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Ассоциация полиморфизмов генов микроРНК MIR146A (rs2910164), MIR758 (rs1885068), MIR33a (rs9620000) с меланомой

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Введение. Накопленные данные показывают, что микроРНК играют решающую роль практически во всех биологических и патофизиологических процессах, таких как дифференцировка клеток, регуляция клеточного цикла, липидный обмен, воспаление, неврологические, сердечно-сосудистые и метаболические расстройства и рак, включая меланому.
Цель: поиск ассоциации между полиморфными вариантами *MIR146A* (rs2910164), *MIR758* (rs1885068), *MIR33a* (rs9620000) и риском развития меланомы.
Методы. В качестве материала для исследования использовали парафиновые блоки (FFPE) 82 лиц с меланомой и периферическую кровь 35 доноров контрольной группы. ДНК выделяли из срезов FFPE с помощью коммерческого набора QIAamp DNA FFPE Tissue Kit (QIAGENE, Германия). Праймеры для ПЦР подбирали с помощью программы WASP. Геномную ДНК выделяли набором QIAamp DNA Blood mini kit (Qiagen, Germany). Генотипирование проводили методом аллель-специфичной ПЦР смесью qPCRmix –HS («Евроген» России) на приборе Real-time CFX96 Touch (США). Анализ равновесия Харди-Вайнберга и различия в распределении вариантов аллелей между группами пациентов и контроля оценивали с помощью критерия χ^2. Для оценки риска развития меланомы использовали коэффициенты отношения шансов (ОШ).
Результаты. Установлено, что аллель А гена *MIR146A* (rs2910164) (ОШ = 2,24, 95% ДИ = 1,24–4,03; p=0,02) и генотип ТТ гена *MIR33a* (rs9620000) (ОШ = 2,98, 95% ДИ = 1,17–7,60; p=0,03) ассоциированы с повышенным риском развития меланомы. Наличие полиморфного аллеля гена *MIR758* (rs1885068) не ассоциировано развитием меланомы.
Заключение. Таким образом, результаты исследования подчёркивают важность поиска диагностических биомаркеров в некодирующей области генома.

Ключевые слова: меланома кожи; микроРНК, *MIR146A, MIR758, MIR33a*.

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Association of polymorphisms of microRNA genes MIR146A (rs2910164), MIR758 (rs1885068), MIR33a (rs9620000) with melanoma

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Introduction. Accumulated data show that microRNA plays a crucial role in almost all biological and pathophysiological processes, such as cell cycle regulation, cell differentiation, lipid metabolism, neurological, cardiovascular and metabolic diseases and cancer, including melanoma.
Aim: to search for associations between polymorphic variants *MIR146A (rs2910164)*, *MIR758 (rs1885068)*, *MIR33a (rs9620000)* and the risk of melanoma.
Methods. Paraffin blocks of 82 people with melanoma and peripheral blood of 35 donors of the control group were used as the material for the study. DNA was used from paraffin block sections (FFPE) using a commercial QIAamp DNA FFPE Tissue Kit (QIAGENE, Germany). Primers for PCR were selected using the WASP program. Genomic DNA is popular with the QIAamp DNA Blood mini kit (Qiagen, Germany). Genotyping of animals using the allele-specific PCR mixture qPCRmix –HS (Eurogen, Russia) on the Real-time CFX96 Touch

(USA). Analyze the Hardy-Weinberg equilibrium and differences in the distributed alleys of variants between experimental patients and control measurements using the χ^2 criterion. To assess the risk of developing melanoma, we used odds ratios (OR).
Results. The study found that the allele A of the *MIR146A* gene (rs2910164) (OR = 2.24, 95% CI = 1.24–4.03; p = 0.02) and the TT genotype of the MIR33a gene (rs9620000) (OR = 2.98, 95% CI = 1.17–7.60); p = 0.03) are associated with an increased risk of melanoma. The presence of a polymorphic allele of the MIR758 gene (rs1885068) is not associated with the development of melanoma.
Conclusion. Thus, the results of the study emphasize the depth of the search for diagnostic biomarkers in the non-coding region of the genome.

Keywords: melanoma; microRNA, MIR146A, MIR758, MIR33a.

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Introduction

Melanoma is a highly lethal cutaneous cancer with the tendency for early invasion and metastasis. MicroRNAs (miRNAs) are a class of short nucleotide sequences of non-coding RNA (21—27 nucleotides) that participate in transcriptional and post-transcriptional regulation of gene expression, but not in protein synthesis [2].

MiRNA-33a is a tumor suppressor miRNA that has been widely studied in recent years. MiR-33 exhibits an abnormal expression in many tumor types. In gliomas, miR-34a expression decreases with increasing clinical grade, which closely correlates with decreased survival time [3,4]. In a mouse model, miR-33 mimic given by tail vein injection or subcutaneous injection reduces distant metastases of osteosarcoma, breast cancer, lung cancer, liver cancer and other tumors [5]. Interestingly, miR-33 antagonism in a Ldlr-/- mouse model of melasnoma prevented melanoma progression [6] as well as regression of established melanoma [7]. miR34a inhibits melanoma tumor growth by directly targeting the FLOT2 gene [8]. Similar to *MIR33*, *MIR758* inhibits *ABCA1* expression in human and mouse macrophages and reduces cellular cholesterol efflux via ApoA1. *MIR-758* is expressed in the brain, heart, aorta and to a lesser extent in the liver [9-12]. Li.e et al. (2017) noted in their study that *MiR758-5p* reduces CD36 expression at both the protein and mRNA levels by targeting the 3'UTR of CD36 in foam cells derived from THP-1 macrophages. Li.e et al. (2017) suggested that *MIR-758-5p* reduces foam cell lipid accumulation through the regulation of CD36. *MIR758* was found to be lowly expressed in retinoblastoma tissues and cell lines, and restoration of its expression caused significant inhibitions in cell proliferation, invasion and migration capacities, and increased cell apoptosis via targeting paired box protein 6 (PAX6) [13]. Previous studies have shown that *MIR146a* is closely associated with apoptosis in various cell types. *MIR146a* can promote apoptosis of nucleus pulposus cells by targeting FRS2 [14]. *MIR146a* regulates RGC-5 cell growth through caspase-dependent apoptosis [15]. In a Parkinson's mouse model study, it was shown that *MIR146a* expression could inhibit iNOS expression and dopaminergic neuron apoptosis by inactivating the MAPK signaling pathway through targeting CACNG5 [16].

Despite previous research findings, the role of miRNA33a, 758, 146a as prognostic biomarkers for melanoma risk remains unclear. Therefore, the aim of our study was to search for an association between polymorphic variants of miRNA *MIR146a (rs2910164), MIR758 (rs1885068), MIR33a (rs9620000)* and the risk of developing melanoma.

Materials and methods

The study included biomaterial (blood from the ulnar vein) from 117 residents of the Rostov region in equal percentages between men and women, aged 31 to 55 years. The study was approved by the Ethics Committee. Before DNA extraction, the tumor cell content in the section was determined to be no less than 20%. Genomic DNA was isolated on columns using the commercial QIAamp DNA FFPE Tissue Kit (QIAGENE, Germany). Genomic DNA for molecular genetic studies was isolated from the peripheral blood of study participants using the QIAamp DNA Blood mini kit (Qiagen, Germany). Genotyping was performed using the allele-specific PCR mixture qPCRmix –HS (Euro-

gen, Russia). Amplification was performed on a Real-time CFX96 Touch device (USA). Primers for PCR were selected using the WASP Web-based Allele Specific Primer program http://bioinfo.biotec.or.th/WASP. The primer sequences and their characteristics are presented in **Table 1**.

Hardy-Weinberg frequency equilibrium analysis was performed using the equilibrium calculator (Rodriguez et al. 2009) http://oege.org/software/hardy-weinberg.html. The odds ratio (OR), confidence interval and χ2 were calculated in the program «Gen-expert» http://gen-exp.ru/calculator_or.php

Results

As a result of genotyping and statistical analysis, we determined the allele and genotype frequencies of the polymorphic regions *MIR146A (rs2910164)*, *MIR758 (rs1885068)*, *MIR33a (rs9620000)* in 82 patients with melanoma and 35 control group. The distribution of the observed genotype frequencies in the control group corresponds to the Hardy-Weinberg equilibrium.

The results of genotyping in the control group showed that the wild-type TT genotype was identified in 42.9% of donors, while TG and GG were present in 34.3% and 22.9% of subjects, respectively. The allele frequencies in the control group T and G were 60% and 40%, respectively. At the same time, the analysis of the distribution of genotype frequencies in case gtroup with melanoma showed that the wild type TT was present in 45.1% of patients, and TG and GG were recorded in 39% and 15.9%, respectively. The frequency of T and G alleles was 64.6% and 35.4%, respectively. In residents of the Rostov region, healthy individuals and patients with melanoma, the ratio of normal and mutant alleles does not differ, that is, no association was established – *MIR758 rs1885068 T / G* (n.2086-912T> G) with melanoma (P> 0.05) (**Table 2**).

Analysis of the distribution of genotype frequencies in the control group showed that the CC genotype prevailed 54.3%, while the AA and AC genotypes were found in 17.1% and 28.6% of subjects, respectively. The frequencies of alleles in the control group A and C were 31.4% and 68.6%,

Таблица 1. Последовательности праймеров.

Table 1. Primer sequences.

miRNA	Primer direct norm 5'/ melting temperature	Primer direct mutant 5'/ melting point	Primer general reverse 5'/ melting point
MIR758 (rs1885068)	CCACCAAAGGTCCTGCCA / 63.12	CCACCAAAGGTCCTGCCC / 63.84	CCAAATGTGGCTGAGTTGGA / 62.04
MIR146A (rs2910164)	GAGCCTGGGCTGCTGGTAT / 62.67	GAGCCTGGGCTGCTGGTAG / 63.39	AGGACCATCCATCTCCTTGC / 61.40
MIR33a (rs9620000)	GAAAGGTGCAGGTAGAAACAAG / 58.06	GAAAGGTGCAGGTAGAAACAAA / 58.43	TGAGTGGGAGCTGAGTTGG / 59.96

Таблица 2. Частоты генотипов и аллелей *MIR758* rs1885068

Table 2. Frequencies of genotypes and alleles *MIR758 rs1885068*

Genotypes	Case Melanoma	Control group	χ^2	*P*	OR	
	(n=82)	(n=35)				95%CI
TT	0.451	0.429	0.85	0.66	1.10	0.49 – 2.44
TG	0.390	0.343			1.23	0.54 – 2.80
GG	0.159	0.229			0.64	0.24 – 1.71
Alleles						
T	0.646	0.600	0.45	0.5	1.22	0.69 – 2.17
G	0.354	0.400			0.82	0.46 – 1.46

Note: n – number of subjects; P – significance level; OR – odds ratio; χ2 – chi-square, * – statistically significant differences.

respectively. In the case group, the distribution of genotype frequencies was as follows: AA=31.7%, AC=37.8%, CC=30.5%. The frequency of alleles A and C was 50.6% and 49.4%, respectively. It is interesting that the high significance of the polymorphic variant rs2910164 *MIR146A* in melanoma was noted in residents of Western Asia (residents of Korea (C=0.00384), Vietnam (C=0.023). The distribution of genotypes between case group and control is statistically significant (χ2 =5.65; P=0.02) (**Table 3**).

The results of *MIR33a* (rs9620000) T>C (c.2907 + 201T> C) genotyping in the control group showed that the TC genotype was observed in 57.1% of donors, while the wild-type TT and mutant CC genotypes were present in 20% and 22.9%, respectively. The frequencies of T and C alleles were 48.6% and 51.4%, respectively. In the case group, the TT and TC genotypes were distributed equally by 42.7%, and CC was recorded in 14.6%, respectively. The frequencies of T and C alleles were 64% and 36%, respectively. For Mir33a, a statistically significant association was established (χ2 = 4.74; P = 0.03) (**Table 4**).

Discussion

We conducted a systematic analysis of the literature and databases to search for polymorphic variants in miRNA genes suitable for searching for an association with the risk of developing melanoma. Due to the fact that the number of such SNPs is limited by the high conservation of miRNA genes, we selected only three polymorphic regions: rs2910164 in the *MIR146A* gene, rs1885068 in the *MIR758* gene, rs9620000 in the MIR33a gene, the association of which has been demonstrated in several studies.

Of the three polymorphic variants studied, only rs1885068 *MIR758* did not reveal an association. The lack of statistical significance in the difference between the distribution of genotypes for the n.2086-912T> G polymorphism of the *MIR-758* gene among patients with melanoma and control group may be due to the relatively small sample size of the study. However, a number of studies have demonstrated the association of this polymorphic locus in the Asian population [17]. The dis-

Таблица 3. Частоты генотипов и аллелей *MIR146A* rs2910164

Таблица 3. Frequencies of genotypes and alleles *MIR146A* rs2910164

Genotypes	Case Melanoma	Control group	χ2	P	OR	
	(n=82)	(n=35)				95%CI
AA	0.317	0.171	5.65	0.02	2.24	0.83 – 6.07
AC	0.378	0.286			1.52	0.64 – 3.59
CC	0.305	0.543			0.37	0.16 – 0.83
Аллели						
A	0.506	0.314	7.30	0.007	2.24	1.24 – 4.03
C	0.494	0.686			0.45	0.25 – 0.81

Note: n – number of subjects; P – significance level; OR – odds ratio; χ^2 – chi-square, * – statistically significant differences.

Таблица 4. Частоты генотипов и аллелей *MIR33a* (rs 9620000)

Table 4. Frequencies of genotypes and alleles *of MI-33a (rs 9620000)*

Genotypes	Case Melanoma	Control group	χ2	*P*	OR	
	(n=82)	(n=35)				95%CI
TT	0.427	0.200	4.74	**0.03**	2.98	1.17 – 7.60
TC	0.427	0.571			0.56	0.25 – 1.24
CC	0.146	0.229			0.58	0.21 – 1.57
Аллели						
T	0.640	0.486	4.86	**0.03**	1.88	1.07 – 3.32
C	0.360	0.514			0.53	0.30 – 0.94

Note: n – number of subjects; P – significance level; OR – odds ratio; χ2 – chi-square, * – statistically significant differences.

crepancy in the results may be due to ethnic differences in the frequency of the mutant allele or to the sample size. Despite the small sample size, it is worth noting that one of the main advantages of our study was the carefully formed groups of representatives of the same population, which excludes interpopulation differences that can complicate the assessment of the functional significance of the studied polymorphisms. There are also some limitations in the study, firstly, we did not take into account lifestyle and environmental factors, secondly, our conclusion cannot be extended to other ethnic groups, thirdly, additional functional experiments are needed to confirm the results of this study.

Thus, the AA genotype of the *MIR146A* gene (rs2910164) and the TT genotype of the *MIR33a* gene (rs9620000) are associated with an increased risk of melanoma. The presence of a polymorphic allele of the *MIR758* gene (rs1885068) is not associated with the development of melanoma. However, our results need further verification in future studies with a larger sample size and more diverse ethnic groups.

Conclusions

The results of the study showed that there are significant associations of microRNA polymorphisms in the non-coding region of the genome in the population of the Rostov region, which can further be used as prognostic markers for the diagnosis of melanoma.

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