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Next-generation sequencing of a large uveal melanoma with whole genome doubling and a *PBRM1* mutation

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ABSTRACT

Purpose: To report a large uveal melanoma with extra-scleral extension which underwent spontaneous infarction and its unique molecular signature profile.

Observations: An 81-year-old female presented with a blind, painful eye. Intraocular pressure was 48 mm Hg. There was a large subconjunctival melanotic mass overlying a choroidal melanoma with anterior extension involving the ciliary body and the iridocorneal angle and iris. Ultrasonography confirmed a dome-shaped anterior cilio-choroidal mass with extra-scleral extension. The patient underwent enucleation and pathologic evaluation confirmed cilio-choroidal melanoma. The posterior half of the tumor involving the ciliary body and the extra-scleral component were spontaneously infarcted and were composed of large melanophages. Next-generation sequencing demonstrated a splice site mutation in *PBRM1* and whole-genome doubling in addition to a *GNAQ* hotspot mutation, chromosome 3 loss and 8q gain.

Conclusions and importance: This case of a large, auto-infarcted uveal melanoma demonstrates a *PBRM1* mutation and whole-genome doubling.

Introduction

Uveal melanoma is the most common primary intraocular malignancy of adulthood and most commonly involves the choroid. Ciliary body involvement, while less common, is associated with increased metastatic potential.[1] Survival prognostication is aided by multivariate analysis considering patient age, sex, and the American Joint Committee on Cancer (AJCC) clinical stage, tumor histology, and genetic profile. Of all the prognostic biomarkers, genetic information is most predictive of survival.[2] We report a case of a large uveal melanoma with extra-scleral extension and a unique genetic profile with tumor doubling and a novel *PBRM1* mutation.

Case report

An 81-year-old female with a history of tobacco use, hypothyroidism, hypertension, hyperlipidemia, and dementia presented with progressive right eye pain and vision loss. Visual acuity was hand motion in the right eye and 20/250 pinhole 20/70 in the left eye. The right eye had an afferent pupillary defect. Intraocular pressure by pneumotonometry was 48 in the right eye and 15 in the left eye. The right eye had both diffuse and microcystic corneal edema, a hyperchromic and heterochromic iris, and a large subconjunctival melanotic mass with dilated episcleral vessels (Fig. 1). The view to the posterior segment was poor due to the corneal edema. Gonioscopy demonstrated melanocytic tumor invasion into the right iridocorneal angle. Ultrasound bio-microscopy confirmed a dome-shaped choroidal mass lesion with involvement of the ciliary body and extra-scleral extension (Fig. 2).

The clinical diagnosis was choroidal melanoma with involvement of the ciliary body and irido-corneal angle and extra-scleral extension with secondary glaucoma. Baseline metastatic imaging with abdominal magnetic resonance imaging (MRI) and chest computed tomography (CT) with contrast were unremarkable. The patient was staged as AJCC stage IIIC (cT4e, Nx, M0). Enucleation was performed. Histopathologic

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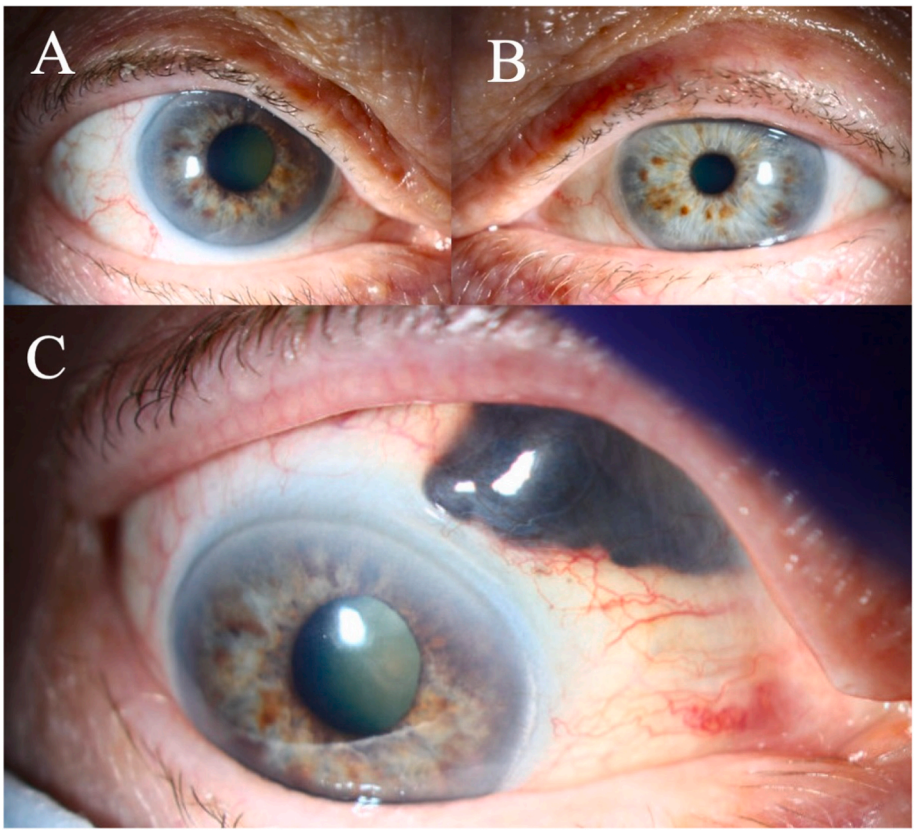

Fig. 1. Slit lamp examination of the right and left eyes
The right iris (A) is hyperchromic and heterochromic compared to the left iris (B), which has numerous iris nevi. Elevation of the right upper eyelid reveals a subconjunctival melanotic mass with sentinel vessels (C).

examination of the globe revealed mixed epithelioid and spindle cell melanoma involving the anterior choroid and ciliary body with invasion of the iris, iridocorneal angle, and Schlemm's canal circumferentially. The extra-scleral pigmented mass was connected to the intraocular melanoma through the pars plana, but was composed entirely of large macrophages with melanin (Fig. 3A). Immunohistochemistry demonstrated SOX10 staining, confirming the diagnosis of melanoma, and retained BAP1 expression, arguing against a *BAP1* gene mutation (Fig. 3B and C). The posterior half of the tumor involving the bulk of the ciliary body in the pars plana was replaced by macrophages and a devitalized debris consistent with auto-infarction of the extraocular extension. Pathologic staging according to the size and distribution of the viable tumor would be pT3b, but if considering the extra-scleral tissue, would be pT4e.

Paired tumor-germline, capture-based next-generation DNA sequencing was performed at a CLIA certified, clinical molecular pathology laboratory using an assay which targets all coding exons of 529 cancer-related genes, select introns, and upstream regulatory regions of 73 genes. This enables detection of structural variants and DNA segments at regular intervals along each chromosome for genome-wide copy number and zygosity analysis (UCSF500 Cancer Panel; Supplementary Table 1) with a mean sequencing depth of 765x. Specifically, this assay covers all exons of the *GNAQ*, *GNA11*, *SF3B1*, *EIF1AX, BAP1*, *SRSF2*, and *PLCB4* genes frequently altered in uveal melanocytic neoplasms. Sequencing of the formalin-fixed paraffin-embedded tumor tissue demonstrated a *GNAQ* hotspot missense mutation (NM_002072.3, p.Q209L, mutant allele frequency of 41%) and a damaging splice site mutation in *PBRM1*on chromosome 3p21 with loss of the remaining wildtype allele (NM_018313.4, c.236+1G > C, mutant allele frequency of 88%). Copy number analysis demonstrated losses of chromosomes 3 and 6q and gain of chromosome 8q in the background of whole-genome duplication (Fig. 4). The patient's germline sample (peripheral blood) harbored a mutation in *NTHL1* (NM_002528.5, p.Q90*, c.268C > T, mutant allele frequency of 47% in germline and 55% in tumor).

Discussion

We describe a patient with a large uveal melanoma with extra-scleral extension, which underwent spontaneous infarction and its unique tumor genetic findings.

Uveal melanoma has been thought to have a low mutation burden.[2] Most tumors show mutations that activate the G-protein alpha-q signaling cascade, affecting *GNAQ* or *GNA11*, but these alone are insufficient for development of malignant melanoma.[2] Frequent collaborating mutations involve the *BAP1, SF3B1* and *EIF1AX* genes, and *BAP1* mutations associated with loss of the remaining wildtype allele on chromosome 3p21 are present in most metastasizing tumors.[2–4] Cytogenetic changes of prognostic relevance in uveal melanoma include chromosome 3 loss ('monosomy 3'), chromosome 8q gain, and chromosome 6p alterations.[2,5] This patient's tumor had chromosome 3 loss and 8q gain, which portend a high metastatic and mortality risk,[5] but lacked *BAP1* gene mutation and showed retained BAP1 protein expression.[3] While it is possible that large intragenic deletions involving *BAP1* may be missed by this assay, retained BAP1 protein expression by immunohistochemistry supports wildtype *BAP1*.

Given the genetic analysis was performed as a paired tumor and germline tissue using a comprehensive next-generation sequencing assay, we were able to identify other genetic alterations less frequently seen in uveal melanomas.[6] This tumor harbored a damaging splice site mutation in *PBRM1*, an epigenetic regulator involved in chromatin remodeling. *PBRM1* mutations have been previously reported in a small subset of uveal melanomas, and are associated with metastatic uveal

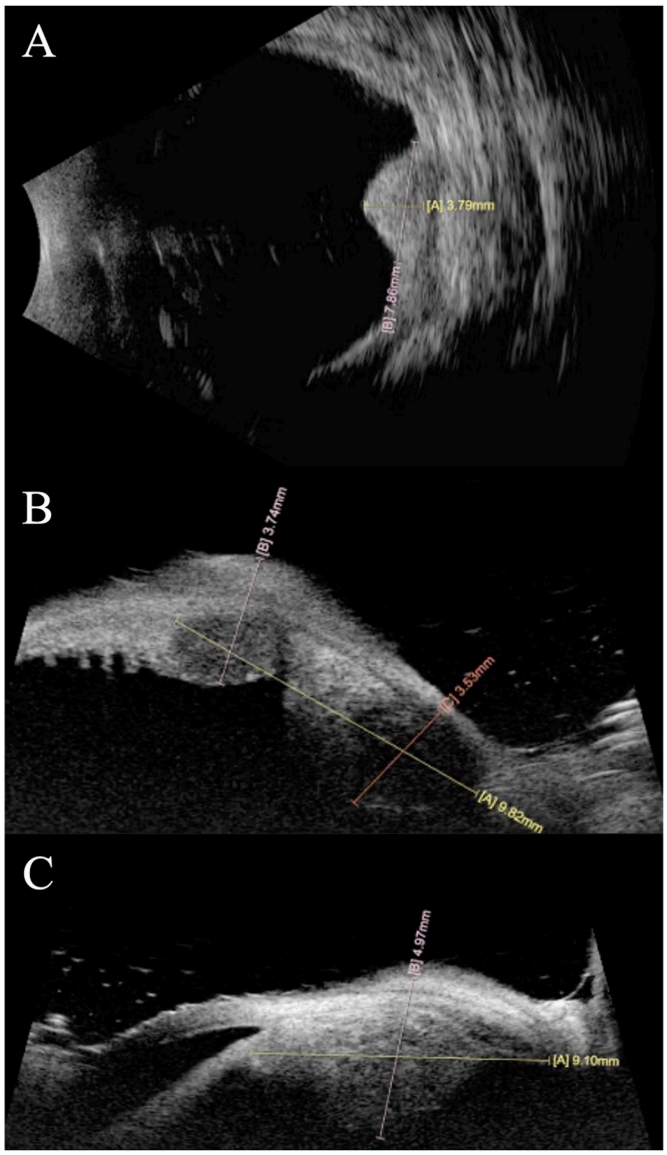

Fig. 2. Ultrasound biomicroscopy of the right eye
There is a dome-shaped choroidal mass lesion anterior to the equator in the superior-nasal quadrant (A) that is contiguous with a multi-lobulated ciliary body mass lesion (B). The lesion has extra-scleral extension (C) measuring 13 mm circumferentially, 9 mm radially, and 5 mm in height.

melanoma.[7,8] PBRM1 mutations in the literature have been largely considered as late events, and some were subclonal.[7,9] Based on the mutant allele frequencies of *GNAQ* and *PBRM1* in this case, *PBRM1* was considered clonal and interpreted as the driver alteration. There was also whole-genome doubling, which has been observed in 5% of tumors in the Cancer Genome Atlas Project (TCGA) dataset, typically in association with *BAP1* mutations and monosomy 3.[4] To the best of our knowledge, whole-genome doubling has not been previously reported in

Fig. 3. Histopathology and immunohistochemistry
Intraocular tumor (seen on the left side of the image) is composed of spindle and epithelioid melanoma cells with patchy weak cytoplasmic pigment while the extra-scleral pigmented mass (on the right side of the image) is composed of large macrophages filled with melanin (A). SOX10 stain highlights the melanoma cells and is negative in macrophages (B). BAP1 stain shows retained nuclear expression in tumor cells (C).

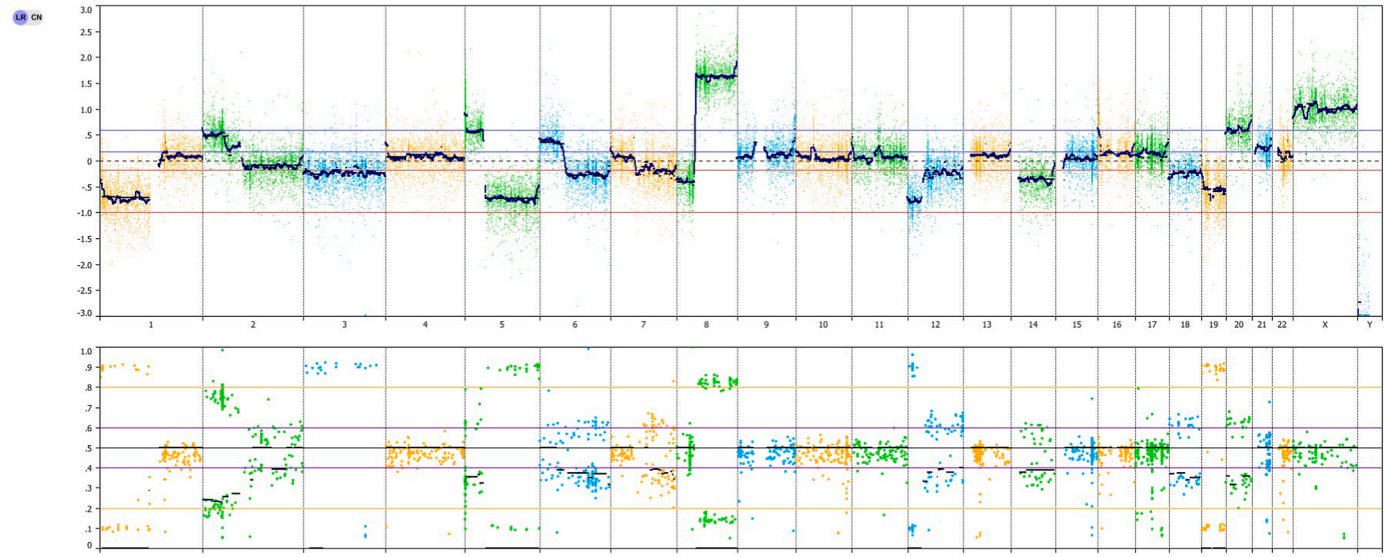

Fig. 4. Copy number alterations and B allele frequency plot
The copy number (top panel) displays a complex pattern of chromosomal gains and losses across the genome, demonstrating whole-genome duplication with the corresponding effects on heterozygosity (bottom panel).

conjunction with the *PBRM1* mutation. *PBRM1* loss of function mutations are most common in clear cell renal cell carcinoma,[10,11] where they are present in a mutually exclusive fashion with *BAP1* and *SETD2* mutations, likely causing similar impacts on chromatin remodeling. Given this, biallelic inactivation of *PBRM1* may contribute to the chromatin instability and aneuploidy in this tumor.

The patient's germline tissue demonstrated a mutation in *NTHL1*, making her an asymptomatic carrier for an autosomal recessive colonic polyposis syndrome.[12] However, there was no loss of the wild-type allele in the tumor, arguing against its role in the pathogenesis of this tumor. The patient is surveilled with biannual abdominal MRI and annual chest CT, and she is free of recurrence at 16 months' follow-up.

Conclusions

Detailed tumor genetic profiling with next-generation sequencing enables detection of less frequent alterations in uveal melanoma, aiding in metastatic risk stratification and facilitating customized screening.

Patient consent

Consent to present case images was obtained.

Disclosures

No conflicting relationship exists for any author. All authors attest that they meet the current ICMJE criteria for Authorship. Supported by That All May See, Inc., San Francisco, CA, an unrestricted grant and a Career Development Award (ARA) from Research to Prevent Blindness, New York, NY, and The National Eye Institute, Bethesda, MD (EY002162, Core Grant for Vision Research, and K23EY027466 to ARA).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ajoc.2023.101861.

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