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Research Paper

Detection of BRAF Gene Mutation in Primary Choroidal Melanoma Tissue

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KEY WORDS

BRAF, PI3K, uveal melanoma

ABBREVIATIONS

PCR polymerase chain reaction

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ABSTRACT

Numerous *BRAF* mutations have been detected in melanoma biopsy specimens and cell lines. In contrast, several studies report lack of *BRAF* mutations in uveal melanoma including primary and metastatic choroidal and ciliary body melanomas. To our knowledge, for the first time, here we report a case of choroidal melanoma harboring the *BRAF* mutation (V600E). The activation of RAF/MEK/ERK pathway, although independent of *BRAF* mutation, was reported in uveal melanoma. The presence of V600E mutation indicates that the RAF/MEK/ERK pathway, in addition to cutaneous melanoma progression, may play a role in the choroidal melanoma development.

INTRODUCTION

Uveal melanoma is the most common primary intraocular tumor in adults. Uveal melanomas arise in the iris in approximately 3% of patients, the ciliary body in 5–10%, and the choroid in about 90%, with about 40% extending to within 3 mm of optic disc and/or fovea. They tend to cause exudative retinal detachment, astigmatism, cataract, secondary glaucoma from angle invasion, and, in the advanced stages, neovascular glaucoma and uveitis.[1] Extraocular extension can occur along scleral channels for posterior ciliary arteries, vortex veins and drainage vessels.[1] Metastatic disease occurs by hematogenous spread and is invariably fatal, usually within a year of the onset of symptoms.[2]

B-Raf is a kinase that activates the RAF/MEK/ERK signal transduction cascade. Increased activity of the RAF/MEK/ERK pathway prevents apoptosis and induces cell cycle progression.[3,4] Mutation of *BRAF* has been proposed to contribute to melanoma development. Numerous *BRAF* mutations have been detected in melanoma biopsy specimens and cell lines.[5-8] V600E accounts for more than 90% of *BRAF* mutations in melanoma. This mutation causes a substantial increase in B-Raf kinase activity.[6] Other *BRAF* mutations in melanoma at the same residue are V600D, V600G, V600K, and V600R. *BRAF* residue 469 is another amino acid that is frequently mutated. G469E, G469R, and G469S have each been observed in melanoma. In contrast, several studies report lack of *BRAF* mutations in uveal melanoma including primary and metastatic choroidal and ciliary body melanomas.[9-12] Cutaneous and uveal melanoma both arise from neural crest-derived melanocyte, the activation of RAF/MEK/ERK pathway, independent of *BRAF* mutation, has been reported in uveal melanoma.[12,13] However, this mechanism remains to be clarified. In addition an interaction between the MAPK and PTEN pathways has been recently found in cutaneous melanoma.[14] Therefore; in this study, a tumor biopsy specimen from a patient with primary choroidal melanoma was analyzed to detect *BRAF* and *PI3K* gene mutations.

MATERIAL AND METHODS

Primary choroidal melanoma specimen. Tumor biopsy specimen was isolated from a 51-year-old woman diagnosed with choroidal melanoma by the Department of Pathology of the Vittorio Emanuele II Hospital in Catania, Italy. The tumor sample was fixed with formalin then embedded with paraffin.

***BRAF* and *PI3K* mutation analysis.** The biopsy specimen was screened in duplicate for *BRAF* and *PI3K* mutations within exons 11 and 15, 9 and 20, respectively. Genomic DNA was isolated with the QIAgen Tissue Kit (Qiagen, Valencia, CA, USA). Primers used to amplify parts of *BRAF* and *PI3K* are shown in Table 1. DNA was denatured for seven minutes at 94°C then subjected to 35 amplification cycles. Each amplification cycle consisted of: (1) denaturation for 30 seconds at 94°C, (2) annealing for 30 seconds at 57°C, and (3) extension for 30 seconds at 68°C. DNA was incubated at 72°C for an additional nine minutes after completion of the final amplification cycle.

PCR products were separated by electrophoresis in 2% agarose then stained with ethidium bromide. Purified PCR products were sequenced with an ABI Prism 3100 Genetic Analyzer (PerkinElmer, Foster City, CA, USA) by the dye terminator protocol. The sequence of each PCR product was compared with that of GenBank.

Table 1 **PCR primers for *BRAF* and *PI3K* genes**

Gene	Exon	Primer name	Primer Sequence
BRAF	11	ex 11F	5′-TCCCTCTCAGGCATAAGGTAA-3′
		ex 11R	5′-CGAACAGTGAATATTTCCTTTGAT-3′
BRAF	15	ex 15F	5′-TCATAATGCTTGCTCTGATAGGA-3′
		ex 15R	5′-GGCCAAAAATTTAATCAGTGGA-3′
PI3K	9	ex 9F	5′-TAAATCATCTGTGAATCCAGAGGGG- 3′
		ex 9R	5′-CATGCTGAGATCAGCCAAATTCAGT-3′
PI3K	20	ex 20F	5′-GACATTTGAGCAAAGACCTGAAGG-3′
		ex 20R	5′-ATTCCTATGCAATCGGTCTTTGCC-3′

RESULTS AND DISCUSSION

Amplification of *ras* proto-oncogenes and activating mutations that lead to the expression of constitutively-active Ras proteins have been extensively studied in human cancer.[6,15] The mutation frequency of the *RAS* gene family in cutaneous melanoma has been estimated to be 20-30%.[6,15] In this regard, controversial results have been reported in choroidal melanomas. Several authors indicate that *BRAF* mutations are not detected in primary uveal melanoma;[11,12] while, in vitro studies suggest that such mutations play roles in uveal melanoma cell growth.[13,14,16]

Cutaneous and choroidal melanomas share a common embryological origin, the neural crest, and similar histological features, but they differ in epidemiological and cytogenetic aspects. To our knowledge, for the first time, in the present study the *BRAF* mutation (V600E) was detected in choroidal melanoma tissue (Fig. 1). While, no mutations within exon 11 were detected in this sample. Similarly, no mutations were found in both exon 9 and 20 of *PI3K* gene (Fig. 2). The choroidal melanoma sample analyzed in the present study showed both epithelioid and spindle cell types and marked pigmentation as revealed in the hematoxylin-eosin-stained section (Fig. 3).

Our results appear to be in contrast with those already reported.[11,12] On the other hand, our data support previous findings showing the presence of *BRAF* mutation (V600E) in primary uveal melanoma cell lines.[13] This mutation was also detected in three choroidal melanoma cell cultures derived from primary uveal melanoma.[16] The same authors also demonstrated that constitutive activation of the MEK/ERK module in melanoma cells is directly linked to the presence of mutated BRAF protein.[16]

The presence of V600E mutation indicates that the RAF/MEK/ERK pathway, in addition to cutaneous melanoma progression, may play a role in the choroidal melanoma development. Activating *BRAF* mutations alter the RAS signaling pathway downstream of many growth factors receptors, contributing to cell autonomy from external growth signals. Previous studies, by western blot analysis and immunohistochemistry have demonstrated the activation of RAF/MEK/ERK pathway in both primary uveal cell line and uveal melanoma specimens independent of *RAS* and *BRAF* mutations.[13]

The detection of *BRAF* mutation in choroidal melanoma suggests that agents that interfere with *BRAF* signalling are likely to provide important new strategies for the management of this disease. Recently, the BAY43-9006, a Raf kinase inhibitor,[17] has been shown to inhibit also both wild-type and mutant B-Raf mediated growth of a human melanoma cell line carrying the *BRAF* V600E mutation.[18] However, it has been suggested that the compound's efficacy in advanced melanoma is lower on activated mutated forms of B-Raf than on wild-type B-Raf.[18,19]

In conclusion, the lack of *PI3K* mutations suggests that in uveal melanoma other mechanisms may cause tumor development. The presence of *BRAF* mutation revealed in a single case of choroidal melanoma indicate that other studies may define if this genetic abnormality is associated with choroidal melanoma patients from a specific geographic area such as the Mediterranean area.

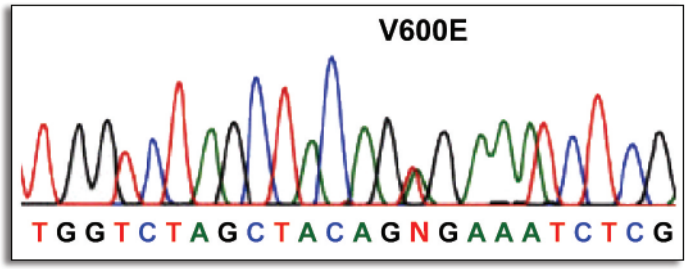

Figure 1. Sequence showing the *BRAF* exon 15 (V600E) mutation in choroidal melanoma DNA.

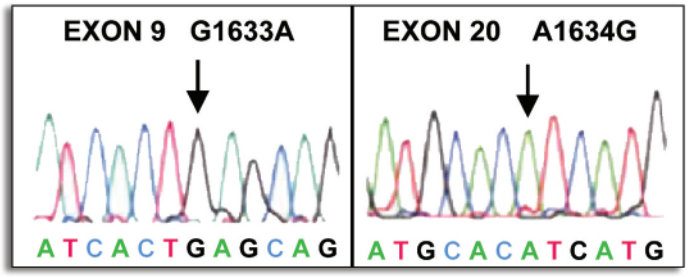

Figure 2. Sequence showing wild-type exons 9 and 20 of the *PI3K* gene. These exons contain the two major "hot spots" located in the helical and catalytic domains recently found to be mutated in human cancer.[20]

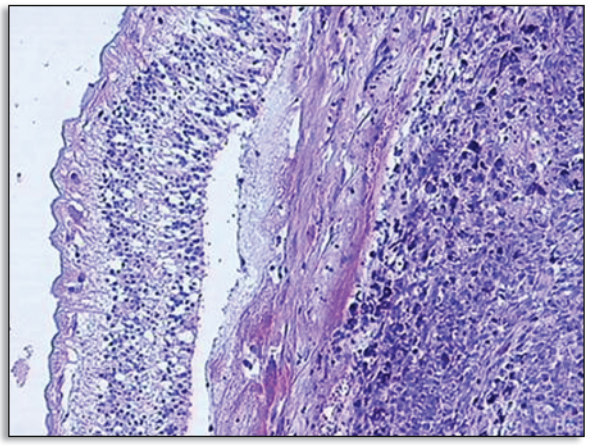

Figure 3. Choroidal melanoma section stained with hematoxylin and eosin.

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