DNA Methyltransferase *DNMT1* rs2228611 and rs2228612 polymorphisms and their effect on breast cancer pathomorphological characteristics and patient prognosis

6th Kaunas / Lithuania International
Hematology / Oncology Colloquium
28 May 2021

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Objective

Breast cancer is the most frequently cancer and the leading cause of cancer related death among women worldwide. The number of new cases and mortality is expected to grow rapidly with population growth. Furthermore, populations adopt lifestyle behaviors that are known to increase cancer risk, such as smoking, physical inactivity, excess body weight and poor diet, different reproductive patterns and et. There is still a need of biomarkers, which could be used for disease phenotype prognostification and for evaluation of the disease outcomes.

Epigenetic regulation plays a major role in supervising the cellular RNA expression patterns, which are important for the normal biological functions in multicellular organisms. DNA methylation is one of epigenetic modification, it has a role in genomic imprinting, X chromosome inactivation, regulation of gene expression and tumorigenesis. DNA methyltransferases (DNMTs) have key role in establishing and maintaining DNA methylation patterns. Abnormal DNA methylation patterns are present in the process of malignant transformation. The aim of this study was to identify DNA sequence variation in *DNMT1* and to analyse their effect on tumor phenotype and breast cancer patient prognosis.

Methods

- In this study there were 100 participators with breast cancer.
- The study research protocol was approved by Kaunas Regional Biomedical Research Ethical Committee (protocol number BE-2-10 and BE-2-10/2014).
- Patient peripheral blood samples were used for genomic DNA extraction. Polymerase chain reaction restriction fragment length polymorphism analysis (PCR-RFLP) was performed for rs2228611 and rs2228612 polymorphism testing (Figure 1-2)
- The patient's clinical information was collected from medical documentation.
- The associations between analyzed SNPs and tumor pathomorphological parameters and the cause of the disease were investigated. The statistical data analysis was performed with SPSS program.

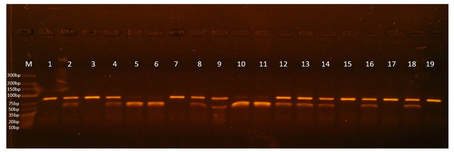

Figure 1. Agarose gel electrophoresis of PCR-RFLP product for DMNT1 (rs2228611)
•Lane M - DNA molecular marker GeneRuler Ultra low range DNA ladder (Thermo Fisher Scientific Baltics, Lithuania); Lanes 1,3,7,15,17 and 19 - AA genotype; Lanes 2,4,8,9,12,13,14,16 and 18 - AG genotype; Lanes 5,6,10 and 11 - GG genotype.

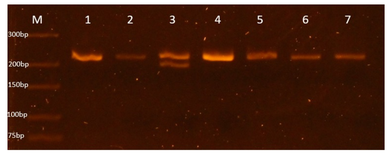

Figure 2. Agarose gel electrophoresis of PCR-RFLP product for DMNT1 (rs2228612)
•Lane M - DNA molecular marker GeneRuler Ultra low range DNA ladder (Thermo Fisher Scientific Baltics, Lithuania); Lanes 1,2,4,5,6, and 7 - AA genotype; Lane 3 - AG genotype.

Results

- In the rs2228612 polymorphism analysis, the A allele (94.6%) was more frequent than the G allele (5.4%). The distribution of genotypes was as followed: AA - 89.1% and AG - 10.9%. In rs2228611 polymorphism the A and G alleles had almost the same frequencies: A allele - 49%, G allele- 51%. The distribution of genotypes was as followed: AA - 24.3%, AG - 49.5%, GG - 26.2%.
- In the association analysis it was determined that patients with *DNMT1* (rs2228611) polymorphism AG and GG genotypes had lower probability of tumor vascular infiltration than patients with AA genotype. In *DNMT1* (rs2228612) polymorphism analysis the association between G allele and lymph node status was observed. The carriers of G allele were 5.3 times more likely to have positive lymph nodes than the non-carriers of G allele.
- None of the polymorphisms showed any significant association with overall survival (OS), progression-free survival (PFS) and metastasis-free survival (MFS).

Dependent	SNP	Covariant	Model No.1			Model No.2		
			Odds	95% CI	p	Odds	95% CI	p
Positive vascular infiltration	*DMNT1* (rs2228611)	Positive ER vs negative	2.126	0.851-5.313	0.106	0.826	0.258-2.643	0.747
		Positive PR vs negative	0.418	0.175-1.000	0.050	1.011	0.319-3.200	0.986
		Positive HER2 vs negative	0.935	0.429-2.040	0.867	0.726	0.240-2.194	0.570
		Age at the time of diagnosis	1.011	0.996-1.026	0.162	0.975	0.952-0.999	0.045
		AG versus AA	0.261	0.133-0.515	**0.000**	0.239	0.093-0.615	**0.003**
		GG versus AA	0.529	0.247-1.134	0.102	0.315	0.107-0.927	**0.036**
		Tumor size T1+T2 vs T3+T4				2.349	1.005-5.492	0.049
		Positive lymph nodes involvement vs negative				0.245	0.084-0.710	0.010
		Tumor grade G1+G2 vs G3+G4				0.716	0.251-2.043	0.533
		Positive lymphatic infiltration vs negative				64.569	19.850-210.036	0.000

Table 1. Multivariate logistic regression analysis. The adjusted odds ratio for associations between SNP and tumor vacular infiltration.

Dependent	SNP	Covariant	Model No.1			Model No.2		
			Odds	95% CI	p	Odds	95% CI	p
Positive lymph nodes involvement	*DMNT1* (rs2228612)	The carriers of G allele versus the non-carriers	4.065	1.517-10.891	**0.005**	5.300	1.471-19.099	**0.011**
		Positive ER vs negative	3.282	1.308-8.237	0.011	1.581	0.492-5.081	0.442
		Positive PR vs negative	0.447	0.190-1.049	0.064	1.252	0.413-3.796	0.692
		Positive HER2 vs negative	1.591	0.732-3.458	0.241	2.042	0.738-5.646	0.169
		Age at the time of diagnosis	0.956	0.935-0.978	0.000	0.903	0.870-0.937	0.000
		Tumor size T1+T2 vs T3+T4				4.807	2.118-10.909	0.000
		Tumor grade G1+G2 vs G3+G4				0.736	0.263-2.059	0.559
		Positive N vs negative				30.718	9.368-100.725	0.000
		Positive V vs negative				0.593	0.195-1.806	0.358

Table 2. Multivariate logistic regression analysis. The adjusted odds ratio for associations between SNP and lymph node status.

Conclusions

Our results suggest that *DNMT1* rs2228612 and rs2228611 polymorphisms are important for breast cancer development and tumor spread. More research on the subject is needed as it can provide us with additional information on prognostic and predictive value of studied polymorphisms for more individualized patient approach.

Key words: breast cancer, polymorphisms, *DNMT1*.