Lack of BRAF Mutation in Primary Uveal Melanoma

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PURPOSE. BRAF T1796A activating mutations have been found in a high proportion of cutaneous melanomas, cutaneous nevi, and papillary thyroid carcinoma and in a small fraction of other cancers. This study was designed to investigate the incidence of BRAF T1796A mutation in uveal melanoma.

METHODS. Twenty-nine formalin-fixed, paraffin-embedded posterior uveal melanomas were included in the study. DNA was extracted from the paraffin sections followed by PCR amplification of exon 15 and detection of the common BRAF missense mutation (T→A transversion at nucleotide 1796) using restriction enzyme analysis.

RESULTS. Although positive cutaneous melanoma control cell lines harbored the T1796A BRAF mutation, none of the 29 uveal melanomas harbored the mutation.

CONCLUSIONS. These data suggest that BRAF T1796A activating mutation is not common in primary uveal melanoma. These findings are in accord with known differences in tumorigenesis between uveal and cutaneous melanomas. (Invest Ophthalmol Vis Sci. 2003;44:2876–2878) DOI:10.1167/iovs.02-1329

Uveal melanoma is the most common form of primary eye cancer in adults, with an annual incidence of six to seven cases per million.1 It accounts for 80% of the noncutaneous melanomas and for 15% of all deaths caused by melanoma.2 This tumor carries up to a 50% 5-year mortality from metastasis that is associated with both histologic and demographic prognostic factors such as cell type, tumor diameter and location, chromosomal aberration, age, and sex.1,3,4

Uveal and cutaneous melanomas originate from a common precursor cell, the melanocyte, which migrates from the neural crest to the respective site during the embryonic development period.3 The similar genetic background and some common histologic characteristics suggest a similar pathogenesis in these tumors.3,6 Although risk factors such as fair skin complexion and blue iris color may be shared,4 UV radiation and sunlight effect appears to play a significant role only in cutaneous melanoma.8 Furthermore, uveal melanoma metastasizes hematogenously, with the liver frequently affected,9 whereas cutaneous melanoma tends to spread through the lymphatic system, usually affecting the regional lymph nodes.10

Unlike cutaneous melanoma, little is known about the underlying molecular pathogenesis of uveal melanoma. Loss of gene function due to deletions or inactivating mutations, as well as gain-of-function mutations is the hallmark of cancer cells.11 To date, no oncogenes or tumor-suppressor genes have been linked to uveal melanoma. Cytogenetic analyses of uveal melanoma have identified chromosome 3 monosomy and increased chromosome 8, short arm, copy number in more than 50% of the tumors.12,13 This alteration also correlates significantly with metastasis and decreased survival.1,14

The importance of oncogenic mutations in the RAS/RAF/MEK/ERK pathway has been well documented in human cancer. More than 15% of all human cancers harbor point mutations of RAS.15 Constant activation of this pathway provides a potent promitogenic force, resulting in abnormal proliferation and differentiation in many human cancers.16 The association between RAS mutations and human uveal melanomas was investigated in several studies.17,18 Soparker et al.18 screened Ha-ras, Ki-ras, and N-ras at codons 12, 13, and 61 and could not find any mutations. It is still not known whether other genes in the RAS/RAF/MEK/ERK pathway have a role in the development of uveal melanoma.

Mutations in one of the RAF genes, BRAF, have been recently discovered in the majority of cutaneous melanomas,15 cutaneous nevi,16 and papillary thyroid carcinoma20 and to a lesser extent in other cancers.15,21 The predominant mutation reported in cutaneous melanoma and cutaneous nevi was a thymine-to-adenine (T→A) transversion at nucleotide position 1796 (corresponding to an amino-acid swap of glutamate for valine at residue 599; V599E). This transversion resulted in constant activation of BRAF and, in turn, of the MEK/ERK pathway. To screen for a possible shared etiologic factor between uveal and cutaneous melanomas, we screened 29 cases of primary posterior uveal melanoma tumors for the T1796A BRAF mutation.

MATERIALS AND METHODS

Pathologic Specimens

Formalin-fixed, paraffin-embedded sections from 29 posterior uveal melanomas were included in the study. The sections were collected from the Wilmer Eye Institute at the Johns Hopkins University School of Medicine (Baltimore, MD) and the ophthalmic pathology laboratory of the Hadassah University Hospital (Jerusalem, Israel). All tumor samples were removed as part of the patient’s treatment and with local ethics committee approval for use of the tissue in this study. The study protocol adhered to the tenets of the Declaration of Helsinki.

DNA Extraction

Tumor tissue was microdissected from an area in the sections with more than 75% malignant cells. DNA was purified by standard phenol-chloroform extraction followed by ethanol precipitation.15

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BRAF Exon 15 PCR Amplification

Approximately 100 ng of total cellular DNA was used in each PCR amplification. The PCR was performed using specific BRAF exon 15 primers: forward primer, TCATAATGCTTGCTCTGATAGGA; reverse primer, GGCACAAATAATTACGTTGGA, as described elsewhere.15 A step-down PCR protocol was used as follows: 95°C for 2 minutes, 1 cycle; 95°C for 1 minute, 60°C for 1 minute, 72°C for 1 minute, 2 cycles; 95°C for 1 second, 58°C for 1 minute, 72°C for 1 minute, 2 cycles; 95°C for 1 minute, 56°C for 1 minute, 72°C for 1 minute, and 30 cycles and a final extension at 72°C for 5 minutes.

Analysis of BRAF T1796A Mutations

Analysis of BRAF T1796A mutations was performed as described previously.20 Briefly, 10 μL of PCR product was incubated with 1 μL of TspRI restriction enzyme (10 U/μL New England Biolabs, Beverly, MA) in a 30-μL reaction volume overnight at 65°C. The same reaction replacing the enzyme with deionized (d)H2O was used as negative control. The samples were loaded and run on a nondenaturing 10% polyacrylamide gel. The gels were stained with ethidium bromide, and the bands were visualized under a UV lamp.

RESULTS

To detect BRAF mutations in posterior uveal melanoma, we screened the gene for the T1796A transversion by PCR and restriction enzyme analysis. TspRI digestion of the PCR fragment yielded three major bands at 125, 87, and 12 bp in the wild-type allele. The T1796A mutation abolished one restriction site, resulting in a prominent 212-bp band from the mutant wild-type allele. The T1796A mutation abolished one restriction site, resulting in a prominent 212-bp band from the mutant wild-type allele. The T1796A mutation abolished one restriction site, resulting in a prominent 212-bp band from the mutant wild-type allele.

DISCUSSION

Although advances in molecular genetics have made possible the identification of genetic changes and particular mutant genes in human tumors, relatively little is known about the molecular genetic alterations leading to the development of uveal melanoma. In an attempt to better understand the molecular events that lead to uveal melanoma, we searched for a BRAF mutation that has been found to occur in up to 80% of cutaneous nevi and cutaneous melanomas.15,19

Although common in cutaneous nevi and cutaneous melanoma, the T1796A BRAF mutation was absent in uveal melanomas. Because we did not sequence the complete exon 15 in the present study, we cannot exclude completely the presence of other less common BRAF mutations (V599D, V599K, V599R)15,19 in uveal melanoma. However, the very common T1796A (V599E) BRAF mutation in cutaneous nevi and cutaneous melanomas does not play a role in the pathogenesis of uveal melanoma. This result is in accord with the known epidemiologic and histologic differences previously described between these two melanoma subtypes.5,9,22 It is conceivable that uveal melanoma arises from a series of genetic changes divergent from those of cutaneous melanoma. Although chromosome 3 monosomy and 8q trisomy are common in uveal melanoma, they are rarely observed in cutaneous melanoma.2 Integrin expression, which is essential for growth and metastatic capacity of cutaneous melanoma cells, as well as the expression of melanoma-associated antigens and the melanocortin-1 receptor, differ markedly between uveal and cutaneous melanomas.3,23–25 Our findings thus support further genetic diversity between cutaneous and uveal melanomas.

REFERENCES