JBUON 2018; 23(4): 936-940
ISSN: 1107-0625, online ISSN: 2241-6293 • www.jbuon.com
E-mail: editorial_office@jbuon.com

ORIGINAL ARTICLE

Polymorphism RAD51 172G>T in Serbian patients with colorectal cancer

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Summary

Purpose: *The RAD51 gene plays an important role in homologous strand exchange in DNA repair. Two common single nucleotide polymorphisms in this gene, 135G>C and 172G>T, were associated with altered gene transcription. While 135G>C was already linked to breast and colorectal cancers in certain populations, 172G>T is far less investigated, although sporadic studies showed it could be a prognostic factor for some cancer lesions.*
The purpose of this study was to investigate RAD51 172G>T polymorphism in Serbian population, its association with colorectal carcinoma, as well as correlation with disease characteristics and response to neoadjuvant chemoradiotherapy.

Methods: *The 172G>T polymorphism was evaluated by PCR-RFLP method in blood samples of 209 colorectal cancer patients and 43 healthy subjects who served as controls. The distribution of genotypes was also analyzed in respect to several tumor characteristics in cases where histopathological data were available.*

Results: *A significant association between the RAD51 172G>T polymoprhism and desmoplastic reaction of colorectal cancer was demonstrated. The 172G allele was found to be significantly more frequent in patients with more intensive desmoplastic response of the tumor tissue.*

Conclusions: *The results of our study suggest that the 172T allele of RAD51 may be a favorable prognostic factor in Serbian patients with colorectal cancer, although larger prospective studies are required to confirm this finding.*

Key words: *colorectal cancer, desmoplastic reaction, polymorphism, RAD51*

Introduction

Colorectal cancer is the third most commonly occurring cancer in men and the second in women [1]. Its incidence in both genders is similar, but the geographic distribution can vary up to 10-fold worldwide, with the highest rate in Australia/New Zealand (45/100,000), and the lowest in Western Africa (4/100,000). About 55% of colorectal cancers occur in more developed regions, but the mortality is higher in less developed ones (52%). Although there is a great variability in incidence across the world, there is less variability in mortality rates, with the highest estimated in Central and Easter Europe and lowest in Western Africa. The incidence of colorectal cancer in Serbia was 27/100,000 in 2013, with mortality of 16.6/100,000, and similar gender distribution [2,3].

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Received: 28/12/2017; Accepted: 21/01/2018

Many factors are associated with increased risk of colorectal cancer: age, colonic polyps, lifestyle, history of previous and/or hereditary cancers and genetic factors [4-6].

Genome instability is one of the hallmarks of cancer and a crucial feature in tumor development. It is known that the single unrepaired DNA double-strand breaks can initiate chromosomal rearrangement or cell death [7]. The major mechanism responsible for genome stability is homologous recombination by restoration of lost sequences in DNA double-strand break [8,9]. Large protein complexes recognize the lesion site and perform resynthesis based on homology to sister chromatid [10,11]. The acquisition of genomic instability in colorectal carcinogenesis can occur through three pathways: chromosomal instability, microsatellite instability, and CpG island methylation. It is known that mutations in DNA double-strand break repair genes are associated with tumor genesis, and defects in this mechanism can lead to cancer development and affect the severity of disease.

The RAD51 gene is located on chromosome 15 at position q15.1. It plays an important role in homologous strand exchange in DNA repair through homologous recombination by binding to single and double-stranded DNA. It catalyzes the recognition of homology end strand exchange between homologous DNA partners to form a joint molecule between a processed DNA break and the repair template. The RAD51 protein binds to single-stranded DNA to form nucleoprotein filaments which are essential for the homology search and strand exchange [12,13].

In the process of fixing damaged DNA, RAD51 interacts with many different proteins, including BRCA1 and BRCA2. These proteins are keeping the stability of the genetic information by helping the DNA repair [14]. Any mistake in this process can lead to chromosomal instability. Changes in RAD51 gene expression have been associated with colorectal cancer [15]. Two common single nucleotide polymorphisms in the RAD51 gene, 135G>C and 172G>T, were associated with altered gene transcription [16]. These functional polymorphisms have been studied as risk factors for different cancers. Polymorphism 135G>C has been associated with head and neck cancer and breast carcinoma, while 172G>T was shown to be a prognostic factor of precancerous lesions, predicting triple-negative breast carcinoma, but also a good prognostic factor in head and neck carcinoma [17,18].

We have investigated RAD51 172G>T polymorphism in Serbian population, its association with colorectal carcinoma, as well as correlation with disease characteristics and response to therapy.

Methods

Subjects

Samples of peripheral blood were collected from 209 patients who underwent surgical removal of colorectal cancer at the First Surgical Clinic, Clinical Center of Serbia, between 2014 and 2016. The study has included 128 men and 81 women, with age range between 28 and 86 years. For all the patients included in the study, the presence of colorectal adenocarcinoma was confirmed by standard pathological examination. The control group consisted of 43 healthy individuals. The study was approved by the local ethical committee and all patients provided written informed consent.

Genotyping of RAD51 172G>T polymorphism

Genomic DNA was extracted from peripheral blood using the salting-out method. The presence of 172G>T polymorphism was determined by polymerase chain reaction - restriction fragment length polymorphism (PCR-RFLP) analysis. The segment of the RAD51 gene containing 172G>T polymorphism (131bp long) was amplified using previously published primers [19]. The PCR was conducted in a 50μL reaction mixture containing 1X KAPA Taq Buffer A (KAPA Biosystems), 0.3mM MgCl2, 0.2mM each dNTP, 10pmol of each primer, 2U of KAPA Taq DNA Polymerase (KAPA Biosystems) and approximately 300ng of DNA. The amplifications were performed as follows: initial denaturation at 95°C for 5 min; 30 cycles consisting of 95°C for 30 s, 65°C for 45 s and 72°C for 50 s , and final extension at 72°C for 10 min. The obtained PCR fragments (131bp long) were digested with PdiI (ThermoFisher Scientific). The products were separated in 3% agarose gel. The TT genotype remains undigested displaying single band (131bp), the GG genotype produces two bands (110bp and 21bp), while the GT genotype displays all three bands (131bp, 110bp and 21bp).

Statistics

Statistical analyses were performed using Statistical Package for Social Sciences 20.0 (SPSS Inc., Chicago, Illinois, USA). Data were expressed as percentages and means±standard deviation (SD) for continuous variables and percentages for categorical variables. To test the normality of parameters one sample Kolmogorov-Smirnov test was used. Differences between groups for categorical data were tested by x^2 test, while for continuous data the following teste were used: Independent Sample t-test, Independent Samples Mann-Whitney U test, Kruskal-Wallis test and Related Samples Wilcoxon Signed Rank test. P value less than 0.05 was considered statistically significant.

Results

The study has included a group of 182 patients with colorectal cancer for whom histopathological data were available (Table 1).

The distribution of genotypes was analyzed in respect to several tumor characteristics.

Table 1. Demographic and clinical characteristics of patients with colorectal cancer

Characteristics	
Total number	182
Age, mean±SD (years)	62.0±11.7
Gender, male (%)	61.5
CEA (ng/mL) (mean±SD)	6.1±19.1
Mucin production (%) (mean±SD)	11.9±22.3
Tumor localization (%)	
Rectum	59.9
Colon	40.1
left colon	4.4
right colon	14.3
sigmoid colon	17.0
transverse colon	4.4
Tumor size (%)	
T0	1.3
T1	1.3
T2	16.0
T3	81.4
Positive lymph nodes (%)	
N0	45.3
N1	30.9
N2	23.8
Metastasis (%)	19.4
Chemotherapy (%)	21.0
Radiotherapy (%)	21.5
Dukes (%)	
A	9.1
B	34.7
C	49.1
D	7.1
Astler Coller (%)	
B	44.6
C	55.4
Differentiation grade (%)	
1	70.5
2	24.3
3	5.2

All study subjects were genotyped for 172G>T polymorphism in the RAD51 gene by PCR-RFLP. The 172G allele was present with a frequency of 44.7%, while the frequency of 172T allele was 55.3%. The distribution of genotypes was 23.9% for 172GG, 41.6% for 172GT and 34.5% for 172TT. The distribution of alleles and genotypes was similar in the control group.

The distribution of genotypes was also analyzed in respect to several tumor characteristics in 182 patients for whom histopathological data were available. In patients with desmoplastic response of the tumor tissue the 172G allele was found to be significantly more frequent than in patients in whom desmoplastic reaction was less intensive (p=0.001). Other investigated tumor characteristics were the presence of metastases, tumor grade (using TNM, Dukes and Astler-Coller classification), differentiation grade and mucus production. Neither of the obtained p values was statistically significant.

Within this study group, 37 patients (17.7%) were subjected to neoadjuvant chemoradiotherapy and for this subgroup the presence of the polymorphism was correlated with response to treatment. The response was evaluated based on the scores of the Rectal Cancer Regression Grade (RCRG). The presence of the polymorphism was found not to correlate with response to therapy.

Discussion

Double-stranded breaks in DNA induced by various environmental factors can cause harmful rearrangement of the genome, especially during the time of cell division. Genome integrity is preserved by homologous recombination, and impairments of this process may lead to carcinogenesis [20,21]. RAD51 protein plays an essential role in this process, and cells deficient in this protein show genomic instability and exibit faulty homologous recombination [22]. Two single nucleotide polymorphisms in the regulatory region of this gene, 135G>C and 172G>T, are connected with mRNA stability and expression [23,24]. The role of polymorphism 135G>C in colorectal cancer has been extensively investigated, while far less data is available on the polymorphism 172G>T.

So far, most of the studies on RAD51 polymorphisms in colorectal cancer have concentrated on the polymorphism 135G>C, while 172G>T was rarely associated with colorectal cancer. We have investigated the association of 172G>T variant with colorectal cancer in Serbian patients, as well as its potential as prognostic and predictive biomarker. The 172G allele was found to be associated with more intensive desmoplastic reaction (p=0.001). No correlation was found between the presence of the polymorphism and other tumor characteristics. There was also no significant correlation between the presence of the polymorphism and pathologic response to neoadjuvant chemoradiotherapy.

The role of polymorphisms RAD51 135G>C and 172G>T in carcinogenesis has been analyzed in different malignancies. The study performed in a Pakistani population showed that the genotype 172TT was associated with 16-fold higher chance of developing head and neck cancer cancer [25]. Some studies showed similar results for ovarian,

breast and squamous head and neck cell carcinoma [26-28]. 172G>T variant has an important role in protein stability and expression, and can influence mRNA stability or translational ability, resulting in the changes of the RAD51 protein levels and function [16]. Homozygosity for T allele was found to be significantly higher in smokers, which indicates that there is a combined effect of inefficient DNA repair and carcinogenic exposure in carcinogenesis [25]. Both RAD51 135G>C and 172G>T variants were associated with an increased risk of endometrial carcinoma in Polish women [29]. A meta-analysis published in 2015 regarding ethnicity, smoking and drinking habits in relation to RAD51 expression and its influence on head and neck cancer showed that the 172G>T polymorphism may play a protective role against head and neck carcinoma among Caucasians [18]. In our study there was uniform ethnicity, but we did not consider hazardous habits in relation to colorectal cancer.

One of a few studies that investigated RAD51 polymorphisms in colorectal cancer has shown significant association of 135G>C variant with the disease in Polish population [19]. In this study, 172G>T polymorphism was not found to be associated with colorectal cancer. Other, recently published studies, investigated RAD51 expression in colorectal cancer tissue. The expression of RAD51 was found to be significantly higher in poorly differentiated colorectal cancers and in lymph node metastases, linking it to malignant histophenotypes, disease agressiveness and poor prognosis [30]. Correlation of RAD51 overexpression with poor prognosis was also observed in patients with non-small lung cancer and ductal breast carcinoma [31,32].

Overexpression of RAD51 has also been studied in the evaluation of the effect of chemoradiotherapy in advanced rectal cancer, showing a significant reverse correlation between its expression and pathological responses [33]. This suggested that RAD51 expression could be one of the most important predictive biomarkers of response to neoadjuvant treatment in advanced rectal cancer. In our study, no association was found between the 172G>T polymorphism and response to therapy. However, the number of patients subjected to radiotherapy in our study was relatively small to show significance in local response to neoadjuvant chemoradiotherapy.

Significant overrepresentation of 172G allele in the group of patients with desmoplastic reaction was found in our study. Desmoplasia, as a recation to an insult such as injury or tumor, is proliferation of connective tissue (fibroblasts) with subsequent secretion of collagen [34-36]. The synthesized collagen provides an environment for local cell proliferation, including cancer cells. The newly formed stroma contains extracellular matrix components such as proteoglycans and glycosaminoglycans which bind growth factors and cytokines. Cancer cells secrete matrix degrading enzymes, such as matrix metalloproteinases that degrade the matrix, thereby releasing growth factors that provide way for the growth of cancer cells. They also degrade matrix to provide space for tumor vascular vessels, migration of cells and their proliferation. The presence of a reactive stroma in cancer is a diagnostic sign of poor prognosis.

In conclusion, the results of our study suggest that the 172T allele of RAD51 may be a favorable prognostic factor in patients with colorectal cancer in Serbian patients, although larger prospective studies are required to confirm this finding. Evidence that RAD51 expression plays an important role in carcinogenesis exists, as it appears to influence the development of tumors of different origins and susceptibility to specific therapeutic regimens. Our study pointed towards a possible protective role of RAD51 172T allele in colorectal cancer and it should be taken under consideration in larger prospective studies of this malignancy.

Acknowledgements

This study was supported by the project 41033 of the Ministry of Education, Science and Technological Development of Republic of Serbia.

Conflict of interests

The authors declare no conflict of interests.

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