

Malat1 RNA Genetic Factor and T1 Bladder Cancer

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ABSTRACT

Bladder cancer is the most common malignant tumor of the urinary tract. This disease has a pronounced tendency to relapse and progress, is characterized by a severe course and a high degree of disability. Non-invasive bladder cancer - superficial tumors of the mucous membrane of the bladder with possible germination in the submucosal layer, but without muscle invasion (pTa and pT1). Invasive bladder cancer - with germination in the muscle layer (\geq pT2). Currently, there is an extensive literature on genetic data with RMP T2-T4, but there is not enough information about the early form of RMP - T1. It was discovered that almost 90% of the human genome is actively transcribed, while only 2% are protein-coding genes, and the majority of the transcripts are non-coding RNAs. Molecules of non-coding RNA, depending on their size, are divided by length. A lot of data has been published regarding the effect of short variants of these molecules on the development of oncopathology by inhibiting mRNA expression. At the same time, we did not find large studies devoted to the study of the influence of long non-coding RNAs. Also now, the MALAT1 dnaRNA is attracting the attention of scientists. Concurrently, researchers are particularly interested in studying the relationship of single nucleotide polymorphisms of the MALAT1 gene with the appearance of oncopathologies of different localization, as well as the study of its association with different characteristics and stages of the tumor process. In this study, we collected overview data regarding the possible association of rs 3200401 polymorphism of the MALAT1 gene in patients with a single focus and multifocal bladder cancer stage T1.

Keywords: bladder cancer, genetic polymorphism, non-coding RNA, multifocality.

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INTRODUCTION

Popularization of next-generation sequencing methods has allowed for large-scale analysis of genetically diverse tumors.

In these terms, bladder cancer is the most common malignant tumor of the urinary tract. This disease has a pronounced tendency to relapse and progress, is characterized by a severe course and a high degree of disability.

Traditionally, bladder tumors are classified into superficial and muscle-invasive, which have a pronounced biological heterogeneity. These differences are determined not only by the morphological variant and degree of tumor invasion, but also by the molecular profile, as well as genetic aberrations. The data of large-scale studies of the Beijing Institute of Genomics (Rouprêt et al. 2018) and the Cancer Genome Atlas, TCGA (Babjuk et al. 2018), allowed us to expand our understanding of the genetic basis of muscle-invasive bladder carcinomas. It was found that during T2-T4 carcinogenesis mainly those genes mutate whose products regulate the cell cycle: TP53 (the “genome keeper” is right on cue) and RB1 (the retinoblastoma oncosuppressive protein encoding gene), CDKN1A, CDKN2A (products of these genes are inhibitor proteins of 1A and 2A cyclin-dependent kinases), CCND1 (encodes cyclin D1, the cell cycle regulator during the transition from G1 to S-phase), MDM2 (its product is a p53-specific E3 ligase that negatively regulates p53 by proteasome

degradation and decreased transcriptional activity) (Rouprêt et al. 2018; Babjuk et al. 2018). The probable pathways of carcinogenesis, considering the system of genetic regulation, are presented in Fig. 1.



FIGURE 1: Pathways for carcinogenesis of bladder cancer (Mariappan et al. 2010)

Non-invasive bladder cancer is superficial tumors of the mucous membrane of the bladder with possible submucosal but not muscle invasion (pTa and pT1).

Invasive bladder cancer - with muscle invasion (\geq pT2). In non-invasive tumors, a high frequency of FGFR3 mutations and RAS family genes was noted:

FGFR3 (fibroblast growth factor receptor 3) is a gene encoding the fibroblast growth factor receptor 3. Its overexpression in the non-invasive bladder cancer provides the activity of the RAS/RTK/RAF cascade with amplification of mitogenic signals and proliferation of cell clone.

TERT is a gene encoding telomerase reverse transcriptase (TERT, telomerasereversetranscriptase), which is part of the telomerase enzyme. High telomerase activity leads to stable cell survival.

Loss of heterozygosity (LOH) of the chromosome region 9q. The essence of LOH is the loss (structural or functional) of one of the alleles of the heterozygous genotype. Loss of the allele allows fatal recessive mutations to occur in the remaining allele.

RAS/RAF/RTK signaling pathway is a protein and kinase chain: rat sarcoma protein (RAS), mitogen activated protein kinase (RAF), tyrosine kinase growth factor receptors (RTK). This cascade controls cell proliferation, cell cycle and migration ability.

KDM6A is a gene encoding an enzyme – histone demethylase, which cleaves methyl groups from trimethylated and dimethylated lysine. PIK3CA – phosphatidylinositol-3-kinase, which promotes the transmission of AKT signals (cell survival signaling pathway), enhances growth, protein synthetic activity and resistance to apoptosis.

STAG2 is a gene encoding stromal antigen 2 or a subunit of a cohesin complex. It controls the process of separation of sister chromatids in cell division.

CDKN2A is an inhibitor of cyclin-dependent kinase 2A.

TP53 is an oncosuppressive gene for TP53 transcription factor. Loss of heterozygosity (LOH) of the chromosome region 9p.

RB1 is a gene encoding a retinoblastoma protein (oncosuppressor).

PTEN (homologue of phosphatase and tensin) is a tumor suppressor encoding gene. Its activity inhibits the cellular pathway of AKT survival by dephosphorylation of signaling molecules.

KMT2D is a gene producing histone lysine N-methyltransferase 2D. Its function is epigenetic activation of transcription (Veskimäe et al. 2017; Guyatt et al. 2008a; Sarafraz et al. 2018; Ece & Tünay 2018)

Literature provides extensive genetic data on T2-T4 bladder cancer, but a few information about the early form of bladder cancer (T1 bladder cancer). Today it is established that almost 90% of the human genome is actively transcribed, while only 2% are protein-coding genes. Accordingly, a large particle of transcripts is non-coding RNA (RNA), which regulate the expression of more than 75% of human genes (Veskimäe ET AL. 2017). Non-coding RNA molecules, depending on their size, are divided

into short (less than 200 nucleotides) and long (more than 200 nucleotides). At that time, quite a few data were published regarding the effect of short variants of these molecules, especially microRNA, on the development of oncopathology by inhibiting the expression of mRNA (mRNA); the effect of long non-coding RNA is less studied and less understood (Guyatt ET AL. 2008a).

Scientists are particularly interested in long non-coding RNAs (MALAT1) (metastasis associated lung adenocarcinoma transcript), also known as NEAT2 (noncoding nuclear-enriched abundant transcript 2) (Guyatt et al. 2008b).

MALAT1 was first identified in 2003 in non-small cell lung cancer cells, where an excessive level of its expression was observed (Guyatt et al. 2008c).

A number of works have been published on the association of changes in MALAT1 expression with the onset of various types of cancer, including breast cancer, endometrial cancer, cervical cancer, liver cancer, bladder cancer, neuroblastoma, osteosarcoma, prostate cancer, pancreatic cancer, stomach cancer, and lung cancer (Howick et al. 2009). Rare cases of chromosomal translocations involving MALAT1 have been reported in kidney carcinoma cells (Guyatt ET AL. 2008a). Along with this, the researchers are particularly interested in studying the single nucleotide polymorphisms of MALAT1 with the onset of oncopathologies of different localization, as well as the study of its association with different characteristics and stages of the tumor process (including metastasis).

The objective of this study is to search for a possible relation of rs 3200401 polymorphism of MALAT1 in patients with a single focus and T1 multifocal bladder cancer.

MATERIAL AND METHODS

The research material was venous blood of 45 men with T1 transitional bladder cancer (average age [\pm SD] 65.40 \pm 11.3 years).

All patients had concomitant benign prostatic hyperplasia, I degree, without chronic prostatitis and age-related androgen deficiency.

All patients were examined and/or treated from 2011 to 2017 in the urology unit of the Regional State Budgetary Healthcare Institution “Belgorod City Hospital No.2”. Twenty patients had a single tumor focus, 25 patients had multiple foci of bladder cancer.

The final morphological diagnosis of transitional bladder cancer was established according to the recommendations of the European Association of Urology. All patients had clinical stage I cancer according to the TNM classification of malignant tumors. The study group excluded individuals with type 2 diabetes mellitus, a hereditary pathology, diseases of unknown etiology, and with tumors of other locations.

The study protocol was approved by the Ethics Committee of the Medical Institute of the Belgorod State University (No. 3/5/12/11) and complied with the Helsinki declaration. All participants provided their written informed consent.

For genotyping, whole venous blood was sampled in 2.7 ml containers with the addition of 11.7 mM EDTA (Sarstedt, Germany). DNA was extracted from the blood by phenol-chloroform extraction (Mathew, 1984) in two stages.

The distribution of alleles of the polymorphic site rs3200401 of MALAT1 was determined using polymerase chain reaction (PCR) of DNA synthesis. Polymerase chain reaction (PCR) was carried out on an IQ5 amplifier by Bio-Rad using DNA polymerase *Thermus aquaticus* by Sileks-M (Sileks-M), and oligonucleotide primers and probes synthesized by Syntol. Genotyping was carried out by discrimination of alleles.

During polymerase chain reaction in a thermocycler with fluorescence detection (using an IQ5 thermocycler), genotyping was carried out using the TagMan method according to the RFU (relative fluorescence level) values of each probe.

Amplification of the desired region of MALAT1 containing the rs3200401 polymorphic locus consisted of 50 cycles: initial denaturation – 95°C (20 sec), denaturation – 95°C (30 sec) hybridization and elongation – 60.0°C (30 sec). Analysis of the data obtained during the polymerase chain reaction was carried out using 7500 Fast Realite PCR Software.

Statistical data analysis was performed using the SPSS software package (version 17.0). The correspondence of the allele distribution with the rs3200401 Hardy-Weinberg equilibrium locus and the distribution of genotypes by the

studied locus in different groups were compared using the Pearson criterion χ^2 . In order to determine the risk of metastasis in patients with transitional bladder cancer depending on the specific genotype of the rs 3200401-site, the odds ratio (OR) and 95% confidence interval (CI) were calculated using different logistic regression models and binary logistic regression. To analyze the relationship of the rs 3200401 locus of MALAT1 with the risk of metastasis of transitional cell carcinoma of the bladder considering the age and gender of the patients, their smoking alcohol abuse habits, multivariate logistic regression was used. A value of $P > 0.05$ was considered statistically significant.

RESULTS

Table 1 presents the clinical characteristics of patients with T1 transitional cell bladder carcinoma with monolocalization and polyfocality. The groups did not differ in terms of age ($P = 0.631$) and the number of smokers ($P = 0.935$). However, the number of alcohol abusers was significantly higher among patients with single tumors ($P = 0.021$).

As a result of the genotyping of the examined patients with the polymorphic site rs3200401 of MALAT1, the distribution of C and T alleles and three different variants of the genotypes – SS, CT, and TT (Table 2) were obtained. Allele frequencies in both groups corresponded to Hardy Weinberg equilibrium ($P > 0.05$). A comparative analysis of the distribution of genotypes at the rs3200401 locus of MALAT1 between patients with monolocalization and polyfocality showed the absence of a significant difference, nevertheless, the parameter P was close to the level of statistical significance ($P = 0.074$). Moreover, the frequencies of the T and C alleles differed significantly between the control groups ($P = 0.015$).

Table 1: Distribution of alleles and genotypes with rs3200401-polymorphism of MALAT1 among patients with transitional bladder cancer in relation to single/multifocal T1 urinary bladder tumors

	Single	Multiple	P		
	(n = 20)	(n = 25)	n	%	
Genotypes					
CC	15	65	14	83	0.075
CT	6	27.0	7	22	
TT	2	17	6	18	
Alleles					
C	19	82	24	89	0.017
T	3	21	5	14	

Note: *n* is the number of patients; *P* is an indicator of statistical reliability.

Table 3 shows the results of an aggression analysis to establish the relative risk of polyfocality in patients with transitional bladder cancer, depending on the specific genotype rs3200401 of MALAT1. A significant relationship was identified in the framework of the dominant model ($P_{obs. (observed)} = 0.048$). It was found that carriers of minor

T-allele with transitional cell carcinoma of the bladder have 2.2 times higher risk of polyfocality than patients with the CC genotype (OR comp. = 2.207; 95% CI = 1.006-4.844). Nevertheless, as adjusted for age, the presence of smoking and alcohol abuse habits, their statistical significance was lost ($P_{adj.} = 0.279$; OR adj. = 0.678; 95% CI = 0.335-1.371).

Table 2: Analysis of the genotypic association of the rs3200401 site of MALAT1 with a risk of polyfocal development in patients with transitional cell bladder cancer

Model	P observed	OR observed (95% CI)	P _{adjustment}	OR _{adjustment} (95% CI)
Dominant	0.049	2.211 (1.006-4.844)	0.283	0.680 (0.338-1.375)
recessive	0.1516	5.824 (0.650-50.22)	0.088	0.384 (0.374-2.653)
supra-dominant	0.833	1.074 (0.556-2.083)	0.884	1.054 (0.527-2.113)

Note: TCBC - transitional cell bladder cancer; R obs. - the observed value of P non-adjusted for covariates; OR obs. - the observed odds ratio; P_{adjustment} - P indicator after adjusting for age, alcohol abuse and smoking habit; OR_{adjustment} - odds ratio after adjustment for covariates; 95% CI - 95% confidence interval.

The MALAT1 dnRNA gene is localized on 11 chromosomes (11q13.1), consists of 8708 base pairs and contains 2 exons (Witjes et al. 2017). 5558 polymorphic sites of MALAT1 are currently known. One of the most studied in terms of the association with the onset of oncological pathologies is the polymorphic locus rs3200401.

In 2003, long noncoding RNA – MALAT1 was identified as a transcript associated with metastasis in patients with early stage non-small cell lung cancer (Guyatt et al. 2008b). It is believed that the main function of MALAT1 is to regulate the expression of genes whose products are involved in the formation of metastases (Ferlay et al. 2013). Zhangetal *et al.* showed MALAT1 expression to correlate with tumor sizes and stage in lung cancer patients (Burger et al. 2013). Along with this, its leading role in the processes of alternative splicing and epigenetic modulation of gene expression has been proven (Ferlay et al. 2012).

SUMMARY

Wang *et al.* showed that patients with lung cancer who were carriers of T-allele with MALAT1 rs3200401 polymorphism had a significantly longer average life expectancy than homozygous CC patients (Chavan et al. 2014). Peng *et al.* showed that heterozygous CT women with the polymorphic locus rs3200401 have a lower risk of breast cancer when compared with dominant CC homozygotes (Peng et al. 2017).

The results of this work, on the contrary, showed that in patients with transitional cell bladder cancer, minor T-allele with MALAT1 rs3200401 polymorphism increases the risk of multifocal development. Nevertheless, the analysis under adjustment for other risk factors for the development of the tumor process did not show a reliable relationship.

Thus, according to the results of the studies, we can state that the studied population has a relationship between the MALAT1 rs3200401 polymorphism and the development of multiple foci in patients with T₁ transitional bladder cancer. And carriers of minor T-allele have a higher risk of T₁ multifocal bladder cancer in comparison with homozygotes with the main C-allele.

CONFLICT OF INTEREST

None

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