One of the major causes of carcinogenesis is loss of genome stability. RAD51 in process of homologous recombination (HR) played crucial role in maintenance integrity of genome through initiate of DNA double strand breaks repair. Presence of single nucleotide polymorphism (SNP) in RAD51 gene could change the capacity of DNA repair and altered the response to damaging agents. Research on potential impact of genetic variability on development and progression CRC may contribute to setting new genetic markers or determined individual susceptibility to CRC.

**The aim of the study.** This study was designed to evaluate the effect of 135 G/C (rs1801320) RAD51 polymorphism located in the 5′ untranslated region on the risk and progression of CRC.

**Material and methods.** The subjects consisted of histologically confirmed colorectal cancer (n = 200) and controls (n = 200) with lack of previous history of cancer. The distribution of genotypes was determined by restriction fragment length polymorphism PCR (RFLP – PCR). Statistical analysis was based on multivariate regression model.

**Results and conclusion.** Our study reveal no significance association of 135 G/C RAD51 polymorphism with occurrence and progression of colorectal cancer.

**Key words:** polymorphism, 135G/C RAD51 gene, colorectal cancer

Colorectal cancer (CRC) is the most common case of cancer in the European population, especially in the developed countries. Moreover, mortality data indicate that CRC is the second cause of death among people suffering from cancer (1). The worldwide estimations also rank CRC at the forefront of frequency list of cancer occurrence, behind lung and breast cancer (2). The main risk factor for CRC includes age of maturity, male gender, long-term medication of non-steroidal anti-inflammatory drugs (3), dietary habits characterized by high consumption of meat and parallel low level of fibre, folate, calcium. Another risk determinant embrace number of non-specific environmental conditions as smoking cigarettes, alcohol abuse, obesity.

The above-mentioned factors are associated with development of sporadic CRC, being around 90% of cases. Remaining percentage of cases (about 10%) carrying hallmark of hereditariness, thus inherited CRC can be divided into two types: hereditary nonpolyposis colorectal cancer (HNPCC) and familial adenomatous polyposis (FAP). Adenomatous polyposis coli (APC) suppressor gene disorder and accompanying biallelic mutation of DNA glycosylase (MYH) gene are identified in FAP, whereas HNPCC type of CRC is involved with appearance of an autosomal-dominant mutation within genes encode proteins of DNA miss-match repair, resulting instability of microsatelite (4, 5). Loss of genome integrity is essential in the process of carcinogenesis,
mainly through the potential impact of rearrangements on activation oncogenes and silenced suppressor gene.

Particularly, double strand breaks (DSB) of DNA pose a threat to genome integrity. The source of DSB are exogenous agents such as ionizing radiation as well as endogenous processes generating reactive oxygen species, mechanical stress or replication forks arrest and collapse (6, 7). Proper course of cell cycle provides DNA damage checkpoints. Recognition of DNA breaks switch appropriate mechanism of repair or lead to apoptosis pathway, depending of the degree of damage (8). The crucial role in maintenance integrity of genome ensure homologous recombination process (HR). The most efficiency of HR repair occurs during S and G2 phase of cell cycle, then cell is preparing to division, genome is replicated and particularly exposed to damaging agents. Using undamaged sister chromatids as a template in HR repair reduces the risk of loss of heterozigosity and result in accurate repair (9). Among several proteins carried out HR, RAD51 seems to play significant role. In accompanied RAD51 paralogs, and BRCA2 mediation protein, RAD51 forms DNA nucleoprotein filament (NF) on previously processed ssDNA (10). NF also called presinaptic filaments, captures homologous undamaged section of dsDNA and performs invasion, leading to formation Holliday junction. As a result of the availability of homologous DNA template, there is enable strand exchange process and re-synthesis missing sequence (7, 11, 12, 13).

RAD51 gene located at position 15q15.1 of about 30 kbp length, consists of 10 exons (14). Lack of RAD51 is embryonic lethality, as in investigation on mice with RAD51 knock-out reveals (15). Regard for significant influence genetic factors on colorectal cancer development, it seems to be important consider very common genetic variability such as single nucleotide polymorphism (SNP). Wang and co-workers localized SNP of RAD51 in 135G/C (rs1801320) position at the 5’UTR region (16). The area of occurrence 135G/C polymorphism is associated with transcriptional activity. Applying transfected U2-OS cells indicates that substitution G allele significantly enhanced transcription RAD51 gene (17). Over the last decade, many results have been published reveal association 135G/C polymorphism with occurrence various cancers. The vast majority of these results concerned breast cancer, especially in conjunction with BRCA2 mutation (18). Constantly increasing amount of reports supporting significant role of inefficiency HR in tumourigenesis has led us to consider SNP variability of RAD51 as potential risk factor.

The aim of the present study was to assessed possible effect of the 135G/C RAD51 gene (rs1801320) polymorphism on development and course of CRC. The scope of our research included determination of genotype among patient and control group, then statistical analysis based on multivariate regression.

MATERIAL AND METHODS

Subject

In case-control study performed on polish population, there was examined 200 patients with diagnosed CRC and 200 control subject. Control group consisted of persons with diagnosed lack of cancer was matched according to age and gender. DNA was isolated from peripheral blood lymphocytes. All blood samples were obtained from Department of General and Colorectal Surgery, Medical University in Łódź. The distribution of age, sex and stage of disease presented in tab. 1.

Genotyping

DNA was isolated using QIAamp DNA Blood Mini Kit for isolation of high-molecular-weight
DNA (Qiagen, Chatsworth, CA, USA). The genotypes were determined by restriction fragment length polymorphism (RFLP-PCR) technique as described previously (19). The volume of reaction mixture was 10μl with following content: 10 ng genomic DNA, PCR Master Mix (Fermentas, Vilnius, Lithuania) and 250 nM of each primer (Sigma-Aldrich, St. Louis, MO, USA). Applied primers had sequence respectively: sense, 5’-TGGGAACTGCAACTCATCTGG-3’ and antisense, 5’-GCGCTCCTCTCTCAGCAG-3’. Polymerase chain reaction was performed in thermal cycler T100 BIO-RAD (Herkules, CA, USA) on follows condition: 95˚C by 3 minutes, then 34 cycle of 95˚C for 30 second, 64˚C for 30 second, 72˚C for 40 seconds and final elongation step 72˚C for 5 minutes. The amplified fragment containing polymorphic site, had length of 157 pb. Entire product was digested with 2U of BstNI restriction enzyme (NE Bio labs, New England, MA, USA) at overnight. Obtained fragments were separated on 3% agarose gel for 1 hour at 110V. DNA was stained with ethidium bromide and visualized under UV light. There were three type of band pattern: 157 pb, 86/71 pb, 157/86/71 bp being equivalents of C/C, G/G, G/C genotypes.

**Statistical analysis**

Assessment of a study population for compliance with Hardy-Weinberg Equilibrium was checked using X<sup>2</sup> test. Values of the odds ratio (OR) and confidence interval (CI 95%) were estimated based on multivariate regression model.

**RESULTS**

Genotype distribution of the 135G/C RAD51 polymorphism, in study subject was in agreement with Hardy-Weinberg Equilibrium ($X^2$ = 3.47; p= 0.06), whereas in control group, genotypes were not fitted to this equilibrium ($X^2$ = 3.89; p= 0.049). The level of frequency of particular genotypes and alleles, was comparable both in patients and controls. As revealed in Table 2, analysis of the obtained values of odds ratio and probability (p), indicate a lack of direct association between appearance of 135G/C polymorphism and colorectal cancer risk. Apart from case-control estimations, there were performed assessment of the risk of progression. Based on cancer stages according to American Joint Committee on Cancer classification takes into account size of tumor and spread of cancer to regional lymph nodes, we compared group of patients in noninvasive first stage (I˚), with more advanced second (II˚) and third (III˚) stages. Evaluation of statistics also indicate no effect on tumor progression as summarized in tab. 3.

**DISCUSSION**

Genomic instability is one of the leading cause of tumorigenesis. The period of cell division is particularly critical in terms of exposure to adverse rearrangement in the genome, induced by DNA DSBs. Homologous recombination process is essential for maintaining genome integrity for reason of high-intensity activities during S and G2 cell cycle. However, apart from involvement of HR in DSBs repair, a repair replication fork arrested seems to be more important, as shown by a recent report (20). A pivotal component of HR is RAD51 protein, due to formation of presinaptic filaments in the break site. It ensure branch invasion on homologous double strand, then initiate exchange reaction between ssDNA and undamaged ds-DNA, finally re-synthesis lost sequence (13).

<table>
<thead>
<tr>
<th>Genotype /allele</th>
<th>Patients n = 200</th>
<th>Controls n = 200</th>
<th>OR (95% CI)*</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>no frequency</td>
<td>no frequency</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G/G</td>
<td>161 0,8</td>
<td>157 0,79</td>
<td>1ref</td>
<td></td>
</tr>
<tr>
<td>G/C</td>
<td>34 0,17</td>
<td>37 0,19</td>
<td>0,9 (0,54 – 1,5)</td>
<td>0,68</td>
</tr>
<tr>
<td>C/C</td>
<td>5 0,03</td>
<td>6 0,03</td>
<td>0,81 (0,24 – 2,71)</td>
<td>0,74</td>
</tr>
<tr>
<td>allele G</td>
<td>356 0,89</td>
<td>351 0,88</td>
<td>1ref</td>
<td></td>
</tr>
<tr>
<td>allele C</td>
<td>44 0,11</td>
<td>49 0,12</td>
<td>0,89 (0,57 – 1,37)</td>
<td>0,58</td>
</tr>
</tbody>
</table>
The subject of the presented research was 135G/C RAD51 polymorphism located at 5'UTR region. Phenotypic effect of above-mentioned polymorphism is still not defined. Presumably, 135G/C variation could be responsible for mRNA stability through modulatory interactions with regulatory elements (21). Moreover, 5'UTR is the site of interaction with crucial suppressor p53 protein being responsible for negatively regulating RAD51 (22). Hasselbach and colleagues, based on in vitro studies demonstrated increase transcription for G allele in 135G/C polymorphism (17).

Outcomes of our case-control study have indicated lack of association 135G/C polymorphism with CRC risk among Polish population. No statistical significance is also observed in association with the progression risk, which could affect low amount of patients with non-invasive cancer in relation to advanced stages. A similar case-control study, carried out on Polish population have shown an increased risk of CRC for G/C genotype (OR= 0.29 95% CI 0.08 – 0.98) (23), whereas association analysed by using wild-type homzygous as reference group pointed out protection effect for C/C (OR= 0.06 95% CI 0.02 – 0.22) genotype in the same Polish population (24). These reports are contrary to our results, odds ratio values obtained by us, disclosed the lack of relation with CRC for the G/C (OR= 0.9 95% CI 0.54 – 1.5) as well as C/C (OR= 0.81 95% CI 0.24 – 2.71) genotype. Presumably, the divergence could be due to difference in the size of the case groups which was only 100 patients (23, 24) in contrast to 200 patients engaged in our trial. The remaining literature data concerned the other types of tumor. A study of gastric cancer revealed a possible influence 135 G/C polymorphism on occurrence of stomach cancer among individuals with high level of oxidative damage, through regulation cellular response to oxidative damage and efficiency of DNA repair (25). Implication of RAD51 in tumorigenesis have been described extensively in relation to breast cancer, nevertheless in strictly correlation with polymorphism of BRCA2 (16, 18) and BRCA1 (26). That notifications could be important in view of further research on CRC in the context of HR disorder. Apparently, the association between RAD51 and BRCA1 polymorphisms is controversial. The most common mutation in BRCA1, 5382insC affected on length protein but intact RAD51 binding site, as reveal Jakubowska and co-workers (26). On the other hand, Wang's study, indicate that primary mutation 185delAG also result in truncated protein but simultaneously abolished RAD51–BRCA1 binding site (16). Therefore, protective effect of RAD51 135 G/C polymorphism should be consider in circumstances of availability RAD51–BRCA1 binding site, thus the C allele have almost twice reduction breast cancer risk(26). Other trials of the 135G/C polymorphism were those investigating patients with acute myeloid leukemia (AML). The examination the frequency of two variants in the 5' UTR of RAD51: 135G/C and 172G/T in a case–control study indicated protective effect for C-G haplotype (27). It may suggest significant contribution of 172G/T (rs1801321) RAD51 polymorphism in carcinogenesis in conjunction with 135G/C.

Indeed, RAD51 play pivotal role in homologous recombination, nevertheless it is a compound and multi-step process requires co-operation large collective of proteins In prospects of better cognition of the role HR in development CRC, the analysis of variability of other genes whose protein products accompany RAD51 in HR seems necessary. The emphasis should be put on BRCA1/2 and XRCC3, some of their variations are associated in CRC risk (28).
REFERENCES