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Molecular Characteristics of Conjunctival Melanoma Using Whole-Exome Sequencing

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Abstract

IMPORTANCE—Conjunctival melanoma (CM) is a highly aggressive ocular cancer for which treatment options are limited; the molecular pathogenesis is poorly understood.

OBJECTIVE—To identify the molecular characteristics of CM using next-generation whole-exome sequencing (WES).

DESIGN, SETTING, AND PARTICIPANTS—Whole-exome sequencing was performed on tumor DNA extracted from the archived specimens of 5 patients with CM who had been treated with surgical excision between 2006 and 2011. These samples were analyzed at a tertiary academic ocular oncology referral center using a customized bioinformatic pipeline.

MAIN OUTCOMES AND MEASURES—Sample analyses were designed to detect driver mutations, chromosome copy number aberrations, and mutation signatures.

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RESULTS—The study's 5 patients ranged in age from 51 to 77 years. Four of the 5 were female, and all were white. Mutations were detected in known oncogenes, including *BRAF*, *NRAS*, *NF1*, *EGFR*, *ALK*, *TERT*, and *APC*. None of the mutations associated with uveal melanoma were found. All samples demonstrated a C→T mutation signature typical of UV-induced DNA damage. The most common CNA was a gain in chromosome 6p.

CONCLUSIONS AND RELEVANCE—In these 5 patients, WES allowed identification of mutations that can be targeted with therapy and supported the role of UV light in CM pathogenesis. These findings indicate a need for larger studies to evaluate the diagnostic, prognostic, and therapeutic value of WES for CM.

Conjunctival melanoma (CM) is a rare but potentially deadly ocular malignant condition, with a 10-year disease-specific mortality of 9% to 35%.[1] Primary treatment of CM consists of local surgical excision with wide margins and adjuvant therapy (cryotherapy, brachytherapy, and/or topical application of mitomycin C). However, regional and systemic metastasis occurs in approximately 30% of patients within 3 years, and there are no effective treatments for metastatic disease.[1] Conjunctival melanoma appears to be a distinct entity compared with other mucosal melanomas. In contrast to these malignant conditions, CM incidence is often associated with UV sunlight exposure. Conjunctival melanoma is also associated with a higher 5-year survival rate (86%) compared with melanomas of the gastrointestinal tract (4%–33%), urogenital tract (7%–22%), and respiratory mucosal tissues (0%–31%); this difference is possibly related to earlier detection or differences in the innate aggressiveness of the tumor.[2]

The molecular attributes of CM remain poorly characterized, which is a problem that has hindered the development of novel therapies. One study[3] reported mutations in *BRAF* and *NRAS* in 29% and 18% of CMs, respectively, but the technology used in this study did not allow for a comprehensive assessment of driver mutations, chromosome copy number aberrations (CNAs), and mutational signatures. In the present study, whole-exome sequencing (WES) permits more comprehensive characterization of the molecular biology of CM.

Methods

Five formalin-fixed, paraffin-embedded, archival CM specimens were selected based on the availability of sufficient tissue for testing. Tumor DNA was extracted from all 5 and prepared for WES. Ethical approval was obtained from the University of Miami institutional review board for this study, which adheres to the tenets of the Declaration of Helsinki and is compliant with the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Per institutional review board approval, the use of archival samples obviated the need for the informed consent of the included patients.

The University of Miami Sequencing Core facility conducted the WES using the customized bioinformatic pipeline of the present study, with a mean exome coverage target of ×60. Sequences were aligned to the human reference genome GRCh37/hg19 using NovoAlign (Novocraft Technologies Sdn Bhd). Since matched blood samples were not available, we used a panel of normal tissue samples (n = 117), a high-coverage blood sample, and somatic

single-nucleotide and insertion and deletion (indel) variant-caller MuTect2 to call out tumor variants and filter out likely silent germline polymorphisms.[4] To minimize artifacts introduced by the specimen archiving process, we filtered out all genetic variants present in less than 20% of sequencing reads. Additionally, variants were excluded if they were outside of coding or splicing regions, had fewer than 3 alternate reads, were present in greater than 0.5% of the population, or were predicted to be non-damaging using ANNOVAR (Children's Hospital of Philadelphia, Pennsylvania). Chromosome copy number aberrations were assessed using CNVkit (University of California, San Francisco) and Genomic Identification of Significant Targets in Cancer (GISTIC) version 2.0 (Broad Institute), whereas mutation signatures were analyzed using pmsignature (the University of Chicago) in R (R Development Core Team). Additional details are available in the Supplement in the eAppendix.

Results

All patients were white, and none had a history of cutaneous melanoma or uveal melanoma. Two of the 5 patients (40%) had CM that arose from primary acquired melanosis; all lesions arose on sun-exposed areas. Four of the 5 patients had bulbar CM (located within the conjunctiva covering the globe) and the remaining individual had palpebral CM (located within the tarsal conjunctiva of the eyelid). None of the 5 patients had had treatment of their disease prior to receiving care at the center where the archiving of their samples occurred. Clinical features are further summarized in the Table.

Deleterious mutations were identified in all 5 CM samples (Figure). These mutations included *BRAF* V600E, *BRAF* V600K, and *NRAS* Q61R, which have been reported previously in CM and cutaneous melanoma.[5,6] One sample (patient 2) harbored a mutation in the tumor suppressor *NF1* (without systemic manifestations), which previously has been reported in CM only in association with neurofibromatosis.[7] Mutations previously unreported in CM occurred in other cancer-associated genes, including *APC* (n = 2), *EGFR* (n = 1), *CBL* (n = 1), and *ALK* (n = 1). In cutaneous melanoma as well as CM,[8] *TERT* mutations have been found to occur in the promoter sequence, there by altering messenger RNA expression, whereas the *TERT* mutation in the present case was a missense alteration within the coding sequence and was there fore predicted to alter protein function. Mutations were also found in epigenetic regulators, including *TET2* (n = 1), *ATRX* (n = 1), and *ASXL1* (n = 1). The gene *ASXL1* encodes a protein-binding partner of *BAP1*, a gene that is commonly mutated in uveal melanoma[9]; however, no samples contained mutations in *BAP1* or other genes commonly mutated in uveal melanoma.[10]

Discussion

Several of these mutations could nominate new therapeutic options. The mutations in *BRAF*, *NRAS*, and *NF1* activate the mitogen-activated protein kinase (MAPK) pathway, which can be pharmacologically inhibited with mitogen-activated protein kinase kinase (MEK) inhibitors.[11] The mutations in *APC*, *EGFR,* and *ALK* can be inhibited with other targeted molecular agents.[12]

The role of UV light in the pathogenesis of CM has been the subject of discussion.[13] However, consistent with a recent report,[14] we observed in all 5 cases a prominent C→T mutation signature associated with UV light-induced DNA damage (Figure), which strongly implicates UV light as a mutagen in CM. The C→T mutations are important in UV-driven malignant conditions. Notably, non–UV-exposed conjunctival melanomas with *KIT* mutations have also been described, particularly in Chinese populations.[2]

The only CNA identified in all 5 samples was a chromosome 6p gain, which is also common in cutaneous melanoma and uveal melanoma. However, other recurrent CNAs were observed (Figure; eTables 1–4 in the Supplement). Samples from patients 1, 3, and 5 appeared to have a lower mutational burden than those found inmucosal melanomas in other locations, as previously described.[15] Interestingly, the 2 samples from individuals who experienced rapid recurrences within a year (patient 2 and patient 4) had the greatest number of mutations (Figure) and the largest amount of CNAs (Figure), suggesting that WES data may be valuable in predicting clinical outcome. This is an interesting observation that deserves further evaluation.

Limitations

Limitations of the present study include the small number of samples. In addition, 2 of 5 cases (40%) arose from primary acquired melanosis, which is thought to serve as the origin of malignant transformation in 75% of CM cases.[1] It is possible that the mutational signatures of de novo CM observed in this study may differ from those arising from primary acquired melanosis. In addition, the location of the tumor (bulbar vs palpebral) may carry distinct signatures as well, given that the areas experience differences in exposure to UV light. All of the CM samples in this series occurred in sun-exposed areas. Larger studies would be appropriate for further evaluation of these potential distinctions.

Conclusions

Whole-exome sequencing is a genome-wide comprehensive approach for identifying mutations and chromosomal alterations. This study demonstrates the potential value of WES for understanding the pathogenesis of CM and potentially providing precision medicine in the future for patients with this disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Key Points

Question

What mutations can be identified with whole-exome sequencing (WES) of conjunctival melanoma (CM)?

Findings

With WES, CM was found to harbor mutations in *BRAF*, *NRAS*, and *NF1*; previously unreported mutations in *EGFR*, *APC*, *TERT* and other cancer-associated genes; and the C→T mutation signature consistent with UV-induced DNA damage. The most common chromosomal alteration was 6p gain.

Meaning

Whole-exome sequencing might enable the detection of molecular mutations targetable by cancer therapies and provide insight into the pathogenesis of CM.

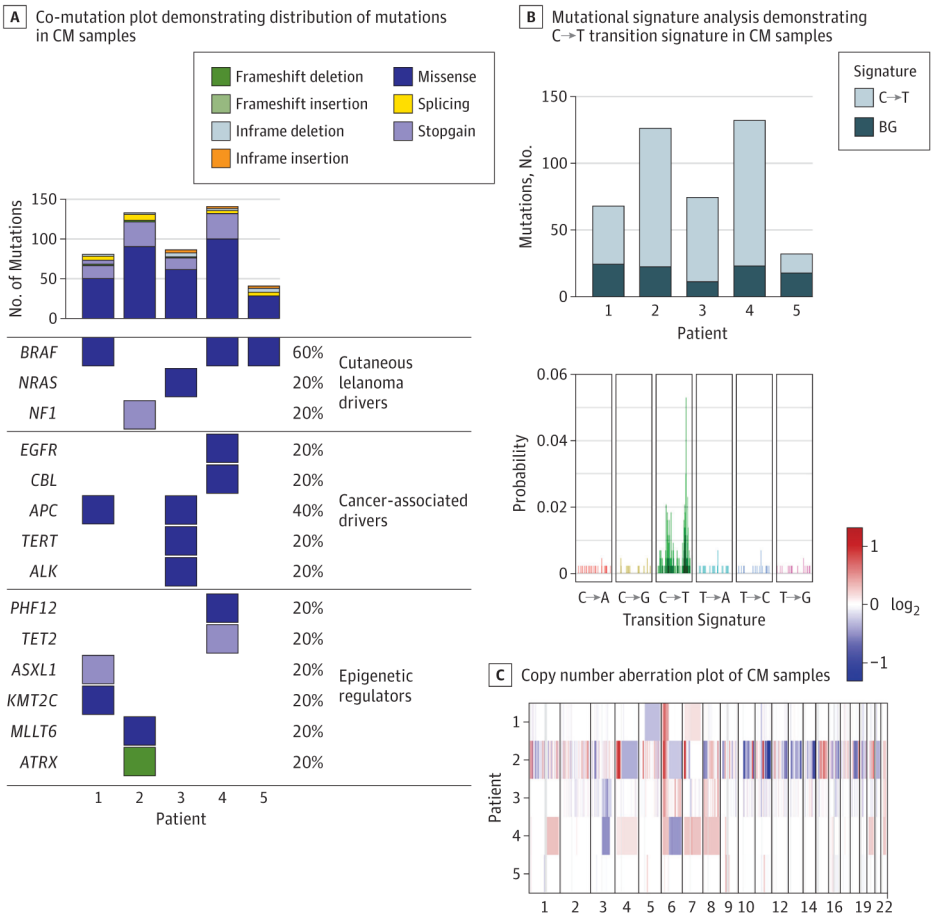

Figure. Co-Mutation Plot, Mutational Signature Analysis, and Copy Number Aberration Plot of Conjunctival Melanoma (CM) Tissue Samples

A, Top bar plot demonstrates number of predicted detrimental mutations in coding and splicing regions; mutations were categorized into clusters associated with cutaneous melanoma, cancer-driving mutations, and epigenetic mutations. Mutations in genes associated with uveal melanoma were absent. B, Bottom plot shows probability of mutations in single nucleotides. Top plot shows frequency of C→T transition signature in each patient in study sample. C, Patients 2 and 4, whose samples demonstrated the largest number of copy number aberrations and mutations, experienced rapid recurrence of disease following primary tumor excision.

Table

Demographic and Clinical Features of Study Population

Patient No./Sex/Age, y	Tumor Location	Tumor Characteristics	Tumor Area, mm^2	Adjuvant Therapy	Recurrence After Primary Excision	Time to Recurrence	Treatment of Recurrence
1/M/68	Temporal bulbar, right eye	Separate focus of PAM; diffuse disease	24	Cryo plus MMC	Local recurrence	3 y	MMC or IFN plus surgery
2/F/65[a]	Plica and inferior palpebral, right eye; caruncle, right eye	Diffuse growth into tarsus/ caruncle + large circumscribed palpebral lesion	78	Cryo plus IFN	Local recurrence	10 mo	Exenteration
3/F/52	Temporal bulbar, right eye	Limbus, overlying cornea	24.5	Cryo	No	NA	NA
4/F/51	Nasal bulbar, left eye	Limbus, overlying cornea	15	Cryo plus MMC	Local recurrence	3 mo	MMC or IFN plus surgery
5/F/77	Inferotemporal bulbar, left eye	Arising from PAM, diffuse along bulbar conjunctiva	3	Cryo	No	NA	NA

Abbreviations: cryo, cryotherapy; IFN, interferon-alfa-2b; F, female; M, male; MMC, mitomycin C; NA, not applicable; PAM, primary acquired melanosis.

[a]Patient died.