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Polymorphic Variants of Genes RAD51B and XRCC1 Influence on Breast Cancer Development

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***Abstract* – The present work studies the polymorphic variants of XRCC1 (rs1799782) and RAD51B (rs10483813) genes, which are associated with the risk in developing a number of oncology diseases, particularly it relates to women with breast cancer, the place as location of their living we consider the Chechen Republic. A reliably significant risk for breast cancer development was detected, if 470T> C gene XRCC1 polymorphism among women (p = 0.04) is presented, which indicates the mutation involvement in malignancy pathogenesis. The RAD51 gene family plays the critical roles in the extensive genetic instability caused by the loss of their activity. The missense-variant A> G of RAD51B was identified in two cases among the patients (5.8 %) and two healthy women (2.7 %). The analysis towards the correlation of this mutation with malignant transformation of breast cells showed the significance of the minor G allele in odds ratio (OR = 2.04 with 95 % CI = 0.29–14.51). However, the differences did not reach the statistical meaning (p> 0.621).**

Keywords – gene polymorphism, repair genes, XRCC1, RAD51B, breast cancer, Chechen population.

I. INTRODUCTION

Currently, breast cancer is the most common form of tumors and the main cause of mortality among women in the world [6]. It is known that tumor formation is caused with a failure in a genetic program. In this vein, the study of genetic determinants has become number one in oncology. A large number of polymorphic genes that impact development of breast cancer have been studied. However, the most studied ones in many populations the polymorphisms of BRCA1 and BRCA2 genes explain only about 20 % of hereditary breast cancer [12]. The convergence of these genes in general reveals the basic biology of these diseases and suggests existing polymorphisms of other breast cancer genes. Thus, mutations in CHEK2, ATM, NBS1, RAD50, BRIP1, PALB2 [14; 17], p53 and PTEN [3], RAD51 [15] are associated with a double risk towards breast cancer development. The cause of familial breast cancer that amounts to 50 % remains under question, which cannot be attributed to any of the most studied genes [18].

The most studies addressing polymorphisms of genes involved in risk development towards breast cancer give conflicting results. There has been shown the increase in case frequency, in which minor alleles are presented within a number of polymorphic variants of DNA repair genes among the Chechen women. Thus, the significant protective effect was demonstrated for C3310G polymorphic variant of ERCC5 gene [2]. In the Spanish population the mono-allelic mutations in reparation genes RAD51C and RAD51D were identified that are susceptible to breast and ovarian cancer [9]; in the Finnish population RAD51B mutations are rare, but largely associated with familial risk towards breast cancer development [11]. At the same time, the meta-analysis of Johnson J and et al. (2011) did not reveal any mutations of RAD51B gene when studying breast cancer in family-related context [7].

Population studies show that the marker loci in the same genes can vary greatly depending on ethno-geographical features of a person [1; 4] Therefore, the study of gene polymorphisms with taking account some population differences is the relevant direction in genetics and epidemiological research.

In this paper we studied prevalence in polymorphic variants of reparation genes among breast cancer patients in the Chechen Republic.

II. METHODS AND MATERIALS

To conduct the molecular genetic studies, we used the single peripheral blood of patients with breast cancer, diagnosed clinically (n = 114). The control group consisted of 100 healthy women. The material was collected with the voluntary consent of the patients registered in their residence place. The consent was taken after the diagnosis was made and the needed chemotherapy procedures (in 89 % of cases after resection of the mammary glands) were undertaken. The patients were under treatment in the Republican Cancer Center, in Grozny. For DNA extraction, DiatomTM DNA Prep200 reagent kits were used (produced by Izogen LLC, Moscow). To determine the mutant variants of CHEK2 gene, DNA-technology OncoGenetic CHEK2 reagent kits were used based on the real-time polymerase chain reaction method and the melting curve analysis method. To determine single nucleotide polymorphism (SNP), the real-time PCR method was used to evaluate the results by allelic discrimination method, when the differences between heterozygotes and wild and minor variants were determined with differences in amplification reactions going within the corresponding primers.

III. RESULTS

In the present work we introduced the study results on the most common polymorphisms of XRCC1 (rs1799782) and RAD51B (rs10483813) genes in the Russian population, the presence of which is associated with the risk in cancer diseases development.

The population studies have shown the significant relation between specific genetic polymorphism of reparation genes and susceptibility to breast cancer. Of all the mutations of XRCC1 gene, polymorphism rs1799782 is the most common. Some researchers note that the frequency of its occurrence is higher among healthy individuals compared to those who are suffer from cancer. In other studies no evidence was obtained in favour that XRCC1 rs1799782 is associated with the risk to breast cancer development [10; 13]. However, in the study done by Loizidou MA and et al. (2008) it is noted that homozygous carriers of XRCC1 280His had an increased risk of breast cancer (ratio is 4.68; 95 % CI 1.01–21.7; P = 0.03 [8].

Below we introduce the results on genotyping of the patients with breast cancer in the Chechen Republic within the genes 470T>C XRCC1 **(**rs1799782) (table 1).

TABLE I. MUTATION FREQUENCY IS 470T>C IN GENE XRCC1 (RS1799782) IN THE EXPERIMENTAL GROUP (WITH BC) AND IN THE CONTROL GROUP (HEALTHY WOMEN)

Gene 470T>C RCC1 (rs1799782)	**Genotypes**						**Allele**	
	C/C		***T/C***		***T/T***		***C***	***T***
	abs.	***%***	***abs.***	***%***	***abs.***	***%***	***abs.***	***%***
BC (N=114)	1	0,9	8	7,00	105	92,1	4,4	95,6
Control (N=100)	0	–	0	–	100	100	100	100

TABLE II. ASSOCIATIVE ANALYSIS RESULTS BETWEEN POLYMORPHIC VARIANT 470T>C OF GENE XRCC1 AND BREAST CANCER

Genotypes	**experimental**	**control**	**p**	**OR**	**95 % CI**
TT	105	100	0,04	18,10	1,05-310,66
TC	8	0			
CC	1	0			

The polymorphic variant 470T> C in XRCC1 gene was detected in a homozygote of one patient (0.9 %) and 8 people were carriers of this allele (7.0 %).

According to the results of this study, all respondents in the control group (n = 100) were homozygous along the major allele, whereas, the minor allele in this sampling was not registered.

The analysis we conducted on correlation of the detected mutation showed a high risk of breast cancer, the odds ratio (OR) was 18.10 with 95 % of confidence interval from 1.05 to 310.66. The results achieved the statistical meaning (P = 0.04).

Thus, the data from this study indicate that 470T> C mutation in XRCC1 (rs1799782) may be involved in the pathogenesis of breast cancer among the experimental patients.

Members of RAD51 family are evolutionarily conserved proteins that are necessary for DNA repair by homologous recombination. It has been shown that this protein forms a stable heterodimer with a member of RAD51C family, which additionally interacts with other members of the family, such as RAD51, XRCC2 and XRCC3. It was found that over expression of this gene causes G1 cell cycle retention and cell apoptosis, which indicates the role of this protein in DNA structure disorders. In recent times, it has been found that homologous recombination is a key mechanism in human cells for repairing serious DNA damage, such as double-stranded breaks. RAD51 gene family, including RAD51 and five RAD51-like genes (XRCC2, XRCC3, RAD51L1, RAD51L2, RAD51L3), is known to play the critical roles in the extensive genetic instability caused by the loss of their activity. So far, such data are presumptive about the possible high risk of developing breast cancer [5, 16].

In this work the analysis on the single nucleotide polymorphism (SNP) of RAD51B c.452 + 3A> G gene (rs753393344) was conducted with Real-Time PCR; the results evaluation is performed through the allelic discrimination method, when differences between heterozygotes, wild and minor homozygotes were determined by differences in the flow amplification reactions of the corresponding primers.

The study has shown SNP changes happening in conformity with the criterion of polymorphic allele frequency of RAD51B gene (Table 3).

TABLE III. FREQUENCY IN ALLOCATION OF GENOTYPES AND POLYMORPHIC VARIANT ALLELE OF RAD51B (RS753393344) GENE

Gene RAD51B c.452 + 3A > G (rs753393344)	**Genotyping**						**Allele**	
	A/A		***A/G***		***G/G***		***A***	***G***
	abs.	***%***	***abs***	***%***	***abs.***	***%***	***%***	***%***
experimental	2	5,8	16	47,05	16	47,05	33,33	66,67
control	2	2,7	22	40,27	41	56,94	20,0	80,00

According to genotyping results, we identified the missense variant of RAD51B p.452 + 3A> G in two female patients (5.8 %) and two clinically healthy ones (2.7 %)

TABLE IV. ASSOCIATIVE ANALYSIS RESULTS BETWEEN POLYMORPHIC VARIANT OF GENE RAD51B (RS10483813) AND BREAST CANCER

Genotypes	experimental	control	p	OR	95 %CI
AA	47,05	56,94	0,621	2,04	0,29-14,51
AG	47,05	40,27			
GG	5,8	2,7			

From the data presented in the table, it is clear that the frequency of the minor allele in the studied sampling of individuals with breast cancer reaches 33.33 %, respectively, the frequency of the major allele is 66.67 %.

In the control sampling, where at the moment of the blood draw the diagnosis 'breast cancer' has not been made and they do not have a history of familial cancer, the frequency of the allele was 20.0 %, the frequency of the wild type C allele, respectively, was 80.00 %.

The results of genotyping in the group of healthy women showed that the frequency of homozygotes along the major allele is 56.94 %, whereas, homozygotes along the minor allele were observed only in two cases (2.7 %). Accordingly, the frequency of the heterozygous genotype was 40.27 % in the control group (Table 4).

In the experimental group (women with malignant pathologies of mammary gland), the genotypes are allocated according to allele discrimination as follows: the frequency of homozygotes for the polymorphic variant A and heterozygous carriers of the minor allele were allocated in this group with the same frequency of 47.05 %, while the frequency of the homozygous genotype according to allele G amounted to 5.8 %.

The comparative analysis of genotypes frequency revealed some negative associations of polymorphism of RAD51B gene, manifested in an increased frequency of the heterozygous genotype 1.2 times and the homozygous GG genotype 2.15 times compared to the control group (Table 3). The statistical analysis on differences in frequency of polymorphic variants of RAD51B gene (rs10483813) between both groups showed that these groups are in equilibrium according to Hardy – Weinberg formula.

The analysis towards conjugation of the polymorphic variant of RAD51B gene (rs10483813) with malignant transformation of breast cells showed the significance of the minor G allele in risk of developing breast cancer. The strength of associations was assessed through values of the odds ratio (OR = 2.04, 95 % CI = 0.29–14.51), but the differences did not reach the statistical meaning (p> 0.621) (Table 4).

Thus, the study initiated towards understanding the role of polymorphic variants of XRCC1 and RAD51B repair genes in pathogenesis of breast cancer revealed the ambiguous nature of the influence of the polymorphisms relating to risk of developing malignant breast tumors and demonstrated the need for more extensive studying. This study provides evidence to support the theory that DNA repair genes are associated with risk of developing breast cancer, and also provides additional information to fully understand the etiology of breast cancer, to identify potential biological mechanisms that link DNA repair, ethnicity, environmental factors and risk development of malignant pathologies. All these undoubtedly are requiring further studying.

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