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Abstract **УДК 616.831-005.1-06:616.12-005.4:575.113.2(043.5)**

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ASSOCIATION OF MALAT1 RS3200401 GENE POLYMORPHISM WITH KIDNEY CANCER IN UKRAINIAN POPULATION

Introduction. As it is known, only 2 % of the human genome encodes proteins, while the greater part of the genome corresponds to non-coding sequences. In the recent years among a large variety of non-coding sequences special attention of researchers was drawn to long non-coding RNA MALAT1. This sequence first was detected in 2003 in non-small cell lung cancer cells. Today the results of numerous experiments revealed that *MALAT1* is one of the major genes involved in various types of cancer, including kidney cancer.

Purpose. To study the association between *MALAT1* rs3200401 polymorphism and kidney cancer development in Ukrainian population.

Materials and methods. 101 patients with kidney cancer (renal cell carcinoma) was enrolled into the study. The final diagnosis was based on anamnesis data, clinical, biochemical and instrumental examinations according to the recommendations of European Association of Urology. All participants were treated in the hospital of Sumy Regional Oncology Center. The control group included 100 healthy individuals without personal cancer history. The study of *MALAT1* rs3200401 SNP was perfprmed by Real-time PCR using 7500 Fast Real-time PCR System (Applied Biosystems, Foster City, USA) and Taq-Man Assays (Taq-Man®SNP Assay C_3246069_10). Statistical analyses were performed using Statistical Package for Social Science software (SPSS, version 17.0, Chicago, IL, USA) and online resource "SNIPKA".

Results. The distribution of CC-homozygotes, CT-heterozygotes and TT-homozygotes in patients with kidney cancer was 71 (70.3%), 29 (28.7 %) and 1 (0.99 %), respectively. In the control group, distribution of genotypes was 59 (59.0 %), 32 (32.0 %) and 9 (9.0 %), respectively. The significant difference in genotypes distribution between kidney cancer patients and the control group was found (P = 0.022). Genotypic analysis has shown that T-minor allele carriers had less risk of kidney cancer development compared to patients with CC-genotype (P = 0.031, OR = 0.101, 95 % CI = 0.013–0.814).

Conclusion. There is statistically significant link between *MALAT1* rs3200401 polymorphism and kidney cancer development in Ukrainian population. Individuals with minor T-allele (TT- and CT-genotypes) have less risk of renal cell carcinoma development compared to major homozygotes (CC).

Keywords: MALAT1, long non coding RNA, single nucleotide polymorphism, renal cell carcinoma.

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Резюме

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ЗВ'ЯЗОК ПОЛІМОРФІЗМУ RS3200401 ГЕНУ MALAT1 З РАКОМ НИРКИ В УКРАЇНСЬКІЙ ПОПУЛЯЦІЇ

Наведено результати вивчення поліморфізму rs3200401 гену MALAT1 у 101 хворого на рак нирки та 100 осіб контрольної групи. Виявлено статистично значиму різницю у розподілі генотипів за поліморфізмом rs3200401 гену MALAT1 у пацієнтів з раком нирки та осіб без цієї недуги. У носіїв мінорного алелю (генотипи T/T та C/T) за поліморфізмом rs3200401 гену MALAT1 ризик розвитку раку нирки менший у порівнянні з домінантними гомозиготами (C/C). Мінорний алель (T) є протективним.

Ключові слова: MALAT1, довга некодуюча РНК, однонуклеотидний поліморфізм, рак нирки.

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Introduction

Today more and more scientific articles report about various transcripts that do not encode amino acid sequences. In humans, about 20,000 genes encoding proteins have been detected. These genes presenting no more than 2% of the whole genome. Wherein almost 90% of our genome is actively transcribed. Thus, most of transcripts are represented by non-coding RNAs that regulate the expression of more than 70% human genes [1].

The non-coding RNA molecules, depending on their length, are divided into short (less than 200 nucleotides) and long (more than 200 nucleotides). While the influence of short versions of these molecules, especially miRNAs, on cancer development by inhibiting of RNA expression has already been described, the effect of long non-coding RNAs is less clear [2].

Particular attention is paid to MALAT1 transcript (metastasis associated lung adenocarcinoma transcript), also known as NEAT2 (noncoding nuclear-enriched abundant transcript 2), which belongs to class of long non-coding RNA [3]. *MALAT1* gene was firstly identified in 2003 in non-small cell lung cancer cells, which had the high level of *MALAT1* expression [4]. Numerous publications shown the link between *MALAT1* expression and various types of cancer development including endometrial cancer, breast cancer, cervical cancer, colorectal cancer, hepatocellular carcinoma, liver cancer, neuroblastoma, osteosarcoma, pancreatic cancer, prostate cancer, bladder cancer, stomach cancer, undifferentiated embryonic liver sarcoma and lung cancer [5]. Rare cases of chromosomal translocation involving MALAT1 have been reported in kidney carcinoma cells [6]. Along with this, studies about association of *MALAT1* gene polymorphisms with kidney cancer development are absent both in Ukrainian population and in other populations and ethnic groups of the world.

Objective. To study the association between *MALAT1* rs3200401 gene polymorphism and kidney cancer development in Ukrainian population.

Materials and methods. The study was conducted using venous blood of 101 kidney cancer (renal cell carcinoma) patients. The final diagnosis was established on the basis of anamnesis, clinical, biochemical and instrumental methods data in accordance with European Association of Urologists (EAU) recommendations. The control group consisted of 100 clinically healthy donors who had no oncological diseases in history.

The research was carried out in compliance with the basic provisions of Convention on Human Rights and Biomedicine, Declaration of Helsinki of the World Medical Association (1964, with subsequent amendments, including version 2000) and the Order of the Ministry of Health of Ukraine No. 690 dated 23.09. 2009. All participants signed an informed consent to participate in studies with subsequent venous blood using for genetic analysis.

Determination of MALAT1 rs3200401 gene polymorphism was carried out by real time polymerase chain reaction (Real-Time PCR) method using the TaqMan®SNP Assay C_3246069_10 components. Amplification mode: primary denaturation – 20 s at 95°C, denaturation – 30 s at 95°C, annealing with elongation –

30 seconds at 60°C (only 50 cycles). The amplification and results analysis were carried out using 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, USA) and 7500 Fast Real-time PCR Software.

Statistical data processing was performed using SPSS 17.0 software package and "SNIPKA" online resource (https://thething.shinyapps.io/SNPcalc). $P < 0.05$ was considered as significant.

Results. The distribution of rs3200401 genotypes in renal cell carcinoma patients and control group individuals corresponded to Hardy-Weinberg equilibrium (Table 1).

Table 1 – Frequency of *MALAT1* rs3200401 gene polymorphism alleles and genotypes in case and control groups

	Case	**Control**
Homozygotes CC, n (%)	71 (70.3)	59 (59)
Heterozygotes CT, n (%)	29 (28.7)	32 (32)
Homozygotes TT, n (%)	1 (1)	9 (9)
C-allele	0.85	0.75
T-allele	0.15	0.25
χ^2	1,12	2.15
P	> 0.05	> 0.05

Note: n is the number of patients, χ^2 and P represent the deviations of each group from Hardy-Weinberg equilibrium

Among kidney cancer patients the number of main allele homozygotes (CC) was 71 (70.3%), heterozygotes (CT) – 29 (28.7%), recessive allele homozygotes (TT) – 1 (1%). In control group the distribution of genotypes was as follows: main allele homozygotes (CC) – 59 (59%), heterozygotes (CT) – 32 (32%), minor homozygotes – 9 (9%). The alleles frequency was also calculated. In the case group the frequency of main and minor alleles was 0.85 and 0.15, respectively. In the control group the main allele frequency was 0.75, the minor allele frequency – 0.25. Comparative analysis showed that difference in genotypes distribution and alleles frequencies between two groups is statistically significant (P = 0.022 and P = 0.016, respectively) (Figure 1).

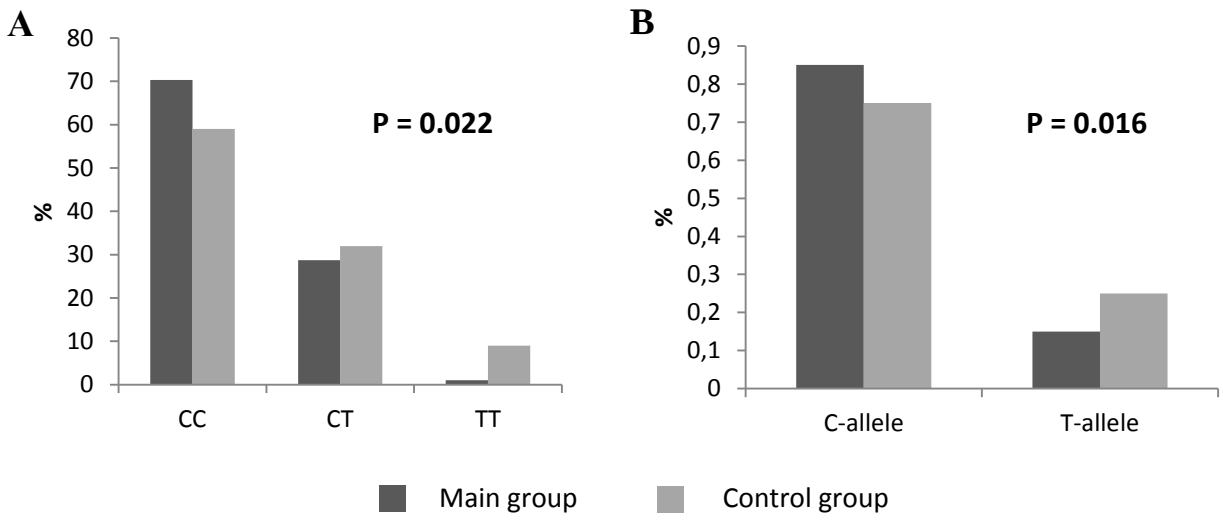

Figure 1 – Comparison of rs3200401 genotypes (A) and alleles (B) frequencies in main (dark columns) and control (light column) groups

In order to determine the risk of kidney cancer development depending on rs3200401 genotypes, the odds ratio (OR) and 95% confidence interval (CI) for the four main models of inheritance were calculated (Table 2). Recessive model had the lowest Aikaike index. According to this, the minor allele carriers (CT- and TT-genotypes) have the lower kidney cancer risk (P = 0.031, OR = 0.101,

95% CI = 0.013-0.814) compared to CC-homozygotes.

Discussion. MALAT1 is highly conserved long non-coding RNA, which firstly was associated with lung tumors with high metastasis propensity [4]. *MALAT1* gene is located on 11q13.1 and consists of 8,829 nucleotides (according to genecards.org). According to the base of single-nucleotide polymorphisms (dbSNP) there are 5558 single nucleotide polymorphisms within *MALAT1* gene [7].

MALAT1 gene is often associated with various tumors development. Hai-min Zhang et al. revealed that MALAT1 transcript expression correlates with size, stage of tumors and metastases in lymph nodes in patients with lung cancer. No reliable association with distant metastases was detected. It was also found that among patients with high *MALAT1* gene expression 16 had tumor size less than 4 cm and 30 had tumor size greater or equal to 4 cm. Among patients with low *MALAT1* expression 49 had tumor size less than 4cm, and 11 patients had tumor size greater or equal to 4 cm [8].

Table 2 – Analysis of *MALAT1* rs3200401 gene polymorphism association with kidney cancer under different models of inheritance

Model	P	OR (95% CI)	AIC
Dominant	0.095	0.608 (0.339-1.090)	21.58
Recessive	0.031	0.101 (0.013-0.814)	16.61
Overdominant	0.612	0.856 (0.469-1.563)	24.14
Additive[1]	0.362 0.026	0.753 (0.409-1.386) 0.092 (0.011-0.750)	17.78

Note: 95% CI – 95% confidence interval; OR – odds ratio; AIC – Akaike Information Criterion.
[1]The first line in additive model describes comparison of CT-genotype with CC-genotype, the second line – comparison of TT-genotype with CC-genotype

The genotyping of lung cancer patients for *MALAT1* rs3200401 polymorphism was performed by Wang et al. It was found that patients with TT- and CT-genotypes had significantly higher average life expectancy compared to homozygotes CC. Authors concluded T-allele is linked to longer life expectancy among patients with lung cancer [11]. Peng et al., studying the effects of several *MALAT1* gene polymorphisms on breast cancer development, found that rs3200401 heterozygotes (CT) had lower breast cancer risk compared to dominant homozygotes (CC) [12].

Our results revealed the statistically significant association between *MALAT1* rs3200401 gene polymorphism and kidney cancer development. It has been established that T-allele is protective, and T-allele carriers (CT and TT genotypes) have lower risk of kidney cancer development compared to homozygotes CC.

In recent years the role of long non-coding RNAs, in particular MALAT1, in cardiovascular diseases pathogenesis was actively studied. Qian Li et al. studied the association of *MALAT1* gene polymorphisms with congenital heart diseases development in Chinese population. Scientists have established the significant association between rs619586 polymorphism and risk of congenital heart diseases development. According to dominant model of inheritance patients with AG- and GG-genotypes had significantly lower congenital heart diseases risk compared to individuals with AA-genotype. Newborns with rs619586 GG-genotype also had a lower risk of congenital heart diseases development. The protective effect of this SNP under additive model was also significant. Wherein significant association between rs11227209, rs3200401 polymorphisms and congenital heart defects development was not detected [9]. Wang et al. demonstrated that rs619586G-allele is protective against coronary arteriosclerosis. Also statistical analysis showed that coronary artery atherosclerosis patients with rs11227209G-, rs619586G- and rs3200401T-alleles have lower total cholesterol level [10].

Conclusions

1. There is the link between MALAT1 rs3200401 gene polymorphism and kidney cancer development in Ukrainian population.

2. The carriers of MALAT1 rs3200401 gene polymorphism minor allele (genotype TT and CT) have the less risk of renal cell carcinoma development compared to homozygotes CC.

Prospects for further research

Today is shown that MALAT1 gene and its polymorphisms are associated with many types of oncological processes. Therefore, our further research will be aimed at detecting the association of rs3200401 SNP with other types of oncological nosologies.

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