Lack of Oncogenic GNAQ Mutations in Melanocytic Lesions of the Conjunctiva as Compared to Uveal Melanoma

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PURPOSE. Somatic mutations in codon 209 of the GNAQ gene are the first initiating events to be identified in uveal melanoma. The purpose of this study was to search for GNAQ209 mutations in conjunctival melanocytic lesions.

METHODS. Forty archival samples of conjunctival melanocytic lesions (conjunctival nevi, primary acquired melanosis, and conjunctival melanoma), 27 samples of uveal melanoma, and 11 samples of uveal melanoma metastases to the liver (3 of which matched primary uveal melanoma samples)—a total of 78 samples from 75 patients—were examined for the presence of GNAQ209 mutations by using chip-based, matrix-assisted laser-desorption time-of-flight (MALDI-TOF) mass spectrometry. Direct sequencing was also performed.

RESULTS. The GNAQ209 mutation was identified in 12 (44.5%) uveal melanoma samples and 4 (36.5%) of the 11 metastases of uveal melanoma. It was not detected in any of the other melanocytic lesions.

CONCLUSIONS. The GNAQ209 mutation rate in uveal melanoma in this study is in line with the rate in other reports. The finding of the same genotype in the primary tumors and their metastases suggests that mutation in GNAQ is an early event in uveal melanoma tumorigenesis. The lack of GNAQ mutations in conjunctival melanocytic lesions suggests the involvement of a different tumorigenic pathway from that of uveal melanoma. (Invest Ophthalmol Vis Sci. 2010;51: 6180–6182) DOI:10.1167/iovs.10-5677

Somatic mutations in codon 209 of the ras-like domain of the heterotrimeric G protein α-subunit (GNAQ) gene are the first initiating oncogenic events identified to date in uveal melanoma.1 The reported rate of the mutation in uveal melanoma is 46%.2 GNAQ mutations have also been identified in blue nevi (83%), and malignant blue nevi (50%).2

Earlier studies of melanocytic lesions in the eye focused on the RAS/RAF/MEK/ERK pathway,2–4 which is involved in the transduction of mitogenic signals from the cell membrane to the nucleus. The BRAF and NRAS genes serve as common targets for somatic mutations in benign and malignant neoplasms that arise from melanocytes.1 Mutations in the BRAF gene have been identified in 40% of conjunctival nevi and in the NRAS gene, in 50% of conjunctival melanomas.3 However, a search for BRAF and NRAS mutations in other melanocytic lesions of the eye yielded negative results for uveal melanoma4;4 as well as for conjunctival primary acquired melanosis,5 although two later studies reported BRAF mutations in some of the tumor cells of uveal melanoma.5,6

Mutations in BRAF (common nevocellular nevi), NRAS (congenital nevi), HRAS (Spitz nevi), and GNAQ (blue nevi) can all cause activation of the mitogen-activated protein kinase (MAPK) signaling pathway, which plays a role in the initiation of melanocytic tumors. Yet, by themselves, they are insufficient to induce progression toward melanoma.6

A recent systematic study of the mutational profile of exon 5 of the GNAQ gene in a panel of tumors (glioblastoma; gastrointestinal stromal tumors; acute myeloid leukemia; bladder, breast, colorectal, lung, ovarian, pancreas, and thyroid carcinomas; skin melanoma; and blue nevi) yielded positive findings only for blue nevi.8 These results were supported by reports of a common finding of a somatic mutation in GNAQ209 in primary melanocytic neoplasms,9 and of a GNAQ-activating mutation in iris melanomas (22%).2 The lack of a GNAQ mutation in skin melanoma highlights its different pathogenesis from uveal melanoma, suggested by earlier reports of differences between these two lesions in the prevalence of BRAF mutations.10–12

Prompted by the relatively high occurrence of GNAQ209 mutations as an initiating event in uveal melanoma, the present study was conducted to search for GNAQ mutations in conjunctival melanocytic lesions.

METHODS

The study protocol was approved by the national and institutional review boards. The research complied with the tenets of the Declaration of Helsinki.

Samples

Review of the archives of the Ophthalmic Pathology Laboratories of Hadassah-Hebrew University Medical Center, Jerusalem, and the Rabin Medical Center, Petah Tiqwa, from 1995 to 2005, yielded 40 surgically removed samples of conjunctival melanocytic lesions available for study (29 nevi, 4 melanosomes, and 7 primary acquired melanosis, in addition to 27 samples of uveal melanoma and 11 samples of liver metastases that arise from melanocytes.1 Mutations in the BRAF gene have been identified in 40% of conjunctival nevi and in the NRAS gene, in 50% of conjunctival melanomas.3 However, a search for BRAF and NRAS mutations in other melanocytic lesions of the eye yielded negative results for uveal melanoma4;4 as well as for conjunctival primary acquired melanosis,5 although two later studies reported BRAF mutations in some of the tumor cells of uveal melanoma.5,6

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metastases (matching 3 of the primary uveal melanoma samples), for a total of 78 samples from 75 patients.

Sample Preparation

DNA was isolated from the archival formalin-fixed, paraffin-embedded tissues, as previously described. In brief, hematoxylin-eosin–stained 10-μm-section slides were first reviewed by a pathologist. Areas with an estimated content of >75% tumor cells were separated by microdissection from five consecutive, 10-μm, unstained, paraffin-embedded sections of each block with a no. 11 surgical blade. After deparaffinization, the microdissected tissues were incubated overnight in 1% SDS and 0.5 mg/mL proteinase K. DNA was purified by phenol-chloroform extraction and ethanol precipitation and dissolved in 50 μL of distilled water, as previously described.

Mutation Analysis

The GNAQ gene was analyzed for mutations with the chip-based, matrix-assisted laser-desorption time-of-flight (MALDI-TOF) mass spectrometer (Sequenom, San Diego, CA). Specific primers flanking the mutation sites and extension primers that bind adjacent to the mutation site were designed using assay design software (Sequenom; see Table 1 for the list of primers). After the region of interest was amplified, the primer extension reaction was performed, including sequence-specific hybridization and sequence-dependent termination. This process generated different products for the mutated and wild-type alleles, each with a unique mass value. Genotyping was performed by spotting the extension products onto silicon chips that were preloaded with proprietary matrix (SpectroChip; Sequenom) and were subsequently read by the MALDI-TOF mass spectrometer. This method is considered highly sensitive, with detection of as little as 1% mutation compared to the 15% needed for direct sequencing.

Sequencing

Direct sequencing of the PCR products was performed with GNAQ2 primers (Table 1) and dye termination chemistry reagents (Big Dye

<table>
<thead>
<tr>
<th>GNAQ Mutation</th>
<th>Forward</th>
<th>Reverse</th>
<th>Extension</th>
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<tbody>
<tr>
<td>627 A&gt;T</td>
<td>ACG TTG GAT GAC ACT ATT GGT CGA TGT AGG</td>
<td>ACG TTG GAT GGA GGT GAC ATT TCC AAA GCA G</td>
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<tr>
<td>626 A&gt;C</td>
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<td>ACG TTG GAT GGA GGT GAC ATT TCC AAA GCA G</td>
<td>ATG TTC TCT CTC TGA CCT T</td>
</tr>
</tbody>
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Table 1. Primers

![Figure 1](https://example.com/figure1.png)

**Figure 1.** The GNAQ209 mutation as identified by MALDI-TOF analysis: heterozygous mutation AC (A) and AT (B). Direct sequencing for detection of the GNAQ209 mutation: AC (C, arrow) and AT (D, arrow).
RESULTS
The results are shown in Figure 1. No mutations in the GNAQ gene were detected in any of the samples of conjunctival primary acquired melanosis, conjunctival nevus, or conjunctival melanoma. It is of note that 40% of the conjunctival nevus and melanoma tested positive for BRAF mutations in a previous study.¹³

Twelve of the 27 (44.5%) samples of uveal melanoma and 4 (36.5%) of the 11 metastases were positive for GNAQ209 mutations. In all three of the uveal primary melanoma tumors for which metastases were available, paired analysis yielded GNAQ mutation in both the metastases and their corresponding primary tumors.

DISCUSSION
We speculated that the relatively high occurrence of somatic GNAQ initiating mutations in uveal melanomas² might indicate the presence of the mutation in conjunctival melanocytic lesions, as well. However, we found GNAQ mutations in 44.5% of the uveal melanoma samples, in accordance with previous studies, but in none of the samples of conjunctival melanocytic lesions. Furthermore, on paired analysis, all three metastases of uveal melanoma had the same GNAQ genotype as their primary tumor, supporting GNAQ mutation as an early event in uveal melanoma,¹ unrelated to tumor spread.

The GNAQ positivity of all three primaries included in the paired analysis, relative to 4 of the 11 uveal melanoma primaries in the study (a rate commensurate with the literature), was probably coincidental.

The guanine nucleotide-binding proteins (G proteins) are a family of heterotrimeric proteins that couple cell surface, seven-transmembrane, domain receptors to intracellular signaling pathways. In mice and humans, the α-subunit (Gq class) is an essential component of G protein interaction with receptors and effectors.¹³ The Gg class is one subfamily of the G protein α-subunit’s multigene family. It comprises four genes: GNAQ, GNA11, GNA14, and GNA15. The GNAQ gene maps on the long arm of chromosome 9, region 21, and encodes a heterotrimeric GTP-binding protein α-subunit that couples G-protein-coupled receptor signaling to the MAPK pathway, which is essential for early melanocyte survival.¹⁴

The frequent somatic mutations in the heterotrimeric G protein α-subunit GNAQ in blue nevi (83%) and uveal melanoma (46%) occur exclusively in codon 209 in the ras-like domain and result in constitutive activation, turning GNAQ into a dominant acting oncogene.² In this study, we attempted to genetically identify initiating GNAQ mutations in pigmented conjunctival lesions. However, the results were negative. This finding appears to be in line with the benign behavior of conjunctival nevi, as suggested by their histopathologic and long-term clinical features.¹³,¹⁴ Growth is documented in only 4% of conjunctival nevi,¹⁵ with fewer than 1% evolving to melanoma.¹⁷ Zamir et al.¹⁶ defined a subset of childhood nevi characterized by a confluent growth pattern and a lack of maturation, which could also prove useful in predicting which primary acquired lesions will progress to melanoma.¹³,¹⁸ The molecular events leading to the development of conjunctival melanoma are poorly understood. Damato and Coupland¹⁸ suggested that primary acquired melanosis of the conjunctiva with atypia and conjunctival nevi is associated with a higher risk of conjunctival melanoma. Invasive conjunctival melanoma can also arise de novo or from a nevus. After treatment, the tumor recurs locally in 50% of patients, and fatal metastases develop in 20% to 30%.¹⁹ The lack of a GNAQ mutation in atypical primary acquired melanosis and conjunctival melanoma in the present study suggests that other pathways are involved in their tumorigenesis.

References