Molecular Genetic Study of the Allelic State of the Cell Cycle Genes *(TP53, BRCA1)* and Features of the Regulation of the Cytokine Cascade in Breast Cancer

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Abstract: This article contains the analysis of mutations in genes that regulate the cell cycle (*TP53* and *BRCA1*) and classification relating to tumor suppressor. Shown that the "risk" alleles of these genes may contribute to tumor development, but the activation of the immune system cytokine spectrum of patients can prevent their destructive degeneration. The authors proposed a personalized approach to the study for the prevention of possible proliferative processes. This is confirmed by reversal of "risk" alleles studied genes in tumors in operated patients with cytokine physiologically normal status.

Keywords: Breast cancer, tumor suppressor, cytokines, nucleotide substitutions, predisposition.

INTRODUCTION

Malignant transformation of cells due to the cell cycle derangement and apoptosis inhibition [1] is the basis of carcinogenesis, irrespective of tumor localization. The molecular pathogenesis of cancer involves a great number of genetic and epigenetic events leading to activation of oncogenes and inactivation of tumor suppression genes [2, 3]. One of the key tumor growth suppressor genes are *TP53* and *BRCA1,* the protein products of which implement a wide range of cellular processes, regulation of the cell cycle, induction of apoptosis, permanent supervision of genome condition and malignant transformation of cells [4-7]. The disorder of the functional activity of these proteins may provoke genetic instability, transcriptional, signal and mitochondrial functions disturbance [8]. The proliferative growth of transformed cell and the shift to autocrine or paracrine mechanism of growth regulation is possible in case of immune system oppression. It has been demonstrated that some solid neoplasms cytokines are able to affect tumor cells growth, altering the expression of pro- and anti-apoptotic proteins, various oncogenes and markers of cell proliferative activity [9]. The self-defense system plays crucial role for implementing the immune response and maintenance of homeostasis. In particular, pro- and anti-inflammatory serumal cytokines imbalance tends to develop pathological processes including malignant neoplasms [10, 11]. Thus, during the process of case-finding of the genetic predisposition to the cancer diseases, the integrated approach is the most prospective and actual area of researches for molecular mechanisms of malignant cells transformation.

MATERIALS AND METHODS

Study Materials

For this work were used DNA of 1044 persons aged 18 to 72 (median 35.75), living in the Republic of Bashkortostan. These include: 956 healthy individuals without burdened cancer anamnesis and 88 breast cancer patients who underwent hospital treatment in national oncology clinic of Bashkortostan Republic. The research involved patients with different disease stages, corresponding to the Classification of Malignant Tumors (TNM): T1-3N0-2M0.

Research Methods

The standard method of Phenol–chloroform extraction was used for the DNA isolation. The DNA isolation from tumor tissue was carried out using sets made by «Fermentas» company. Genotyping of polymorphic loci examined genes was conducted by the polymerase chain reaction (PCR) method and restriction analysis that were followed by DNA paragraph electrophoresis in 7% polyacrylamide gel. Gels staining was accomplished by Ethidium bromide solution (1%), following visualization with the help of Gel Imager. Cytokine concentration (IL-1β, IL1RA, IL2, IL4, IL6, IL10, α-TNF) in Blood serum of healthy

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individuals and breast cancer patients (stages T1-3N0-2M0) was defined by imunnoenzyme method using reagents made by «Vector-best» company (Novosibirsk). The blood serum was taken before the tumor operation and during postoperative period.

The Design of the Study

In the first phase of the study was conducted on three genotyping of polymorphic variants of the *TP53* gene (rs1042522, rs1625895, dup 16 bp in 3 intron), since, according to the literature, these polymorphic loci are the most significant and affect the functioning of both the gene and the encoded their protein [7, 8, 12]. According to the results of genotyping was formed the control group, which consisted of 143 women who are carriers of the 'normal' allele of the *TP53* gene. Appropriateness of the control group with the allelic status of the *TP53* gene due to the fact that scientific studies have proven the fact that the carrier cancer patients "risk" alleles of the *TP53* gene [13-16]. Therefore, to exclude from the control group of people potentially susceptible to cancer, was used similar genetic "filter".

DNA samples from women with breast cancer (n = 88) and women (n = 143), selected in the control group were studied for two gene loci *BRCA1* (rs80357629, rs1799966). *BRCA1* gene product plays a dual role in the pathogenesis of breast cancer: *BRCA1* protein deficiency increases the rate of mutation of genes, including *TP53* [17], in addition, *BRCA1* protein is involved in hormonal regulation and as a result in the development of hormone-dependent tumors of the breast, ovarian and prostate cancers [18, 19]. Results showed that patients with breast cancer are carriers of risk alleles of *BRCA1*, and in the control group, found 56 women who are carriers of alleles that determine the proper conformation of the protein *BRCA1*.

The next phase of the study was to determine the concentrations of cytokines in the serum of women with breast cancer and 56 healthy, non-carriers of normal alleles of the *TP53* gene.

At the final stage, 15 of women with breast cancer study of DNA samples of tumor tissue.

Statistical processing was carried out using t-test. Differences between parameters were considered statistically significant at $p<0,05$. To determine the statistical parameters used in the program MS Excel and Statistica 6.0. Univariate analysis of variance (ANOVA) was performed using the statistical package SPSS (version 13.0). Linkage analysis was performed using two LD, gaplotipichesky analysis - using the EH.

RESULTS AND DISCUSSION

The analysis of genotypes frequency distribution according to polymorphic genes loci cell cycle *TP53* and *BRCA1* from healthy individuals and breast cancer patients

The analysis of genotypes frequency distribution according to explored polymorphic genes loci *TP53* (rs1042522, rs1625895, DUP16BP in 3 intron) and *BRCA1* (rs80357629, rs1799966) drew out significant difference. Thus, according to polymorphic locus rs1042522 (G/C or w/m on the Figures **1** and **2**) in *TP53* gene was found proved elevation of heterozygous genotype frequency GC (w/m) in the group of healthy individuals ($p=0,005$, $\chi2=8,30$) and in the group of cancer patients heterozygous genotype GG (w/w; $p=0,023$, $\chi2=5,22$) occurred more often. According to the studies of patients, *G (*w) allele codes the protein, possessing a greater capability of mitochondrial translocation in comparison with protein form, which is encoded by allele *C (*m), which correlates with differences in pro-apoptosis activity, encoded by these alleles form of protein. P53 protein contains a number of functionally significant domains. Nucleotide substitution G/C in polymorphic locus rs1042522 leads to the replacement of arginine amino acid (Arg) for proline (Pro) in 72 position of protein sequence [20]. The section formed by amino acid leftovers from 43^{rd} to 73^{rd}, creates assident transcriptional domain [21] which is presumably involved in the apoptosis process [22, 23]. In particular, P53 protein with Arg leftover in the 72 position evokes the apoptosis induction in the most effective way and its quantity in mitochondrial faction is an order of magnitude higher in containing of P53 protein than that of containing Pro leftover [24]. Thus, the polymorphic condition of the *TP53* gene according to explored locus (rs1042522, G/C) defines the formation of the low-level protein. During the analysis of the frequency distribution of genotypes and alleles frequencies of polymorphic loci rs1625895 (G / A or w / m in Figures **1** and **2**) in the *TP53* gene was revealed that the genotypes w / m and m / m, and *m allele were significantly more frequent in the group of cancer patients ($p=0,0005$, $\chi2=100,03$; $p=0,0014$, $\chi2=11,59$; $p=0,0005$, $\chi2=95,49$ correspondingly). Whereas, in the group of healthy individuals genotype w / w and allele *w ($p=0,0005$, $\chi2=117,36$; $p=0,0005$, $\chi2=95,49$

correspondingly) predominated. A polymorphic variant of the sixth intron was studied rather widely in the course of breast cancer study and is associated with Li-Fraumeni syndrome [25]. It was established that this polymorphic variant can alter the expression of the *TP53* gene [26]. Biros *et al*. [27] also a significant increase in the frequency of heterozygotes for the sixth intron by lung cancer patients compared to controls was shown. No significant differences were found in the distribution of genotypes and alleles frequencies of polymorphic loci DUP16BP *TP53* gene possessed by healthy individuals and cancer patients. Although some authors suggest that the polymorphic variant is highly significant, as in the third intron is found MDM2, the product of which binds to the amino terminal end of the P53 molecule and stimulates ubiquitination and proteasomal degradation of P53 [28]. Therefore, changing of the structure of the third intron can lead to the disruption of the activation processes of transcription of the target genes required for the cell cycle block and the startup of the apoptosis [28].

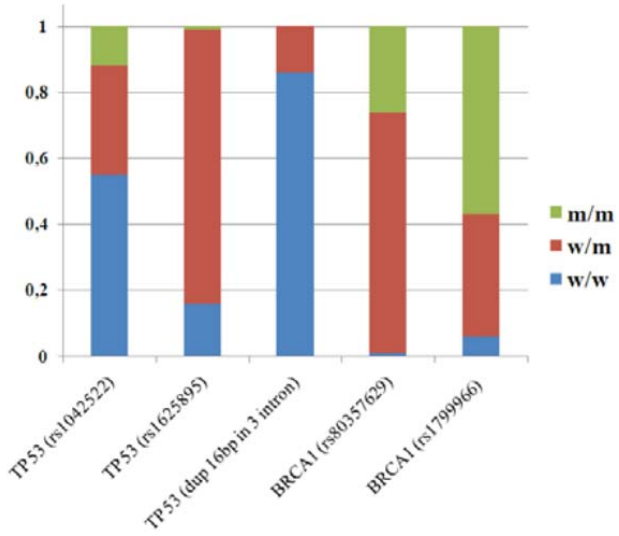

Figure 1: The distribution of genotype frequencies of polymorphic loci and *TP53* genes and *BRCA1* possessed by patients with breast cancer.

Note: On Figures **1**, **2** w - «normal» allele, m - mutant allele

According to the results of the frequency distribution of genotypes according to polymorphic loci *TP53* gene among healthy individuals a group was formed in order to compare the results of the distribution of genotypes and allele frequencies of polymorphic loci gene *BRCA1* (rs803357629, G / A; rs1799966, A / G) analysis. The comparative analysis of the distribution of studied polymorphic loci (rs803357629, G / A (w / m); rs1799966, A / G (w / m) in the gene *BRCA1*. A significant increase of the frequency of the homozygous genotype GG (w / w) and allele *G (*w) in the group of healthy individuals relative to the group of cancer patients (p=0,0005, χ2=49,40; p=0,0005, χ2=66,10 correspondingly) polymorphic loci rs803357629 (G / A) in the gene *BRCA1* (Figures **1**, **2**) was discovered. Homozygous genotype AA (m / m) was discovered only in case of cancer patients (p=0,0005, χ2=66,31). According to our sources, that structural changes in the 11th exon of the *BRCA1* gene lead to a decoupling of BRCA1 protein interaction with SWI /SNF transcription factor complex, as well as blocking the stimulation of P53-mediated transcription [29]. The analysis of the frequency distribution of genotypes and allele frequencies of polymorphic loci rs1799966 (A / G or w / m) in the *BRCA1* gene identified reliably significant difference between patients and healthy individuals. Thus, the homozygous genotype GG (m / m) and allele *G (*m) were significantly more frequent in the group of cancer patients (p=0,0005, χ2=96,48; p=0,0005, χ2=98,14 correspondingly). Whereas, in the group of healthy individuals genotypes AA (w / w) and AG (w / m), as well as allele *A (p=0,0005, χ2=75,46; p=0,006, χ2=7,85; p=0,0005, χ2=98,14, correspondingly) dominated.

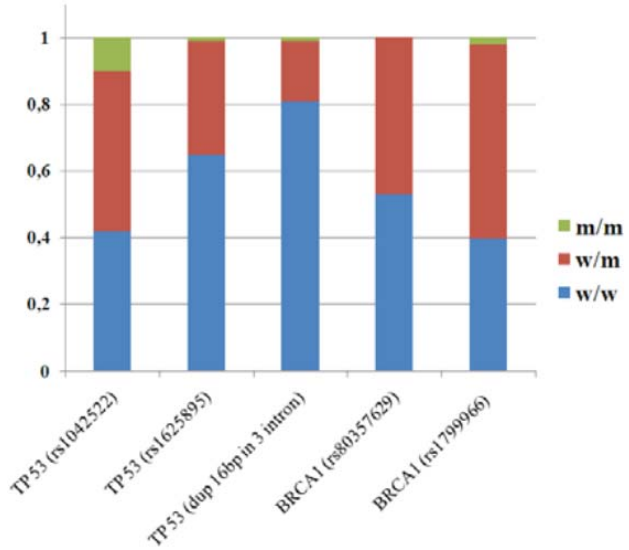

Figure 2: The distribution of genotype frequencies of polymorphic loci and *TP53* genes and *BRCA1* polymorphic loci in the group of healthy individuals.

Healthy Individuals and Breast Cancer Patient Group Alleles Frequency Distribution and Polymorphous Genes Variants of Cytokine System Genotypes Analysis

Comparative analysis of genotypes and alleles frequency distribution by polymorphic locus *rs1143634*

in gene *IL-1 β* detected significant raise of homozygous «risk» genotype *E2E2* (p=0,05, χ^2=3,81) and allele **E2* (p=0,006, χ^2=7,65) frequency in oncological patients group.

While typing by minisatellite in receptor gene of interleukin-1 (*IL1RA*), oncological patients significantly often have genotype *IL-1RA *II/*II* with frequency 24,39% (p=0,0005, χ2=36,24) and allele *IL-1RA*II* with frequency 51,83% (p=0,0005, χ2=22, 36). According to literature data, the production raise of IL1RA *in vitro* and *in vivo* [30, 31] is registered in allele *IL1RA*II,* furthermore, presence of allele *IL1RA**II is associated with production raise of IL-1β *in vitro* [32]. Some authors consider carriage of allele *IL1RA*II* as genetic determinant in development of cancer, specifically breast cancer [33], carcinoma of uterine cervix [34] and ovarian carcinoma [35].

Also statistically significant differences were detected between healthy individuals group and cancer patients by polymorphic locus *rs1800796* in promoter region of interleukin-6 gen**.** The genotype GC significantly often appears in cancer patient group (69,32%, p=0,0005, χ^2=30,25). According to literature data, allele *C of gene *IL6* carriers have higher production of proinflammatory interleukin-6 [36]. In turn, IL-6, being vigorous type-high and differentiation factor of B-cells, also has angiogenic action and takes main part in growth malignant cell in cancer [37-39], as well as increasing dissemination of tumor cells [10, 40, 41].

Analysis of Alleles' Inheritance of Oncosupression System's Gens (*TP53*, *BRCA1*)

Was formerly held by linkage analysis [42] of polymorphic loci in gens *TP53* (*rs1625895, rs1042522, DUP 16bp)* and *BRCA1* (*rs80357629, rs1799966).* In cancer patients identified risk haplotypes **G/***m/*D/*G*, ***C/*w/*D/***A** and ***C/***m/*D/***A** *(rs1042522, TP53 / rs1625895, TP53 / DUP 16BP, TP53 / rs80357629, BRCA1* corresponding*)*, were detected in cancer patients groups, which are absent in comparison group [42].

The Analysis of Genotype Polymorphous Loci Combination in Gene: Interleukin-1 and *TP53* Family

In populations the activity of the protein P53 substantially modified by genetic polymorphism. The most significant and examined are three polymorphous variants of gene *TP53*: dotted replacement of guanine by cytosine in 72nd codon of exon (*rs1042522*, Arg72Pro).Depending on that, which amino-acid residue situated in position of 72nd polypeptide chain protein P53 changes the ability of P53 to start apoptosis [43]. Protein P53 with resedue of Arg in position 72 more effective to bring on apoptosis induction than protein containing resedue Pro [24, 44]; polymorphous locus in 6th intron (*rs1625895*) and insertion/deletion 15 bp in 3^{d} intron changes «dose» of gene, thus influence on activity of protein P53 [45].

On the basis of above mentioned, the distribution character of genotypes frequency of three examined genes of the family interleukin-1 (*IL-1β* (*rs1143634*)*, IL1Ra (VNTR), IL1RI* (*rs2287047)*) in group of breast cancer patients, which are genotype carriers *Pro/Pro* of gene *TP53 (rs1042522,* Arg72Pro*).* 7 combinations of 27 possible were detected (Figure **3**). The share of the genotype combination *E1**E2**//I//**II**//T**C*** had 25%, genotype combination of *E1E1//I/**II**//T**C**, E1E1//**II**/**II**//T**C***, and *E1E2**//I//I//T**C*** appeared with 16,6% frequency each and with 9% frequency combinations of *E1E1//I/**II**//**CC**, E1E1//**II**/**II**//TT and **E2E2**//**II**/**II**//T**C***.

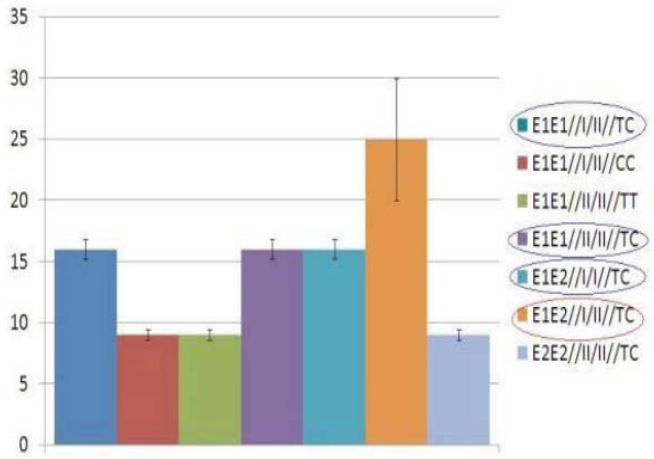

Figure 3: Frequencies distribution of genotypes combination *IL-1β/IL1RA/IL1RI* in genotype carriers group *Pro/Pro* by gene *TP53* in cancer patients group, %.

The Analysis of Haplotypes Association by Genes Alleles *IL-1β, IL1RA, IL1RI*

In group of cancer patients the individuals were singled out who are carriers of «risk» alleles of polymorphic loci *(*Pro* (*rs1042522), *m (rs1625895), *196* (*Dup 16BP*) of gene *TP53*. The comparison group was entered by the individuals without oncological anamnesis, which are carriers of normal alleles of gene *TP53*. The monitoring group was forming not accidently as shown that «risk» alleles of gene *TP53* are detected with frequency from 50 to 86% with different forms of cancer [13, 46-48].

The analisis of haplotypes frequency distribution by gene allele *IL-1β, IL1RA, IL1RI* (Figure **4**) in group of apparently healthy individuals and cancer patients detected significant haplotype frequency raise (p=0,004, χ^2=8,71, carrying «favorable» alleles combinations **E1/*I/*T* in group of apparently healthy individuals.

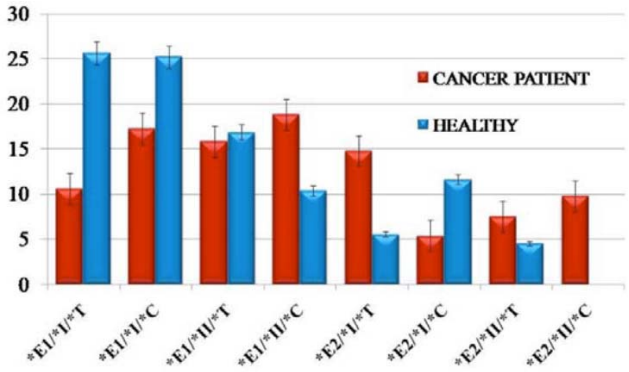

Figure 4: Comparative analysis of haplotypes frequency distribution on the based of loci analysis *IL-1β* (*rs1143634*), *IL1Ra (VNTR), IL1RI* (*rs2287047) i*n breast cancer group and a healthy individuals group, %.

The haplotype ****E2/*I/*T*** which has one allele with changed function (*IL-1β*E2),* which associated with high production interleukin-1, appears in group of cancer patients more often than in healthy individuals group (p=0,02, χ^2=4,73). The haplotype, carrying three «unprotective» alleles ****E2/*II/*C,*** appears only in group of cancer patients (p=0,0034, χ^2=9,14). Our investigations have revealed by alleles combinations that individuals have significant higher rates of interleukin-1β productions and antagonist in serum. According to Hurme M. *et al*., [31], such ratio cytokines can show itself as cytodifferentiation factor.

According to the results of the molecular genetic analysis of the number of healthy women were selected individuals who are the carriers of the 'normal' allele studied cell-cycle genes (*TP53*, *BRCA1*). The number of patients was 56, which later was related to as a control group during the comparative analysis of cytokine concentration in the serum.

Enzyme Multiplied Immunoassay of Cytokines Concentration in Serum

The analysis of the content of the main pro-and anti-inflammatory cytokines in the serum of patients with breast cancer shows a significant change in their level compared to healthy individuals (Figure **5**).

A significant decrease in pro-inflammatory IL-2 cytokine in patients with breast cancer was found. It is known that IL-2 is an inducer of apoptosis [49], therefore, these patients have depressed process of elimination of the damaged cells. In patients with breast cancer was found increased production of proinflammatory cytokines IL-6. It is established that IL-6 has a great influence on the regulation of the immune response: it stimulates the proliferation and differentiation of B - cells, enhances the production of antibodies, it is involved in the production of multipotent colony-forming factors and megakaryocytes, can inhibit neutrophil apoptosis [49].

In addition, IL-6 acts as a carcinogenesis trigger, including a self-sustaining chain that causes the initiation and maintenance of the malignant state in mammalian cells [50]. Some authors refer IL-6 to the cytokines that inhibits apoptosis, which promotes tumor growth and neoangiogenesis [37, 51]. Concentrations

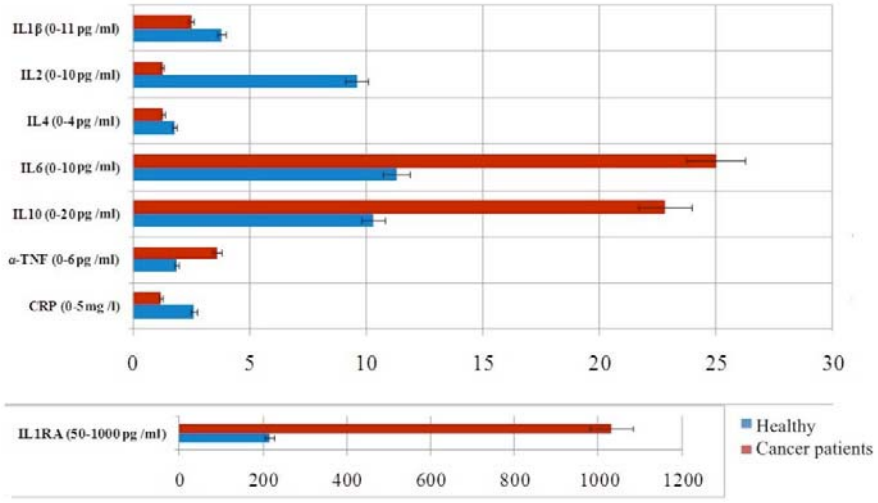

Figure 5: Cytokine status of breast cancer patients before surgery and healthy individuals, pg /ml (CRP-mg /l).
Note: The clinical standard values are given in parentheses.

Table 1: Cytokine Status Indices in Breast Cancer Patients Group Before Operation and During Postoperative Period

Cytokines	before operation		during postoperative period		p
	n	$\bar{x}$ ±m	n	$\bar{x}$ ±m	
IL-1β	88	2,5±0,21	88	1,78±0,13	0,7
IL1RA	88	1031,43±75,71	88	1010,5±86,05	0,4
IL2	88	1,26±0,06	88	-	0,08
IL4	88	1,3±0,09	88	1,55±0,07	0,4
IL6	88	25±1,96	88	30,8±1,85	0,8
IL10	88	22,8±1,85	88	18,2±0,92	0,19
α-TNF	88	3,62±0,22	88	3,31±0,16	0,37

Note: significance of differences was estimated by t-criterion.

of other pro-inflammatory cytokines were at the minimum values of the physiological norm (Figure **5**), which can be explained by the weak immunoreactivity. Enzyme multiplied immunoassay of cytokines in the serum of patients with breast cancer which was conducted after surgery showed no change in concentrations of pro-and anti- cytokines (Table **1**).

The concentration of pro-and anti-inflammatory cytokines in healthy individuals was at the physiological ratio according to their functional relationships.

The Analysis of the Association of Polymorphic Gene Markers with the Average Values of Cytokine (ANOVA) Quantitative Indicators in Breast Cancer Patients

With the help of one-way analysis of variance (ANOVA) in breast cancer patients was revealed the association of reduced concentration of IL-2 allele with * m (rs1625895) of gene *TP53* (p <0,001). The pathogenesis of cancer involves the processes of malignant transformation of cells arising from an inhibition of the functioning of the tumor suppressor gene *TP53* and mechanisms of immune control oppression due to the low immunoreactivity.

By means of one-way analysis of variance (ANOVA) in patients with breast cancer was discovered the association of low values of IL-10 with allele BRCA1 * A (rs80357629, p = 0.038). Changing the BRCA1 protein function by nucleotide substitution in the polymorphic locus rs80357629 of gene *BRCA1* reduces the efficiency of DNA repair, and as a consequence, leads to the genetic instability. In combination with a reduction in apoptosis inducers (IL-10, IL-2, IL-4), these two processes serve to the progression of transformed cells and the development of cancer.

Thus, the disruption of the cell-cycle genes together with the imbalance ratio of serum cytokines involved in the induction of apoptosis is a factor of poor prognosis for the development of breast cancer.

Typing of Polymorphic Loci *TP53* and *BRCA1* in DNA Genes of Peripheral Blood and Tumor Tissue of Breast Cancer Patients

During the genotyping of polymorphic loci, rs1042522, rs1625895, DUP 16bp, *TP53* gene and rs80357629, rs1799966 *BRCA1* gene in DNA of peripheral blood leukocytes and tumor tissue in patients with breast cancer the changes of genotypes was revealed in seven out of the fifteen biopsies.

In tissue samples number 2 (Figure **6**) was revealed a homozygous genotype ww (rs1625895) in the *TP53* gene, as reflected by the emergence of recognition site for the restriction nuclease MspI.

Histological presentation of the tumor tissue was corresponding to the benign tumors characteristics (Figure **7**, 21). Supposedly the genetic constitution of the *TP53* wm gene predisposing to the cancer pathology, contributed to malignant degeneration of cells, but the reversion of *TP53* allele (wm → ww) in the tumor has determined a large number of healthy tissue.

In two samples (№ 4 and 3, Figure **6**) the discrepancies in genotypes in four out of the five loci were found. The individual analysis showed that these patients had stage II of disease without metastasis (T2N0M0). Histological studies have identified the stage of tumor infiltrative ductal carcinoma (Figure **8**, 21).

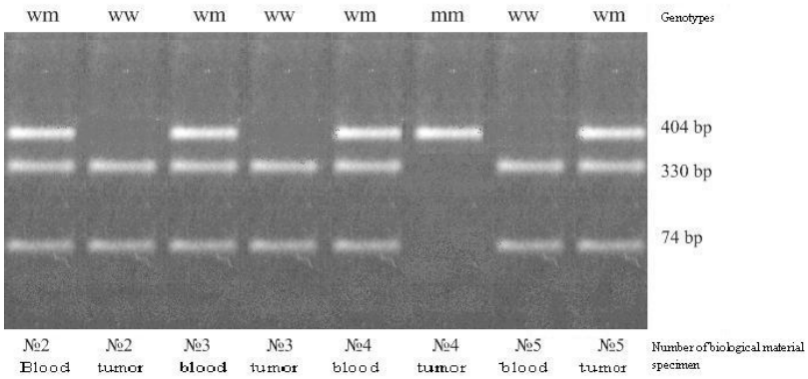

Figure 6: Electrophoregram of genotyping polymorphic locus rs1625895 *TP53* gene samples of peripheral blood and tumor tissue results.

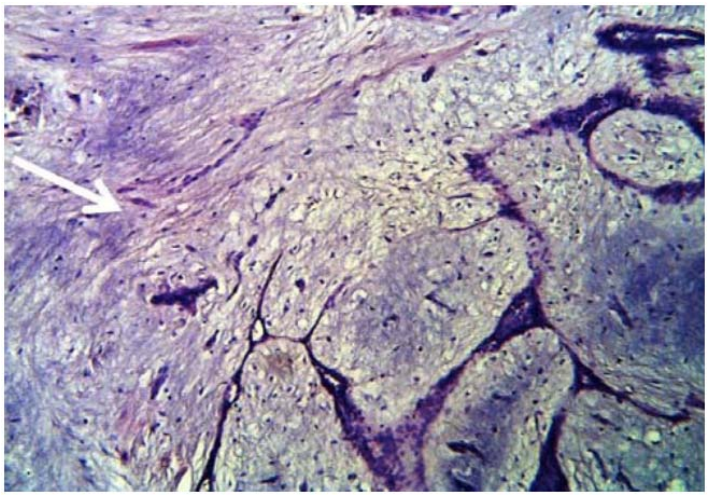

Figure 7: Intracanalicular fibroadenoma with a marked stromal myxomatosis *micronecrosis* and polymorphism of individual cells of the stroma. Staining was carried out by using hematoxylin and eosin, × 100.

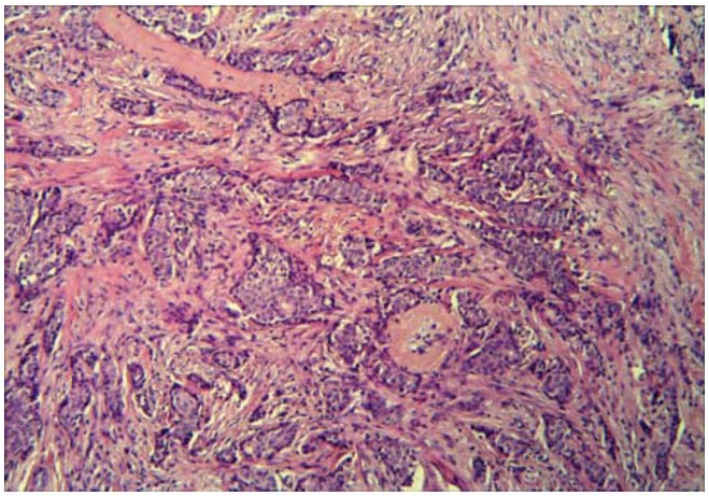

Figure 8: Infiltrating ductal carcinoma. Stained with hematoxylin and eosin, × 100.
Note: in a dense fibrous stroma tumor cells form a solid-serous structure. Tumor cells of different size and shape, hyperchromic nuclei, single mitosis.

Five risk alleles in genes *TP53* and *BRCA1* that are able to start cell proliferation are identified in the patient number 3. The personalized analysis of cytokine profile showed that the concentration of anti-inflammatory cytokine IL-10 was significantly increased, indicating the hard work of the immune system (Table **2**).

Table 2: The Indicators of Cytokine Status in Women, Possessing Nucleotide Changes in the Tumor Tissue

№ specimen	Concentration of cytokines, pg/ml							
	IL-1β (0-15)	IL1RA (to 1500)	IL2 (0-10)	IL4 (to 4)	IL6 (to 10)	IL10 (to 20)	α-TNF (0-6)	CRP (до 5)
2	1	271,8	0	0,93	2	2	0	1,44
3	1,6	314,2	1,87	0,2	6,5	38,5	4,8	1,14

Note: The clinical standard values of cytokine production are given in parentheses.

In support of this conclusion we may refer to the following facts: the metastases are not identified, and two alleles in the *TP53* gene were recovered to the «normal» level. It should be noted that these patients didn't undergo pre-surgical chemotherapy and radiotherapy.

These results make it possible to point out a key role of the allelic state of tumor suppressor genes (*TP53* and *BRCA1*) in the risk formation for malignant transformation of cells. In the pathogenetic link between cancer genetic predisposition and its clinical manifestations the cytokines, regulating the cell-cell interactions are included. It is shown that a pathophysiological role in the development and metastasis of tumor plays a high production of interleukin-6 receptor antagonist, interleukin-1β and interleukin-10, and the low production of interleukin-2, which finally leads to an imbalance of the cytokine regulatory network, which aims to inhibit apoptosis and suppress the immune response. Changes in the functioning of cytokine regulation in tandem with carriage of risk alleles in the genes *BRCA1* and *TP53* are the factors conditioning not only the appearance, but also the development of cancer.

REFERENCES

[1] Logan D. The mitochondrial compartment. J Exper Bot 2004; 2: 9.

[2] Croce A, D'Agostino L, Moretti A, Augurio A. Parotid surgery in patients over seventy-five years old. Acta Otorhinolaryngol Ital 2008; 28(5): 231-38.

[3] Payne H, Pelz F, Brooks R, Horrocks L, Kemp A, Webb E, *et al*. Benefits of interprofessional learning: an interprofessional MSc in child health. Hospital Med 2005; 66: 239-41.

[4] Kopnin BP. Diverse P53: diversity of forms, functions, tumorsuppressive and oncogenic activities. Clin Oncohaematol 2008; 1(1): 2-9.

[5] Phillips H. The role of the p53 tumour suppressor gene in human breast cancer. Clin Oncol 1999; 11(3): 148-55. http://dx.doi.org/10.1053/clon.1999.9032

[6] Vousden K, Lane D. p53 in health and disease. Nat Rev Mol Cell Biol 2007; 8(4): 275-83. http://dx.doi.org/10.1038/nrm2147

[7] Zheltuhin AO, Chumakov PM. Routine and inducible functions of gene P53. Biol Chem Succes 2010; 50: 447-54.

[8] Chumakov PM. P53 protein and its universal functions in multicellular organism. Biol Chem Success 2007; 47: 3-52.

[9] Ellis RE, Yuan J, Horvitz HR. Cytokines in ascites fluid from ovarion carcinoma. Cancer Lett 2002; 61: 243-53.

[10] Berezhnaya NM. Immunity system cells' role in tumor's microenvironment. Immunity system cells' cooperation with other microenvironment components. Oncology 2009; 11(2): 86-93.

[11] Durum SK, Oppenheim JJ. Proinflammatory cytokines and immunity. Fundamental Immunol 1993; 1: 801-35.

[12] Soussi T, Fromentel C, May P. Structural aspects of the p53 protein in relation to gene evolution. Oncogene 1990; 5: 945-52.

[13] Denisov EV, Litvyakov NV, Slonimskaya EM, Malinovskaya EA, Babishkina NN, Stegniy VN, *et al*. Investigation of Arg72Pro, polymorphism and gene TP53 somatic mutation correlation in breast cancer patients group. Mol Biol Technol Pract Med 2009; 13: 66-74.

[14] Galikeeva GF, Vasilyeva EM, Kayumova LR, Gumerova OV, Vorobyeva EV, Gorbunova VY. Analisys of polymorphous genes variations of xenobiotics *GSTM1* and *p53* biotransformation in breast cancer patients group. Orenburg State University Herald 2009; 4: 659-61.

[15] Kinzler KW, Vogelstein B. Life (and death) in a malignant tumour. Nature 1996; 379: 19-20. http://dx.doi.org/10.1038/379019a0

[16] Weinberg RA. How cancer arises. Sci Amer 1996; 275: 32-41. http://dx.doi.org/10.1038/scientificamerican0996-62

[17] Deng W, Wu Y, Luo L, Wang S, Tang W, Zhang Y. Expression of estrogen sulfotransferase in breast tissues and the regulation of ESTmRNA by estrogen. Zhonghua Yi Xue Za Zhi 2003; 83(21): 1891-94.

[18] Crowe D, Lee M. New role for nuclear hormone receptors and coactivators in regulation of BRCA1-mediated DNA repair in breast cancer cell lines. Breast Cancer Res 2006; 8: R1. http://dx.doi.org/10.1186/bcr1362

[19] Park J, Irvine R, Buchnan G. Breast cancer susceptibility gene 1 (BRCA1) is a coactivator of the androgen receptor. Cancer Res 2000; 60: 5946-49.

[20] Zhu B, Cong W, Wang H. WAF1/CIP1 gene expression in human hepatocellular carcinoma and its relationship with p53 mutation. Zhonghua Gan Zang Bing Za Zhi (Journal of Hepatology) 1999; 7(4): 217-20.

[21] Walker K, Levine A. Identification of a novel p53 functional domain that is necessary for efficient growth suppression. Proc Natl Acad Sci USA 1996; 93(26): 15335-40. http://dx.doi.org/10.1073/pnas.93.26.15335

[22] Sakamuro D, Sabbatini P, White E, Prendergast G. The polyproline region of p53 is required to activate apoptosis but not growth arrest. Oncogene 1997; 15(8): 887-98. http://dx.doi.org/10.1038/sj.onc.1201263

[23] Venot C, Maratrat M, Dureuil C, Conseiller E, Bracco L, Debussche L. The requirement for the p53 proline-rich functional domain for mediation of apoptosis is correlated with specific PIG3 gene transactivation and with

transcriptional repression. Eur Mol Biol Organ J 1998; 17(16): 4668-79.

[24] Dumont P, Leu J, Della-Pietra A, George D, Murphy M. The codon 72 polymorphic variants of p53 have markedly different apoptotic potential. Nat Genet 2003; 33(3): 357-65. http://dx.doi.org/10.1038/ng1093

[25] Barel D, Avigad S, Cohen I. A novel germline p53 insertion/duplication mutation in intron 6 in a Li-Fraumeni Family. Cancer Res 1994; 54:1298-304.

[26] Wu X, Zhao H, Amos C. p53 Genotypes and haplotypes associated with lung cancer susceptibility and ethnicity. J Natl Cancer Instit 2002; 94(9): 681-90. http://dx.doi.org/10.1093/jnci/94.9.681

[27] Biros E, Kalina I, Kohut A. Germ line polymorphisms of the tumor suppressor gene p53 and lung cancer. Lung Cancer 2001; 31: 157-62. http://dx.doi.org/10.1016/S0169-5002(00)00188-4

[28] Liang H, Lunec J. Characterisation of a novel p53 downregulated promoter in intron 3 of the human MDM2 oncogene. Gene 2005; 361: 112-18. http://dx.doi.org/10.1016/j.gene.2005.07.018

[29] Bochar D, Wang L, Beniya H, Kinev A, Xue Y, Lane W, *et al*. BRCA1 is associated with a human SWI/SNF-related complex: linking chromatin remodeling to breast cancer. Cell 2000; 102(2): 257-65. http://dx.doi.org/10.1016/S0092-8674(00)00030-1

[30] Daly AK, Day CP, Donaldson PT. Polymorphisms in Immunoregulatory Genes. Am J Pharmacogenomics 2002; 2: 13-23. http://dx.doi.org/10.2165/00129785-200202010-00002

[31] Hurme M, Santtila S. IL-1 receptor antagonist (IL-1RA) plasma levels are co-ordinately regulated by both IL-1RA and IL-1β genes. Eur J Immunol 1998; 28: 2598-602. http://dx.doi.org/10.1002/(SICI)1521-4141(199808)28:08<2598::AID-IMMU2598>3.0.CO;2-K

[32] Santtila S, Savinainen K, Himure M. Presence of the IL-1RA allele 2 (IL1RN*2) is associated with enhanced IL-1beta production *in vitro*. Scand J Immunol 1998; 47: 195-98. http://dx.doi.org/10.1046/j.1365-3083.1998.00300.x

[33] Grimm C, Kantelhardt E, Heinze G, Polterauer S, Zeillinger R, Kölbl H, *et al*. The prognostic value of four interleukin-1 gene polymorphisms in Caucasian women with breast cancer: a multicenter study. Cancer 2009; 6: 78.

[34] Mustea A, Sehouli J, Könsgen D, Stengel D, Sofroni D, Lichtenegger W. Interleukin 1 receptor antagonist (IL-1RA) polymorphism in women with cervical cancer. Anticancer Res 2003; 23: 1099-102.

[35] Sehouli J, Mustea A, Koensgen D, Lichtenegger W. Interleukin-1 receptor antagonist gene polymorphism in epithelial ovarian cancer. Cancer Epidemiol Biomarkers Prev 2003; 11: 1205-13.

[36] Brull DJ, Montgomery H, Sanders J, Dhamriat S, Luong L, Rumley A, *et al*. Intrleukin – 6 gene -174G.>C and -572 G>C promoter polymorphisms are strong predictors of plasma intrleukin – 6 levels after coronary artery bypass sugrey. Arterioscler Thromb Vask Biol 2001; 21: 1458-63. http://dx.doi.org/10.1161/hq0901.094280

[37] Carpi A, Nicolini A, Antonelli A, Ferrari P, Rossi G. Cytokines in the management of high risk or advanced breast cancer: an update and expectation. Curr Cancer Drug Targets 2009; 9(8): 888-903. http://dx.doi.org/10.2174/156800909790192392

[38] Drachenberg DE, Elgamal AA, Rowbotham R. Circulating levels of interleukin-6 in patients with hormone refractory prostate cancer. Prostate 1999; 41: 127-33. http://dx.doi.org/10.1002/(SICI)1097-0045(19991001)41:2<127::AID-PROS7>3.0.CO;2-H

[39] Hoosein N, Abdul M, McCabe R. Clinical significance of elevation in neuroendocrine factors and interleukin-6 in metastatic prostate cancer. Urol Oncol 1995; 1: 246-51. http://dx.doi.org/10.1016/1078-1439(96)00012-9

[40] Lu C, Rak JW, Kerbel RS. Interleukin-6 in progression of human solid tumors: transitional changes in the regulation of cell growth, apoptosis and angiogenesis. Cancer J 1997; 10: 256-61.

[41] Teletaeva GM. Cytokines and antitumor immunity. Pract Oncol 2007; 8(4): 211-18.

[42] Gantsev SK, Gorbunova VY, Galikeeva GF, Vorobyeva EV, Vasilyeva EM, Rustamhanov RA. Oncosuppression genes functioning (*TP53, BRCA1*) and their cooperation with cytokines during breast cancer. Creative oncology and surgery – electronic theoretical and practical magazine 2012 (April); 23099 signs. url:http:/eoncosurg.com/?p=2273.

[43] Thomas M, Kalita A, Labrecque S. Two polymorphic variants of wild-type p53 differ biochemically and biologically. Mol Cell Biol 1999; 19(2): 1092-100.

[44] Bourdon JC, Fernandes K, Murray-Zmijewski F. p53 isoforms can regulate p53 transcriptional activity. Genes Dev 2005; 19(18): 2122-37. http://dx.doi.org/10.1101/gad.1339905

[45] Whibley C, Pharoah PD, Hollstein M. p53 polymorphisms: cancer implications. Nat Rev Cancer 2009; 9(2): 95-107. http://dx.doi.org/10.1038/nrc2584

[46] Dimitrakakis C, Konstadoulakis M, Messaris E, *et al*. Molecular markers in breast cancer: can we use c-erbB-2, p53, bcl-2 and bax gene expression as prognostic factors. Breast 2002; 11(4): 279-85. http://dx.doi.org/10.1054/brst.2002.0445

[47] Feki A, Irmgard Irminger-Finger. Critical reviews in oncology. Hematology 2004; 52(2): 103-16. http://dx.doi.org/10.1016/j.critrevonc.2004.07.002

[48] Oliver M, Hussain SP, Caron de Fromentel C, Hainaut P, Harris CC. TP53 mutation spectra and load: a toll for generating hypotheses on the etiology of cancer. IARC Sci Publ 2004; 157: 247-70.

[49] Kadagidze ZG. Cytokines. Pract Oncol 2003; 3: 131-39.

[50] Rokavec M, Wu W, Luo J. Il-6 mediated suppression of miR-200c directs constitutive activation of inflammatory signalling circuit driving transformation and tumorigenisis. Mol Cell 2012; 45(6): 777-89. http://dx.doi.org/10.1016/j.molcel.2012.01.015

[51] Sangaletti S, Tripodo C, Ratti C. Oncogene-driven intrinsic inflammation induces leukocyte production of tumor necrosis factor that critically contributes to mammary carcinogenesis. Cancer Res 2010; 70: 7764-75. http://dx.doi.org/10.1158/0008-5472.CAN-10-0471

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