Acta Dermatovenerol Croat 2019;27(4):225-230 CLINICAL ARTICLE

Clinicopathological Characteristics of BRAF V600E Mutated Melanomas in the Dalmatian Region of Croatia

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Received: September 19, 2018
Accepted: November 19, 2019

ABSTRACT A high proportion of cutaneous melanomas harbor activating mutations of the BRAF or NRAS genes, which are components of mitogen-activated protein kinase (MAPK) signal transduction pathway. The importance of BRAF V600E mutation in melanoma is not only related to the possibility of the administration of the targeted therapy, but also to the fact that BRAF V600E mutated melanomas have distinct clinicopathological features. We investigated the clinicopathological features of 80 primary skin melanomas with known BRAF V600E mutation status excised in the Dalmatian region of Croatia, with comparison of these features between the mutated and wild-type group. The frequency of BRAF V600E mutation was 47.5%. In comparison with wild-type melanomas, BRAF V600E mutated melanomas were significantly associated with younger age and female sex (P=0.014 and P=0.011, respectively). The mutated melanomas were more often located on the extremities, of a nodular type, ulcerated, and with higher median of mitotic index but without significant difference in comparison with wild-type tumors. There were no differences in the depth of invasion and the presence of lymphovascular invasion, tumor infiltrating lymphocytes, and regression between the investigated groups. The frequency of BRAF V600E mutation in our cohort of primary skin melanomas and the clinicopathological features of mutated tumors were similar to those reported in the literature, except for the higher proportion of women observed in our group with mutation.

KEY WORDS: cutaneous melanoma, BRAF V600E, mutation, histopathology

INTRODUCTION

The majority of skin tumor deaths are related to melanoma, and the worldwide incidence rate of melanoma has been increasing (1). In the countries of South-Eastern Europe the incidence rates of melanoma, particularly in those aged over 50, have also increased, while mortality rates have increased less prominently and with a diversity of trends across different countries (2). Melanoma comprises 3% of all newly diagnosed malignant tumors in Croatia, with an incidence rate of 13.7 per 100 000 (14.8 per 100 000 in men, and 12.7 per 100 000 in women) (3).

The risk factors for cutaneous melanoma are well-established and include a history of intermittent sun exposure and sunburns (especially during childhood), pale skin phenotype, red or blond hair, large number of common melanocytic nevi, three or more atypical

nevi, family history of melanoma, existence of non-melanocytic skin tumors or lesions related to actinic damage, and sunbed use (4-7).

The recent findings of causative genetic alterations in melanoma have provided a link between skin actinic damage and some frequent gene mutations. Melanomas occurring in non-chronically sun-damaged skin frequently harbor BRAF mutation, while melanomas occurring in chronically sun-damaged skin usually harbor NRAS or KIT mutations with infrequent BRAF mutation (8-10). Identification of these frequent changes of genes involved in cell signal transduction pathways is a prerequisite for the administration of the targeted therapy with BRAF and BRAF/MEK inhibitors (11).

The aim of this study was to determine the frequency of BRAF V600E mutation in a group of primary skin melanomas in Dalmatia and to compare the clinicopathological characteristics of the melanomas with and without this type of mutation.

PATIENTS AND METHODS

Clinicopathological features of 80 primary cutaneous melanomas with known BRAF V600E status excised in the period from 2004 to 2017 were retrieved from the databases of four Dalmatian hospitals (Split, Zadar, Šibenik, Dubrovnik). The analysis included 22 patients with stage 3 melanoma and 58 patients with stage 4 melanoma. BRAF V600E mutation analysis was performed at the Institute of Pathology and Cytology, Clinical Hospital Center Split, Croatia, in the period from 2013-2017. The analysis included 38 BRAF V600E mutation cases and 42 BRAF V600E wild type cases of cutaneous melanoma.

The following clinicopathological features were analyzed: patient age, tumor size, patient sex, tumor site, tumor histological type, Clark and Breslow staging, mitotic index, ulceration, lymphovascular invasion, presence of tumor infiltrating lymphocytes, and presence of regression. All aforementioned features were presented in a standardized pathohistological report (12). Pathohistological features, except for the histological types, were assessed according to recent AJCC cancer staging criteria (13). The tumors were histologically classified according to WHO criteria (14).

Hematoxylin and eosin (H&E) stained slides from each submitted sample were reviewed by a pathologist for DNA extraction analysis, and tumor tissue was also identified for analysis. For all tissue specimens, DNA was extracted from 10 mm thick sections using the cobas® DNA Sample Preparation Kit (Roche Molecular Diagnostics) following the manufacturer's protocol.

Table 1. Clinicopathological features of the study group of primary skin melanomas

Feature	Value /range / or number (%)
Age* (years ± SD)	59.5±15.6 /25-84/
Tumor size* (cm ± SD)	1.93±1.15 /0.5-7.5/
Sex	
male	59 (73.7)
female	21 (26.2)
Tumor site	
head	9 (11.3)
trunk	37 (46.2)
extremities	30 (37.5)
hand	2 (2.5)
foot	2 (2.5)
Tumor histological type	
nodular	38 (47.5)
superficial spreading	21 (26.2)
acral lentiginous	4 (5)
other	1 (1.3)
unclassifiable	16 (20)
Clark	
2	1 (1.3)
3	10 (12.6)
4	56 (70)
5	13 (16.3)
Breslow	
1	1 (1.3)
2	6 (7.5)
3	23 (28.7)
4	50 (62.5)
Mitotic index** (number per mm^2)	7
Ulceration	
present	49 (61.2)
absent	31 (38.7)
Lymphovascular invasion	
present	12 (15)
absent	68 (85)
Tumor infiltrating lymphocytes	
absent	42 (52.5)
nonbrisk	15 (18.8)
brisk	23 (28.7)
Regression	
present	4 (5)
absent	76 (95)
BRAF mutation status	
mutated	38 (47.5)
wild type	42 (52.5)

*Mean
**Median
SD: Standard Deviation

The amount of genomic DNA was quantified using the Qubit® 2.0 Fluorometer (Life Technologies) and adjusted to a fixed concentration to be added to the amplification/detection mixture.

Table 2. Comparison of clinicopathological features between the groups with mutated BRAF V600E and with BRAF V600E wild-type melanomas

Feature	Value /range/ or number (%)		*P****
	BRAF mutated	BRAF wild type	
Age* (years ± SD)	54.9±16.6 /25-84/	63.7±13.6 /34-84/	**0.014**
Tumor size* (cm ± SD)	1.96±1.06 /1-4.5/	1.92±1.25 /0.5-7.5/	0.89
Sex			
male	23 (60.5)	36 (85.7)	
female	15 (39.5)	6 (14.3)	**0.011**
Tumor site			
head	3 (7.9)	6 (14.3)	
trunk	15 (39.5)	22 (52.4)	
extremities	19 (50)	11 (26.2)	
hand	0	2 (4.8)	
foot	1 (2.6)	1 (2.4)	0.18
Tumor histological type			
nodular	20 (52.6)	18 (42.9)	
superficial spreading	8 (21.1)	13 (31)	
acral lentiginous	1 (2.6)	3 (7.1)	
other	0	1 (2.4)	
unclassifiable	9 (23.7)	7 (16.7)	0.50
Clark			
2	1(2.6)	0	
3	4 (10.5)	6 (14.3)	
4	26 (68.4)	30 (71.4)	
5	7 (18.4)	6 (14.3)	0.67
Breslow			
1	1 (2.6)	0	
2	2 (5.3)	4 (9.5)	
3	10 (26.3)	13 (31)	
4	25 (65.8)	25 (59.5)	0.6
Mitotic index** (number per mm^2)	7	6	0.15
Ulceration			
present	25 (65.8)	24 (57.1)	
absent	13 (34.2)	18 (42.9)	0.43
Lymphovascular invasion			
present			
absent	5 (13.2)	7 (16.7)	
	33 (86.8)	35 (83.3)	0.66
Tumor infiltrating lymphocytes			
absent			
non-brisk	17 (44.7)	25 (59.5)	
brisk	9 (23.7)	6 (14.3)	
	12 (31.6)	11 (26.2)	0.37
Regression			
present	2 (5.3)	2 (4.8)	
absent	36 (94.7)	40 (95.2)	0.92

*Mean
**Median
*** χ^2 test except t-test for age and size, and Mann-Whitney test for mitotic index
SD: Standard Deviation

For mutation analysis, the target DNA was amplified and detected on the cobas® z 480 analyzer using the amplification and detection reagents provided in the cobas® 4800 BRAF V600 Mutation Test kit, according to the manufacturer's protocol.

The cobas® results were reported as follows: V600E

mutation detected, V600E mutation not detected, or invalid (i.e. no result was obtained on the cobas® test).

The comparison of the clinicopathological features between the groups of melanomas with and without BRAF V600E mutation was performed using the χ^2 test, Mann-Whitney, and t-test. The significance of the differences was calculated as *P* value with probabilities lower than 5% ($P<0.05$) considered significant. Statistical analysis was performed using the MedCalc ver. 11.5.1.0 statistical software for Windows (MedCalc Software, Ostend, Belgium).

RESULTS

Clinicopathological features of the study group of stage 3 and 4 primary cutaneous melanomas are summarized in Table 1. The group predominantly consisted of male patients (mean age of 60 years) with the tumors of the average size of 1.9 cm. The majority of the tumors were located on the trunk and extremities (46.2% and 37.5%, respectively), and were predominantly of the nodular histological type (47.5%). The majority of the melanomas were of Breslow 4 and Clark 4 levels of invasion (62.5% and 70%, respectively), with high mitotic count (average mitotic index of 8/mm^2), with ulceration (61.2%) and no lymphocytic infiltration (52.5%). The lymphovascular invasion of melanoma cells and regression were rare events (15% and 5%, respectively). BRAF V600E mutation was observed in 38 patients (47.5%).

The comparison of the prevalence of the investigated clinicopathological features between the group of BRAF V600E mutated and the group of BRAF V600E wild type cutaneous melanomas is shown in Table 2. The patients with BRAF V600E mutated melanomas were significantly younger than patients with melanomas without BRAF V600E mutation (55 years vs. 64 years, respectively; $P=0.014$). We also observed a significantly higher percentage of women in the group of patients with BRAF V600E mutated melanomas than in the group of patients with BRAF V600E wild type melanomas (39.5% vs. 14.3%, respectively; $P=0.011$). In the group of BRAF V600E mutated melanomas we also observed a higher percentage of melanomas located on the extremities, higher percentage of nodular melanomas, and higher mitotic activity, but these differences did not reach statistical significance in the comparison with the group of melanomas without BRAF V600E mutation. There was no difference between this two groups of melanomas in tumor size, Breslow and Clark levels of invasion, presence of ulceration, presence of lymphovascular invasion, amount of tumor infiltrating lymphocytes, and presence of regression.

DISCUSSION

BRAF is a serine/threonine protein kinase which is an essential part of the mitogen-activated protein kinase (MAPK) pathway. It is physiologically activated by RAS, but in mutated form BRAF becomes constitutively active due to molecular conformational change, with subsequent persistent activation of downstream cytoplasmic and nuclear proteins (MEK, ERK, ETS) which ultimately leads to gene expression that promotes cell growth and survival (10,15). Inhibition of the altered MAPK pathway by BRAF inhibitors and combined BRAF/MEK inhibitors in BRAF mutated, unresectable, or metastatic melanoma has become the standard therapeutic approach (11).

The most common among the BRAF mutations in melanoma is the V600E mutation (over 90% of all BRAF mutations), which is a single nucleotide mutation at codon 600 causing amino acid substitution (glutamic acid for valine). The other types of BRAF mutations (V600K, V600R, V600E2, and V600D) are infrequent (11,15).

The frequency of codon 600 mutation in BRAF among cutaneous melanoma is 40-60% (11,15,16). The frequency of BRAF V600E mutation observed in this study is 47,5%. Katunarić *et al.* found a frequency of 52% for BRAF V600E mutations among patients with melanoma in the Rijeka County, while Kožaj *et al.* found that 43% of cases in their cohort harbored the same type of mutation (17,18).

Investigation of clinical characteristics of BRAF V600E mutated melanomas in this cohort found that the mutated tumors were significantly associated with earlier age of onset and female sex in comparison with wild-type tumors ($P=0.014$ and $P=0.011$, respectively). Several studies have found that BRAF V600E mutation is associated with the presentation of melanoma at an earlier age, indicating that the BRAF mutation is an early molecular event in melanoma evolution (18-21,23). Although the majority of studies have found no difference in sex between BRAF V600E mutated and wild-type melanomas or male predominance in the group of mutated melanomas, in the present study we found a significantly higher proportion of women in the group of mutated tumors ($P=0.011$) (18,21,22). Only a Japanese cohort of primary cutaneous melanomas showed female predominance in the group of BRAF V600E mutated tumors, but with borderline significance ($P=0.087$) (23). BRAF V600E mutated melanomas are related to intermittent sun exposure and usually arise on covered, truncal part of the skin (19,21,22). We did not find any significant difference in primary tumor site or in the average size of the tumors between the

BRAF V600E mutated and wild-type group, although our BRAF V600E mutated tumors were larger and in more commonly located on the extremities than wild-type tumors. Menzies *et al.* also reported that BRAF (V600E and non-V600E) mutation status is not associated with anatomic site in their large group of 301 primary cutaneous melanomas, but the authors found a significantly higher proportion of tumors located on the extremities in the BRAF V600E mutated tumors subgroup (20).

Many studies have investigated the correlation between BRAF status in melanoma and various pathohistological variables. Sehdev *et al.* found that BRAF mutation was significantly associated with higher Breslow thickness, higher mitotic rate, and presence of ulceration on multivariate analysis (22). Long *et al.* reported significant association of superficial spreading or nodular histological subtype, presence of mitosis, and histological signs of chronic sun damage with BRAF (V600E and non-V600E) mutation, and no association with Breslow thickness and presence of ulceration in their cohort of 197 cutaneous melanomas (19). Baiter *et al.* also showed an association between BRAF (V600E and non-V600E) mutation and superficial spreading and nodular histological subtypes (24). In the present study we also observed a higher percentage of nodular subtype and ulcerated tumors as well as higher median mitotic index in the group of BRAF V600E mutated melanomas, but without significant differences in the comparison with wild-type tumors. There was no difference between the mutated and wild-type group in presence of lymphovascular invasion, tumor infiltrating lymphocytes, regression, and the depth of invasion.

Some other, more subtle morphological features may be related to BRAF status. Viros *et al.* found significant association between BRAF codon 600 mutation and increased upward migration and nest formation of intraepidermal melanocytes, thickening of the involved epidermis, sharper demarcation of BRAF mutated melanoma from the surrounding skin, and larger epithelioid melanoma cells with abundant pigment (25). We were not able to investigate some of these features in our cohort of primary skin melanomas because none of these features were listed in standardized pathohistological reports of melanoma in Croatia (12).

CONCLUSION

Herein we have reported a frequency of BRAF V600E mutation of 47.5% in the group of 80 primary skin melanomas excised in the southern part of Croatia (Dalmatia). Comparison of the clinicopathological features between mutated BRAF V600E and wild-type melanomas showed that mutated tumors were significantly associated only with younger age and female sex. The mutated melanomas were more often located on the extremities, of the nodular type, ulcerated, and with a higher median of mitotic index, but without significant difference in comparison with wild-type tumors.

References:

1. Garbe C, Peris K, Hauschild A, Saiag P, Middleton M, Spatz A, *et al.* European Dermatology Forum; European Association of Dermato-Oncology; European Organization of Research and Treatment of Cancer. Diagnosis and treatment of melanoma. European consensus-based interdisciplinary guideline--Update 2012. Eur J Cancer. 2012;48:2375-90.
2. Barbaric J, Sekerija M, Agius D, Coza D, Dimitrova N, Demetriou A, *et al.* Disparities in melanoma incidence and mortality in South-Eastern Europe: Increasing incidence and divergent mortality patterns. Is progress around the corner? Eur J Cancer. 2016;55:47-55.
3. Croatian National Cancer Registry, Croatian National Institute of Public Health. Cancer incidence in Croatia 2014. Bulletin No 39. Zagreb (Croatia): Croatian National Institute of Public Health; 2016.
4. MacKie RM, Hauschild A, Eggermont AM. Epidemiology of invasive cutaneous melanoma Ann Oncol. 2009;Suppl 6:vi1-7.
5. Gandini S, Sera F, Cattaruzza MS, Pasquini P, Abeni D, Boyle P, *et al.* Meta-analysis of risk factors for cutaneous melanoma: I. Common and atypical naevi. Eur J Cancer. 2005;41:28-44.
6. Gandini S, Sera F, Cattaruzza MS, Pasquini P, Picconi O, Boyle P, *et al.* Meta-analysis of risk factors for cutaneous melanoma: II. Sun exposure. Eur J Cancer. 2005;41:45-60.
7. Gandini S, Sera F, Cattaruzza MS, Pasquini P, Zanetti R, Masini C, Boyle P, *et al.* Meta-analysis of risk factors for cutaneous melanoma: III. Family history, actinic damage and phenotypic factors. Eur J Cancer. 2005;41:2040-59.
8. Curtin JA, Fridlyand J, Kageshita T, Patel HN, Busam KJ, Kutzner H, *et al.* Distinct sets of genetic alterations in melanoma N Engl J Med. 2005;353:2135-47.
9. Curtin JA, Busam K, Pinkel D, Bastian BC. Somatic activation of KIT in distinct subtypes of melanoma. J Clin Oncol. 2006;24:4340-6.
10. 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.

11. Fedorenko IV, Gibney GT, Sondak VK, Smalley KS. Beyond BRAF: where next for melanoma therapy? Br J Cancer. 2015;112:217-26.
12. Herceg D, Buzina DS, Ceović R, Dotlić S, Ilić I, Orehovec SS, *et al.* Croatian society for medical oncology clinical guidelines for diagnosis, treatment and follow-up of patients with melanoma. Lijec Vjesn. 2016;138:22-9.
13. Gershenwald JE, Scolyer RA, Hess KR, Thompson JF, Long GV, Ross MI, *et al.* Melanoma of the skin. In: Amin MB, editor-in-chief. AJCC Cancer Staging Manual. 8th edition. Chicago: American Joint Committee on Cancer and Springer; 2017. pp. 563-85.
14. De Vries E, Bray F, Coebergh JW, Cerroni L, Ruiter DJ, Elder DE, *et al.* Malignant melanoma: Introduction. In: LeBoit PE, Burg G, Weedon D, Sarasin A, editors. World Health Organisation classification of tumours. Pathology and genetics of skin tumours. Lyon: IARC Press; 2006. pp. 52-65.
15. Ascierto PA, Kirkwood JM, Grob JJ, Simeone E, Grimaldi AM, Maio M, *et al.* The role of BRAF V600 mutation in melanoma. J Transl Med. 2012;10:85.
16. Dhomen N, Marais R. BRAF signaling and targeted therapies in melanoma. Hematol Oncol Clin North Am. 2009;23:529-45.
17. Katunarić M, Jurišić D, Zamolo G, Grahovac B. BRAF gene mutations in malignant melanoma in Rijeka county. In: Proceedings of the 3rd Pannonia Congress of Pathology; 2014 May 15-17; Bled, Slovenia.
18. Kožaj S, Ulamec M, Vučić M, Dabelić N, Marić-Brozić J, Haramustek S, *et al.* BRAF V600E and V600K mutations in Croatian patients with melanoma. In: Ozretić P, Levanat S, editors. HDIR-4: "From Bench to Clinic", Fourth Meeting of the Croatian Association for Cancer Research with International Participation; 2016 November 3-4; Zagreb, Croatia.
19. Long GV, Menzies AM, Nagrial AM, Haydu LE, Hamilton AL, Mann GJ, *et al.* Prognostic and clinicopathologic associations of oncogenic BRAF in metastatic melanoma. J Clin Oncol. 2011;29:1239-46.
20. Menzies AM, Haydu LE, Visintin L, Carlino MS, Howle JR, Thompson JF, *et al.* Distinguishing clinicopathologic features of patients with V600E and V600K BRAF-mutant metastatic melanoma. Clin Cancer Res. 2012;18:3242-9.
21. Heppt MV, Siepmann T, Engel J, Schubert-Fritschle G, Eckel R, Mirlach L, *et al.* Prognostic significance of BRAF and NRAS mutations in melanoma: a German study from routine care. BMC Cancer. 2017;17:536.
22. Sehdev A, Hayden R, Kuhar MJ, Cheng L, Warren SJ, Mark LA, *et al.* Prognostic role of BRAF mutation in malignant cutaneous melanoma. J Clin Oncol. 2018;Suppl 36:15.
23. Shinozaki M, Fujimoto A, Morton DL, Hoon DS. Incidence of BRAF oncogene mutation and clinical relevance for primary cutaneous melanomas. Clin Cancer Res. 2004;10:1753-7.
24. Baiter M, Schuler G, Hartmann A, Schneider-Stock R, Heinzerling L. Pathogenetic implications of braf mutation distribution in stage IV melanoma Patients. Dermatology. 2015;231:127-33.
25. Viros A, Fridlyand J, Bauer J, Lasithiotakis K, Garbe C, Pinkel D, *et al.* Improving melanoma classification by integrating genetic and morphologic features. PLoS Med.2008; 5:e120.