Driver Mutations in Uveal Melanoma Associations With Gene Expression Profile and Patient Outcomes

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IMPORTANCE Frequent mutations have been described in the following 5 genes in uveal melanoma (UM): BAP1, EIF1AX, GNA11, GNAQ, and SF3B1. Understanding the prognostic significance of these mutations could facilitate their use in precision medicine.

OBJECTIVE To determine the associations between driver mutations, gene expression profile (GEP) classification, clinicopathologic features, and patient outcomes in UM.

DESIGN, SETTING, AND PARTICIPANTS Retrospective study of patients with UM treated by enucleation by a single ocular oncologist between November 1, 1998, and July 31, 2014.

MAIN OUTCOMES AND MEASURES Clinicopathologic features, patient outcomes, GEP classification (class 1 or class 2), and mutation status were recorded.

RESULTS The study cohort comprised 81 participants. Their mean age was 61.5 years, and 37% (30 of 81) were female. The GEP classification was class 1 in 35 of 81 (43%), class 2 in 42 of 81 (52%), and unknown in 4 of 81 (5%). BAP1 mutations were identified in 29 of 64 (45%), GNAQ mutations in 36 of 81 (44%), GNA11 mutations in 36 of 81 (44%), SF3B1 mutations in 19 of 81 (24%), and EIF1AX mutations in 14 of 81 (17%). Sixteen of the mutations in BAP1 and 6 of the mutations in EIF1AX were previously unreported in UM. GNAQ and GNA11 mutations were mutually exclusive. BAP1, SF3B1, and EIF1AX mutations were almost mutually exclusive with each other. Using multiple regression analysis, BAP1 mutations were associated with class 2 GEP and older patient. EIF1AX mutations were associated with class 1 GEP and the absence of ciliary body involvement. SF3B1 mutations were associated with younger patient age. GNAQ mutations were associated with the absence of ciliary body involvement and greater largest basal diameter. GNA11 mutations were not associated with any of the analyzed features. Using Cox proportional hazards modeling, class 2 GEP was the prognostic factor most strongly associated with metastasis (relative risk, 9.4; 95% CI, 3.1-28.5) and melanoma-specific mortality (relative risk, 15.7; 95% CI, 3.6-69.1) (P < .001 for both). After excluding GEP class, the presence of BAP1 mutations was the factor most strongly associated with metastasis (relative risk, 10.6; 95% CI, 3.4-33.5) and melanoma-specific mortality (relative risk, 9.0; 95% CI, 2.8-29.2) (P < .001 for both).

CONCLUSIONS AND RELEVANCE BAP1, SF3B1, and EIF1AX mutations occur during UM tumor progression in an almost mutually exclusive manner and are associated with different levels of metastatic risk. These mutations may have value as prognostic markers in UM.

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Uveal melanoma (UM) is the most common primary cancer of the eye and has a propensity for fatal hematogenous metastasis. Uveal melanomas can be stratified by gene expression profile (GEP) classification into 2 prognostically significant molecular classes. Class 1 UM have a low metastatic risk, whereas class 2 UM have a high metastatic risk. Class 1 tumors retain a differentiated melanocytic phenotype, whereas class 2 tumors exhibit a dedifferentiated stem cell-like phenotype. After it was shown by multiple groups that the prognostic accuracy of GEP outperforms clinicopathologic features and chromosomal gains and losses, our group developed a GEP classifier for routine clinical use in which expression of 12 discriminating genes and 3 control genes is measured by quantitative polymerase chain reaction (PCR) on a microfluidics platform after targeted amplification. The result was an ultrahigh-performance assay that accurately measures gene expression from fine-needle biopsy samples that are too small to be reliably assessed using chromosome-based assays. A prospective multicenter study was performed, which confirmed the assay’s prognostic accuracy and showed it to be superior to chromosome 3 testing. To date, this assay is the only prognostic test for UM ever to undergo prospective multicenter validation, which is required for a cancer biomarker to achieve the highest level I evidence according to the National Comprehensive Cancer Network Task Force on cancer biomarkers and the Tumor Marker Utility Grading System.

Consequently, this assay has been made commercially available (DecisionDX-UM; Castle Biosciences, Inc), which has become the standard care for molecular prognostic testing in many ocular oncology centers. The class 2 profile is strongly associated with inactivating mutations in the BAP1 (OMIM 603089) tumor suppressor gene. Four other genes are frequently mutated in UM, including EIF1AX (OMIM 300186), GNA11 (OMIM 139313), GNAQ (OMIM 600998), and SF3B1 (OMIM 605590). Herein, we describe the associations between mutations in these 5 genes, GEP molecular class, clinicopathologic features, and patient outcomes in 81 primary UMs treated by enucleation.

**Methods**

**Tissue Samples**

This study was conducted in a Health Insurance Portability and Accountability Act of 1996–compliant manner in accord with the tenets of the Declaration of Helsinki. Approval was obtained from the Institutional Review Board of the University of Miami. Written informed consent was attained from each patient. Tumor samples were taken at enucleation between November 1, 1998, and July 31, 2014, from patients with UM arising from the ciliary body, choroid, or both. Samples were snap frozen and stored at −80°C. Baseline clinical and pathologic information, as well as patient outcomes, were recorded.

**Molecular Analyses**

Molecular prognostic class assignments (class 1 or class 2) were obtained using a prospectively validated 12-gene classifier, as previously reported. Genomic tumor DNA was prepared for sequencing with a purification kit (Wizard Genomic DNA; Promega) following the manufacturer’s protocol, and target regions were amplified using PCR. The PCR was performed on a thermal cycler (Veriti; Applied Biosystems) in a reaction volume of 25 μL. Thermocycling was performed in the following conditions: initial denaturation at 95°C for 3 minutes and 30 rounds of amplification at 95°C for 15 seconds, touchdown PCR ramping from 65 to 55°C for 30 seconds, 72°C for 45 seconds, and a final extension step at 72°C for 7 minutes. The PCR products were visualized on a 2% agarose gel, and the remaining volume was purified using resin (SOP; Edge BioSystems). One microliter of clean PCR product was then sequenced using a cycle sequencing kit (BigDye Terminator, version 3.1; Applied Biosystems) according to the manufacturer’s protocol. Products were combined with a plate (Sephadex; GE Healthcare Biosciences AB) for purification and loaded on a sequencer (ABI 3730; Applied Biosystems) for 25 cycles. Mutations in BAP1 and SF3B1 were initially identified with exon sequencing in a subset of tumors, and then the mutation status of additional samples was determined by Sanger sequencing, as previously described. For this study, sequencing of BAP1 included all coding regions and splice junctions, as previously described. For GNAQ and GNA11, the 2 mutation hot spots at R183 and Q209 were sequenced. For SF3B1, the mutation hot spot at R625 was sequenced. For EIF1AX, the hot spot regions containing reported mutations within exons 1 and 2 were sequenced. Mutation calling was performed by aligning the genomic sequence traces using sequence analysis software to the hg19 genome build (Sequencher, version 4.1.2; Gene Codes).

**Statistical Analysis**

Statistical analysis was performed using a computer program (MedCalc, version 14.10.2; MedCalc Software bvba). The Fisher exact test was used to evaluate discrete dichotomous variables, Cox proportional hazards modeling was used to assess variables associated with metastasis and melanoma-specific mortality, and multiple regression was used for identifying clinicopathologic features associated with mutations in BAP1, EIF1AX, GNA11, GNAQ, and SF3B1. P ≤ .05 was considered statistically significant.

**Key Points**

**Question** What are the associations between driver mutations, gene expression profile classification, clinicopathologic features, and patient outcomes in uveal melanoma?

**Findings** This retrospective study of patients with uveal melanoma treated by enucleation found that BAP1, SF3B1, and EIF1AX mutations were almost mutually exclusive with each other. BAP1 mutations were associated with poor prognostic factors, and EIF1AX and SF3B1 mutations were associated with good prognostic factors.

**Meaning** BAP1, SF3B1, and EIF1AX mutations may have value as prognostic markers in uveal melanoma.
Results

Baseline Data
Clinicopathologic and patient outcome data are summarized in Table 1. Molecular and genetic data are summarized in Figure 1. Among 81 participants, the GEP classification was class 1 in 35 (43%), class 2 in 42 (52%), and unknown in 4 (5%). Among those with DNA samples available for sequencing, BAP1 mutations were identified in 29 of 64 (45%), GNAQ mutations in 36 of 81 (44%), GNA11 mutations in 36 of 81 (44%), SF3BI mutations in 19 of 81 (24%), and EIF1AX mutations in 14 of 81 (17%). Sixteen of the mutations in BAP1 were previously unreported in UM or any other cancer (Table 2). Six of the mutations in EIF1AX, found in 10 tumors, were novel in UM, and 2 of the 6 mutations were not previously reported in any cancer (Table 3). One of the novel mutations was a splicing change predicted to lead to loss of exon 2.

During a mean follow-up of 34.1 months (median, 24.4 months) among 81 participants, metastasis was detected in 28 (35%), and melanoma-specific mortality occurred in 21 (26%). Among primary tumors that metastasized, mutations were detected in BAP1 in 17 of 24 (71%), GNAQ in 13 of 28 (46%), GNA11 in 12 of 28 (43%), SF3BI in 3 of 28 (11%), and EIF1AX in none.

Associations Between Mutations
Statistical analysis for the associations between different mutated genes was performed using the Fisher exact test, and the results are shown in Figure 2. Mutations in GNAQ and GNA11 were mutually exclusive (P < .001). Mutations in BAP1 and SF3BI were almost mutually exclusive (P = .007), as were mutations in BAP1 and EIF1AX (P = .03). Mutations in SF3BI and EIF1AX were also almost mutually exclusive, with only one tumor having a mutation in both, but this association did not achieve statistical significance (P = .17).

Mutations vs Clinicopathologic Features
The associations between mutations and clinicopathologic features were analyzed for statistical significance using multiple regression analysis (eTable in the Supplement). BAP1 mutations were associated with class 2 GEP (P < .001) and older patient age (P = .007). EIF1AX mutations were associated with class 1 GEP and the absence of ciliary body involvement (P = .03 for both). SF3BI mutations were associated with younger patient age (P = .006). GNAQ mutations were associated with the absence of ciliary body involvement (P = .008) and greater largest basal diameter (P = .04). GNA11 mutations were not associated with any of the analyzed features.
Survival Analysis

Cox proportional hazards modeling was used to analyze the prognostic value of GEP class, gene mutations, and clinico-pathologic features vis-à-vis time to metastasis and to melanoma-specific mortality (eTable in the Supplement). When all of these variables were considered together, class 2 GEP was the prognostic factor most strongly associated with metastasis (relative risk, 9.4; 95% CI, 3.1-28.5) and melanoma-specific mortality (relative risk, 15.7; 95% CI, 3.6-69.1) (P < .001 for both). After excluding GEP class, the presence of BAP1 mutations was the factor most strongly associated with metastasis (relative risk, 10.6; 95% CI, 3.4-33.5) and melanoma-specific mortality (relative risk, 9.0; 95% CI, 2.8-29.2) (P < .001 for both).

Table 2. Somatic BAP1 Mutations Previously Unreported in Primary Uveal Melanoma

<table>
<thead>
<tr>
<th>Tumor ID</th>
<th>GEP Class</th>
<th>Metastasis</th>
<th>Mutation Position</th>
<th>Mutation Nucleotide Change (hg19)</th>
<th>Predicted Functional Change</th>
<th>Mutation Type</th>
<th>Exon Involved</th>
</tr>
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<tr>
<td>MM004</td>
<td>NA</td>
<td>No</td>
<td>3:52437275</td>
<td>T→A</td>
<td>p.Q590L</td>
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<td>14</td>
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<td>MM129</td>
<td>2</td>
<td>Yes</td>
<td>3:52442047</td>
<td>A→C</td>
<td>p.L101R</td>
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<td>5</td>
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<tr>
<td>MM137</td>
<td>2</td>
<td>Yes</td>
<td>3:52443889-52443927</td>
<td>delATTACATCTCCGGGGCGCCCTAGCCATGTTCC</td>
<td>Removal of start site</td>
<td>Deletion</td>
<td>5′ Untranslated region, exon 1</td>
</tr>
<tr>
<td>MM144</td>
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<td>No</td>
<td>3:52442595</td>
<td>delG</td>
<td>p.F50LfsX22</td>
<td>Premature termination</td>
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</tr>
<tr>
<td>MM151</td>
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<td>Yes</td>
<td>3:52440918-52440925</td>
<td>delAGG GCCCT</td>
<td>Deletion of splice donor and 6 bases of exon</td>
<td>In-frame deletion</td>
<td>Splice donor, exon 8</td>
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<tr>
<td>MM161</td>
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<td>No</td>
<td>3:52439813-52439814</td>
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<td>p.R300GfsX6</td>
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<td>MM162</td>
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<td>Yes</td>
<td>3:52537431; 3:52437433</td>
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<td>Splice donor, exon 14</td>
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<tr>
<td>MM175</td>
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<td>No</td>
<td>3:52439825-52439845</td>
<td>delAGCACGGGGGACCTGTG</td>
<td>p.S289fsX41</td>
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<td>MM179</td>
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<td>Yes</td>
<td>3:52443876</td>
<td>C→A</td>
<td>p.E007*</td>
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<tr>
<td>UMM002</td>
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<td>Yes</td>
<td>3:52437252-52437291</td>
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<td>No</td>
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<td>No</td>
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<td>UMM101</td>
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<td>delC</td>
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<td>7</td>
</tr>
</tbody>
</table>

Abbreviations: fs, frameshift; GEP, gene expression profile; ID, identification; NA, not available; p, protein.

Table 3. Somatic EIF1AX Mutations Previously Unreported in Primary Uveal Melanoma

<table>
<thead>
<tr>
<th>Tumor ID</th>
<th>GEP Class</th>
<th>Metastasis</th>
<th>Mutation Position</th>
<th>Mutation Nucleotide Change (hg19)</th>
<th>Predicted Functional Change</th>
<th>Mutation Type</th>
<th>Exon Involved</th>
<th>Cosmic ID</th>
</tr>
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<td>MM041</td>
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<td>Missense</td>
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<tr>
<td>MM068</td>
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<td>No</td>
<td>X:20156732</td>
<td>C→G</td>
<td>p.G9R</td>
<td>Missense</td>
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<tr>
<td>MM074</td>
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<td>No</td>
<td>X:20156732</td>
<td>C→G</td>
<td>p.G9R</td>
<td>Missense</td>
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<td>MM082</td>
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<td>No</td>
<td>X:20156731</td>
<td>C→T</td>
<td>p.G9D</td>
<td>Missense</td>
<td>2</td>
<td>COSM3372213</td>
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<tr>
<td>MM094</td>
<td>1</td>
<td>No</td>
<td>X:20156737</td>
<td>T→C</td>
<td>p.K7R</td>
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<td>MM105</td>
<td>1</td>
<td>No</td>
<td>X:20156742</td>
<td>T→C</td>
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<td>Splice site</td>
<td>Intron 1</td>
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<tr>
<td>MM131</td>
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<td>No</td>
<td>X:20156731</td>
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<td>UMM003</td>
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<td>Missense</td>
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Abbreviations: GEP, gene expression profile; ID, identification; NA, not available.
mortality. These findings suggest that mutational analysis of BAPI may have value as a biomarker for poor prognosis, whereas EIF1AX and SF3BI may be useful markers of good prognosis, as previously suggested.20 Our group recently reported that expression of the oncogene PRAME identifies class 1 UMs with intermediate metastatic risk, and these class 1 PRAME-positive UMs often harbor SF3BI mutations.21 As such, we anticipate that SF3BI mutations will be associated with a newly identified subclass of UM associated with metastatic risk that is intermediate between UMs with BAPI mutations (high risk) and UMs with EIF1AX mutations (low risk).

A limitation of this study was that it included only UMs treated by enucleation, which was a matter of necessity to obtain adequate amounts of tumor tissue for the various molecular analyses that were performed. As such, the findings of our study and others that are limited to enucleation specimens may not be representative of smaller UMs that are treated by globe-sparing procedures. With recent advances in next-generation sequencing technology, all of these mutations can be detected from a single fine-needle aspiration biopsy sample. A second limitation was that tumor DNA adequate for sequencing all coding regions of the entire BAPI gene (which was not required for the other 4 genes) was available in only 64 of 81 (79%) participants. However, most other studies have relied on immunohistochemistry for the presence of the BAPI protein as a surrogate marker for BAPI mutations,22 which is associated with considerable false-positive and false-negative results, so our study provides important new insights based on validated mutations. A third limitation was that we performed targeted sequencing of only the 5 commonly mutated genes. With multiple groups reporting their whole-exome sequencing results for UM, rare mutations in other genes have been found and will likely continue to be discovered.11,16,23 The importance of these additional infrequent events will need to be assessed in future studies. A fourth limitation was the retrospective design and its effect on the clinical outcomes obtained. We are planning a prospective multicenter study to evaluate these and other genetic abnormalities in UM.

Conclusions

Five common mutations in UM have been identified to date. Two of these mutations (GNAQ and GNA11) occur early in tumor formation and are not associated with prognosis, whereas the other 3 (BAPI, SF3BI, and EIF1AX) likely occur later in tumor progression and are prognostically significant. These findings suggest that the mutation status of BAPI, SF3BI, and EIF1AX is of clinical value in the application of precision medicine in UM. Because the GEP classification was prognostically superior to the mutation status of these genes, the role of mutational analysis for prognostication will likely be as a supplement to GEP. While GEP remains the most accurate prognostic biomarker, it is possible that continued research will show that the inclusion of mutational information with GEP could increase the prognostic accuracy or suggest specific treatment choices, such as an MEK inhibitor for GNAQ and GNA11 mutations or an epigenetic modulator for BAPI mutations.24,25

Discussion

The identification of driver mutations has become a centerpiece of cancer precision medicine for diagnostic, prognostic, and therapeutic decision making in individual patients with cancer.17 Thus far, only the following 5 genes have been found to be commonly mutated in UM: GNAQ, GNA11, BAPI, SF3BI, and EIF1AX. In this study, we found mutation frequencies of 89% (72 of 81) for GNAQ and GNA11, 45% (29 of 64) for BAPI, 23% (19 of 81) for SF3BI, and 17% (14 of 81) for EIF1AX, which is similar to previous reports.11-16,18,19 Sixteen mutations in BAPI and 6 mutations in EIF1AX were previously unreported in UM. GNAQ and GNA11 mutations were mutually exclusive with each other, they were present in similar proportions in class 1 and class 2 UMs, and they showed no association with tumor size or patient outcomes, suggesting that these mutations occur early in tumorigenesis.12 Mutations in BAPI, SF3BI, EIF1AX were almost mutually exclusive with each other, suggesting that they may represent alternative downstream molecular events during tumor progression. Of particular interest, BAPI mutations were associated with poor prognostic factors (class 2 GEP and older patient age) and high metastatic risk. In contrast, EIF1AX and SF3BI mutations were associated with good prognostic factors (EIF1AX mutations with class 1 GEP and the absence of ciliary body involvement and SF3BI mutations with younger patient age).

Consistent with previous work,8 class 2 GEP demonstrated prognostic accuracy that was superior to all other variables that were examined. After excluding GEP class, the next most accurate prognostic factor was the presence of BAPI mutations for both time to metastasis and to melanoma-specific
Driver Mutations in Uveal Melanoma

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Author Contributions: Ms Decatur and Dr Harbour had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Garg, Bowcock, Harbour.
Acquisition, analysis, or interpretation of data: Decatur, Ong, Garg, Anbunathan, Bowcock, Field.
Drafting of the manuscript: Decatur, Ong, Field.
Critical revision of the manuscript for important intellectual content: All authors.

Statistical analysis: Garg, Field, Harbour.
Obtaining funding: Bowcock, Harbour.
Administrative, technical, or material support: Bowcock, Anbunathan, Bowcock.
Study supervision: Bowcock, Harbour.

Conflict of Interest Disclosures: All authors have completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Drs Bowcock and Harbour reported being coinventors of intellectual property related to the gene expression profile technology used in the study, reporting being a paid consultant for Castle Biosciences, Inc (which licensed this intellectual property), and reported receiving royalties from its commercialization. No other disclosures were reported.

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