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The Matrix Metalloproteinase-21 Gene 572C/T Polymorphism and the Risk of Breast Cancer

ELENA I. SHAGISULTANOVA[1], INNA A. NOVIKOVA[2], YURI S. SIDORENKO[2], GEORGE N. MARCHENKO[1], ALEX Y. STRONGIN[1] and SERGEI R. MALKHOSYAN[1]

[1]*The Burnham Institute, 10901 North Torrey Pines Road, La Jolla, CA 92037, U.S.A.;*
[2]*Rostov Oncological Institute, 14-ya Liniya, 63, Rostov-on-Don, 334037, Russia*

Abstract. *Background: Matrix metalloproteinases (MMPs) contribute in multiple ways to all stages of tumor development, and a number of DNA polymorphisms in the MMP genes are associated with an increased risk of cancer. We previously identified the MMP-21 gene 572C/T polymorphism leading to Ala191Val substitution within the enzyme's catalytic domain. We performed a case-control study to test association between this polymorphism and the risk of breast cancer. Patients and Methods: 572C/T polymorphism was analyzed by RFLP method in 396 unrelated Russian females: 76 breast cancer patients and 320 disease-free blood donors. Results: The frequencies of C/C, T/C and T/T genotypes in patients (69.7%, 25.0% and 5.3%) did not differ significantly form those in controls (61.9%, 34.7% and 3.4%); the polymorphism was not associated with the increased tumor size and the presence of metastases. Conclusion: The MMP-21 gene 572C/T polymorphism has no significant effect on the development and progression of breast cancer.*

Matrix metalloproteinases (MMPs) are enzymes directly involved in the turnover and degradation of the extracellular matrix and the basement membrane. Ample evidence indicates that MMPs synthesized either by tumor cells or surrounding host cells contribute in multiple ways to all stages of malignant progression, including tumor invasion, blood vessel penetration, metastases and tumor angiogenesis (1). Furthermore, MMPs can cleave surface adhesion receptor molecules, such as tumor suppressor E-cadherin, and release active growth and angiogenic factors from the cell surface and extracellular matrix (2). Thus, MMPs contribute to tumor initiation by stimulating cell signaling. It has been found that MMPs are consistently up-regulated in all epithelial cancers (1).

A number of DNA sequence polymorphisms have been identified for different MMPs, some of which were linked to an increased risk of cancer (3-5). In particular, a single guanine insertion polymorphism in the MMP-1 gene promoter region creates a binding site for Ets family transcription factors, causing an increase in transcriptional activity. This polymorphic locus is associated with the risk of lung cancer and metastatic colorectal cancer (3, 4). A single adenine insertion/deletion polymorphism (5A/6A) in the MMP-3 gene promoter region is linked to the enhanced transcription and the risk of metastatic breast cancer (5).

We have recently characterized the gene and the protein of a novel human MMP-21 (6). The MMP-21 gene consists of seven exons and is located on the chromosome 10q26.2. It is expressed in a variety of fetal and adult normal tissues, as well as in some tumor tissues and tumor cell lines, including breast, ovary, lung and prostate carcinomas, epidermoid carcinomas and osteosarcomas (6, 7).

There is a 572C/T single nucleotide polymorphism in exon 2 of the MMP-21 gene that leads to an Ala191 to Val191 substitution within the N-terminal part of the enzyme's catalytic domain (6, 7). Despite a structural similarity between the Ala and Val aminoacids, Ala to Val mutations can negatively affect the protein functionality, *e.g.*, cause inherited disorders: hemophilia A and B, cystic fibrosis and phenylketonuria (8-11). We hypothesized that because the 572C/T polymorphic site is located within the MMP-21 gene region that encodes the catalytic domain, the Ala to Val substitution can cause an alteration of the protein enzymatic properties, which, in turn, can affect the development and progression of malignancy.

Here we report the results of a case-control study designed to test a putative association between the MMP-21 gene 572C/T polymorphism and breast cancer occurrence and progression.

Correspondence to: Sergei R. Malkhosyan, PhD, The Burnham Institute, 10901 North Torrey Pines Road, La Jolla, CA 92037, U.S.A. Tel: (858) 7136275, Fax: (858) 6463190, e-mail: smalkhosyan@burnham.org.

Key Words: Matrix metalloproteinases, MMP-21, polymorphism, breast cancer.

Table I. *Characteristics of the breast cancer patients and the disease-free individuals.*

	Cases	Controls
Number of subjects	76	320
Age (mean±SE), years	54.7±1.4	36.9±0.6
Age range, years	29-82	18-59
Clinical stage:		
stage I	41	
stage IIa	30	
stage IIb	3	
stage III	1	
stage IV	1	
Histopathologic type of the tumor:		
invasive ductal carcinoma	60	
invasive lobular carcinoma	7	
invasive mucinous carcinoma	4	
invasive papillary carcinoma	4	
invasive tubular carcinoma	1	
Histopathologic grade of the tumor:		
moderately-differentiated	76	
Presence of metastases:		
localized tumors (no metastases)	68	
metastatic tumors (lymph node or distant metastases)	8	

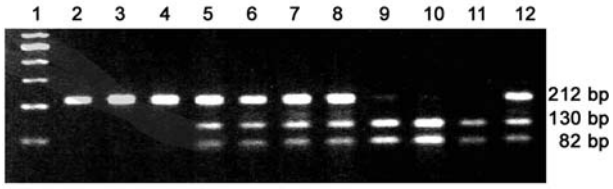

Figure 1. *RFLP analysis of 572C/T polymorphism of MMP-21. Lane 1, GeneRuler 100-bp DNA ladder; lanes 2-4, T/T genotype; lanes 5-8 and 12, T/C genotype; lanes 9-11, C/C genotype.*

Patients and Methods

The study included 396 unrelated Caucasian females (Russians): 76 breast cancer patients and 320 disease-free individuals (Table I), recruited at the Rostov Oncological Institute (Rostov-on-Don, Russia). The patients underwent surgical treatment at the Breast Surgery Division and had a histopathologically confirmed diagnosis of invasive breast carcinoma; the controls were recruited among the disease-free blood donors. Informal consent was obtained from all participants in the study. Genomic DNA was extracted according to standard phenol-chloroform technique from the normal breast tissue of the breast cancer patients and from the blood of the control individuals.

The 572C/T single nucleotide polymorphism is located within a SacII restriction endonuclease recognition site. The restriction fragments length polymorphism (RFLP) method was used to discriminate between the T (SacII resistant) and C (SacII sensitive) alleles. The fragment of the MMP-21 gene sequence that contains the polymorphic site was amplified by PCR. The PCR primers, forward 5'-CGGGCGCCGCTGTCCTTGTC-3' and reverse 5'-GCCAGGTCCTCGCGGAAGTCCAG-3', were designed with the PrimerSelect software (DNASTAR Inc., Madison, WI, USA). PCR was performed in a 10 μl reaction containing 1 U of Taq DNA polymerase, 1 x Taq PCR buffer, 2.5 mM of $MgCl_2$, 125 μM of each dNTP (Sigma, St. Louis, MO, USA), 1.0 μl of GC-melt (Clontech, Palo Alto, CA, USA), 0.14 μM of each primer and 40 ng of genomic DNA. Amplification was done using a Robocycler PCR machine (Stratagene, La Jolla, CA, USA). The following PCR cycles were used: 94˚C, 4 min; 33 times of 95˚C, 30 sec and 72˚C, 1 min; 72˚C, 7 min.

Ten μl of each PCR product was digested with 14 U of SacII restriction endonuclease (New England Biolabs, Beverly, MA, USA) at 37˚C for 16 h. The restriction products were separated on a 2.0% agarose gel, stained with ethidium bromide and visualized in UV light (Figure 1).

The genotype and allele frequencies were compared by two-sided Fisher exact test $p(F_2)$, and odds ratio (OR) with 95% confidence interval (CI) using the Statview 5.0.1 software (SAS). Because of the difference between the average age of the patients and the controls, the ORs and the corresponding CIs were adjusted for age using the logistic regression model. In the breast cancer patients, the average tumor size was calculated as a mean±SE for T allele carriers (either the T/T or the T/C genotype) and for individuals with the C/C genotype. These values were compared by the *t*-test.

Results and Discussion

The C/C, T/C and T/T genotypes were found in the breast cancer patients with frequencies of 69.7%, 25.0% and 5.3%, respectively; the corresponding values for the disease-free individuals were 61.9%, 34.7% and 3.4% (Table II). The observed genotype frequencies within the female control group complied with the Hardy–Weinberg proportions ($p=0.752$). The frequency of the T allele did not differ significantly between the patients and the controls: 17.8% and 20.8%, respectively; $p(F_2)=0.434$. A logistic regression analysis indicated that neither the T/C genotype (OR=0.52, 95% CI 0.25-1.09) nor the T/T genotype (OR=1.18, 95% CI 0.27-5.27) was associated with the risk of breast cancer. No significant difference in the frequency of T allele carriers (either the genotype T/T or T/C) was found between the patients and the control individuals (OR=0.58, 95% CI 0.29-1.16).

We hypothesized that, because MMPs play a role in tumor invasiveness and angiogenesis, the 572C/T polymorphism of the MMP-21 gene can influence breast cancer progression, even though it showed no association with breast cancer incidence. Thus, we tested a putative association between the 572C/T polymorphism and clinico-

Table II. *Genotype and allele frequencies of 572C/T polymorphism in breast cancer patients and control individuals.*

572C/T	Cancer patients (N=76)		Control individuals (N=320)		$p(F_2)$[a]	OR (95%CI)[b]
	n	(%)	n	(%)		
Genotype						
C/C	53	(69.7)	198	(61.9)	0.234	1 (ref)
T/C	19	(25.0)	111	(34.7)	0.135	0.52 (0.25-1.09)
T/T	4	(5.3)	11	(3.4)	0.501	1.18 (0.27-5.27)
T/T + T/C	23	(30.3)	122	(38.1)	0.234	0.58 (0.29-1.16)
Allele						
T	27	(17.8)	133	(20.8)	0.434	
C	125	(82.2)	507	(79.2)	0.434	

[a]$p(F_2)$, two-sided Fisher exact test
[b]OR and 95% CI were adjusted for age and calculated using logistic regression model with the C/C genotype as a reference category

pathological parameters of the tumors. Although the average tumor size of the T allele carriers was higher than that of the patients with the C/C genotype (2.56±0.39 cm *vs.* 2.02±0.13 cm), the difference was not statistically significant (*t*-value of difference 1.299, two-sided *p*-value 0.205). Twenty percent (4 out of 20) of the tumors derived from the T-allele carriers were metastatic, whereas only 8.3% (4 out of 48) of the tumors obtained from the individuals with C/C genotype had metastases. However, this difference did not reach statistical significance ($p(F_2)$=0.253, OR=2.55, 95% CI 0.52-12.50).

In conclusion, the current study is the first to explore a relationship between the structural polymorphism of the MMP-21 gene and the risk of cancer development. Our data showed that the 572C/T polymorphism of the MMP-21 gene has no significant effect on the development and progression of breast cancer.

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