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Research Article

Molecular Profile of Cutaneous Melanoma

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Abstract

Objectives: Cutaneous melanoma (CM) is characterized by molecular heterogeneity. The aim of the study was to clarify clinical and pathological characteristics associated with gene mutations in the MAPK signaling pathway in Russian CM patients.

Methods: *BRAF, NRAS, KIT*, and *PDGFRA* mutations were evaluated in tumor DNA from 214 CM patients with Sanger sequencing of PCR products.

Results: Analysis of 173 non-acral CM revealed somatic mutations in *BRAF* (61.3%), *NRAS* (15.0%), *KIT* (1.1%), *PDGFRA* (1.1%), while 41 metastatic melanomas with unknown primary sites demonstrated a lower frequency of BRAF (46.3%) and *NRAS* (12.2%) mutations. The spectrum of *BRAF* and *NRAS* mutations differs among CM specimens, depending on tumor location and UV exposure. BRAFV600E was found in 90.4% of BRAF+ melanomas, that is, 52.8% of all CM cases, among them in 70% of patients aged under 30 years. *KIT* exon 11 mutations (p.V559A and p.Q556_W557del) were detected in CM, affecting the skin areas exposed to UV insolation (lower lip and shoulder). Somatic *PDGFRA* mutations (p.R558C and p.S847L) were found in patients with metastatic nodular CM of shin and back. Substitution c.2472C>T *PDGFRA* (silent mutation p.V824V or functional synonymous SNP rs2228230:C>T) was detected in CM cases with low expression of immunohistochemical diagnostic markers (poorly differentiated CM).

Conclusion: Molecular genetic study has revealed the prevalence of BRAF, NRAS and KIT (italics) gene mutations which were associated with primary non-acral CM location, whereas PDGFRA (italics) alterations were detected in a few metastatic poorly differentiated CM cases.

Keywords: Cutaneous melanoma, *BRAF, NRAS, KIT*, and *PDGFRA* mutations, *PDGFRA* SNP rs2228230 gene polymorphism

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Melanoma represents a significant and increasing public health burden. Although melanoma accounts for only 1% of diagnosed skin cancers, it is the cause of most skin cancer-related deaths.[1] Until recently, patients with advanced melanoma had few effective treatment options. The new therapeutic strategy includes treatments targeted specifically to gene mutations in patients' tumors as well as immune checkpoint inhibitors. The importance of the genetic background of melanoma cells for the individual susceptibility to treatment has become apparent.

Melanoma is a complex, heterogeneous oncological disease with multiple signaling pathways implicated in its

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molecular pathogenesis. A key advance in the understanding of melanoma carcinogenesis was the discovery of mutations in the *BRAF* gene encoding BRAF kinase involved in the RAS–RAF–MEK–ERK signaling pathway. Such activated mutations are the most common activating genetic event detected in cutaneous melanoma (CM).[2–4] The study of clinically annotated specimens has identified significant associations of *BRAF* mutations with clinical and pathological features, including melanoma subtype, primary tumor location, and prognosis.[4, 5] Close associations of *NRAS* and *KIT* mutations with certain clinical characteristics have also been identified.[4, 6] These findings support the concept that analysis of mutation status contributes to the identification of therapeutic targets and provides insights into molecular pathogenesis and the origin of melanoma subtypes.

CM is the most aggressive form of skin malignancies. During the last two decades, the incidence of melanoma in Russia, as well as worldwide, has increased. The absolute number of patients has increased by 23% in males and 16% in females in 5 years. Thus, the CM patient number per 100 000 of the Russian population continuously increased from 44.5 in 2009 up 66.9 in 2019. The proportion of CM identified at stage I–II from the firstly revealed malignant tumors also increased from 68.6% to 80.8% in 2009. However, during these 10 years, the proportion of CM detected at stage IV, among the firstly revealed malignant tumors, decreased from 28.6% to 17.8%.[7]

Previously, we have analyzed the oncogene mutation spectrum in cutaneous, acral, and mucosal melanoma in Russian patients.[8] Our results have shown that the pattern of activating genetic abnormalities varies among these melanoma subtypes and confirmed biological and molecular heterogeneity of melanoma. CM predominantly contained tumor cells carrying *BRAF* mutations. Moreover, the rate of *BRAF* mutations was significantly higher in non-chronic sun-induced damage (non-CSD) tumors, whereas CSD tumors had a higher frequency of *NRAS* mutations. In this study, we focused on cutaneous and metastatic melanoma cases, where *BRAF, NRAS, KIT*, and *PDGFRA* gene alterations were tested and novel data were obtained.

Methods

A total of 214 melanoma tissue samples obtained from patients who were followed and treated at N.N. Blokhin National Medical Research Center of Oncology were included in the study. Of 214 patients, 173 had primary (51) or metastatic (122) non-acral CM, and in 41 patients with metastatic melanoma, the primary melanoma site was unknown. The patients were 14–87 years old, and the number of female patients prevailed over the male ones (117, 54.6% vs. 97, 45.4%). The exact localization of the primary tumors was established for 173 patients: 85 patients had CM affecting trunk (63 – back, 22 – chest, abdomen), 15 patients had CM on head and neck, and 73 patients had CM of extremities (28 cases – upper limbs, 45 cases – lower limbs). The preexisting nevus was in anamnesis of 31 patients, nodal melanoma was diagnosed in 29 patients, and amelanotic/hypomelanotic melanoma in 53 patients.

All of the procedures were approved by the Ethics Committee of N.N. Blokhin National Medical Research Center of Oncology guidelines according to the Ministry of Health of Russia and were conducted in adherence to the principles of the Declaration of Helsinki. Informed consent for the use of specimens was obtained from all the patients under study.

Immunohistochemical Analysis

Morphological characterization and histological verification of tumor tissues were performed by pathologists. The study was performed using paraffin-embedded formalin-fixed tumor specimens. Four micrometer thick sections derived from patient samples were fixed on silane-coated glass slides, deparaffinized, and subjected to antigen retrieval. Antibodies against the following proteins were used: S100 (Cell Marque), melan A (BioGenex), HMB 45 (Dako), MITF (Cell Marque), tyrosinase (Cell Marque), H-caldesmon (Dako), CD99 (Cell Marque), vimentin (BioGenex), PanCKAE1/AE3 (Cell Marque), CK5/6(Cell Marque), P63 (Epitomics), EMA (Cell Marque), and chromogranin A (BioGenex). Immunohistochemical analysis was performed using standard procedures according to the manufacturer's protocol followed by microscopic visualization and estimation of labeled antibodies (EnVision, Helicon and MATLAB program for image analysis).

Mutation Detection

We looked for somatic hotspots mutations in *BRAF, NRAS, KIT*, and *PDGFRA* genes with PCR followed direct sequencing as described previously.[8] Genomic DNA was isolated using proteinase K (Novagen, USA) from tumor cells manually macrodissected from 5 micrometer thick deparaffinized tissue sections. Oncogene mutations were analyzed by PCR using specific primers and PCR conditions (Table 1). PCR products were separated by electrophoresis in 2% agarose gel, isolated using Wizard® PCR Preps DNA Purification System (Promega, USA) according to the manufacturer's recommendation and sequenced by ABI PRISM 3100Avant, using the ABI PRISM® Big Dye™ Terminator Vol.3.1 reagents.

The identity of DNA amplicon sequences was confirmed by database search (NCBI database, www.ncbi.nlm.nih.gov). Sequences were analyzed by Chromas 2.6.6. (Technely-

Table 1. Oncogenes primer sequences and PCR conditions.

Gene	Primer name	Primer sequence	Amplicon length	T (° C)
BRAF	*ex15_F*	*acc-taa-act-ctt-cat-aat-gct*	173 bp	56
	ex15_R	*aca-act-gtt-caa-act-gat-gg*		
NRAS	*ex2_F*	*ttg-ctg-gtg-tga-aat-gac-tga*	173 bp	64
	ex2_R	*ccg-aca-agt-gag-aga-cag-gat*		
NRAS	*ex3_F*	*aat-tga-act-tcc-ctc-cct-ccct*	158 bp	68
	ex3_R	*tgt-cct-cat-gta-ttg-gtc-tct-c*		
KIT	*ex11_F*	*tag-ctg-gca-tga-tgt-gca-tt*	295 bp	58
	ex11_R	*tgg-aaa-gcc-cct-gtt-tca-ta*		
PDGFRA	*ex12_F*	*tcc-agt-cac-tgt-gct-gct-tc*	241 bp	62
	ex12_R	*gca-agg-gaa-aag-gga-gtc-tt*		
PDGFRA	*ex18_F*	*ttc-ctt-ttc-cat-gca-gtg-tgt-cc*	210 bp	68
	ex18_R	*gaa-gca-aca-cct-gac-ttt-aga-ga*		

sium DNA Sequencing Software) and compared with the Encembl/BLAST database. For the detection of gene mutation, we also used the COSMIC database (the Catalogue of Somatic Mutations in Cancer, http://cancer.sanger.ac.uk/cosmic) and the BLAST database (Basic Local Alignment Search Tool, https://blast.ncbi.nlm.nih.gov/Blast.cgi).

Statistical analysis of the data was performed using Fisher's exact test. A p-value of less than 0.05 was considered statistically significant.

Results

Genetic analysis of 214 melanoma specimens identified mutations in *BRAF* (58.4%), *NRAS* (14.4%), *KIT* (0.9%), and *PDGFRA* (0.9%). Of them, 173 non-acral CM revealed mutations in *BRAF* (61.3%), *NRAS* (15.0%), *KIT* (1.1%), and *PDGFRA* (1.1%), whereas 41 metastatic melanomas with unknown primary sites demonstrated the lower frequency of *BRAF* (46.3%) and *NRAS* (12.2%) mutations (Table 2). Among 173 patients with CM, there were 51 patients with primary tumors: *BRAF* mutations were revealed in 30 (58.8%), *NRAS* mutations in 7 (13.7%), and *KIT* mutations in 2 (3.9%) melanoma cases.

The spectrum of mutations differs among CM specimens depending on primary tumor location and UV exposure. *BRAF* mutations were detected in 68.2% (58/85) of tumors located on skin areas (back and chest) not chronically exposed to UV light (cutaneous non-CSD melanoma of trunk) and in 40.0% (6/15) of tumors on UV-exposed areas (cutaneous CSD melanoma of the face, head, and neck) (p=0.023). *BRAF* mutations were found in 57.5% (42/73) of melanomas of extremities. These results confirm the previously published data that *BRAF* mutations are typical for superficial melanoma of skin areas protected from UV light.[6, 8, 9]

BRAF mutations were detected in 125/214 (58.4%) melanoma cases. Of them, BRAFV600E (c.1799 T>A) was found in 113 patients (90.4% of all *BRAF* mutations), that is, 52.8% (113/214) of CM patients. In addition, 12 non-V600E substitutions were found: 7 cases – p.V600K (c.1798_1799GT>AA), 2 cases – p.K601E (c.1801A>G), and 3 cases – p.L597Q (c.1790T>A). The frequency of BRAFV600E (52.8%) mutations in Russian patients was higher than in other studies.[9, 10] The highest frequency of BRAFV600E mutations was in

Table 2. Frequency of *BRAF, NRAS, KIT and PDGFRA* mutations in cutaneous melanoma specimens

Tumors	Number of cases	Frequency of mutations			
		BRAF	***NRAS***	***KIT***	***PDGFRA***
Total melanoma cases	214	125 (58.4 %)	31 (14.4%)	2 (0.9%)	2 (0.9%)
Cutaneous melanoma	173	106 (61.3%)	26 (15.0%)	2 (1.1%)	2 (1.1%)
back	63	42 (66.6%)	10 (15.9 %)	1 (1.6%)	1 (1.6%)
chest and abdomen	22	16 (72.7 %)	2 (9.1%)	0	0
face, head and neck	15	6 (40.0%)	3 (20.0%)	1(6.7%)	0
upper limbs	28	14 (50.0%)	5 (17.8 %)	0	0
lower limbs	45	28 (62.2%)	6 (13.3%)	0	1 (2.2%)
Melanoma metastases with unknown primary site	41	19 (46.3%)	5 (12.2%)	0	0

melanoma patients aged under 30 years (70%, 49/70), which corresponds to the notion that a history of sunburn, especially in childhood and youth, increases the personal risk of melanoma several times.[3, 6] Of the patients older than 50 years, BRAFV600E mutation was found in 43%. On the contrary, BRAFV600K mutation was more frequently seen in elders as only 2 CM specimens from male patients of 32 and 49 years old had V600K substitutions. Other non-V600E BRAF mutations were found in 10 patients in the age group 53–82 years. The level of BRAF kinase activity, associated with rare non-V600 mutations, constitutes only 30% of the activity of the BRAFV600 enzyme. CM carrying BRAFV600E and V600K mutations are sensitive to the BRAF inhibitors vemurafenib[11] and dabrafenib,[6] while tumors with p.L597Q or p.K601E mutations are resistant to BRAF inhibitors, but sensitive to treatment with the MEK inhibitor trametinib.[12]

According to the data obtained, *NRAS* mutations were found in 15.0% (26/173) of CM cases; 13.8% (7/51) from them were revealed in primary tumors and 12.2% in metastases of melanoma with unknown primary tumor sites. The frequency of *NRAS* gene mutation varied from 9.1% in chest melanomas (non-CSD tumors) up to 20% in head/neck melanomas and 17.8% in upper limb melanomas (Table 2). Thus, it was confirmed that *NRAS* mutations were more frequent in CSD tumors.[13]

Mutations p.Q61K (c.181C>A) and p.Q61R (c.182A>G) encoded *NRAS* exon 3 were more frequent, while p.Q61L (c.182A>T), p.Q61P (c.182A>C), and p.Q61H (c.183A>C) substitutions were revealed only in one case each. Tumors with *NRAS* mutations in exon 3 were observed mainly in older patients (aged 50–76 years), excluding three patients of 30–33 years old. Substitution p.G12C (c.34G>T) encoded *NRAS* exon 2 was found in 2 melanomas with a history of preexisting nevus in patients of 22 and 32 years old. *NRAS* mutations affecting codon p.G12 and the cause of NRAS GTP-ase hyperactivation are involved in melanoma initiation, while substitutions in codon p.Q61 appeared during tumor metastasizing as the result of UV exposition.[13] Both BRAF p.L597Q and NRAS p.Q61R mutations were revealed in lymph node metastasis of melanoma with an unknown primary site in a 63-year-old woman.

The molecular profile of CM with different clinical characteristics was studied. Mutation profiles did not differ significantly between superficial spreading melanomas and 29 CM cases with vertical tumor growth (nodal melanoma), but *NRAS* mutations were more frequent in the last one: *BRAF* (51.7%) and *NRAS* (17.2%). The highest frequency of *BRAF* (67.7%) or *NRAS* (19.3%) mutations was detected in 31 melanoma cases that developed from preexisting nevi. Among 214 CM, there were 53 (24.8%) amelanotic/hypopigmented mostly metastatic tumors. Of them *BRAF* mutations were found in 28 (52.8%) and *NRAS* – in 4 (7.5%) melanomas.

Thus, RTK/RAS/MAP kinase signaling pathway was affected by *BRAF*/*NRAS* driver mutations in 74% of CM cases, while 26% BRAF/NRAS-negative melanomas were analyzed analyzed for mutations in KIT- and PDGFRA-tyrosine kinases.

KIT exon 11 mutations were detected in two patients with primary CM, which affected the skin areas exposed to UV insolation. *KIT* substitution c.1676 T>C (p.V559A) was found in spindle cell melanoma with nodal growth on the lower lip of a 33-year-old woman. *KIT* deletion c.1666_1671del (p.Q556_W557del) was detected in pigmented epithelioid cell melanoma of the upper back (shoulder) of a 41-year-old man.

PDGFRA mutations were observed in metastatic nodal CM cases in two female patients: p.S847L (c.2540C>T) encoded *PDGFRA* exon 18 was detected in metastasis of melanoma of shin in a 37-year-old woman. The substitution p.R558C (c.1672C>T) encoded *PDGFRA* exon 12 was detected in metastasis of CM of back in a 78-year-old woman. This nodal metastatic melanoma lesion revealed a low level of diagnostic markers (Table 3, cases 1 and 2).

Analysis of 22 *BRAF*/*NRAS*/*KIT* negative CM identified the substitution c.2472C>T in *PDGFRA* exon 18 (a silent mutation p.V824V, GUC>GUT, ID COSV57264143 or COSM 22413, pathogenic score 0.88, COSMIC Database) in 6 CM cases (Table 3). However, the substitution c.2472C>T could mean the functional synonymous SNP rs2228230:C>T (BLAST Database). We tried to test SNP, but normal blood DNA was available only for 1 CM patient, and in this case, the rs2228230:T allele was found and the functional SNP *PDGFRA* was confirmed (Table 3, case 8).

Thus, the substitution c.2472C>T *PDGFRA* was detected in 5 CM cases and 4 of them revealed poor expression of immunohistochemical diagnostic markers (Table 3, cases 3–6). Remarkably, patient #2 with somatic mutation p.R558C encoded *PDGFRA* exon 12 also had low expression of diagnostic markers. There were problems with the diagnosis of metastatic tumors with low expression of melanocytic markers. All these tumors were *BRAF*/*NRAS*/*KIT* WT large metastatic nodular melanomas located in the soft tissues of the back or shin. Three patients had metastatic melanomas with the unknown primary site and some CM cases were amelanotic while other tumors were pigmented. Their pathological evaluation was complicated by alternative differential diagnoses including sarcoma and schwannoma (Table 3). These tumors revealed low expression of melanoma diagnostic markers S100, HMB 45, melan A, MITF, and tyrosinase, while the absence of cytokeratins,

Table 3. Characteristics of *BRAF/NRAS/KIT* wild type CM with *PDGFRA* alterations

N0	Age/Sex	Primary site	Primary diagnosis	Pigmentation	Histology	Differentiation	Imunohistochemical staining	*PDGFRA* alteration
1	37 F	shin	melanoma	yes	epithelioid	well		S847L
2	78 F	back	melanoma	yes	spindle nodular	poor	S100+/-, Melan A+/-, HMB 45-, MITF , nucl-tyrosinase+,PanCKAE1/AE3-, vimentin+/-P63-, chromogranin-	R558C
3	47 F	NA	lymphoma	no	mixed	poor	S100+/-, Melan A+/-, HMB 45+/-, MITFnucl+,chromogranin+/-	V824V c.2742C>T
4	38 F	NA	sarcoma, melanoma	no	epithelioid	poor	S100+/-, Melan A+/-, HMB 45+, tyrosinase+,SMA-, EMA-, CD99+	V824V c.2742C>T
5	27 F	NA	rhabdomio-sarcoma	no	mixed	poor	S100+/-, Melan A+/-, HMB 45+/-, MITF nucl+, tyrosinase+, PanCKAE1/AE3-, P63-, EMA-, cytokeratin 5|6-, vimentin-, CD99+	V824V c.2742C>T
6	34 M	back	melanocytic shwannoma melanoma	yes	mixed nodular	poor	S100+, Melan A+, HMB 45+, cytokeratin5|6-, vimentin +	V824V c.2742C>T
7	50 M	back	melanoma	yes	spindle nodular	well		V824V c.2742C>T
8	68 F	shin	melanoma	no	epithelioid nodular	well		rs2228230:T

NA: Not available.

p63, and smooth muscle actin expression supported the diagnosis "melanoma." This CM cohort was discussed in detail in our previous study.[14] Melanoma is a very heterogeneous disease entity that includes several molecular and clinical pathological variants. Some melanomas are characterized by divergent differentiation (plasticity).[15] When melanoma loses its typical morphophenotype, revealed by routinely used diagnostic markers, it is defined as "dedifferentiated melanoma."[16]

Discussion

The relationship of the genetic background of melanoma cells with individual susceptibility to treatment has become apparent. Molecular testing of *BRAF*, *NRAS*, and *KIT* gene mutations is now performed routinely for patients with advanced melanoma. Significant prevalence of *BRAF* mutations over many other activated genes was shown in CM patients who underwent next-generation sequencing analysis. Mutations affecting the V600 site of BRAF kinase and hotspots in *NRAS* were the most frequent mutations among 46 genes tested in a large cohort of 699 patients.[9]

Ethnic differences are conspicuous in melanoma. Really, the *BRAF* gene is one of the most frequently mutated genes in Caucasians (40%–60%), whereas only about 25% of Asian patients were reported harboring *BRAF* mutations. [6] Previously, we investigated oncogene mutations in cutaneous, acral, and mucosal melanoma specimens from Russian patients. It was shown that the frequency of *BRAF* mutations was rather high in Russian melanoma patients (55%), while it was distinct in cutaneous (59%), acral (20%), and mucosal (14%) melanomas.[8]

In this study, *BRAF* mutations were found in 61.3% of non-acral CM specimens. *BRAF* mutations were detected in 68.2% of cutaneous non-CSD melanoma of the trunk and in 40.0% of tumors on UV-exposed areas of the head and neck. Most of the patients were younger than 30 years old. Substitution p.V600E was found in 90.4% of BRAF+ melanoma cases, that is, 52.8% from all Russian CM patients. Thus, the frequency of V600E mutations was higher in Russian patients than in other studies, where *BRAF* mutations were found in 30%–50% of melanoma cases and BRAFV600E substitution was revealed in 75%–80% of all *BRAF* patients (less than in 30% of melanoma patients).[9] It is worth to note that BRAFV600E substitution in Asian patients was found in 95.7% of BRAF+ melanoma cases, but only 23% of melanoma patients had *BRAF* mutation.[17] These data are important as genetic background to the individual susceptibility to treatment. Therapies by targeted agents and immune checkpoint inhibitors have changed CM prognosis. Thus, the study of the molecular genetic basis for the development of novel treatment approaches is an actual problem. The data on the frequency and distribution of *NRAS* mutations are also important for treatment strategy as new drugs and lines of treatment are adopted.[18]

Though *KIT* mutations are rarely detected in CM, we have found *KIT* exon 11 mutations in two patients with CM of the lower lip and of the suprascapular area of the back (shoulder), namely on skin lesions exposed to UV insolation. It is important for targeted therapy by several KIT-kinase inhibitors that may be used for such melanoma patient treatment.[6]

PDGFRA mutations are very rare in melanomas, *PDGFRA* encodes a cell surface tyrosine kinase receptor, which binds to three forms of PDGF and plays role in cell proliferation, differentiation, survival, and tumor progression.[19, 20] It was shown that mutations increase *PDGFRA* expression and MAPK and PI3K/AKT pathway activation in melanoma. Few publications reported *PDGFRA* mutations in melanoma patients. *PDGFRA* mutations were absent in primary melanoma in a limited number of Caucasian[21] and Asian[22] patients. Later, *PDGFRA* mutations in exons 12, 14, and 18 were found in 1.4% (40/2793)[17] and in 4.6% (16/351)[23] of Asian patients. *PDGFRA* mutations were detected mostly in acral or mucosal melanoma and in non-CSD melanoma only, all in *KIT* wild-type tumors. The analysis of the whole exons of *PDGFRA* in 225 melanomas from mostly Caucasians (TCGA database) has revealed 23 different *PDGFRA* mutations in 21 melanomas (9.3%; 21/225). However, in this cohort, the frequency of *PDGFRA* mutations in exons 12, 14, and 18 was 1.3% (3/225).[24]

In our study, *PDGFRA* mutations (p.R558C and p.S847L) were revealed in 2 of 173 (1.1%) non-acral CM patients. Additionally a silent mutation *PDGFRA* p.V824V (c.2472C>T) or functional synonymous SNP rs2228230 was found in 6 of 22 (27.2%) tested *BRAF*/*NRAS*/*KIT* wild-type CM cases (Table 3). Unfortunately, the SNP rs2228230 was confirmed only for 1 patient with a well-differentiated amelanotic melanoma. Therefore, 4 from 5 melanoma cases with *PDGFRA* c.2472 C>T demonstrated low expression of diagnostic markers, which means they were poorly differentiated tumors. Additionally, mutation p.R558C was also found in the low differentiated CM. These cases are from our cohort of low differentiated melanoma cases with atypical histological characteristics.[14] The clinical and histological presentations of metastatic melanoma vary greatly, and it is characterized by phenotypic heterogeneity and plasticity.[15, 16]

According to the BLAST database, the rs2228230:T allele frequency in the European population is 14.51% (https://blast.ncbi.nlm.nih.gov). The same frequency of the rs2228230:T allele was shown for acral (15.4%) and CM (16.7%) cohorts of Asian patients.[24] As the higher frequency of *PDGFRA* alterations (mutations and/or polymorphisms) was observed in low differentiated CM cases, we can assume that *PDGFRA* mutations may be a characteristic feature of low-differentiated ("dedifferentiated") non-CSD melanoma as well as acral or mucosal.

SNP rs2228230 was mapped to the ATP-binding site and polypeptide substrate-binding site in a conserved PDGFRA protein kinase domain. The genotype of rs2228230 affects the mRNA secondary structure and expression of *PDGFRA*. It is mapped within the exon splicing binding sites, which may disrupt mRNA splicing and affect protein function.[25] It was shown that the rs2228230:T allele can reduce the expression and function of *PDGFRA* by altering the stability of its mRNA and protein, which reduces the signaling activity of the MAPK and PI3K/AKT pathways. The rs2228230:T allele was associated with better survival in acral melanoma patients, but not in CM patients from Asia and Europe.[25] It should be noted that, in our study, metastatic melanoma patients with *PDGFRA* alterations also had low survival.

Thus, we found that the frequency of *PDGFRA* c.2621C>G (the rs2228230 genotype or mutation p.V824V) was elevated in some CM with low expression of diagnostic markers but such melanoma cases are very seldom. The significance of synonymous *PDGFRA* alteration for CM phenotypic plasticity and prognosis should be studied in an extended set of patients in the future.

Conclusion

A molecular genetic study revealed the correlations of gene mutation status with clinical and pathological characteristics in Russian CM patients. The prevalence of *BRAF*, NRAS, and *KIT* gene mutations was significantly associated with primary tumor location that justified an application of targeted therapy. *PDGFRA* mutations were detected in a few metastatic CM cases mostly with a low level of diagnostic markers that are poorly differentiated.

Disclosures

Ethics Committee Approval: For all of the patients who participated in the study, written informed consent was obtained. It was approved by the Ethical Committee of N.N. Blokhin Cancer Research Center according to the Ministry of Health of Russia and was conducted according to the Guidelines of the Declaration of Helsinki.

Peer-review: Externally peer-reviewed.

Conflict of Interest: None declared.

Authorship Contributions: Concept – N.N.M.; Design – N.N.M.; Supervision – N.N.M.; Materials – I.N.M., L.V.D., A.A.L., O.O.A; Data collection and processing – I.V.T., A.A.L., O.O.A.; Analysis and interpretation – N.N.M., I.V.T., A.A.L., I.N.M.; Literature search – N.N.M.; Writing – N.N.M., A.A.L.; Critical review – I.V.T., I.N.M., O.A.A.

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