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Uveal Melanomas with *SF3B1* Mutations

A Distinct Subclass Associated with Late-Onset Metastases

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Purpose: To investigate the prevalence and prognostic value of *SF3B1* and *EIF1AX* mutations in uveal melanoma (UM) patients.

Design: Case series.

Participants: Cohort of 151 patients diagnosed with and treated for UM.

Methods: *SF3B1* and *EIF1AX* mutations in primary tumors were investigated using whole-exome sequencing (n = 25) and Sanger sequencing (n = 151). For the detection of *BAP1* mutations, a previously reported cohort of 90 patients was extended using *BAP1* sequencing or immunohistochemistry.

Main Outcome Measures: The status of *SF3B1*, *EIF1AX*, and *BAP1* in tumors of patients were correlated to clinical, histopathologic, and genetic parameters. Survival analyses were performed for patients whose tumors had *SF3B1*, *EIF1AX*, and *BAP1* mutations.

Results: Patients with tumors harboring *EIF1AX* mutations rarely demonstrated metastases (2 of 28 patients) and overall had a longer disease-free survival (DFS; 190.1 vs. 100.2 months; $P < 0.001$). Within the patient group with disomy 3, UM patients with an *SF3B1* mutation had an increased metastatic risk compared with those without an *SF3B1* mutation (DFS, 132.8 vs. 174.4 months; $P = 0.008$). Patients with such a mutation were more prone to demonstrate late metastases (median, 8.2 years; range, 23−145 months). Patients with UM and loss of BAP1 expression had a significantly decreased survival (DFS, 69.0 vs. 147.9 months; $P < 0.001$).

Conclusions: According to our data, patients with UM can be classified into 3 groups, of which *EIF1AX*-mutated tumors and tumors without *BAP1*, *SF3B1*, or *EIF1AX* mutations are associated with prolonged survival and low metastatic risk, *SF3B1*-mutated tumors are associated with late metastasis, and tumors with an aberrant BAP1 are associated with an early metastatic risk and rapid decline in patient DFS. *Ophthalmology 2016;■:1−11 © 2016 by the American Academy of Ophthalmology.*

Supplemental material is available at www.aaojournal.org.

Uveal melanoma (UM) is the most frequent primary tumor in the eye, with an estimated annual incidence between 4.3 and 8.6 cases per 1 million in the Western world.[1,2] This tumor is derived from melanocytes in the choroid, ciliary body, or iris. Approximately 40% of patients demonstrate metastasis with a peak within 4 years after initial treatment, but metastatic disease has been observed even 15 years or longer after diagnosis. This suggests the presence of occult micrometastases at the time of primary treatment of the tumor, because treatment of the primary UM is almost always successful without local recurrence.[3,4]

In addition to clinical features, such as the age of the patient and the tumor size, molecular and genetic markers are used to prognosticate UM patients with low- and high-risk profiles.[5−7] Chromosomal aberrations have been associated with metastatic disease in UM patients, of which loss of chromosome 3 (monosomy 3) is the most prominent.[7] Monosomy 3 is present in approximately half of the tumors and is associated strongly with poor survival.[7] In contrast, tumors with disomy 3 rarely metastasize within the first 3 years of follow-up.[7] A gain of chromosome 8q is associated independently with decreased survival, and this is even more profound in combination with the loss of chromosome 3.[8] In addition to chromosomal abnormalities, RNA expression also has been used to categorize UM patients in low-risk (class 1) and high-risk (class 2) categories with high accuracy.[9]

DNA sequencing led to the identification of recurrent affected genes in UM. Activating *GNAQ* and *GNA11* hotspot mutations were found in most cases of UM, but were not associated with prognosis.[10−12] Hemizygous mutations in the BRCA-associated protein 1 (*BAP1*) gene were found in most monosomy 3 tumors, resulting in an inactivation of the protein and loss of BAP1 expression.[6] Hence, *BAP1* mutations or no detectable BAP1 expression are associated with metastatic disease in UM patients.[13−15]

More recently, 2 other genes, *SF3B1* (splicing factor 3 subunit B1) and *EIF1AX* (eukaryotic translation initiation

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factor 1A), were reported to be mutated in UM patients.[13,16–19] *SF3B1* mutations, almost exclusively in amino acid 625 (R625 located in exon 14), can be found in 10% to 21% of cases of UM.[16–18] Mutations in this gene have been associated with favorable prognostic features in UM patients, such as lower age at diagnosis and tumors with disomy 3, in contrast to patients with *BAP1*-mutated tumors.[16,18] Survival analyses revealed that patients with *SF3B1*-mutated UM had a better survival compared with the *SF3B1* wild-type patients.[16] However, in another study with a longer follow-up, these survival differences between patients with *SF3B1*-mutant tumors and *SF3B1* wild-type tumors did not reach significance.[18] In 16% to 19% of UM patients, *EIF1AX* mutations were observed mainly in disomy 3 tumors.[13,17,19] The patients with *EIF1AX* mutations had a better survival than those with *EIF1AX* wild-type tumors at 48 months of follow-up.[13]

The high prevalence of mutations in these genes and distinct survival patterns of UM patients urged us to investigate the prognostic value of these genes in a large cohort with long follow-up. We performed mutation analyses of *SF3B1* and *EIF1AX* in the tumor DNA of 151 patients. *BAP1* mutation analysis and immunohistochemical detection of BAP1 loss in a subset of 74 tumors was reported previously by our group,[15] and additional immunohistochemistry and mutation data were added. We correlated the mutational status with clinical, histopathologic, and genetic parameters.

Methods

Study Population

Tissue specimens were obtained from 151 UM patients. Patients with UM (n = 144) underwent primary enucleation between 1993 and 2013 at the Erasmus University Medical Centre or the Rotterdam Eye Hospital, Rotterdam, The Netherlands. Seven patients primarily underwent irradiation of the UM, of whom 6 patients underwent secondary enucleation (median, 15 months; range, 3–55 months), and 1 patient underwent biopsy examination 26 months after irradiation. Patient survival data were updated from the patients' charts. After enucleation, tumor material was obtained and partly snap frozen in liquid nitrogen, whereas the remaining tumor was embedded in paraffin. A histopathologic diagnosis of melanoma was made by an experienced ophthalmic pathologist (R.M.V.) conforming to the Royal College of Pathologists guidelines (available at: www.rcpath.org/resourceLibrary/dataset-for-the-histopathological-reporting-of-uveal-melanoma-3rd-edition-.html). Patients with iris melanoma were excluded. The local ethics committee approved this study, and informed consent was obtained before the intervention. This study was performed according to the guidelines of the Declaration of Helsinki.

DNA Extraction and Copy Number Analysis

DNA was extracted directly from fresh tumor tissue or frozen sections using the QIAamp DNA-mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The tumors were processed for fluorescence in situ hybridization and single nucleotide polymorphism array analysis (HumanCytoSNP-12 version 2.1 BeadChip and Illumina 610Q BeadChip; Illumina, San Diego, CA), as described previously.[15] Cutoff limits for deletion (>15% of the nuclei with 1 signal) or amplification (>10% of the nuclei with 3 or more signals) were adapted from the literature.[20] Tumors were examined for ploidy status with fluorescence in situ hybridization using control probes on chromosome 5q (chromosome 5q is usually not altered in UM). Polyploid tumors were excluded from the analyses because chromosomal abnormalities in these tumors require much more detailed analyses.

Whole-Exome Sequencing

Uveal melanoma samples of 25 patients were subjected to whole-exome sequencing. For 19 samples, SureSelect version 4 capture kit (Agilent Technologies, Santa Clara, CA) was used with 1 μg of genomic DNA, followed by sample preparation and sequencing using the HiSeq 2000 system (Illumina). A CLC Cancer Research Workbench (Qiagen, Redwood, CA) was used with default Burrows Wheeler aligner settings for the alignment against human reference genome build (hg19) to generate BAM files (*.bam). For the remaining 6 samples, the ACE Clinical Exome assay (Personalis, Inc., Menlo Park, CA) was used on 1 to 3 μg genomic DNA. Sequencing (*.fastq) and alignment (*.bam) were generated and provided by Personalis, Inc. For all whole-exome sequencing samples, the BAM files were investigated manually for the regions of interest (*BAP1*, *SF3B1*, *EIF1AX*, *GNAQ*, and *GNA11*) using the Integrative Genomics Viewer version 2.3 (Broad Institute, Cambridge, MA). In general, all exons including the flanking regions (up to 25 base pairs) were covered at least 10 times, and for exons with insufficient coverage, additional Sanger sequencing was carried out. Found variants were validated using Sanger sequencing.

Sanger Sequencing

We sequenced exon 14 of *SF3B1* covering codon 625 (n = 151) with polymerase chain reaction. Additionally, we sequenced exons 12 to 16 of *SF3B1* in 106 samples that proved to be wild-type for codon 625. For *EIF1AX*, we amplified the 5′UTR exon 1 and exon 2, including surrounding splice-site sequences. The primers are shown in Table 1 (available at www.aaojournal.org). The polymerase chain reaction and Sanger sequencing protocols are available upon request. Sequences were aligned and compared with reference sequence hg19 from the Ensemble genome database (ENST00000335508 and ENST00000379607). De novo missense mutations found in the genes of interest were evaluated with PolyPhen-2 (available at genetics.bwh.harvard.edu/pph2/index.shtml) and SIFT (available at sift.jcvi.org) for predictions (using default settings) to determine the possible functional impact and pathogenicity of the amino acid change. Mutation analysis of *GNAQ*, *GNA11*, and *BAP1* was carried out as reported previously.[12,15]

cDNA Sequencing

For cDNA sequencing, 5 samples were selected based on the mutation type in *EIF1AX*. As described previously,[21] 1 μg RNA was extracted from fresh frozen tumor material and converted to cDNA with the iScript cDNA Synthesis Kit (Bio-Rad Laboratories, Veenendaal, The Netherlands). This cDNA was amplified and sequenced using the primers shown in Table 1 (available at www.aaojournal.org). Sequences were aligned and compared with the same reference sequence used for genomic mutation analyses.

Immunohistochemical Staining

Immunohistochemistry analysis was performed with an automated immunohistochemistry staining system (BenchMark Ultra; Ventana Medical Systems, Inc., Tucson, AZ), as described previously.[15] For BAP1 staining, we used a mouse monoclonal antibody