original report

Frequency of *BRAF* V600E Mutation in the Mexican Population of Patients With Metastatic Melanoma

abstract

Purpose The *BRAF* V600E mutation has been described in melanomas occurring in the Caucasian, European, and Asian populations. However, in the Mexican population, the status and clinical significance of *BRAF* mutation has not been researched on a large scale.

Methods Consecutive *BRAF*-tested Mexican patients with metastatic melanoma (n = 127) were analyzed for mutations in exon 15 of the *BRAF* gene in genomic DNA by real-time polymerase chain reaction technology for amplification and detection. The results were correlated with the clinical-pathologic features and the prognosis of the patients.

Results The frequency of somatic mutation V600E within the *BRAF* gene was 54.6% (43 of 127 patients). Nodular melanoma was the most prevalent subtype in our population, with *BRAF* mutations in 37.2% (16 of 55 patients). In contrast, superficial spread had a frequency of 18.6% *BRAF* mutation (eight of 24). Other clinicopathologic features were assessed to correlate with the mutation status.

Conclusion This study searched for the most prevalent *BRAF* V600E mutation type in melanoma in a heterogeneous population from Mexico. Nodular melanoma was found to be the most prevalent in metastatic presentation and the presence of *BRAF* V600E mutation, perhaps related to the mixed ancestry; in the north, ancestry is predominantly European and in the south, it is predominantly Asian. The outcomes of the mutation correlations were similar to those found in other populations.

J Glob Oncol 00. © 2017 by American Society of Clinical Oncology

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INTRODUCTION

Melanoma Frequency in the World

Melanoma is the second most common skin cancer and the most aggressive. Prevalence records have shown that the highest rates are in Australia (39 cases per 100,000 inhabitants per year) and New Zealand (34 cases per 100,000 inhabitants), followed by the United States with 17 cases per 100,000 inhabitants.[1,2] As known, melanoma rates are higher among people with fair skin with European descent and considerably lower in those with darker skin (eg, Hispanics and blacks in the United States).[3] Other European populations (eg, those in Great Britain, Germany, the Netherlands, Austria, and France) report rates of four to 10 cases per 100,000 inhabitants. African, Asian, and Pacific non-Caucasian populations report lower rates of three per 100,000 inhabitants.[2]

In Latin America, a prevalence of 1.7 cases per 100,000 inhabitants is estimated by the International Agency for Research on Cancer, with an extensive variability of zero cases per 100,000 in countries such as Belize to 7.6 cases per 100,000 in Uruguay.[1] In Mexico, the actual prevalence of malignant melanoma is unknown; estimations are two cases per 100,000 inhabitants according to International Agency for Research on Cancer[1]; however, national reports in hospital records report a lower incidence of 0.4 cases per 100,000[4] to 1.01 cases per 100,000 inhabitants, according to a retrospective study from the Malignant Neoplasm Histopathological Record.[5]

BRAF Mutation

The *BRAF* gene (v-raf murine sarcoma viral oncogene homolog B1; Mendelian Inheritance of Man no. 164757) is located at the 7q34 chromosome and encodes a serine/threonine kinase proto-oncogene, the normal function of which is to control the proliferation and differentiation through the mitogen-activated protein kinase pathway.

In general, mutations in *BRAF* may be found in 8% of human cancers, including 50% of melanomas, 30% to 70% of thyroid cancers, 30% of low-grade

ovarian cancer, and 10% of colorectal cancer.[6] In the article by Davies et al[7], somatic mutations in the BRAF gene were found in 66% of malignant melanomas, of which 80% corresponded to a simple substitution of a neutral amino acid (valine at position 599 in exon 15) by one negatively charged by glutamic acid. Subsequently, this numeric sequence was changed by V600E because of a discrepancy of a codon in exon 1 of the *BRAF* genetic sequence.[8]

The number of reports of *BRAF* mutations in primary malignant and metastatic melanoma has grown. On average, constitutive mutations in the *BRAF* oncogene are reported in 33% to 47% of primary melanomas and 41% to 55% of metastatic melanomas. V600E mutations have been described in different populations, especially Caucasian, European, and Asian populations.[9-14] In this article, we report our experience in V600E mutation and its clinical significance in the Mexican population.

PATIENTS AND METHOD

Patients and Tumor Tissue Samples

Tumor tissue samples were collected from different oncology centers throughout Mexico. From May 2012 to March 2013, 146 patients diagnosed with melanoma (metastatic or recurrent) were included in the study. Each patient signed an informed consent endorsed by the national institute authorities. Initially, only information about age, sex, histologic subtype, and clinical stage was requested. Afterward, information regarding ulceration degree; sites of metastasis; and treatment received, such as surgery, systemic therapy, and/or radiotherapy, was requested via e-mail. From the 146 samples, 139 could be analyzed for *BRAF* V600E mutation. From this cohort, 11 samples were excluded because of rare subtypes.

Table 1. Clinical and Pathologic Characteristics

Characteristics	Mutant *BRAF* (n = 43)	Wild-Type *BRAF* (n = 84)	*P* Value
Sex			
Female	20 (46.5)	45 (53.6)	.567
Male	23 (53.5)	39 (46.4)	
Age, years, mean ± SD	48.4 ± 13.0	58.3 ± 14.2	.659
Stage			.029
IIIB	2 (4.7)	14 (16.7)	
IIIC	12 (27.9)	12 (14.3)	
IV	22 (51.2)	36 (42.9)	
ND	7 (16.3)	22 (26.2)	
Subtypes			.743
Acral lentiginous	8 (18.6)	16 (19.0)	
Superficial spreading	8 (18.6)	9 (10.7)	
Lentigo maligna	4 (9.3)	8 (9.5)	
Nodular	16 (37.2)	39 (46.4)	
ND	7 (16.3)	12 (14.3)	
Sample site			.007
Metastatic	33 (76.7)	65 (73.9)	
Primary	10 (23.3)	23 (26.1)	
Age, years			.012
⩽ 40	11 (25.6)	9 (10.7)	
40-60	24 (55.8)	47 (47.6)	
> 60	8 (18.6)	40 (41.7)	

NOTE. Data given as No. (%) unless otherwise indicated.
Abbreviations: ND, no data; SD, standard deviation.

DNA Preparation and Mutation Test

Genomic DNA was extracted from paraffin-embedded tissue samples by using the QIAamp DNA FFPE Tissue Kit (catalog no. 56404; Qiagen, Hilden, Germany). For the detection of the mutation, we followed the instructions for and used the cobas 4800 *BRAF* V600 mutation test kit (Roche Molecular Systems, Pleasanton, CA), a real-time polymerase chain reaction-based assay designed to detect the presence of *BRAF* V600E (1799T>A).

Statistical Analysis

All statistical analyses were performed using software SPSS version 23 (IBM, Armonk, NY). Categorical information was described using frequencies and percentages. The continuous information such as age was described by using mean ± standard deviation or mean (range) for information with normal distribution. The χ^2 test or Fisher's exact test was used to differentiate the rates of different groups, and the differences in measurements of two groups were assessed through an unpaired *t* test.

RESULTS

BRAF V600E Gene Mutations in Melanoma

A total of 127 patients with melanoma were included in the study; their cancer was classified according to the American Joint Committee on Cancer as stage IIIB (n = 16), stage IIIC (n = 24), stage IV (n = 58), and unclassified (n = 29). The frequency of somatic mutation V600E in the *BRAF* gene was 54.6% (43 of 127 patients). The analysis of *BRAF* was performed in tumor tissue that was used for the initial pathologic diagnosis.

A descriptive analysis per geographical region of Mexico was performed; more samples were collected in the northern and central regions of the country. The central regions of Mexico focus more attention on melanoma, and these regions contributed more samples. More mutations per case

were recorded in samples from the northwest region (12 of 25 samples).

Clinical Characteristics Related to *BRAF* V600E Mutation

The clinicopathologic characteristics of the tumor samples and their relationship with the mutational stage are summarized in Table 1. The *BRAF* V600E mutation was more frequent in patients 40 to 60 years of age, compared with those younger than 40 and older than 60 years ($P = .012$). There was no association between sex and *BRAF* V600E mutation.

When histologic subtypes were compared, the prevalence of *BRAF* V600E differed from that reported in other series.[15] In our population, the superficial spreading melanoma presented a lower mutation frequency in comparison with that of nodular melanoma (18.6% *v* 37.2%, respectively); lentigo maligna melanoma and acral lentiginous melanoma (the other two subtypes) had mutation frequencies of 9.3% and 6.9%, respectively.

Up to 44% of patients had melanoma located on the lower limbs and only one patient (10%) had the *BRAF* V600E mutation. In 25 patients, previous sun exposure could be determined, showing a similar tendency to that reported in literature in which nonexposed patients had two cases of mutations compared with one case of mutation in those with sun exposure.

Prognostic Significance of *BRAF* V600E Mutation

Overall survival data were obtained from only 25 patients. The median follow-up was 9.38 months (range, 3.6 to 21.4 months). The median overall survival time for patients with mutated *BRAF* was 6.5 months, compared with 13.1 months for patients with wild-type *BRAF* ($P = .174$). Other analyses were difficult to perform because of the size of the sample and the lack of clinicopathologic information.

DISCUSSION

Malignant melanoma in Mexico has an estimated prevalence of 1.2%,[5] but the real prevalence is unknown. This study aimed to determine the frequency of *BRAF* V600E mutations in a heterogeneous population of Mexican patients with malignant melanoma. The result shows a mean frequency of 54.6%, similar to that reported in the Caucasian and European populations, differing from Asian and South American populations. Table 2 lists evidence of the prevalence of *BRAF* mutation in different countries.

In Mexican patients, two previous studies searching for *BRAF* V600E mutations found a distant frequency, from 6.4%[24] to 73%,[25] explained by the heterogeneity of the populations analyzed (from the center and northeast of the country, respectively) and the size of the sample analyzed (< 50 patients). The current study shows the correlation of the mutation with clinical characteristics is similar to those in other populations,[15] although it was not feasible to perform a deeper analysis because of incomplete clinical information. The most frequent histologic subtype in the Mexican population is acral lentiginous melanoma[26]; however, nodular melanoma is the form with the highest number of cases with *BRAF* V600E mutations, consistent with that reported in a previous study.[25] In the present cohort, no mucous melanoma cases or *BRAF* mutation were reported. One of the lines of research of our group, however, has been characterizing melanomas in sinunasal and buccal mucosa in the Mexican population,[27] and we have found a lower distribution than that reported in skin lesions (data not shown). In our study, the central part of the country

Table 2. Prevalence of *BRAF* Mutation in Different Countries

Country	*BRAF* Mutation Prevalence	V600E	Primary	Metastatic
Switzerland[10,11]	43-53	88-96		
France[16]	54.3	45.2	54.6	55.6
Germany[17]	48	82.1	45.5	51.3
Italy[9]	50	92.3	49	51
Sweden[11]	43	88	33	41
Ireland[18]	19			
Belgium	43.3			
Pakistan*[13]	34	98	48.5	57.1
Lebanon*	49		50	50
Syria*	67		29.1	70.8
Saudi Arabia*	54		95.6	4.3
Israel[19]	61			
China[20]	25.5	89.1		
Japan[20]	41.8			
Australia[12]	48			
Africa[21]	8			
Brazil[22]	39	92.1	39	40
Argentina[23]	32		55	12
Mexico	54.6			

NOTE. All values expressed as percentages.
*Excluding the nevi results.

was the region with the highest prevalence of *BRAF* mutation (41.8%), as observed in a previous study.[24] This might be related to the sample supply and the general ethnic mix in the country. In Mexico, larger epidemiologic and educational efforts are needed to determine the current incidence of melanoma, as are better data collection tools and definition of the characteristics of the different regions of the country to perform better studies of clinicopathologic correlation.

DOI: https://doi.org/10.1200/JGO.2016.008912
Published online on jgo.org on June 12, 2017.

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The following represents disclosure information provided by authors of this manuscript. All relationships are considered compensated. Relationships are self-held unless noted. I = Immediate Family Member, Inst = My Institution. Relationships may not relate to the subject matter of this manuscript. For more information about ASCO's conflict of interest policy, please refer to www.asco.org/rwc or ascopubs.org/jco/site/ifc.

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ACKNOWLEDGMENT

The quantitative polymerase chain reaction thermal cycler (CobasZ480) was provided by Roche Mexico.

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REFERENCES

1. Ferlay J, Shin H, Bray F, et al: GLOBOCAN 2008 v1.2, cancer incidence and mortality worldwide: IARC cancer base no. 10. Lyon, France, International Agency for Research on Cancer, 2010
2. Whiteman D, Green A: Epidemiology of malignant melanoma, in Dummer R, Pittelkow MR, Iwatsuki K, Green A, Elwan NM (eds): Skin Cancer–A World-Wide Perspective. Berlin, Germany, Springer, 2011, p 13
3. Merrill RM, Pace ND, Elison AN: Cutaneous malignant melanoma among white Hispanics and non-Hispanics in the United States. Ethn Dis 20:353-358, 2010
4. Schmerling RA, Loria D, Cinat G, et al: Cutaneous melanoma in Latin America: The need for more data. Rev Panam Salud Publica 30:431-438, 2011
5. Jacobo R, Pineda B, Leon G: Melanoma maligno cutáneo. Gac Mex Oncol 2:17-22, 2003
6. Young A, Lyons J, Miller AL, et al: Ras signaling and therapies. Adv Cancer Res 102:1-17, 2009
7. Davies H, Bignell GR, Cox C, et al: Mutations of the *BRAF* gene in human cancer. Nature 417:949-954, 2002
8. Kumar R, Angelini S, Czene K, et al: *BRAF* mutations in metastatic melanoma: A possible association with clinical outcome. Clin Cancer Res 9:3362-3368, 2003
9. Colombino M, Lissia A, Capone M, et al: Heterogeneous distribution of *BRAF*/*NRAS* mutations among Italian patients with advanced melanoma. J Transl Med 11:202, 2013
10. Edlundh-Rose E, Egyházi S, Omholt K, et al: *NRAS* and *BRAF* mutations in melanoma tumours in relation to clinical characteristics: A study based on mutation screening by pyrosequencing. Melanoma Res 16:471-478, 2006
11. Ekedahl H, Cirenajwis H, Harbst K, et al: The clinical significance of *BRAF* and *NRAS* mutations in a clinic-based metastatic melanoma cohort. Br J Dermatol 169:1049-1055, 2013
12. Long G V, Menzies AM, Nagrial AM, et al: Prognostic and clinicopathologic associations of oncogenic *BRAF* in metastatic melanoma. J Clin Oncol 29:1239-1246, 2011
13. Saroufim M, Habib R, Karram S, et al: *BRAF* analysis on a spectrum of melanocytic neoplasms: An epidemiological study across differing UV regions. Am J Dermatopathol 36:68-73, 2014
14. Si L, Kong Y, Xu X, et al: Prevalence of *BRAF* V600E mutation in Chinese melanoma patients: Large scale analysis of *BRAF* and *NRAS* mutations in a 432-case cohort. Eur J Cancer 48:94-100, 2012
15. Liu W, Kelly JW, Trivett M, et al: Distinct clinical and pathological features are associated with the *BRAF* T1799A (V600E) mutation in primary melanoma. J Invest Dermatol 127:900-905, 2007
16. Boursault L, Haddad V, Vergier B, et al: Tumor homogeneity between primary and metastatic sites for *BRAF* status in metastatic melanoma determined by immunohistochemical and molecular testing. PLoS One 8:e70826, 2013
17. Heinzerling L, Baiter M, Kühnapfel S, et al: Mutation landscape in melanoma patients clinical implications of heterogeneity of *BRAF* mutations. Br J Cancer 109:2833-2841, 2013
18. Balint B, van den Hurk K, Toomey S, et al: Low incidence of BRAFV600E mutation among melanoma patients in Ireland. Cancer Res 73, 2013 (suppl 1; abstract 23)
19. Lazarev I, Yakobson SA, Beer-Sheva IL: *BRAF* mutations are frequent in malignant melanoma in Israeli population and predicts poor response to biochemotherapy. ESMO 2012 (abstr 3145)
20. Yamazaki N, Tanaka R, Tsutsumida A, et al: BRAF V600 mutations and pathological features in Japanese melanoma. Melanoma Res 25:9–14, 2015
21. Akslen LA, Puntervoll H, Bachmann IM, et al: Mutation analysis of the *EGFR-NRAS-BRAF* pathway in melanomas from black Africans and other subgroups of cutaneous melanoma. Melanoma Res 18:29-35, 2008
22. Jung JE, Falk TM, Bresch M, Eduardo J, Matias F: *BRAF* mutations in cutaneous melanoma: No correlation with histological prognostic factors or overall survival. J Bras Patol Med Lab 46:487-493, 2010
23. Boggio G, Falco A, Maur Perotti J: Oncogenic BRAF mutation in cutaneous melanoma in Argentina. Poster presented at the 17th ECCO- 38th ESMO - 32nd ESTRO European Cancer Congress (ECC): Amsterdam, The Netherlands, September 27-October 1, 2013
24. Zepeda-Lopez PD, Salas-Alanis JC, Toussaint-Caire S, et al: BRAF mutation (V600E) prevalence in Mexican patients diagnosed with melanoma. Case Rep Oncol 9:241-245, 2016
25. Fajardo-Ramírez OR, Salas-Alanis JC, Guzmán-Huerta E, et al: BRAF mutations among patients from the Northeast of México with malignant melanoma. Rev Invest Clin 66:288-290, 2014
26. Káram-Orantes M, Toussaint-Caire S, Domínguez-Cherit J, et al: Clinical and histopathological characteristics of malignant melanoma cases seen at "Dr.Manuel Gea González" General Hospital [in Spanish]. Gac Med Mex 144: 219-223, 2008
27. Maldonado-Mendoza J, Ramírez-Amador V, Anaya-Saavedra G, et al: Clinicopathological characterization of primary oral and sinonasal melanoma in a referral centre in Mexico City: 2000-2012. Int J Oral Maxillofac Surg 44:427-432, 2015