RESEARCH ARTICLE

BRCA1 and TP53 Gene-Mutations: Family Predisposition and Radioecological Risk of Developing Breast Cancer

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Abstract

Frequencies of polymorphisms of genes BRCA1 and TP53 in breast cancer (BC) patients with a BC family history and radiation history were assessed and compared in the Semey region of Kazakhstan. The study included 60 women directly irradiated by the activities of the Semipalatinsk test site with a calculated effective equivalent dose of 500 mSv and their first generation descendants (group BC+Her+Exp); 65 women with family BC and absence of radiological history - the effective equivalent dose due to anthropogenic sources not exceeding 50 mSv (group BC+Her-Exp). The comparison group consisted of 65 women patients with breast cancer without family and radiological history (BC-Her-Exp). The control group comprised 60 women without breast cancer and without family and radiological history (nonBC). We carried out the genotyping of the polymorphisms c.2311T>C, c.4308T>C and 5382insC of the BRCA1 gene and rs1042522 of the TP53 gene. The frequency of the polymorphism c.2311T>C was significantly higher in patients of the group BC+Her+Exp than in healthy women, and of the polymorphism 5382insC in BC+Her+Exp compared to all other groups. The frequency of the rs1042522 polymorphism of TP53 was significantly higher in all groups of patients with breast cancer compared with the control group. Differences between groups of women with breast cancer were significant only in BC+Her+Exp vs. BC+Her-Exp. Combinations of polymorphisms of the genes BRCA1 and TP53 predominated in women with a family and radiological history.

Keywords: Breast cancer - TP53 - BRCA1 - polymorphism - radiation factor - the Semipalatinsk test site

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Introduction

Oncological diseases are one of the most important problems of modern health care and medicine. Their relevance in the modern world continues to grow (Mckenna et al., 2016). According to WHO, the share of malignant neoplasms in the structure of mortality of the industrialized nations is at the 3rd place after cardiovascular diseases (Aune et al., 2016). Unfortunately, the reason for this situation is the rapid growth of the influence of technical factors which have a direct carcinogenic effect on the human body (Azim and Partridge, 2014).

The risk of breast cancer (BC) may be due to internal and external factors such as reproductive, hereditary and genetic predisposition (presence of cancer in mother or sisters, mutations in the genes, ethnicity, presence of radiation exposure) (Couch et al., 2014).

To date, there are distinguished several genes, defects in which can lead to the development of hereditary breast cancer (Boyd, 2014). Most frequently among the genes associated with the risk of tumor development, regulatory genes are identified which determine the conditions of cell growth or death (Kleibl and Kristensen, 2016). Among the latter, one of the most studied are the genes (TP53, PTEN, BRCA1, BRCA2, CDH1 and LKB1/STK11) (Kuchenbaecker et al., 2015). Hereditary breast cancer is diagnosed in 5-10% of cases, but only 25% of cases can be attributed to the damage in known genes (BRCA1, BRCA2, p53 and others) (Sana and Malik, 2015).

Important risk factors for the development of

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malignant tumors are environmental, particularly the effect of ionizing radiation (Malhotra et al., 2016). The development of oncological pathology in this case is a marker of radiation damages (Mark et al., 2016). It has been determined that ionizing radiation is one of the environmental factors that cause increased frequency of malignant neoplasms at a population level (Wade et al., 2015).

Numerous studies indicate that some tissues are more susceptible to malignant growth than others (Galluzzi et al., 2015). Breast tissues have a relatively high radiosensitivity, as suggested, including through the emergence of mutations (Preston et al., 2007).

Purpose of the research

To compare the frequencies of certain polymorphisms of the genes BRCA1 and TP53 in patients with breast cancer with family and radiation history in Semey region of Kazakhstan.

Materials and Methods

The research meets the requirements of the Declaration of Helsinki of the World Medical Association (http://www.wma.net/en/30publications/10policies/b3/). All the research participants were informed about the objectives and upcoming procedures, an informed written consent to participate in the research was received from everybody.

The research included 250 women aged 40 to 79 years of Kazakh nationality.

Among others, 125 women were studied, they are patients with breast cancer which have a family history. These patients were divided into groups depending on the presence of radiological history. The first group included 25 women directly irradiated by the activities of the Semipalatinsk test site with a calculated effective equivalent dose of 500 mSv and 35 of their descendants of the first generation (aged 40 to 78 years, average age 54.3±2.5 years)

The second group consisted of 65 women with family breast cancer and the lack of the burdened radiological history (effective equivalent dose due to anthropogenic sources doesn't exceed 50 mSv, age from 41 to 79 years, average age - 54.6±2.4 years).

The comparison group consisted of 65 women patients with breast cancer without family and radiological history (aged 43 to 77 years, average age 60.5±2.3 years).

The control group included 60 women without breast cancer and without its family and radiological history (age from 42 to 79 years, average age 61.1±2.5 years).

Genetic studies were conducted in the laboratory of genetics of the Institute of therapy, Siberian branch of RAMS, Novosibirsk. For the research peripheral blood in 5 ml tubes with EDTA (Ethylenediaminetetraacetic acid) was used. The purification of genomic DNA was conducted using the set of "Proba-NK" (DNA-Technology, Moscow, Russia) in accordance with the manufacturer's instructions. The concentration and frequency of DNA was calculated using a NanoDrop 1000 spectrophotometer (optical density at wavelengths 260 and 280 nm). The purified DNA was stored at a temperature of –20ºC.

Genotyping of the polymorphisms c.2311T>C, c.4308T>C, and 5382insC of the BRCA1 gene and rs1042522 of the gene TP53 was conducted by the Real-Time PCR method using ready mixes, primers and TaqMan probes in the presence of a reagent TaqMan Genotyping Master mix and 50 ng of genomic DNA as a matrix. The genotyping was conducted on the device Applied Biosystems 7500 Fast Real-Time PCR System (Technology, USA). The amplification program consisted of a pre-denaturation at 95ºC in 10 minutes, then of 40 cycles 92ºC for 15 seconds and 60ºC for 1 minute (Polakova et al, 2009).

For statistical analysis of frequency differences a unilateral Fisher's exact test was used. $P<0.05$ was accepted as the threshold level for refuting the null hypothesis.

Prevalence of the polymorphisms c.2311T>C, c.4308T>C and 5382insC of the BRCA1 gene

The table 1 presents data on the frequency of the researched polymorphisms of the gene BRCA1 generally for the group studied with regard to their family and radioecological history.

The frequency of the identified polymorphisms was much higher in patients than in healthy women. However, the aspects of significance of differences had peculiarities. In distributing according to the groups, the degrees of statistical significance reached values only in one couple, namely between the patients of the group BC+Her+Exp and nonBC (differences by 2.38 times, p=0.018).

All other couples of values were characterized by a significantly lower level of differences. So, between the first and second groups, the difference was only 1.29 times (p>0.1). Between the first and the third was 1.46 times (p>0.05). Differences of statistical significance were not identified also between groups of patients of the group BC+Her-Exp, the control group and also patients of the group BC-Her-Exp with the control one.

The detection rate of polymorphism c.4308T>C of the gene BRCA1 was almost twice in women of the group BC+Her+Exp compared to groups BC+Her-Exp and BC-Her-Exp. The differences with a group of women without breast cancer was 3-fold. It should be noted that statistically significant differences between groups' data were absent.

A significant excess frequency of the polymorphism 5382insC in the group BC+Her+Exp was also identified. This frequency was 20%, while in the group BC+Her-Exp – 7.7%, BC-Her-Exp – 6.2% and in the control group - 3.3% (p=0.038, p=0.019, p=0.004, respectively). Meanwhile there were no statistically significant differences between the other groups. Even in women with a family history in relation to the control, the value of Fisher's exact test corresponding to p=0,357 was obtained.

The prevalence of the polymorphism rs1042522 of the gene TP53

Totally the presence of the polymorphism rs1042522 was identified in 41 cases out of 250 (16.4 per cent). Frequency distribution for the selected groups depending on the presence of breast cancer, family and radiological history is presented in table 2.

The clear and statistically significant differences primarily according to the parameter of presence of breast cancer were identified in the patients. Thus, the frequency of detection of polymorphism in the group BC+Her+Exp was 5.3 times higher than in the control (p<0.001). The similar differences between the group BC+Her-Exp and the control amounted to 3.08 times (p=0.004). At the same time, women of the group BC-Her-Exp had the frequency of polymorphism moderately higher than in the BC+Her-Exp. The differences between the group BC-Her-Exp and the control one amounted to 3.69 times (p=0.003).

Differences between groups of women with breast cancer was significant only in the couple BC+Her+Exp vs. BC+Her-Exp (frequency ratio was equal to 1.71, p=0,031).

These data seem somewhat paradoxical, as the direct effect of burdened radiological history on the frequency of detection of polymorphism in women with breast cancer cannot be traced.

Combinations of polymorphisms of the genes BRCA1 and TP53

In a number of cases simultaneously multiple polymorphisms have been detected in one patient. The distribution of combinations is presented in Table 3.

Combinations of polymorphisms were prevalent in women with a family and radiological history. Their frequency on average was 20.0% and moreover a combination of one of the polymorphisms of the BRCA1 gene and the researched polymorphism TP53 (8.3 per cent) was the most frequent. In addition, quite frequent were the combinations of c.4308T>C+ 5382insC (5.0 per cent).

The overall frequency of combinations of polymorphisms in the group BC+Her-Exp amounted to 10.8% of its population. Most often, the combinations of one of the polymorphisms of the BRCA1 gene and the studied polymorphism of the gene TP53 (4.6 per cent) were also noted. In addition, there were 2 cases of combinations c.2311T>C+ C.4308T>C and C.2311T>C+ 5382insC (3.1 percent).

In the group of women BC-Her-Exp the occurrence of combinations of polymorphisms was lower than in the group BC+Her-Exp, and amounted to 6.2%. In the vast majority of cases they belonged to the combinations of one of the polymorphisms of the gene BRCA1 and TP53. There were statistically significant differences between groups BC+Her+Exp and BC-Her-Exp (p=0.022).

Thus, the presence of excess frequency of combinations of polymorphisms in irradiated women testified about the role of radiation in causing mutations. A noticeable higher frequency of combinations including the polymorphism rs1042522 might have been associated with greater likelihood of developing breast cancer at their presence. It should be noted that in healthy women of the control group, any combinations of the studied polymorphisms were not identified.

Table 1. Frequencies of Polymorphisms of the BRCA1 Gene

The studied group	c.2311T>C		c.4308T>C		5382insC	
	abs.	%	abs.	%	abs.	%
BC+Her+Exp, n=60	19	31.7	9	15.0	12	20.0
BC+Her-Exp, n=65	16	24.6	5	7.7	5	7.7
BC-Her-Exp, n=65	13	21.7	5	7.7	4	6.2
nonBC, n=60	8	13.3	3	5.0	2	3.3
Total	56	22.4	22	8.8	23	9.2

Table 2. Frequency of Polymorphism rs1042522 of the TP53 Gene

The studied group	With the presence of the polymorphism	%
BC+Her+Exp, n=60	16	26.7
BC+Her-Exp, n=65	10	15.4
BC-Her-Exp, n=65	12	18.5
nonBC, n=60	3	5.0
Total, n=250	41	16.4

Results and Discussion

In recent years, in the periodic literature there have been provided very few results which indicate the role of ionizing radiation in the genesis of breast cancer. Mainly these data relate to the carcinogenic effects of radiation therapy (Berrington et al., 2013; Wijnen et al., 2016).

An increase in the frequency of malignant tumors of different localization in the irradiated due to the combat use of nuclear weapons, their tests and radiological accidents is also known (Hughes et al., 2000; Mangano and Sherman, 2011). In the region of the Semipalatinsk test site exceeding the frequency of certain malignant tumors was observed in directly irradiated persons in the period of 15-25 years since the exposure to medium and high radiation doses due to direct radiation during nuclear explosions and living at the contaminated territories (Bauer et al., 2006). According to some studies, exposure to ionizing radiation, which led to the development of tumors, is not always accompanied by the presence of mutations in genes that suppress the formation of tumors (Nichols et al., 2003).

However, according to our data, the development of breast cancer in patients with family history in populations

Table 3. Frequency of Combinations of Various Gene Polymorphisms BRCA1 and TP53

The studied group	Polymorphisms c.2311T>C+ c.4308T>C		Polymorphisms c.2311T>C+ 5382insC		Polymorphisms c.4308T>C+ 5382insC		All 3 polymorphisms of the gene BRCA1		Any polymorphism of the gene BRCA1+ rs1042522 of the gene TP53	
	Number of patients	%	Number of patients	%	Number of patients	%	Number of patients	%	Number of patients	%
BC+Her+Exp, n=60	2	3.3	1	1.7	3	5.0	1	1.7	5	8.3
BC+Her-Exp, n=65	2	3.1	2	3.1	0	0	0	0	3	4.6
BC-Her-Exp, n=65	0	0	0	0	1	1.5	0	0	3	4.6
nonBC, n=60										
Total, n=250	4	1.6	5	2.0	4	1.6	1	0.4	11	4.4

living at the territories contaminated by radionuclides is accompanied by a significant increase in the frequency of identification of polymorphisms of the genes BRCA1 and TP53. At the same time, the prevalence of individual mutations of these genes in the Kazakh population to the present time has not been defined, which leaves room for different interpretations of the results.

However, in the groups of patients with breast cancer, we observed excess frequencies of all researched polymorphisms over the indicators of the control group. The differences in the frequency of polymorphisms in individuals with and without family and radiation history were identified. The highest was identified in female patients with a combination of adverse prognostic factors - residence in contaminated territories and the presence of breast cancer in close relatives. Judging by the frequency of identifying polymorphisms in patients with breast cancer, the factors of family and radiation history are summarized.

The most likely hypothesis is that the irradiation causes mutations of the genes BRCA1 and TP53 which are inherited. As a result new "hot spots" of family breast cancer are created usually not typical for the Kazakh population.

Both the growth rate of breast cancer among the population of the region in modern conditions (Apsalikov et al., 2016) and frequency increase of family breast cancer in the descendants of the irradiated and identification of increased mutations in them serve as the confirmation of this hypothesis.

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References

Apsalikov BA, Manambaieva ZA, Belikhina TI, et al (2016). The dynamics of morbidity of breast cancer in the Eastern-Kazakhstanskaia Oblast and role of radiation factor. *The Journal of Problems of Social Hygiene, Public Health and History of Medicine*, **24**, 7-10.

Aune D, Keum N, Giovannucci E, et al (2016). Whole grain consumption and the risk of cardiovascular disease, cancer, and all-cause and cause-specific mortality–a systematic review and dose-response meta-analysis of prospective studies. *BMJ*, **353**, 1-14.

Azim HA, Partridge AH (2014). Biology of breast cancer in young women. *Breast Cancer*, **16**, 427.

Bauer S, Gusev BI, Pivina LM, et al (2006). Esophagus cancer and radiation exposure due to nuclear test fallout: an analysis based on the data of the Semipalatinsk historical cohort, 1960-1999. *Radiats Biol Radioecol*, **46**, 611-628.

Berrington de Gonzalez A, Gilbert E, Curtis R, et al (2013). Second solid cancers after radiation therapy: a systematic review of the epidemiologic studies of the radiation dose-response relationship. *Int J Radiat Oncol Biol Phys*, **86**, 224-33.

Boyd J (2014). Genetic predisposition to breast cancer. *Cancer*, **7**, 932-934.

Couch FJ, Nathanson KL, Offit K (2014). Two decades after BRCA: setting paradigms in personalized cancer care and prevention. *Science*, **6178**, 1466-70.

Galluzzi L, Pietrocola F, Bravo-San Pedro JM, et al (2015). Autophagy in malignant transformation and cancer progression. *EMBO J*, **7**, 856-80.

Hughes CK, Tsark JU, Kenni CK, et al (2000). Cancer research studies in native Hawaiians and pacific islanders. *Ann Epidemiol*, **10**, 49–60.

Kleibl Z, Kristensen VN (2016). Women at high risk of breast cancer: Molecular characteristics, clinical presentation and management. *Breast*, **28**, 136-44.

Kuchenbaecker KB, Ramus SJ, Tyrer J, et al (2015). Identification of six new susceptibility loci for invasive epithelial ovarian cancer. *Nature Genetics*, **2**, 164-171.

Malhotra J, Malvezzi M, Negri E, et al (2016). Risk factors for lung cancer worldwide. *Eur Respiratory J*, **359**.

Mangano JJ, Sherman JD (2011). Elevated *in vivo* strontium-90 from nuclear weapons test fallout among cancer decedents: a case-control study of deciduous teeth. *Int J Health Serv*, **41**, 137-58.

Mark A Wade, Felicity EB May, Ken Onel (2016). Does radiation-induced c-MYC amplification initiate breast oncogenesis? *Molecular Cellular Oncol*, **3**, 1-3.

Mckenna Longacre, Nicole A Snyder, Genevieve Housman, et al (2016). Comparative analysis of genetic and epigenetic events of breast and ovarian cancer related to tumorigenesis. *Int J Molekular Sci*, **17**, 759.

Nichols KE, Heath JA, Friedman D, et al (2003). TP53, BRCA1, and BRCA2 tumor suppressor genes are not commonly mutated in survivors of Hodgkin's disease with second primary neoplasms. *J Clin Oncol*, **24**, 4505.

Polakova V, Pardini B, Naccarati A, et al (2009). Genotype and haplotype analysis of cell cycle genes in sporadic colorectal cancer in the Czech Republic. *Hum Mutat*, **30**, 661-8.

Preston DL, Ron E, Tokuoka S, et al (2007). Solid cancer incidence in atomic bomb survivors: 1958–1998. *Radiat Res*, **168**, 1-64.

Sana M, Malik HJ (2015). Current and emerging breast cancer biomarkers. *J Cancer Res Therapeutics*, **3**, 508.

Wade MA, Sunter NJ, Fordham SE, et al (2015). c-MYC is a radiosensitive locus in human breast cells. *Oncogene*, **34**, 4985-94.

Wijnen M, Heuvel-Eibrink MM, Medici M, et al (2016). The role of early life exposures on subsequent cancer risk. *Endocr Relat Cancer*, **23**, 299-321.