ISSN 0891-4168, Molecular Genetics, Microbiology and Virology, 2020, Vol. 35, No. 1, pp. 38–46. © Allerton Press, Inc., 2020.
Russian Text © The Author(s), 2020, published in Molekulyarnaya Genetika, Mikrobiologiya i Virusologiya, 2020, No. 1, pp. 41–49.

EXPERIMENTAL WORKS

The Overall Survival of Breast Cancer Patients Depends on a Combination of Polymorphisms of Tumor Necrosis Factor Gene and HLA Haplotypes

T. F. Malivanova[a, b, *], E. V. Alferova[a, **], A. S. Ostashkin[b, *], T. A. Astrelina[b, ****], and N. N. Mazurenko[a, *****]**

[a] *Blokhin National Medical Research Center of Oncology, Ministry of Health of Russian Federation, Moscow, 115478 Russia*
[b] *Burnazyan Federal Medical Biophysical Center of the Federal Medical Biological Agency, Moscow, 123098 Russia*
**e-mail: tmalivanova@yandex.ru*
***e-mail: skoromyslovalena@yandex.ru*
****e-mail: ostashkin@yandex.ru*
*****e-mail: t_astrelina@mail.ru*
******e-mail: nnmazurenko@mail.ru*

Received August 16, 2019; revised October 17, 2019; accepted December 3, 2019

Abstract—Tumor necrosis factor (TNF) is a proinflammatory cytokine involved in the pathogenesis of a number of diseases, including oncological and autoimmune diseases. The *–308(g/a)TNF* and *–238(G/A)TNF* polymorphisms are included in the extended ancestral haplotypes covering the whole complex of *HLA* genes. We assumed that the previously found effect of these polymorphisms on the overall survival (OS) of breast cancer (BC) patients may be a consequence of cooperation with the genomic environment, namely, with the AH8.1 and B57 haplotypes associated with autoimmune conditions. The archival collection of DNA from 442 primary BC patients and 327 women from the control group with known *–308(g/a)TNF* and *–238(G/A)TNF* genotypes was used in the work. Four hundred and twelve BC patients were tested for AH8.1 and B57 markers. During the study, the association of *–308a* and *–238A* alleles of the *TNF* gene with haplotypes AH8.1 and B57, respectively, was confirmed. Analysis of the results of the study demonstrated that *TNF* gene polymorphisms do not affect the predisposition to BC disease, but significantly decrease the OS of BC patients; moreover, the final effect of the *TNF* gene polymorphisms on the disease prognosis depends on genomic context. At stage II of the disease, the carriers of the *–308ag/–238GG* genotype in the presence of marker AH8.1 alleles and the *–308gg/–238GG* carriers, regardless of AH8.1 markers, had a 10-year OS above 80%, while a 10-year OS was lower than 50% in the *–308ag/–238GG* carriers in the absence of AH8.1 markers and in the *–308gg/–238AG* genotype carriers ($p = 0.0076$). The mechanisms of action of *–308(g/a)TNF* and *–238(G/A)TNF* differ, and a decrease in OS in the carriers of minor *–238A* allele is mediated by its association with *HLA-B*57*, while a decrease in OS in the carriers of *–308a*, on the contrary, is not associated with the ancestral AH8.1 haplotype. Thus, two genetically determined BC patient groups that have an unfavorable prognosis in conditions of standard BC therapy were detected.

Keywords: TNF polymorphism, HLA, ancestral haplotype, breast cancer, overall survival
DOI: 10.3103/S0891416820010061

INTRODUCTION

Tumor necrosis factor (TNF) is a proinflammatory cytokine, which plays a significant role in the pathogenesis of a number of diseases, including oncological and autoimmune diseases [1, 2]. The *TNF* gene is located on chromosome 6 (6p21.3) in a highly polymorphic region of class III *HLA* histocompatibility complex at a distance 250 kb from the class I *HLA-B* locus and 850 kb from the class II *HLA-DR* locus. The *TNF* gene has a number of functional polymorphisms, of which single nucleotide substitutions in the promoter regions *–308(g/a)TNF* (rs1800629) and *–238(G/A)TNF* (rs361525) are most studied [3] (for convenience, *–308(g/a)TNF* and *–238(G/A)TNF* alleles are designated by lowercase and uppercase letters, respectively). It is known that these polymorphisms are representatives of the extended ancestral haplotypes covering the whole complex of *HLA* genes. One of the most common haplotypes, AH8.1 (which is found in 9% of cases in the European population), which includes the *HLA-A*1*, *HLA-B*8*, *HLA-DRB1*3*, and *–308(a)TNF* alleles, is associated with an increased level of TNF synthesis and predisposition to autoimmune diseases [4, 5]. In turn, the *–238(A)TNF* allele

is associated with a low production of the cytokine and forms a unique B57 haplotype with the *HLA-B*5701* allele, which is known to be a protective allele in the pathogenesis of HIV-1 infection [6]. It has been suggested that the effect of *HLA* complex haplotypes can be associated with the level of *TNF* gene expression; however, on the contrary, the effect of *TNF* can be caused by a linkage with the *HLA* complex genes.

TNF can perform multidirectional functions in the pathogenesis of oncological diseases: inducing both apoptosis and proliferation of tumor cells and stimulating tumor infiltration by cells of the immune system and promote the invasion and metastasis of tumor cells [1].

Breast cancer (BC) is the most common oncological disease in women. The antitumor activity of TNF with BC is actively studied in preclinical and clinical studies. Although the clinical use of TNF is limited by systemic toxicity, its use as an adjuvant in combination with in chemo- and radiation therapy is considered promising [7]. However, the effect of the microenvironment and cellular and genomic context on the final effects of TNF should be taken into consideration. The *TNF* gene polymorphism is studied as a factor of risk and prognosis of BC. Previously, we demonstrated the dependence of overall survival (OS) of BC patients on *–308(g/a)TNF* and *–238(G/A)TNF* polymorphisms [8, 9]. In the present study, we tried to answer the question of whether the effect of *TNF* gene polymorphism on OS is a consequence of cooperation with the gene environment, namely, with the ancestral haplotypes of *HLA* complex involved in autoimmune processes.

MATERIALS AND METHODS

An archival collection of DNA from 442 primary BC patients with the confirmed histological diagnosis and 327 women without oncological and autoimmune diseases (clinical control group) with known *–238(G/A)TNF* and *–308(g/a)TNF* genotypes (partially described previously [8–10]) was used in the work. The mean age of BC patients was 54.2 years (from 23 to 80 years); in the control group, the mean age was 53.7 years (from 19 to 89 years). Data on the stage of the disease were available for 420 BC patients. Since only 2.9% of patients had stage IV BC, these patients were included in the group with stage III BC, and the distribution by stages of the disease was in general as follows: I, 25.0%; II, 46.4%; and III, 28.6%. Information about the condition of BC patients during the observation period since the initial hospitalization was obtained in 278 cases.

The *HLA-A*1*, *HLA-B*8*, and *HLA-DRB1*03* alleles were determined by the polymerase chain reaction (PCR) method in 412 BC patients; *HLA-B*57*, in 169 BC patients. The following allele-specific primers were used in the reaction: A1_F: 5'-ggACCAggAgACACggAATA-3', A1_R: 5'-AggTATCTgCggAgCCCg-3'; B8_F: 5'-gACCggAACACACAgATCTT-3', B8_R: 5'-CCgCgCgCTCCAgCgTg-3' [11]; B57_F: 5'-gCTCACATCATCCAggT-3', B57_R: 5'-CgTCTCCTTCCCgTTCTC-3'; B57_ R0101: 5'-ATCCTTgCCgTCgTAggCgg-3', B57_R0102: 5'-ATCCTTgCCgTCgTAGGCAG-3', [12]; DR3_F: 5'-TACTTCCATAACCaggAggAgA-3', DR3_R: 5'-TgCAgTAgTTgTCCACCCg-3' [13]. The alleles of *–238(G/A)TNF* were determined by a PCR-RFLP method as described previously [9].

To determine the *TNF* gene *–308/–238* haplotype in samples heterozygous for both studied sites (*–308ag/–238AG* genotype), a PCR using allele-specific primers was conducted to determine the *–308(g/a)TNF* polymorphism: TNF-308aF 5'-AATAggTTTTgAgggg-CATgA-3', TNF-308gF 5'-ATAggTTTTgAggggCATgg-3', TNF-308R 5'-TCTCggTTTCTTCTCCATCg-3' [14], since this region of PCR product includes the position of the *–238(G/A)TNF* polymorphism (Fig. 1a). The nucleotide sequence of the PCR product of each haplotype was determined by a reverse Sanger sequencing.

When analyzing the data obtained, the groups were compared using a two-sided Fisher's criterion. Pearson's criterion was used to check the compliance of genotype distribution with Hardy–Weinberg equilibrium. The distribution of the *TNF* gene *–238/–308* haplotypes was determined by a direct calculation. To estimate the risk of disease, the odds ratio was calculated and presented as OR (95% confidence interval, CI). The hazard ratio (HR) (95% CI) was a criterion for estimating the prognostic significance of the trait, the indices of 10-year OS was presented as Mean ± SE%, and the comparison of OS curves was conducted by a Kaplan–Meier method using a Logrank test in the GraphPad Prism program (version 4.00). For all criteria, differences were considered significant upon reaching $p < 0.05$.

RESULTS

Data on the *TNF* gene polymorphism were analyzed for 442 BC patients and 327 women from the control group. The distribution of *–308(g/a)TNF* and *–238(G/A)TNF* genotypes corresponded to theoretical Hardy–Weinberg distribution ($p > 0.05$). The distribution of genotypes and haplotypes for these sites had no statistical differences in BC patients and in the control group, and the obtained OR values do not allow to talk about the effect of these polymorphisms on predisposition to BC disease (Table 1).

It is necessary to note that all seven samples (three from the control group and four from BC patients) that were heterozygous both for the *–238(G/A)TNF* site and the *–308(g/a)TNF* site had only one polymorphic substitution in each of the haplotypes of its diploid set (Fig. 1b). Accordingly, the double heterozygous *–308ag/–238AG* genotype was taken into

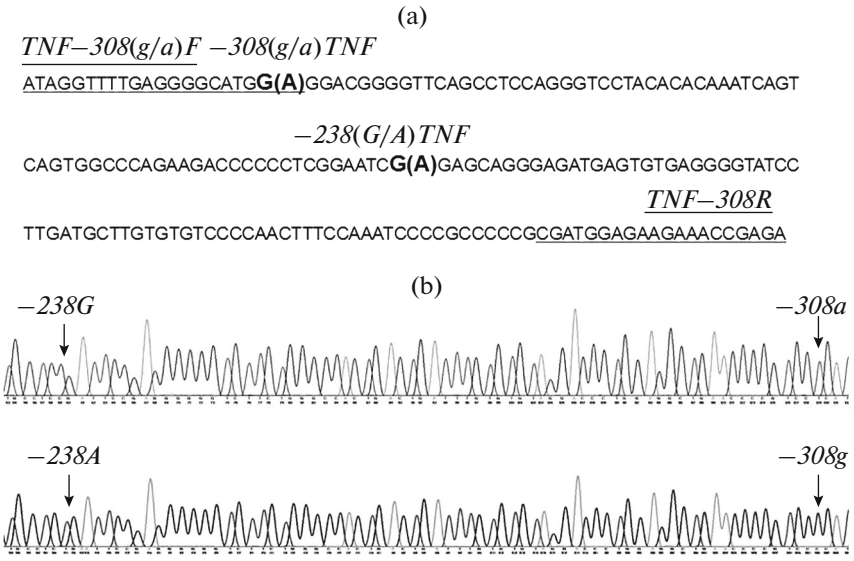

Fig. 1. Results of sequencing of the TNF gene double heterozygous *–308ag/–238AG* genotype. (a) Sequence of PCR product using allele-specific TNF-308a/gF and TNF-308R primers [14] (underlined) includes the sites of single nucleotide substitutions *–308(g/a)TNF* and *–238(G/A)TNF* (highlighted in bold). (b) Fragment of PCR product sequence: above, *TNF* gene *–308a/–238G* haplotype; below, *–308g/–238A* haplotype.

account in the analysis as containing two haplotypes: *–308a/–238G* and *–308g/–238A* (Table 1). Thus, except for these samples, the carriers of three *TNF* gene *–308/–238* haplotypes (*a/G*, *g/G*, *g/A*) in fact correspond to the carriers of *ag/GG*, *gg/GG*, and *gg/AG* genotypes. The carriers of the *aa/GG* genotype (in our sample, 1.4% of BC patients) were included in the group *ag/GG* for subsequent analysis.

Four hundred and twelve BC patients were tested for the markers of extended AH8.1 haplotype (*HLA-A*1*, *HLA-B*8*, and *HLA-DRB1*03* alleles) (Table 2). In 8.5% of cases, three markers were found simultaneously, which corresponds to literature data [15]. The *HLA-B*57* was detected only in the *–238A* allele carriers, but not in the carriers of *TNF* gene *–238GG* homozygote (74.4 and 0%, respectively; $p = 7.1\text{D-}22$). In addition, samples positive for *HLA-B*57*, of which 96% (24 out of 25) had the *HLA-B*5701* allele subtype, were additionally tested in nested PCR.

The distribution of marker AH8.1 alleles in the carriers of *TNF* gene *–308/–238* genotypes (*gg/GG*, *ag/GG*, and *gg/AG*) is presented in Table 2. The group

Table 1. Distribution of TNF gene genotypes and haplotypes in BC patients ($n = 442$) and control group ($n = 327$)

Sample		Control		BC		OR [95% CI]
		n	%	*n*	%	
Genotype *–308(g/a)*	aa	2	0.6	6	1.4	2.2 [0.4; 11.2]
	ag	76	23.2	105	23.8	1.03 [0.7; 1.4]
	gg	249	76.1	331	74.9	0.9 [0.7; 1.3]
Genotype*–238(G/A)*	AG	20	6.1	35	7.9	1.3 [0.7; 2.3]
	GG	307	93.9	407	92.1	0.8 [0.4; 1.3]
Genotype *–308/–238*	aa/GG	2	0.6	6	1.4	2.2 [0.4; 11.2]
	ag/GG	73	22.3	101	22.9	1.03 [0.7; 1.5]
	gg/GG	232	70.9	300	67.9	0.9 [0.6; 1.2]
	gg/AG	17	5.2	31	7.0	1.4 [0.7; 2.5]
	ag/AG	3	0.9	4	0.9	0.9 [0.2; 4.4]
Haplotype *–308/–238*	g/A	20	3.1	35	4.0	1.3; [0.7; 2.3]
	g/G	554	84.7	732	82.8	0.87; [0.66; 1.15]
	a/G	80	12.2	117	13.2	1.1; [0.8; 1.5]

Table 2. Distribution of marker alleles of AH8.1 and B57 haplotypes in BC patients with different *TNF* genotypes

Haplotype markers	Total	A*1	B*8	DRB1*3	M+[1]	AH.8.1[2]	Total	B*57
All BC	$n = 412$	93	55	75	131	35	$n = 165$	
		22.6%	13.3%	18.2%	31.8%	8.5%		
Genotype *TNF*								
−238AG	$n = 33$	10	4	7	15	2	$n = 35$	25
		30.3%	12.1%	21.2%	45.5%	6.1%		71.4%
−238GG	$n = 379$	83	51	68	116	33	$n = 130$	0
		21.9%	13.5%	17.9%	30.6%	8.7%		0.0%
pF −238AG vs −238GG		0.3	1	0.6	0.08	1		7.1D-22
Genotype *TNF*								
−308ag	$n = 107$	47	48	46	64	30	$n = 47$	4
		43.9%	44.9%	43.0%	59.8%	28.0%		8.5%
−308gg	$n = 305$	46	7	29	67	5	$n = 118$	21
		15.1%	2.3%	9.5%	22.0%	1.6%		17.8%
pF −308ag vs −308gg		5.7D-9	2.8D-25	4.0D-13	3.1D-12	9.7D-15		0.16
TNF −308/−238								
gg/AG	$n = 29$	7	1	5	12	0	$n = 31$	21
		24.1%	3.4%	17.2%	41.4%	0.0%		67.7%
gg/GG	$n = 276$	39	6	24	55	5	$n = 87$	0
		14.1%	2.2%	8.7%	19.9%	1.8%		0.0%
ag/GG	$n = 103$	44	45	44	61	28	$n = 43$	0
		42.7%	43.7%	42.7%	59.2%	27.2%		0.0%
ag/AG	$n = 4$	3	3	2	3	2	$n = 4$	4
		3 out of 4	3 out of 4	2 out of 4	3 out of 4	2 out of 4		4 out of 4
pF gg/AG vs ag/GG		0.085	1.6D-5	0.016	0.096	5.4D-4		2.9D-11
pF gg/GG vs ag/GG		1.04D-8	3.4D-23	4.5D-13	9.4D-13	5.2D-13		1
pF gg/GG vs gg/AG		0.17	0.51	0.13	0.016	1		4.7D-16

[1]The genotype contains one or more marker alleles of AH8.1 haplotype (A*1, B*8, DRB1*3); [2]the genotype contains three marker alleles of AH8.1 haplotype (A*1, B*8, DRB1*3).

of *ag/GG* carriers differed significantly in the frequencies of the *HLA-A*1*, *HLA-B*8*, and *HLA-DRB1*03* alleles from the carriers of both *gg/GG* and *gg/AG* genotypes, except for the *HLA-A*1* in the *gg/AG* carriers (Table 2). A high frequency of the *HLA-A*1* allele (30.3%) was registered for the *−238AG* genotype, which corresponds to literature data, since the ancestral B57 haplotype also includes the *HLA-A*1* alleles along with the minor allele of *−238(A/G)TNF* polymorphism and the *HLA-B*57* allele [15, 16]. It should be noted that, all of the four carriers of the *TNF* gene double heterozygous *−308ag/−238AG* genotype were positive for the *HLA-B*57*, while two of them had all three AH8.1 markers. Thus, as was expected, the *−308ag* genotype is associated with AH8.1 markers, while the *TNF* gene *−238AG* genotype is associated with B57 haplotype markers.

We considered OS of BC patients as a characteristic of the final response of polymorphic alleles. We found no statistically significant differences for the whole sample, and 10-year OS was 65.0 ± 10.6% for the *gg/AG* genotype, 73.6 ± 3.3% for the *gg/GG* and 75.0 ± 5.3% for the *ag/G* (Fig. 2a). For the *−308gg/−238AG* genotype carriers, a decrease in 10-year OS till 50.0 ± 17.7% was associated with the *HLA-B*57*, although this dependence was not statistically significant (Fig. 2b). No statistically significant effect of the AH8.1 haplotype on the OS of BC patients was found (Fig. 2c). Since the number of carriers of three marker alleles of the AH8.1 haplotype simultaneously was small (8.5% in our sample), groups for comparison were subsequently formed depending on the presence of at least one of the marker *HLA-A*1*, *HLA-B*8*, and *HLA-DRB1*03* alleles or in the absence of these alleles (designated as M+ and M−, respectively) (Fig. 2d).

Subsequently, we tried to identify the effect of marker AH8.1 (M+) alleles on the OS of the carriers of the *TNF* gene *gg/AG*, *gg/GG*, and *ag/GG* genotypes

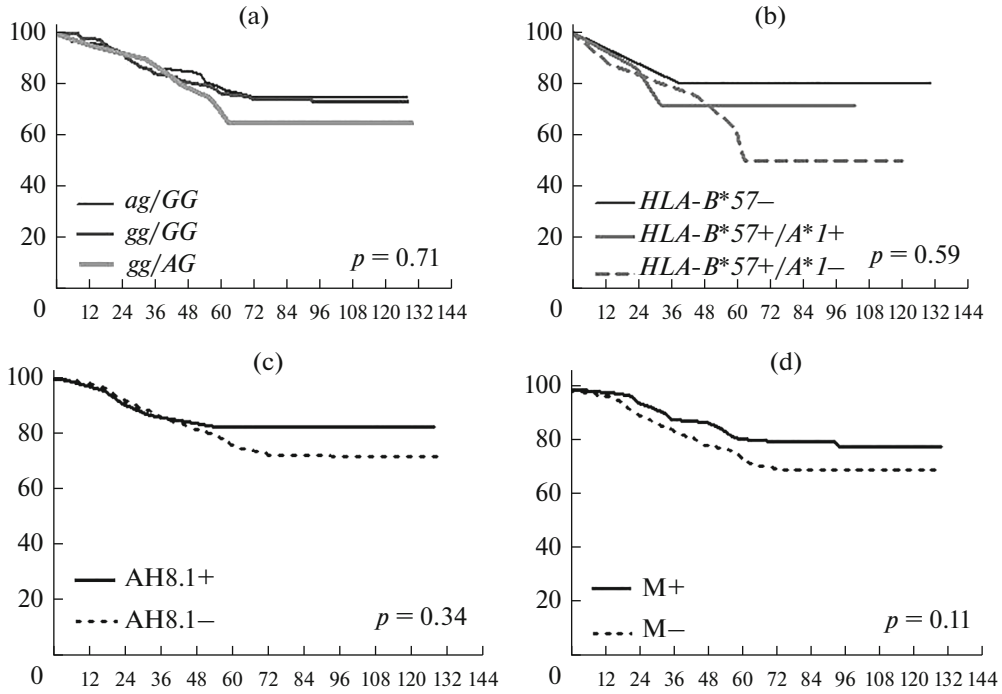

Fig. 2. Overall survival of BC patients depending on TNF gene *–308/–238* genotypes and marker alleles of ancestral AH8.1 and B57 haplotypes. Overall survival curves according to Kaplan–Meier. Abscissa axis, observation time, months. Ordinate axis, overall survival, %. (a) *TNF* gene *–308/–238 ag/GG*, *gg/GG*, *gg/AG* genotypes; (b) overall survival curves for carriers of *TNF* gene *gg/AG* genotype in the presence or absence of *HLA-A*1* and *HLA-B*57* alleles; (c) AH8.1+ contains three marker *HLA-A*1*, *HLA-B*8*, and *HLA-DRB1*3* alleles simultaneously; and (d) M+ contains one or more marker alleles of AH8.1 haplotype.

(Fig. 3a). A significant difference was detected only for the *ag/GG* genotype (10-year OS was 87.2 ± 5.4% in the presence of at least one AH8.1 marker and 56.0 ± 9.9% in the absence of AH8.1 alleles; Logrank test p = 0.0052, HR = 3.8 [1.4; 11.1]) (Fig. 3a). For the *gg/AG* carriers, the 10-year OS had an even larger, but statistically insignificant, difference in the presence of marker AH8.1 alleles and in their absence (88.9 ± 10.5 and 44.4 ± 16.6%, respectively; Logrank test p = 0.064; HR = 5.9 [0.9; 22.9]) (Fig. 3a).

The occurrence of both marker alleles of AH8.1 haplotype and minor alleles of *–238(G/A)TNF* and *–308(g/a)TNF* polymorphisms did not depend on the stage of BC (Fig. 3b). Three groups of survival curves were noted when the BC stage was included in the analysis of OS dependence on *TNF* genotypes (Fig. 3c). The carriers of common *gg/GG* genotype at the stage II of the disease had a high 10-year OS (more than 80%) comparable with the OS of patients with stage I BC. On the contrary, the *gg/AG* genotype carriers in stage II BC had a low 10-year OS (less than 60%) comparable with stage III BC. The intermediate group included patients with stage II and III BC that are carriers of the *ag/GG* genotype. At the same time, the maximum difference was noted between the groups of carriers of three *TNF* genotypes at stage II of the disease (Logrank test p = 0.05; Logrank test for trend p = 0.016) (Fig. 3c).

When analyzing stage II BC, it was found that the 10-year OS of the *TNF* gene *–308ag/–238GG* carriers in the presence of at least one AH8.1 allele (M+) was significantly higher than in their absence (90.0 ± 6.7 and 46.2 ± 13.8%, respectively; Logrank test p = 0.0063; HR = 6.6 [1.7; 26.9]). At the same time, the OS curves came in two groups: the *ag/GG* genotype carriers in the presence of AH8.1 markers and *gg/GG* carriers regardless the AH8.1 markers had 10-year OS more than 80%; 10-year OS was lower than 50% in the *ag/GG* carriers in the absence of AH8.1 markers and in the *gg/AG* carriers (since the frequency of the *gg/AG* carriers was low, the size of this group did not allow the dependence of OS on AH8.1 markers to be analyzed in this case) (Fig. 3d).

DISCUSSION

The effect of *TNF* gene polymorphisms on predisposition to oncological diseases has been quite extensively studied. In a metaanalysis of literature data on the effect of *–238(G/A)TNF* on predisposition to BC (based on eight studies), no association of this polymorphism with BC was detected (including with ranking by ethnicity) [16]. For the *–308(g/a)TNF* polymorphism, no effect on predisposition to BC was found for the whole sample in a metaanalysis of 20 publications; however, their authors draw the conclusion that

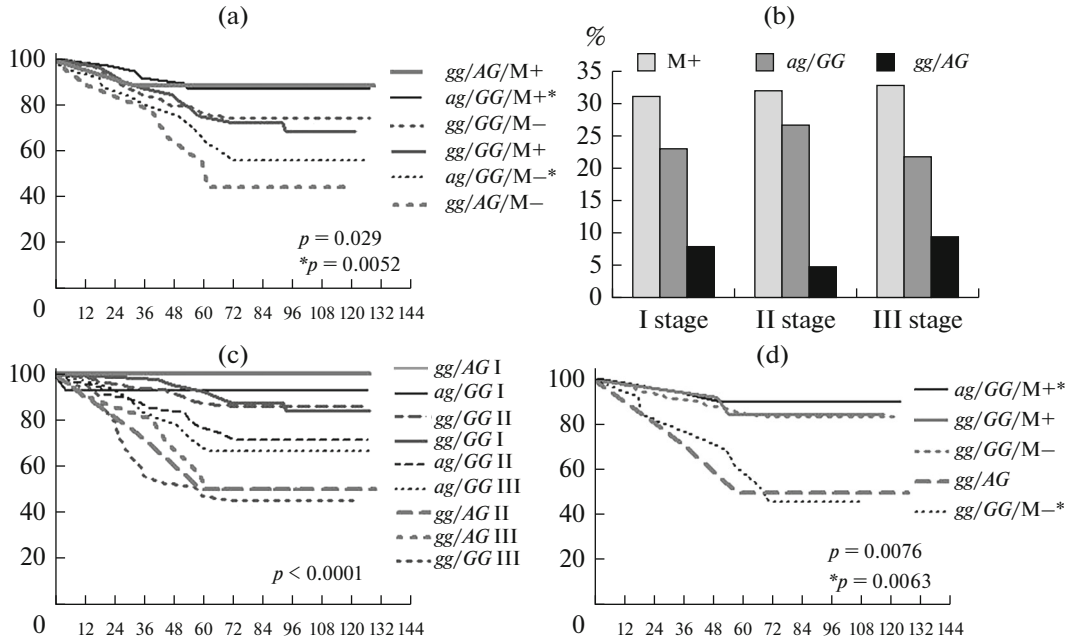

Fig. 3. Overall survival of BC patients depending on genotype and stage of the disease. (a, c, d) Overall survival curves according to Kaplan–Meier. Abscissa axis, observation time, months; ordinate axis, overall survival, %. (b) Dependence of the occurrence of genotypes on stage of BC. Abscissa axis, stage of BC; ordinate axis, occurrence, %. (a, b, c) Whole sample of BC patients. (d) BC patients with stage II. I, II, III, BC stages; *ag/GG*, *gg/GG*, *gg/AG*, *TNF* gene *–308/–238* genotypes; M+ genotype contains one or more marker alleles of AH.8.1 haplotype.

the *–308a* allele may serve as a protective factor in postmenopause, while the *–308aa* genotype may be a risk factor in premenopause [17]. In our study, we found no association with BC for both *–238(G/A)TNF* and *–308(g/a)TNF* genotypes and the *TNF* gene *–308/–238* haplotypes (Table 1).

The effect of the *TNF* gene polymorphisms on the prognosis of BC is much less studied, while the published data are controversial. The association of the *TNF* gene *–308aa* homozygote with an overall and tumor-specific survival was detected [18], while other authors found no such connection for the *–238(G/A)TNF* and *–308(g/a)TNF* polymorphisms [19]. In other works, the minor allele of the *–308(G/A)TNF* polymorphism was associated with both overall and relapse-free survival [20, 21]. Previously, we demonstrated a statistically significant decrease of 5-year OS in the carriers of common *–308gg* genotype in BC patients with stage III [8] and in the carriers of the minor *–238A* allele at stage II of the disease [9]. These previously obtained data can be interpreted as a decrease in OS for the *–308gg/–238GG* and *–308gg/–238AG* genotype carriers in BC patients with stage III and for the *–308gg/–238AG* genotype carriers with stage II of the disease presented in this study (Fig. 3c).

When analyzing the dependence of OS curves on the *TNF* genotype and the stage of the disease, we categorized BC patients into three groups with high, low, and intermediate levels of OS (Fig. 3c), the total indices for which were 86.7 ± 2.9, 70.6 ± 6.4, and 46.3 ± 6.1%, respectively ($p < 0.0001$). Although the difference in the stages of the disease (a prognostic trait used in the clinic) makes the main contribution to such difference, a modulating effect of the *TNF* gene polymorphisms on OS (which is most pronounced in stage II of the disease) should be noted. It is possible that the conditions for the manifestation of functional differences of the *TNF* gene minor alleles are namely created at the stage II of BC. Such conditions can include an imbalance of the immune system with oncological disease. It is known that the expression of class I HLA molecules decreases with the progression of BC; at the same time, the expression of class II HLA appears in the tumor tissue. Class I HLA molecules play a central role in the cellular immune response as an antigen presenting molecules for cytotoxic T lymphocytes. A decrease in the expression of class I HLA up to complete elimination from the surface of tumor cells is a mechanism of the escape of neoplastic cells from antitumor immune surveillance, which leads to dissemination and metastasis and worsens the prognosis of the disease [22]. In turn, class II HLA molecules are required for the presentation of peptides to T helper cells and their expression is responsible for the initiation of the immune response. The presence of such molecules makes the tumor more immunogenic, which yields a good prognosis [23]. Since the AH8.1 haplotype is related to autoimmune processes [6], we supposed that it can affect the intensity of antitumor immune response and BC prognosis.

A maximum effect of the interaction of the *TNF* gene *ag/GG* genotype with marker AH8.1 alleles was registered for stage II BC (Fig. 3d), although it was detected for the whole sample. Regarding the *gg/GG* genotype (which is not affected by the AH8.1 alleles), the presence of marker AH8.1 alleles increases, while their absence decreases OS of the carriers of the *TNF* gene minor *–308a* and *–238A* allele in the total sample (Fig. 3a). However, only a decrease in OS for the *ag/GG* genotype in the absence of marker AH8.1 alleles can be observed for stage II BC. Thus, if the effect of the *gg/GG* genotype (presented in 67.9% of our sample of BC patients) is taken as a reference point, the effect of surrogate AH8.1 haplotype, which includes the *TNF* gene *–308a* allele and at least one of the *HLA-A*1*, *HLA-B*8*, and *HLA-DRB1*3* alleles, will be close to it. It can be supposed that the ancestral AH8.1 haplotype maintains regulation and immune balance at the optimal (favorable) level under standard therapy of BC; at the same time, a combination of the *TNF* gene *–308a* and *–238A* alleles with other *HLA* haplotypes is apparently unfavorable for the prognosis of BC.

A trends toward an increase in OS in the presence of at least one AH8.1 marker was also detected for the carriers of the *gg/AG* genotype, probably due to the association of this genotype with the *HLA-A*1* allele (Fig. 3a). However, in general, the *gg/AG* genotype carriers have a poor prognosis at stage II BC, apparently due to the association with the *HLA-B*57* allele (Fig. 2b). The unique B57 haplotype is known and has been studied in connection with HIV-1 infection. It has been suggested that a low expression of the TNF cytokine in the *–238* carriers can promote the protective effect of the *HLA-B*5701* allele [24]. An increased level of a number of factors with antiviral activity, including those related to the family of *APOBEC3* cytidine deaminases, was found in healthy uninfected *HLA-B*57* carriers [25]. *APOBEC3*-associated mutagenesis has been registered in many tumors; at the same time, only the expression of *APOBEC3B* correlates with the proliferation of tumor cells, while the functions of other members of this family are related to processes in cells of the immune system [26]. It has been demonstrated that an increased level of *APOBEC3B* mRNA correlates with a poor prognosis of the disease [27, 28]. The *HER2* amplification and the loss of *PTEN* suppressor gene activates *APOBEC3B* in vitro and correlates with *APOBEC3*-associated mutagenesis in vivo [29]. Previously, we found in our studies that the *–238(G/A)TNF* polymorphism is associated with activating allele HER2 Ile655Val and can be a risk factor of BC for *–238AG* genotype carriers [10]. Thus, it can be supposed that the increased risk of BC and a decrease in OS in the carriers of minor *–238(G/A)TNF* allele may be mediated by the association of the *HLA-B*57* with the *APOBEC3B*.

A low frequency of the *TNF* gene minor *–238A* allele carriers and, appropriately, small groups of comparison are a limitation of this study. However, although we obtained no statistically significant evidence of the effect of *–238(G/A)TNF* polymorphism on the disease prognosis, the results seem biologically justified to us and require further detailed study. Apparently, this group of BC patients, as well as *-308a* allele carriers in the absence of marker *AH8.1* alleles, need additional treatment, and the use of immunotherapeutic approaches can be efficient for these cases.

CONCLUSIONS

The results of our study demonstrate that the *–308(g/a)TNF* and *–238(G/A)TNF* polymorphisms do not affect predisposition to BC diseases, but both of them significantly decrease the OS of BC patients, especially at stage II of the disease. Apparently, the final effect of the *TNF* gene polymorphisms on the disease prognosis depends on the genomic context. Based on data obtained and the analysis of literature sources, it can be assumed that the mechanisms of the *–308(g/a)TNF* and *–238(G/A)TNF* effects differ. Thus, a decrease in OS in patients with the minor *–238A* allele can be mediated by its association with the *HLA-B*57*, while, on the contrary, a decrease in OS in the *–308a* carriers is not associated with the autoimmune AH8.1 haplotype. Thus, two genetically determined groups of BC patients having an unfavorable prognosis in conditions of standard antitumor therapy of BC were detected, which requires additional study.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest. The authors declare that they have no conflict of interest.

Statement of compliance with standards of research involving humans as subjects. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants involved in the study.

ADDITIONAL INFORMATION

Malivanova T.F., e-mail: tmalivanova@yandex.ru; https://orcid.org/0000-0001-9699-2603

Alferova E.V., e-mail: skoromyslovalena@yandex.ru; https://orcid.org/0000-0002-3423-9816

Ostashkin A.S., e-mail: ostashkin@yandex.ru; https://orcid.org/0000-0002-5809-0075

Astrelina T.A., e-mail: t_astrelina@mail.ru; https://orcid.org/0000-0003-3629-0372

Mazurenko N.N., e-mail: nnmazurenko@mail.ru; https://orcid.org/0000-0003-4767-6983

Corresponding author: Malivanova T.F., e-mail: tmalivanova@yandex.ru

To cite this article:

Malivanova T.F., Alferova E.V., Ostashkin A.S., Astrelina T.A., Mazurenko N.N. "Breast cancer patients' overall survival depends on a combination of the polymorphisms of tumor necrosis factor gene and HLA-haplotypes." *Molekulyarnaya Genetika, Mikrobiologiya i Virusologiya* (*Molecular Genetics, Microbiology, and Virology*), 2020, vol. 38, no. 1, pp. 4–49 (Russian).
https://doi.org/10.17116/molgen20203801141

REFERENCES

1. Waters, J.P., Pober, J.S., and Bradley, J.R., Tumor necrosis factor and cancer, *J. Pathol.,* 2013, vol. 230, no. 3, pp. 241–248. https://doi.org/10.1002/path.4188
2. El-Tahan, R.R., Ghoneim, A.M., and El-Mashad, N., TNF-α gene polymorphisms and expression, *SpringerPlus,* 2016, vol. 5, no. 1, p. 1508. https://doi.org/10.1186/s40064-016-3197-y
3. Hajeer, A.H. and Hutchinson, I.V., TNF-alpha gene polymorphism: Clinical and biological implications, *Microsc. Res. Tech.,* 2000, vol. 50, no. 3, pp. 216–228. https://doi.org/10.1002/1097-0029(20000801)50:3<216::AIDJEMT5>3.0.CO;2-Q
4. Elahi, M.M., Asotra, K., Matata, B.M., and Mastana, S.S., Tumor necrosis factor alpha-308 gene locus promoter polymorphism: An analysis of association with health and disease, *Biochim. Biophys. Acta,* 2009, vol. 1792, no. 3, pp. 163–172. https://doi.org/10.1016/j.bbadis.2009.01.007
5. Aly, T.A., Eller, E., Ide, A., Gowan, K., Babu, S.R., Erlich, H.A., et al., Multi-SNP analysis of MHC region: remarkable conservation of HLA-A1-B8-DR3 haplotype, *Diabetes,* 2006, vol. 55, no. 5, pp. 1265–1269. https://doi.org/10.2337/db05-1276
6. Merino, A.M., Zhang, K., Kaslow, R.A., and Aissani, B., Structure of tumor necrosis factor-alpha haploblocks in European populations, *Immunogenetics,* 2013, vol. 65, no. 7, pp. 543–552. https://doi.org/10.1007/s00251-013-0700-2
7. Martinez-Reza, I., Diaz, L., and Garcia-Becerra, R.J., Preclinical and clinical aspects of TNF-α and its receptors TNFR1 and TNFR2 in breast cancer, *J. Biomed. Sci.,* 2017, vol. 24, no. 1, p. 90. https://doi.org/10.1186/s12929-017-0398-9
8. Malivanova, T.F., Yurchenko, V.A., Skoromyslova, E.V., and Mazurenko, N.N., How *–308(G/A)TNF* polymorphism influences onto overall survival of breast cancer patients, *J. N.N. Blokhin Russ. Cancer Res. Cent. RAMS,* 2012, vol. 23, no. 1, pp. 40–44. https://elibrary.ru/item.asp?id=17901571. Accessed August 12, 2019.
9. Malivanova, T.F., Skoromyslova, E.V., Yurchenko, V.A., Kononenko, I.V., Manzyuk, L.V., and Mazurenko, N.N., Analysis of the *–238(G/A)TNF* polymorphism in breast cancer patients, *Mol. Genet., Microbiol. Virol.,* 2013, vol. 28, no. 2, pp. 52–55. https://doi.org/10.3103/S0891416813020031
10. Malivanova, T.F., Ostashkin, A.S., and Mazurenko, N.N., The connection of polymorphisms *–238(A/G)TNF* and Ile655Val HER2 influences the risk and molecular features of breast cancer, *Mol. Genet., Microbiol. Virol.,* 2017, vol. 32, no. 3, pp. 141–147. https://doi.org/10.3103/S0891416817030053
11. Tonks, S., Marsh, S.G., Bunce, M., and Bodmer, J.G., Molecular typing for HLA class I using ARMS-PCR: further developments following the 12th International Histocompatibility Workshop, *Tissue Antigens,* 1999, vol. 53, no. 2, pp. 175–183. https://doi.org/10.1034/j.1399-0039.1999.530208.x
12. Cascella, R., Strafella, C., Ragazzo, M., Zampatti, S., Borgiani, P., Gambardella, S., et al., Direct PCR: A new pharmacogenetic approach for the inexpensive testing of HLA-B*57:01, *Pharmacogenomics J.,* 2015, vol. 15, no. 2, pp. 196–200. https://doi.org/10.1038/tpj.2014.48
13. Ma, S., Wu, J., Wu, J., Wei, Y., Zhang, L., Ning, Q., and Hu, D., Relationship between HLA-DRB1 allele polymorphisms and familial aggregations of hepatocellular carcinoma, *Curr. Oncol.,* 2016, vol. 23, no. 1, pp. 1–7. https://doi.org/10.3747/co.23.2839
14. Perrey, C., Turner, S.J., Pravica, V., Howell, W.M., and Hutchinson, I.V., ARMS-PCR methodologies to determine IL-10, TNF-alpha, TNF-beta and TGF-beta 1 gene polymorphisms, *Transplant Immunol.,* 1999, vol. 7, no. 2, pp. 127–128. https://doi.org/10.1016/S0966-3274(99)80030-6
15. Williams, F., Meenagh, A., Single, R., McNally, M., Kelly, P., Nelson, M.P., et al., High resolution HLA-DRB1 identification of a Caucasian population, *Hum. Immunol.,* 2004, vol. 65, no. 1, pp. 66–77. https://doi.org/10.1016/j.humimm.2003.10.004
16. Zhang, Q., Zhao, G.S., Yuan, X.L., Li, X.H., Yang, Z., Cui, Y.F., et al., Tumor necrosis factor alpha-238G/A polymorphism and risk of breast cancer: An update by meta-analysis, *Medicine* (Baltimore), 2017, vol. 96, no. 29, p. e7442. https://doi.org/10.1097/MD.0000000000007442
17. Jin, G., Zhao, Y., Sun, S., and Kang, H., Association between the tumor necrosis factor alpha gene *–308G>A* polymorphism and the risk of breast cancer: a meta-analysis, *Tumor Biol.,* 2014, vol. 35, no. 12, pp. 12091–12098. https://doi.org/10.1007/s13277-014-2510-z
18. Mestiri, S., Bouaouina, N., Ahmed, S.B., Khedhaier, A., Jrad, B.B., Remadi, S., and Chouchane, L., Genetic variation in the tumor necrosis factor-alpha promoter region and in the stress protein hsp70-2: Susceptibility and prognostic implications in breast carcinoma, *Cancer,* 2001, vol. 91, no. 4, pp. 672–678. https://doi.org/10.1002/1097-0142(20010215)91:4<672::AID-CNCR1050>3.0.CO;2-J
19. DeMichele, A., Martin, A.M., Mick, R., Gor, P., Wray, L., Klein-Cabral, M., et al., Interleukin-6-174G–>C polymorphism is associated with improved outcome in high-risk breast cancer, *Cancer Res.,* 2003, vol. 63, no. 22, pp. 8051–8056. http://cancerres.aacrjournals.org/content/63/22/8051.full-text.pdf. Accessed August 12, 2019.

20. Duggan, C., Baumgartner, R.N., Baumgartner, K.B., Bernstein, L., George, S., Ballard, R., et al., Genetic variation in TNFα, PPARγ, and IRS-1 genes, and their association with breast-cancer survival in the HEAL cohort, *Breast Cancer Res. Treat.,* 2018, vol. 168, no. 2, pp. 567–576. https://doi.org/10.1007/s10549-017-4621-x

21. Korobeinikova, E., Myrzaliyeva, D., Ugenskiene, R., Raulinaityte, D., Gedminaite, J., Smigelskas, K., and Juozaityte, E., The prognostic value of IL10 and TNF alpha functional polymorphisms in premenopausal early-stage breast cancer patients, *BMC Genet.,* 2015, vol. 16, p. 70. https://doi.org/10.1186/s12863-015-0234-8

22. Park, H.S., Cho, U., Im, S.Y., Yoo, C.Y., Jung, J.H., Suh, Y.J., and Choi, H.J., Loss of human leukocyte antigen class I expression is associated with poor prognosis in patients with advanced breast cancer, *J. Pathol. Transl. Med.,* 2019, vol. 53, no. 2, pp. 75–85. https://doi.org/10.4132/jptm.2018.10.11

23. Axelrod, M.L., Cook, R.S., Johnson, D.B., and Balko, J.M., Biological consequences of MHC-II expression by tumor cells in cancer, *Clin. Cancer Res.,* 2019, vol. 25, no. 8, pp. 2392–2402. https://doi.org/10.1158/1078-0432.CCR-18-3200

24. Simpson, P.D., Moysi, E., Wicks, K., Sudan, K., Rowland-Jones, S.L., McMichael, A.J., et al., Functional differences exist between TNFα promoters encoding the common –237G SNP and the rarer HLA-B*5701-linked A variant, *PLoS One,* 2012, vol. 7, no. 7, p. e40100. https://doi.org/10.1371/journal.pone.0040100

25. Raposo, R.A., Abdel-Mohsen, M., Holditch, S.J., Kuebler, P.J., Cheng, R.G., Eriksson, E.M., et al., Increased expression of intrinsic antiviral genes in HLA-B*57-positive individuals, *J. Leukocyte Biol.,* 2013, vol. 94, no. 5, pp. 1051–1059. https://doi.org/10.1189/jlb.0313150

26. Ng, J.C.F., Quist, J., Grigoriadis, A., Malim, M.H., and Fraternali, F., Pan-cancer transcriptomic analysis dissects immune and proliferative functions of APOBEC3 cytidine deaminases, *Nucleic Acids Res.,* 2019, vol. 47, no. 3, pp. 1178–1194. https://doi.org/10.1093/nar/gky1316

27. Tokunaga, E., Yamashita, N., Tanaka, K., Inoue, Y., Akiyoshi, S., Saeki, H., et al., Expression of APOBEC3B mRNA in primary breast cancer of Japanese women, *PLoS One,* 2016, vol. 11, no. 12, p. e0168090. https://doi.org/10.1371/journal.pone.0168090

28. Sieuwerts, A.M., Schrijver, W.A., Dalm, S.U., de Weerd, V., Moelans, C.B., Ter Hoeve, N., et al., Progressive APOBEC3B mRNA expression in distant breast cancer metastases, *PLoS One,* 2017, vol. 12, no. 1, p. e0171343. https://doi.org/10.1371/journal.pone.0171343

29. Kanu, N., Cerone, M.A., Goh, G., Zalmas, L.P., Bartkova, J., Dietzen, M., et al., DNA replication stress mediates APOBEC3 family mutagenesis in breast cancer, *Genome Biol.,* 2016, vol. 17, no. 1, p. 185. https://doi.org/10.1186/s13059-016-1042-9

Translated by A. Barkhash