TÜBİTAK

Turkish Journal of Medical Sciences

http://journals.tubitak.gov.tr/medical/

Research Article

Turk J Med Sci
(2018) 48: 973-979
© TÜBİTAK
doi:10.3906/sag-1802-80

Clinicopathological characteristics and mutation profile of BRAF and NRAS mutation in cutaneous melanomas in the Western Turkish population

Mustafa Kürşat EVRENOS[1,*], Peyker TEMİZ[2], Fethi Sırrı ÇAM[3], Murat YAMAN[4], Levent YOLERİ[1], Aylin Türel ERMERTCAN[5]

[1]Department of Plastic Reconstructive and Aesthetic Surgery, Faculty of Medicine, Celal Bayar University, Manisa, Turkey
[2]Department of Pathology, Faculty of Medicine, Celal Bayar University, Manisa, Turkey
[3]Department of Medical Genetics, Faculty of Medicine, Celal Bayar University, Manisa, Turkey
[4]Department of Plastic Reconstructive and Aesthetic Surgery, Dr. Lutfi Kırdar Kartal Research and Training Hospital, İstanbul, Turkey
[5]Department of Dermatology, Faculty of Medicine, Celal Bayar University, Manisa, Turkey

Received: 11.02.2018 • **Accepted/Published Online:** 22.09.2018 • **Final Version:** 31.10.2018

Background/aim: Malignant melanoma is the most common cause of death due to skin cancers. The most common mutations in RAF-RAS pathway from tumor oncogenes are BRAF and NRAS. In this study, we analyzed the frequency of BRAF and NRAS gene mutations and investigated their association with clinicopathological features of melanomas in the Turkish population.

Materials and methods: 65 primary cutaneous melanoma were included in the study. The mutations were evaluated with real-time PCR-based PCR-array through allele-specific amplification, and the results were correlated with various clinicopathological characteristics.

Results: 52.3% of the patients were female and 47.7% were male. The mean age of the patients with a mutation was lower than those without mutation. 16 patients had BRAF mutation. 12 patients had NRAS mutation. NRAS mutation was statistically more common in men (P = 0.036). The number of mitoses increased with the increase of the tumor thickness (P = 0.003). There was more mitosis in the presence of ulceration (P = 0.05). A total of 41.7% of NRAS mutations had adjuvant chemotherapy.

Conclusion: We found lower mutation rate when compared to regional studies. NRAS mutation was common in men. This is the first study from our region evaluating the prognostic value of clinical stage and necessity of adjuvant treatment with the presence of BRAF and NRAS mutations.

Keywords: Malignant melanoma, mutation, BRAF, NRAS, real-time PCR

1. Introduction

The incidence of malignant melanoma (MM) of the skin has steadily increased in the past decades (1). New studies regarding the biology of melanoma and its molecular mechanisms have led to new investigations of targeted therapies (2).

Stem cell growth factor receptor (KIT) and mitogen-activated protein kinase (MAPK) cascade including RAS-RAF-MEK-ERK are important pathways mediating cellular responses to growth signals regulating cell proliferation, survival, and differentiation (3). KIT is a cytokine receptor that is expressed on the surface of melanocytes. Altered forms of this receptor are associated with some melanoma types (4). NRAS and BRAF mutations are mainly involved in the pathogenesis of melanoma. BRAF is a member of the RAF kinase family of growth signal transduction protein kinases and it takes part in regulating the MAPK pathway (5). NRAS is a member of RAS family of GTPases and the most commonly mutated isoform in melanoma (6). Mutations in kinases in the MAPK signal transduction pathway have been found about 40% – 70% of melanomas (3, 7).

The information about mutation frequencies of melanoma from the Middle East region is limited. In this study, we aimed to determine the frequency of both BRAF and NRAS mutations, the correlation between the presence of mutations and tumor depth, histological subtypes, growth pattern, the presence of ulceration, regression, tumor localization, and mitosis number per mm^2. Also, we evaluated the prognostic value of the presence of mutation by evaluating the clinical stages at diagnosis, disease-free and diseased survival periods, and adjuvant therapies.

2. Materials and Methods

2.1. Tumor Samples

Formalin-fixed paraffin-embedded (FFPE) blocks of patients diagnosed as MM between 2006 and 2013 were

* Correspondence: mkevrenos@hotmail.com

examined at the department of pathology, Celal Bayar University, Manisa. A total of 81 primary cutaneous melanoma patients with available blocks were selected. Sixteen of the cases were excluded because of low quality or insufficient DNA. At last 65 cases of the primary cutaneous melanoma were included in the study. The study was approved by the Ethics Committee of Faculty of Medicine, Celal Bayar University, Manisa, Turkey.

Clinical information of the patients including age, sex, and tumor localization was obtained from pathology reports. Tumor thickness classified according to Clark level and Breslow level, number of mitosis per square millimeter, and ulceration, growth phase, presence of lypmhocytic and lymphovascular infiltration, and presence of regression were reviewed with hematoxylin and eosin-stained sections. Cases were classified into melanoma in situ (MIS), superficial spreading melanoma (SSM), nodular melanoma (NM), lentigo maligna melanoma (LMM), and acral lentiginous melanoma (ALM) according to the current classification of World Health Organization.

Areas containing viable tumors were marked on the hematoxylin and eosin-stained slides, and tumor tissue was manually dissected from 5 unstained paraffin sections (thickness per section was 8 μm). Two samples were prepared for each patient for both BRAF and NRAS mutations.

2.2. Mutation analysis

2.2.1. DNA extraction

Samples of DNA were extracted from paraffin-embedded tumor tissue via QIAamp DNA FFPE Tissue Kit (cat. 56404; Qiagen, GmbH, Hilden, Germany) according to the manufacturer's protocol. DNA measurements of the purified samples were made with the NanoDrop device, and the DNA samples with the suitable purity and concentration levels were selected to use in BRAF and NRAS mutation analysis.

2.2.2. Mutation Analysis of BRAF and NRAS

BRAF mutations were detected with Easy® BRAF kit (Diatech Pharmacogenetics; cat. no. RT002) which allows detecting the main mutations of codon 600 of the gene BRAF using 5 oligo mixes. NRAS mutation detections were performed with Easy® NRAS kit (Diatech Pharmacogenetics; cat. no. RT004) which allows detecting the main mutations of exon 2 (codons 12, 13), exon 3 (codons 59, 61), and exon 4 (codons 117, 146) of NRAS gene of EGFR gene using 8 oligo mixes. Each mix allows the coamplification of one or more mutated alleles. Related kits allow the detection of low percentages of mutated allele in the presence of high amounts of wild type genomic DNA by real-time amplification with sequence-specific probes marked with FAM and HEX (LOD down to 0.5%).

2.3. Clinical Information

Files of patients who had mutation analysis result were investigated. Clinical stage of the disease at the time of diagnosis was noted. Adjuvant therapy information, disease-free and the overall survival time, and the duration of follow-up were recorded.

2.4. Statistical Analysis

Correlation of mutation status with clinical and pathological features was analyzed by using Pearson X^2, Mann–Whitney U, and one-way analyses of variance (ANOVA) test together with the Log-logistic regression test to calculate statistical significance. All analyses were two tailed and the value of $P < 0.05$ was considered as statistically significant. Statistical analyses were performed by the SPSS 21.0 (Chicago, IL, USA) software.

3. Results

The clinical and pathological data are listed in Table. A total of 34 (52.3%) of the patients were female and 31 (47.7%) were male. The ages of patients ranged between 18 and 80 (mean: 59.9). Tumor histology was melanoma in situ (MIS) in 8 (12.3%) patients, while it was nodular melanoma (NM) in 18 (27.7%), superficial spreading melanoma (SSM) in 12 (18.5%), lentigo maligna melanoma (LMM) in 19 (29.2%), and acral lentiginous melanoma malignant (ALM) in 8 (12.3%) patients. The localization of the tumor was head and neck in 33 (50.7%), trunk in 11 (16.9%), and extremities in 21 patients (32.3%). Tumor thickness ranged between 0.1 and 40 mm (median, 3 mm). The mean number of mitoses per square millimeter was 1.96 (0–13). The number of mitoses increased with the increase of tumor thickness, and mitosis was more common with the presence of ulceration ($P = 0.003$ and $P = 0.05$). Four of the tumors were classified as Clark 1 (6.2%), 8 of the tumors were Clark 2 (16.3%), 8 of the tumors were Clark 3 (16.3%), 33 of the tumors were Clark 4 (50.8%), and 12 of the tumors were Clark 5 (18.5%). Thirty (46.1%) of the tumors had ulceration. Fifty three (81.5%) of the tumors had no regression, 10 (15.3%) had less than 50% regression and 2 (3%) had more than 50% regression. Thirty-three (50.7%) of the tumors had no lymphocytic infiltration, 9 (13.8%) of the tumors were classified as brisk, and 23 (35.3%) of the tumors were classified as nonbrisk. There was no significant difference between tumor infiltrating lymphocyte grade (TIL) and overall BRAF and NRAS mutations ($P = 0.95$, $P = 0.56$, $P = 0.48$, consequently). At the time of diagnosis, 27 of the patients were evaluated as stage 0 or 1 (41.53%), 28 patients were stage 2 (43.07%), 8 patients were stage 3 (12.3%), and 2 patients were stage 4 (3%). There was no significant correlation between mutation status and clinical stage at the diagnosis. Evaluation was performed again for metastatic and nonmetastatic diseases with mutation

Table: Clinicopathological features of mutations and statistical analysis.

	Overall mutation			BRAF			NRAS		
	Mutation, n (%)	Wild, n (%)	P	Mutation, n (%)	Wild n (%)	P	Mutation n (%)	Wild n (%)	P
Total	24 (36.9)	41 (63.1)		16 (24.6)	49 (75.4)		12 (18.5)	53 (81.5)	
Age, yr mean	56.58	61.87	0.068	56.12	61.16	0.182	57.75	60.41	0.343
Sex									
Male	12 (38.7)	19 (61.3)	0.776	6 (19.4)	25 (80.6)	0.258	9 (29)	22 (71)	**0.037**
Female	12 (35.3)	22 (64.7)		10 (29.4)	24 (70.6)		3 (8.8)	31 (91.2)	
Breslow mm									
≤ 1	10 (47.6)	11 (52.4)		6 (28.6)	15 (71.4)		5 (23.8)	16 (76.2)	
1.01-2.0	3 (37.5)	5 (62.5)		1 (12.5)	7 (87.5)	0.808	2 (25)	6 (75)	
2.01-4.0	3 (21.4)	11 (78.6)	0.479	3 (21.4)	11 (78.6)		1 (7.1)	13 (92.9)	0.611
> 4.0	8 (36.4)	14 (63.6)		6 (27.3)	16 (72.7)		4 (18.2)	18 (71.8)	
Ulceration									
Present	13 (54.2)	16 (39)	0.236	8 (50)	21 (42.9)	0.618	8 (66.7)	21 (39.6)	0.084
Absent	11 (45.8)	25 (61)		8 (50)	28 (57.1)		4 (33.3)	32 (60.4)	
Regression									
Absent	19 (79.2)	34 (83.0)		13 (75.5)	40 (24.5)		8 (15.1)	45 (84.9)	
<50%	4 (16.8)	6 (14.6)	0.899	3 (70)	7 (30)	0.667	3 (30)	7 (70)	0.272
>50%	1 (4.2)	1 (2.4)		0 (0)	2 (100)		1 (50)	1 (50)	
Growth Phase									
Radial	7 (53.8)	6 (46.2)		5 (38.5)	8 (61.5)		3 (23.1)	10 (76.9)	
Vertical	4 (23.5)	13 (76.5)	0.234	4 (23.5)	13 (76.5)	0,416	2 (11.8)	15 (88.2)	0.689
Radial + Vertical	13 (37.1)	22 (63.9)		7 (20)	28 (80)		7 (20)	28 (80)	
Mitosis	1.66	2.14	0.484	1.937	1.979	0.956	1.25	2.13	0.300
Localization									
Head&Neck	10 (30.3)	23 (69.7)		7 (21.2)	26 (78.8)		5 (15.2)	28 (84.8)	
Trunk	3 (27.3)	8 (72.7)	0.200	1 (9.1)	10 (91.9)	0.158	3 (27.3)	8 (72,7)	0.666
Extremity	11 (52.4)	10 (47.6)		8 (38.1)	13 (61,9)		4 (19.0)	17 (81.0)	
Subtypes									
MIS	3 (37.5)	5 (62.5)		1 (12.5)	7 (87.5)		2 (25)	6 (75)	
NM	5 (27.8)	13 (72.2)		5 (27.8)	13 (72.2)		2 (11.1)	16 (88.9)	
SSM	5 (41.7)	7 (58.3)		3 (25)	9 (75)		3 (25)	9 (75)	
LMM	6 (31,6)	13 (68.4)	0.518	3 (15.8)	16 (84.2)	0.366	4 (21.1)	15 (78.9)	0.829
ALM	5 (62.5)	3 (37.5)		4 (50)	4 (50)		1 (12.5)	7 (87.5)	
Stage at the diagnoses									
Stage 1	13 (48.1)	14 (51.9)		8 (29.6)	19 (70.4)		6 (22.2)	21 (77.8)	
Stage 2	7 (25)	21 (75)		6 (21.4)	22 (78.6)		3 (89.3)	25 (10.7)	
Stage 3	4 (50)	4 (50)	0.177	2 (25)	6 (75)	0.760	3 (37.5)	5 (62.5)	0.290
Stage 4	0 (0)	2 (100)		0 (0)	2 (100)		0 (0)	2 (100)	
Adjuvant therapy									
None	16 (66.7)	31 (75.6)		11 (68.8)	38 (77.6)		7 (58.3)	42 (79.2)	
CT	6 (25)	6 (14.6)	0.459	3 (18.8)	9 (18.4)	0.469	5 (41.7)	7 (12.2)	0.056
CT + RT	2 (8.3)	4 (9.8)		2 (12.5)	2 (4.1)		0 (0)	4 (7.5)	
Disease free survival (n:58) (month)	37.57 (n:21)	37.45 (n:37)	0.990	36.27 (n:15)	41.00 (n:43)	0.629	42.00 (n:9)	36.67 (n:49)	0.652
Overall survival	34.71	38.41	0.647	40.12	36.04	0.653	33.16	37.92	0.637

status (P = 0.82 for overall mutation, P = 0.71 for BRAF mutation and P = 0.30 for NRAS mutation). The duration of follow-up of the patients ranged between 1 month and 108 months (mean: 36.70). The mean disease-free survival time was 33.46 months and mean overall survival time was 37.05 months. The mean disease-free survival with BRAF mutation was slightly shorter than BRAF wild-type (36.27 months), but overall survival was longer (40.12 months). The results were not significant statistically (P = 0.63, P = 0.65, consequently). Interestingly, the mean disease-free survival with NRAS mutation was slightly longer than NRAS wild-type (42.00 months), but the overall survival was shorter (33.16 months) and the results were not significant statistically (P = 0.65, P = 0.63 consequently). A total of 47 patients (72.3%) did not take adjuvant therapy, 11 patients (16.9%) received chemotherapy (CT), and 4 patients (6.2%) received CT and radiotherapy (RT).

We did not find a statistically significant relationship between tumor thickness and the number of mitoses or the presence of mutations in our study. M/F ratio was 1/1 for overall mutations. Through the evaluation of histological subtypes, mutations were most commonly seen with ALM and SSM (62.5% and 41.7%, respectively) followed by MIS (37.5%), LMM (31.6%), and NM (27.8%).

Twenty-four of the patients had mutations. Sixteen of them had BRAF mutation (24.6%) and 12 of them had only BRAF mutation (18.4%). The mean age of the patients with a mutation (56.58 years) was lower than the patients without mutation (61.87 years) (P = 0.068). Six of the patients with BRAF mutation were men (37.5%) and 10 were women (62.5%). Seven of the tumors with BRAF mutation were located on the head and neck (43.7%), 8 of them were located on extremities (50%), and one was located on trunk (17.3%). The BRAF mutation was more common in melanomas with extremity localization but there was no statistical difference.

Twelve of the patients had NRAS mutation (24.6%) and 8 of them had only NRAS mutation (18.4%). Nine of the patients with NRAS mutation were men (75%) and 3 were women (25%); in other words, NRAS mutation was statistically more common in men (P = 0.036). Five of the tumors with NRAS mutation were located on the head and neck (41.7%), 3 of the tumors were located on extremities (27.3%), and one was located on the trunk (19%). The NRAS mutation was more common in melanomas with head and neck localization but there was no statistical difference. Although there were no significant statistical differences, 67% of the tumors with NRAS mutation had ulceration (P = 0.08). The NRAS mutation was more common in LMM cases but there was no significant difference. Five of the patients with NRAS mutation (41.7%) had CT as adjuvant therapy and this was statistically insignificant (P = 0.056) (Table).

4. Discussion

Malignant melanoma of the skin is mainly caused by UV exposure (chronic or intermittent), which is proved by characteristic base changes in the DNA (C > T transition) of the melanoma (8). Certainly, the most sensitive human oncogene for UV is BRAF and is most frequently mutated in melanoma. The second most frequently UV-mutated oncogene is NRAS. These mutations can be seen with similar rates both in MM and benign tumors of melanocytes as well. In rarer forms of melanoma, such as the lentiginous, acral or mucosal forms, the KIT oncogene is mutated involving several exons, where the UV-induced alteration is less evident (9).

Different mutation rates for various mutations in melanoma studies have been reported ranging between 45% and 75% (10,11). One of the studies, conducted in a common region of our study, reported the overall mutation rate as 64.2% and most of them were BRAF and NRAS mutations (12). The other study from Turkey reported 55.3% as the overall mutation rate. BRAF and NRAS mutation rates were (29.8%) and (21.3%), consequently (2). The overall mutation rate was lower in our study (36.9%).

BRAF mutations in primary melanomas are seen at a rate of 22%–72% and are mostly frequent in SSM and NM (4,12–20). The BRAF mutation rate in our study was 15.8% and it was lower than both of these reports (2,12).

As reported before, the BRAF mutation rate in LMM in Australia and Europe/United States data varies between 8.3% and 27.7% (3,14,21). In the literature, there are so many studies that have reported BRAF mutations are less common in melanomas with chronic sun damaged skin and have higher mutation rate in cases with non ALM (3,12,14,21). Differently, we found 50% of the ALM cases were BRAF mutant with extremity localization. This result should be another evidence showing that BRAF mutations are more likely to develop in tumors located on skin subject to intermittent sun exposure (22). Also, mutation rate was 62.5% in ALM group, but it was not significant statistically.

NRAS mutations are more commonly found in tumors on the skin subject to continuous sun exposure (13,23). Our data was compatible with literature and regional data (2,12,13,23,24). The mean age of the NRAS mutant patients was higher than the patients without a mutation in many reports (2,12,13,20,25). However, in our study, the mean age of the patients with mutation was slightly lower than without mutation, but it was not significant. Most of the patients with mutation were men in our study. NRAS mutations have been reported to develop more commonly in NM cases than in other melanoma subtypes (13,24). However, Wu et al. and Akslen et al. had reported the NRAS mutation at a similar rate in NM and

SSM (15,25). Similarly, we found the rate of mutation in SSM as 25% and 21.1% in LMM. The absence of ulceration with NRAS mutation was correlated in a previous study (26). We found a high incidence of ulceration with NRAS mutation unlike wild-type but it was not statistically significant (P = 0.084). Yaman et al. reported similar findings as in our study (12).

TIL grade was scored as absent, nonbrisk, or brisk using a previously defined grading system in our study (27). Tumor-infiltrating lymphocyte grade was evaluated with melanoma specific survival and with BRAF and NRAS mutation status before (11,24,25). Edlundh-Rose et al. found high TIL in BRAF mutant patients relative to wild-type according to different TIL scale (13). Thomas et al. found low TIL in NRAS mutant patients (26). Greater than half of our patients with or without mutation had no TIL and statistically, there was no correlation between TIL grade and mutation status.

The necessity of adjuvant CT was statistically correlated with the presence of NRAS mutation weakly. Five of the patients with NRAS mutation had adjuvant CT and 3 of them were stage 2C according to histopathological staging. Although CT regimen information was not available, the follow-up period of NRAS mutated patients ended before recent approvals of new systemic agents that alter the natural course of the disease.

Stage of the disease at the diagnosis and the BRAF and/or NRAS mutation status were evaluated in the literature. Also, the overall and disease-free survival times with mutant, wild types and prognostic value were investigated. Previous studies on the associations between BRAF and NRAS mutations and survival of melanoma patients have been inconsistent. (11,23,28–33). In our study, all data items were available for the classification describing the state of the primary tumor in the AJCC TNM (tumor, regional nodes, distant metastasis) clinical melanoma staging system (34). The survival time was accumulated from the diagnosis date until the date of death due to melanoma or the end of follow up. In our study, 83% of the mutations were observed in clinically nonmetastatic patients at diagnosis. There was no significant difference between clinical stage and presence of mutation or mutation type. Also, we found that the overall survival was slightly shorter than disease-free survival among the NRAS mutant patients and overall survival was slightly longer than disease-free survival among the BRAF mutant patients but this data was not statistically significant. This may suggest NRAS mutant advanced clinical stage patients have shorter survival time.

Lymph node in the body region to which a malignant tumor first drains is named as sentinel lymph node (SLN). The importance of SLN sampling in identification of the occult lymph node metastases has been established, and accepted as a prognostic factor (35). A limitation of this study is that we could not evaluate SLN status due to the few numbers of patient populations with SLN biopsy indication. So, we could not determine whether NRAS/BRAF status provides information beyond SLN status for outcome prediction. We also did not obtain information regarding definitive chemotherapies potentially utilized, such as, systemic interferon, or clinical trial participation, which could confound our results. Although our study population is similar to the regional data, the larger population should give more definitive information about the prognostic value of mutations. So, our data showed lower mutation rates than in other regional studies.

In conclusion, we found lower mutation rate when compared to other regional studies. The overall mutation was seen at younger ages. Tumor mitotic rate was higher when the tumor was deeper. NRAS mutation was common in men and NRAS mutant patient needed more adjuvant CT than the one with the wild type after therapeutic surgery.

This is the first study from our region evaluating the prognostic value of clinical stage and necessity of adjuvant treatment according to the presence of BRAF and NRAS mutations.

References

1. Timar J, Vizkeleti L, Doma V, Barbai T, Raso E. Genetic progression of malignant melanoma. Cancer Metastasis Rev 2016; 35: 93-107.
2. Yilmaz I, Gamsizkan M, Kucukodaci Z, Berber U, Demirel D, Haholu A, Narli, G. BRAF, KIT, NRAS, GNAQ and GNA11 mutation analysis in cutaneous melanomas in Turkish population. Indian J Pathol Microbiol 2015; 58: 279-284.
3. Curtin JA, Fridlyand J, Kageshita T, Patel HN, Busam KJ, Kutzner H, Cho KH, Aiba S, Brocker EB, LeBoit PE. et al. Distinct sets of genetic alterations in melanoma. N Engl J Med 2005; 353: 2135-2147.
4. Curtin JA, Busam K, Pinkel D, Bastian BC. Somatic activation of KIT in distinct subtypes of melanoma. J Clin Oncol 2006; 24: 4340-4346.
5. Davies H, Bignell GR, Cox C, Stephens P, Edkins S, Clegg S, Teague J, Woffendin H, Garnett MJ, Bottomley W. et al. Mutations of the BRAF gene in human cancer. Nature 2002; 417: 949-954.
6. Goydos JS, Mann B, Kim HJ, Gabriel EM, Alsina J, Germino FJ, Shih W, Gorski DH. et al. Detection of B-RAF and N-RAS mutations in human melanoma. J Am Coll Surg 2005; 200: 362-370.

7. Scolyer RA, Long GV, Thompson JF. Evolving concepts in melanoma classification and their relevance to multidisciplinary melanoma patient care. Mol Oncol 2011; 5: 124-36.

8. Balch CM, Soong, S-J., Thompson, J. F. The natural history of melanoma and factors predicting outcome. In: J. F. DLM, & B. B. R. Kroon, editor. Textbook of melanoma. London: Taylor & Francis Group; 2004. pp. 181-199.

9. Whiteman DC, Pavan WJ, Bastian BC. The melanomas: a synthesis of epidemiological, clinical, histopathological, genetic, and biological aspects, supporting distinct subtypes, causal pathways, and cells of origin. Pigment Cell Melanoma Res 2011; 24: 879-97.

10. Lovly CM, Dahlman KB, Fohn LE, Su Z, Dias-Santagata D, Hicks DJ, Hucks D, Berry E, Terry C, Duke M. et al. Routine multiplex mutational profiling of melanomas enables enrollment in genotype-driven therapeutic trials. PLoS One 2012; 7: e35309.

11. Barbour AP, Tang YH, Armour N, Dutton-Regester K, Krause L, Loffler KA, Lambie D, Burmeister B, Thomas J, Smithers BM. et al. BRAF mutation status is an independent prognostic factor for resected stage IIIB and IIIC melanoma: implications for melanoma staging and adjuvant therapy. Eur J Cancer 2014; 50: 2668-2676.

12. Yaman B, Akalin T, Kandiloglu G. Clinicopathological characteristics and mutation profiling in primary cutaneous melanoma. Am J Dermatopathol 2015; 37: 389-397.

13. Edlundh-Rose E, Egyhazi S, Omholt K, Mansson-Brahme E, Platz A, Hansson J, Lundeberg J. NRAS and BRAF mutations in melanoma tumours in relation to clinical characteristics: a study based on mutation screening by pyrosequencing. Melanoma Res 2006; 16: 471-478.

14. Viros A, Fridlyand J, Bauer J, Lasithiotakis K, Garbe C, Pinkel D, Bastian BC. Improving melanoma classification by integrating genetic and morphologic features. PLoS Med 2008; 5: e120.

15. Akslen LA, Puntervoll H, Bachmann IM, Straume O, Vuhahula E, Kumar R, Molven A. Mutation analysis of the EGFR-NRAS-BRAF pathway in melanomas from black Africans and other subgroups of cutaneous melanoma. Melanoma Res 2008; 18: 29-35.

16. Venesio T, Chiorino G, Balsamo A, Zaccagna A, Petti C, Scatolini M, Pisacane A, Sarotto I, Picciotto F, Risio M. In melanocytic lesions the fraction of BRAF V600E alleles is associated with sun exposure but unrelated to ERK phosphorylation. Mod Pathol 2008; 21: 716-726.

17. Liu W, Kelly JW, Trivett M, Murray WK, Dowling JP, Wolfe R, Mason G, Magee J, Angel C, Dobrovic A, et al. Distinct clinical and pathological features are associated with the BRAF(T1799A(V600E)) mutation in primary melanoma. J Invest Dermatol 2007; 127: 900-905.

18. Saldanha G, Potter L, Daforno P, Pringle JH. Cutaneous melanoma subtypes show different BRAF and NRAS mutation frequencies. Clin Cancer Res 2006; 12: 4499-4505.

19. Lang J, MacKie RM. Prevalence of exon 15 BRAF mutations in primary melanoma of the superficial spreading, nodular, acral, and lentigo maligna subtypes. J Invest Dermatol 2005; 125: 575-579.

20. Platz A, Egyhazi S, Ringborg U, Hansson J. Human cutaneous melanoma; a review of NRAS and BRAF mutation frequencies in relation to histogenetic subclass and body site. Mol Oncol 2008; 1: 395-405.

21. Bauer J, Buttner P, Murali R, Okamoto I, Kolaitis NA, Landi MT, Scolyer RA, Bastian BC. BRAF mutations in cutaneous melanoma are independently associated with age, anatomic site of the primary tumor, and the degree of solar elastosis at the primary tumor site. Pigment Cell Melanoma Res 2011; 24: 345-51.

22. Maldonado JL, Fridlyand J, Patel H, Jain AN, Busam K, Kageshita T, Ono T, Albertson DG, Pinkel D, Bastian BC. Determinants of BRAF mutations in primary melanomas. J Natl Cancer Inst 2003; 95: 1878-1890.

23. Ellerhorst JA, Greene VR, Ekmekcioglu S, Warneke CL, Johnson MM, Cooke CP, Wang LE, Prieto, VG, Gershenwald, JE, Wei Q, et al. Clinical correlates of NRAS and BRAF mutations in primary human melanoma. Clin Cancer Res 2011; 17: 229-235.

24. Lee JH, Choi JW, Kim YS. Frequencies of BRAF and NRAS mutations are different in histological types and sites of origin of cutaneous melanoma: a meta-analysis. Br J Dermatol 2011; 164: 776-784.

25. Wu S, Kuo H, Li WQ, Canales AL, Han J, Qureshi AA. Association between BRAFV600E and NRASQ61R mutations and clinicopathologic characteristics, risk factors and clinical outcome of primary invasive cutaneous melanoma. Cancer Causes Control 2014; 25: 1379-1386.

26. Thomas NE, Edmiston SN, Alexander A, Groben PA, Parrish E, Kricker A, Armstrong BK, Anton-Culver H, Gruber SB, From L, et al. Association Between NRAS and BRAF Mutational Status and Melanoma-Specific Survival Among Patients With Higher-Risk Primary Melanoma. JAMA Oncol 2015; 1: 359-368.

27. Elder DE, Gimotty PA, Guerry D. Cutaneous melanoma: estimating survival and recurrence risk based on histopathologic features. Dermatol Ther 2005; 18: 369-385.

28. Long GV, Menzies AM, Nagrial AM, Haydu LE, Hamilton AL, Mann GJ, Hughes TM, Thompson JF, Scolyer RA, Kefford RF. Prognostic and clinicopathologic associations of oncogenic BRAF in metastatic melanoma. J Clin Oncol 2011; 29: 1239-1246.

29. Shim JH, Shin HT, Park J, Park JH, Lee JH, Yang JM, Kim DH, Jang KT, Lee DY. Mutational profiling of acral melanomas in Korean populations. Exp Dermatol 2017; 26: 883-888.

30. Jakob JA, Bassett RL, Jr., Ng CS, Curry JL, Joseph RW, Alvarado GC, Rohlfs M L, Richard J, Gershenwald JE, Kim KB, et al. NRAS mutation status is an independent prognostic factor in metastatic melanoma. Cancer 2012; 118: 4014-4123.

31. Pracht M, Mogha A, Lespagnol A, Fautrel A, Mouchet N, Le Gall F, Paumier V, Lefeuvre-Plesse C, Rioux-Leclerc N, Mosser J, et al. Prognostic and predictive values of oncogenic BRAF, NRAS, c-KIT and MITF in cutaneous and mucous melanoma. J Eur Acad Dermatol Venereol 2015; 29: 1530-1538.

32. Mar VJ, Liu W, Devitt B, Wong SQ, Dobrovic A, McArthur GA, Wolfe R, Kelly JW. The role of BRAF mutations in primary melanoma growth rate and survival. Br J Dermatol 2015; 173: 76-82.

33. Hong JW, Lee S, Kim DC, Kim KH, Song KH. Prognostic and Clinicopathologic Associations of BRAF Mutation in Primary Acral Lentiginous Melanoma in Korean Patients: A Preliminary Study. Ann Dermatol 2014; 26: 195-202.

34. AJCC Cancer Staging Atlas: A Companion to the Seventh Editions of the AJCC Cancer Staging Manual and Handbook. New York: Springer-Verlag; 2012. XI, 637 p.

35. Oltulu P, Ince B, Turk N, Uyar M, Kilinc F. High risk factors and sentinel lymph node biopsy in cutaneous squamous cell carcinoma: Analysis of prevalence and recurrence. Selcuk Med J 2018; 34: 112-118.