ASSOCIATION OF POLYMORPHISM OF LYS589GLU EXO1 GENE WITH THE RISK OF COLORECTAL CANCER IN THE POLISH POPULATION*

JACEK KABZIŃSKI1, KAROLINA PRZYBYLOWSKA1, MICHAŁ MIK2, ANDRZEJ SYGUT2, ŁUKASZ DZIKI2, ADAM DZIKI2, IRENEUSZ MAJSTEREK3

Department of Clinical Chemistry and Biochemistry, Medical University in Łódź1
Kierownik: prof. dr hab. I. Majsterek

Department of General and Colorectal Surgery, Medical University in Łódź2
Kierownik: prof. dr hab. A. Dziśki

The incidence of colorectal cancer (CRC) is increasing from year to year. Despite intensive research CRC etiology remains unknown. Studies suggest that at the basis of the process of carcinogenesis can lie reduced efficiency of DNA repair mechanisms, often caused by polymorphisms in DNA repair genes.

The aim of the study was to determine the relationship between gene polymorphism Lys589Glu of EXO1 gene and modulation of the risk of colorectal cancer in the Polish population. Determination of the molecular basis of carcinogenesis process and predicting increased risk will allow qualifying patients to increased risk group and including them in preventive program.

Material and methods. The material used in study was blood collected from 130 patients diagnosed with colorectal cancer. The control group consisted of 135 healthy people. Genotyping was performed by TaqMan method.

Results. The results obtained indicate that the genotype Lys/Glu is associated with an increased risk of colorectal cancer (OR 1.811, 95% CI 1.031-3.181, p = 0.038).

Conclusion. On the basis of these results, we conclude that Exo1 gene polymorphism Lys589Glu may be associated with an increased risk of colorectal cancer.

Key words: colorectal cancer, polymorphisms, EXO1, DNA repair

Colorectal cancer (CRC) is currently the second most common cancer among women and the third among men. Incidence is increasing from year to year, and in spite of intensive research in this area, etiology of the disease remains undetermined. Low responsiveness to therapy and high malignancy are the cause of a very low coefficient of 5-year survival (in Poland about 30%) and make the CRC fourth cause of death from cancer. It is estimated that 15-30% of cases is familial, with about 3% is caused by mutations of strongly predisposed genes. However, despite the fact that approximately 70% of sporadic cancer is caused by environmental factors, studies indicate that it may occur primarily in people with a genetic predisposition, associated mainly with changes in the DNA repair genes.

Four basic systems of DNA repair should be distinguished: base excision repair (BER), nucleotide excision repair (NER), mismatch repair (MMR) and repair of Double-strand breaks (DSB). So far, in the course of the study, irrefutable relationship between the presence of mutations in the MMR system genes and hereditary colorectal cancer unrelated polyposis (Lynch syndrome) has been found. This process is associated with muta-
Polymorphism of Lys589Glu EXO1 gene and the risk of colorectal cancer

The gene product EXO1 – exonuclease 1 – is an enzyme interacting closely MSH2 and MLH1 protein, which can affect the efficiency of the system and the MMR indirectly modulate the risk of CRC. EXO1 gene polymorphisms are currently under investigation in terms of their potential impact on the incidence of colorectal cancer.

The aim of this study was to determine the effect of gene polymorphism Lys589Glu of EXO1 gene on the risk of colorectal cancer in the Polish population.

MATERIAL AND METHODS

Experimental material

Test DNA was isolated from peripheral blood samples collected from 150 unrelated patients. All patients had histologically confirmed colorectal cancer. The study group consisted of 89 men and 61 women (mean age 65 ± 7). To assess the stage of cancer TNM scale was used. A detailed information about the patients illustrates tab. 1. The control group consisted of 150 persons age corresponding to the study group who did not have cancer.

Methods

DNA isolation was performed with commercial kit QIAamp DNA Blood Mini Kit for isolation of high-molecular-weight DNA (Qiagen). The distribution of polymorphic variants EXO1 Lys589Glu was examined using TaqMan method. Test polymorphism refSNP is 1047840.

Statistical analysis

The resulting number of each genotype was compared with the expected value based on Hardy-Weinberg equilibrium. The significance of differences between the frequencies of alleles and genotypes between groups was assessed using χ² test. The risk of an event was assessed using multivariate regression analysis (odds ratio, OR) with corresponding confidence interval 95% (CI 95%).

RESULTS

Table 2 presents the analysis of the distribution of polymorphic variants of the gene Lys589Glu EXO1 and their correlation with the modulation of the risk of colorectal cancer. Research indicates that the genotype Lys/Glu may affect the increased risk of CRC (OR 1.811, 95% CI 1.031-3.181, p=0.038). Also the distribution of patients according to the severity of the tumor according to the scale of the American Joint Committee on Cancer has been made. The results are shown in tab. 3. Analysis of the results shows that none of the genotypes or alleles of tested polymorphism affect the progression of cancer.

DISCUSSION

A long suspected link between polymorphisms of DNA repair genes and the process of carcinogenesis becomes more and more

Table 1. Characteristics of the groups of patients with colorectal cancer

<table>
<thead>
<tr>
<th>Patients</th>
<th>Sex</th>
<th>Stage of tumor according to TNM classification</th>
<th>Stage of tumor according to AJCC classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td></td>
<td>T</td>
<td>N</td>
</tr>
<tr>
<td>150</td>
<td>61</td>
<td>89</td>
<td>21</td>
</tr>
</tbody>
</table>

Table 2. Correlation of Arg399Gln polymorphism of XRCC1 gene and the occurrence of colorectal cancer

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Patients n=150</th>
<th>Controls n=150</th>
<th>OR (95% CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lys/Lys</td>
<td>33</td>
<td>39</td>
<td>1 (ref)</td>
<td></td>
</tr>
<tr>
<td>Lys/Glu</td>
<td>95</td>
<td>62</td>
<td>1,811 (1,031-3,181)</td>
<td>0,038</td>
</tr>
<tr>
<td>Glu/Glu</td>
<td>22</td>
<td>49</td>
<td>0,531 (0,268-1,052)</td>
<td>0,068</td>
</tr>
<tr>
<td>Lys</td>
<td>140</td>
<td>161</td>
<td>1 (ref)</td>
<td></td>
</tr>
<tr>
<td>Glu</td>
<td>160</td>
<td>139</td>
<td>1,324 (0,960-1,825)</td>
<td>0,086</td>
</tr>
</tbody>
</table>
documented every year, through research. Yet still, despite many studies and publications in this field, road to determine irrefutable linkage remains long, since results are often inconclusive and sometimes contradictory. The reasons for this state should be sought in the complexity of the process of carcinogenesis and a wide range of factors, both endo- and exogenous, which may affect the incidence of cancer. There are also important differences between the study populations and the impact of gene-gene interactions affecting the efficiency of DNA repair systems and the process of carcinogenesis. According to current knowledge, it has been determined primarily irrefutable link between the hereditary nonpolyposis colorectal cancer (HNPCC) and defects of the DNA mismatch repair (MMR) (1, 2, 3), which by mutations in Mut proteins interfere genome stability, leading to microsatellite instability observed in most human cancers.

Currently, research is focused on the impact of factors affecting mutations of Mut protein.

The aim of this study was to investigate the effect of gene polymorphism Lys/Glu of EXO1 on the modulation of risk of colorectal cancer.

Exonuclease 1, EXO1 gene product, is the enzyme involved in the repair of DNA damage the interacting closely with Mut proteins – MSH2 (4, 5, 6), and MLH1 (7, 8). Therefore, one should suspect that as a result of single nucleotide polymorphisms (SNPs) that may adversely affect the enzymatic activity of the protein causing a decrease in the efficiency of DNA repair, leading to modulation of the risk of CRC. The study indicates that the genotype Lys/Glu gene of interest may increase the risk of colorectal cancer (OR 1.811, 95% CI: 1.031-3.181, p=0.038). The results are consistent with part of the available literature (9, 10), which confirms the existence of dependencies, but also stand in contrast to other reports (11) that deny the correlation between gene polymorphisms of EXO1 and the incidence of CRC. The reasons for the ambiguity should be found in differences in study populations (eg, different races), and as mentioned in the introduction in interactions with other groups of genes that may modulate the risk of cancer. This is consistent with recent studies suggesting that even in the absence of a clear link between the given polymorphism and the process of carcinogenesis, such a correlation can occur in the case of the analysis of gene-gene interactions having a significant impact on the modulation of risk (12, 13).

At the same time the analysis of the relationship between the studied polymorphisms and progression of CRC according to the classification by the American Joint Committee on Cancer showed that there are no effect of the tested polymorphism on the progression of cancer, which clearly indicates that this gene is involved only in the process of neoplastic transformation, but there is no subsequent impact on its development. Therefore, we believe that the tested EXO1 gene polymorphism is associated with an increased risk of colorectal cancer in the Polish population, however, further studies are needed to determine the effects of intergenic interactions and their impact on the process of carcinogenesis. This will allow in the future for simple analysis and typing of patients with a predisposition to the occurrence of CRC and including them into prevention programs.
REFERENCES

5. Tishkoff DX, Boerger AL, Bertrand P: Identification and characterization of Saccharomyces cerevisiae EXO1, a gene encoding an exonuclease that interacts with MSH2 *Proc Natl Acad Sci USA* 1997 Jul 8; 94.
6. Eccleston J, Yan C, Yuan K: Mismatch repair proteins MSH2, MLH1, and EXO1 are important for class-switch recombination events occurring in B cells that lack nonhomologous end joining. *J Immunol* 2011 Feb 15; 186.

Received: 14.07.2014 r.
Adress correspondence: 90-647 Łódź, Pl. Hallera 1
e-mail: jacek.kabzinski@umed.lodz.pl