed under observation and treated with prophylactics.

The purpose of this paper is to define the correlation of Arg399Gln polymorphism of \textit{XRCC1} gene with the risk of colorectal cancer occurrence.

\textbf{MATERIAL AND METHODS}

Experimental material

DNA for tests was isolated from lymphocytes from peripheral blood from samples taken from 150 unrelated patients. Each patient had histopathologically confirmed colorectal cancer. The studied group included 85 men and 65 women (average age 63 years; ±8 years). The stage of the tumors was established according to TNM scale. Detailed classification is shown in tab. 1. The control group included 170 individuals not diagnosed with cancer and with ages corresponding to the age of the studied group.

Methods

DNA isolations were carried out with a commercial kit QIAamp DNA Blood Mini Kit for isolation of high-molecular-weight DNA (Qiagen).

The occurrence of polymorphic variant Arg399Gln of \textit{XRCC1} was studied with PCR-RFLP technique (Restriction Fragment Length Polymorphism). 25 µl of reaction mixture contained 10 µl of genomic DNA solution, 20 pmol of each starter, 1.5 mM of MgCl₂, 1.5 U of Taq polymerase, 0.2 mM of dNTP (commercial kit Qiagen Taq PCR Core Kit). The sequence of starters used for amplification was: 5'-TTGT-GCTTTCTCTGTGTCCA-3' and 5'-TCCTCCAGCCTTTCTGATA-3'. Reactions were performed as follows: initial denaturation at 94°C for 5 minutes, 30 cycles 94°C – 5 minutes, 61°C – 30 seconds, 72°C – 45 seconds. Final elongation 72°C – 7 minutes. The products of the amplification 615 bp long were digested with the use of 10 U of Mspl enzyme at 36°C for 16 hours, and then electrophoresed in 3% agarose gel, stained with ethidium bromide and photographed in UV light. Homozygote (wild type, Arg allele) was determined by the presence of two stripes in the gel, 374 bp and 221 bp long, and the presence of Gln allele (mutant) was determined by uncleaved stripe 615 bp long (fig. 1).

![Fig.1. Genotyping in amplified fragment Arg399Gln XRCC1. Path – marker of dimension of dimention of separatek fragments of DNA – 100 bp DNA Ladder (Solis Biodyne); patos 2, 4, 7- heterozygote Arg 399 Gln (stripes 221 bp, 374 bp and 615 bp); patos 3, 5 – homozygote Arg399Arg (strip es 221 i 374 bp); path 6 – homozygote Gln399Gln (strip 615 bp)](image)

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Statistical analysis

The acquired number of each genotype was compared to the expected value based on the Hardy-Weinberg principle. The relevance of the differences between the frequency of occurrence of the alleles and genotypes in the groups was evaluated with the $\chi^2$ test. The risk of occurrence was evaluated with multiparameter regression analysis (OR – odds ratio) with confidence interval 95% (CI 95%).

RESULTS

Table 2 shows the analysis of the correlation of polymorphic variant Arg399Gln of XRCC1 gene with the occurrence of colorectal cancer. The study showed, that the occurrence of genotype Gln/Gln may increase the risk of developing colorectal cancer (OR 1.984; CI 95% 1.070-3.677; p=0.029).

Patients were also assigned to categories according to the progression of cancer based on the three-stage scale of the American Joint Committee on Cancer. The results are shown in tab. 3. Analysis of these results shows, that heterozygote Arg/Gln increases the risk of progression of the cancer from stage one to stage two on this American Joint Committee on Cancer scale (OR 3.52; CI 95% 1.157-10.707; p=0.023).

DISCUSSION

The purpose of this paper was to examine the correlation of Arg399Gln polymorphisms of XRCC1 gene with the risk of colorectal cancer occurrence. A correlation between Gln/Gln genotype and an increased risk of developing colorectal cancer (OR 1.984; CI 95% 1.070-3.677; p=0.029) was shown and the influence of Arg/Gln polymorphic variant on cancer progression was defined (OR 3.52; CI 95% 1.157-10.707; p=0.023).

The influence of polymorphisms of DNA repair genes on the risk of developing cancer has been studied for many years, but the published results are not conclusive. The results published so far show a significant correlation between Arg/Gln and Gln/Gln genotypes and the occurrence of colorectal cancer, however there are published data which demonstrate there is no such correlation (1-4). The divergence may be caused by the fact that many different proteins are involved in the DNA
repair process and their activity may be correlated. Therefore correlations of polymorphisms of the studied genes may be related to developing a cancer (5).

It is also suggested that individual ability of DNA repair may depend on the environmental conditions the cell is exposed to (6). This results in differences in the development process of the cancer, despite the presence of the same polymorphism: for example, the risk of developing a cancer in cells which react properly to an apoptosis signal is decreased and the risk in apoptosis-abrogated cells is increased (7). Moreover, recent research shows that in cases where formerly no correlation between polymorphism of DNA repair genes and cancer development was found, a correlation exists if the relations between polymorphisms of individual genes is considered (7). This indicates that the morbidity for colorectal cancer may depend not only on the occurrence of particular polymorphisms of individual DNA repair genes, but on their interactions in the respective DNA repair mechanism, e.g. XRCC1 and XRCC3 in BER (8) or on the interactions of polymorphisms between different DNA repair mechanisms, e.g. XRCC1 and XPD in BER and NER(5). Moreover, a correlation between polymorphisms of XRCC1 gene and other factors which affect cancer development is noticed, particularly the age of patients with colorectal cancer (9). Furthermore, an increased risk of developing colorectal cancer in an urban population was shown (1), and the correlation of XRCC1 polymorphisms and the development of colorectal cancer in a male population (10).

The results of our research indicate that there is a significant correlation of the Arg399Gln polymorphism of XRCC1 gene and the development of colorectal cancer in the Polish population. This suggests the need for further research on the correlation of polymorphisms of BER DNA repair genes with the development of colorectal cancer. The research should include the factors that influence the progression of cancer and, in particular, a study of the intergene correlations. This would enable the recognition of the molecular basis of colorectal cancer development and provide a fast and efficient way of indentifying individuals with a high risk of developing colorectal cancer.

REFERENCES