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Genetic Background of Iris Melanomas and Iris Melanocytic Tumors of Uncertain Malignant Potential

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Purpose: Uveal melanoma (UM) is the most common primary intraocular malignancy in adults. Iris melanoma comprises 4% to 10% of all UMs and has a lower mortality rate. The genetic changes in iris melanoma are not as well characterized as ciliary body or choroidal melanoma. The aim of this study was to gain more insight into the genetic background of iris melanoma and iris nevi.

Design: Multicenter, retrospective case series.

Participants: Patients diagnosed with iris melanoma or iris nevi who underwent surgical intervention as primary or secondary treatment.

Methods: Next-generation sequencing of *GNAQ, GNA11, EIF1AX, SF3B1, BAP1, NRAS, BRAF, PTEN, c-Kit, TP53*, and *TERT* was performed on 30 iris melanomas and 7 iris nevi. Copy number status was detected using single nucleotide polymorphisms (SNPs) included in the next-generation sequencing (NGS) panel, SNP array, or fluorescent in situ hybridization. BAP1 immunohistochemistry was performed on all samples.

Main Outcome Measures: Mutation and copy number status were analyzed. Results of BAP1 immunohistochemistry were used for survival analysis.

Results: In 26 of the 30 iris melanoma and all iris nevi, at least 1 mutation was identified. Multiple mutations were detected in 23 iris melanoma and 5 nevi, as well as mutations in *GNAQ* and *GNA11*. Furthermore, 13 of 30 *BAP1*, 5 of 30 *EIF1AX*, and 2 of 30 *SF3B1* mutations were identified in iris melanoma. No correlation between *BAP1* status and disease-free survival was found. The iris nevi showed 1 *EIF1AX* and 3 *BAP1* mutations. Two of the nevi, with a *BAP1* mutation, were histologically borderline malignant. Mutations in *NRAS, BRAF, PTEN, c-KIT*, and *TP53* were detected in 6 iris melanomas and 4 iris nevi.

Conclusions: Mutations that are often found in uveal and cutaneous melanoma were identified in this cohort of iris melanomas and iris nevi. Therefore, iris melanomas harbor a molecular profile comparable to both choroidal melanoma and cutaneous melanoma. These findings may offer adjuvant targeted therapies for iris melanoma. There was no prognostic significance of BAP1 expression as seen in choroidal melanoma. Consequently, iris melanoma is a distinct molecular subgroup of UM. Histologic borderline malignant iris nevi can harbor BAP1 mutations and may be designated iris melanocytic tumors of uncertain malignant potential. *Ophthalmology 2017;■:1–9 © 2017 by the American Academy of Ophthalmology*

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Uveal melanoma (UM) is the most common primary intraocular malignancy in adults with an incidence of 7 in 1 000 000 people in the Western World.[1] Iris melanomas comprise 4% to 10% of all UM.[1–4] The observed and relative survival is higher compared with UM in general.[5] There is no difference in incidence between men and women, but UMs occur more often in the white population.[4,6] Treatment includes surgical resection, enucleation, brachytherapy, and proton beam irradiation.[7,8] Currently, no studies on targeted adjuvant therapies in primary or metastatic iris melanoma exist. The choice of treatment depends on tumor size, localization, and patient preference. Diffuse iris melanomas are difficult to recognize, causing a delay in diagnosis. Moreover, they have a greater risk of metastasis than nodular iris melanoma.[9,10] Other clinical risk factors for metastasis include elevated intraocular pressure, iris root or angle involvement, increased tumor thickness, older patient age, and extraocular tumor extension. The metastatic rate of iris melanoma is quoted as 1% to 10% at 5 years, 2% to 10% at 10 years, and 10% at 20 years of follow-up.[6,10] A metastatic rate of 11% at 5 years was described in a series of biopsied iris melanoma.[11] However, gene expression profiling of iris melanoma showed that 67% of iris melanoma exhibit a class I (low metastatic risk) gene expression profile and 33% exhibit a class II profile (high metastatic risk).[12]

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Chromosomal abnormalities of iris melanoma are poorly characterized. Partial and complete loss of chromosome 3 were found in 41% to 45% and 15% to 29%, respectively.[7,13,14] Monosomy 3 was correlated with increasing patients' age.[13] Although chromosome 3 loss is described in UM as a risk factor for metastatic disease,[15] in iris melanoma this was only associated with a progressive disease in a univariate analysis. Chromosome 9p loss was reported in 35%.[7] Furthermore, loss of 1p and 6q, and gain of 6p, 8, and 8q have been described.[7,14] Abnormalities of chromosomes 5 and 18 have been reported.[16]

Mutations in genes encoding the guanine nucleotide-binding protein G subunit alpha q and 11 (*GNAQ* and *GNA11*) and the genes *BAP1*, *SF3B1*, and *EIF1AX* are typical for UM.[17,18] *GNAQ* mutations are more common in ciliary body and choroid UM compared with iris melanoma.[19] The aim of this study was to elucidate the genetic background of iris melanoma and iris nevi and to ascertain whether iris melanoma constitutes a distinct molecular group among UM. Next-generation sequencing (NGS) and immunohistochemistry were used to identify mutations in genes that are involved in both uveal and cutaneous melanoma.

Methods

Inclusion

Tissue was collected from patients with iris melanoma or iris nevi from The Royal Hallamshire Hospital (Sheffield, UK) and the Rotterdam Ocular Melanoma Study Group (ROMS) database. The ROMS is a collaboration between the Erasmus Medisch Centrum (Rotterdam, The Netherlands) and The Rotterdam Eye Hospital (Rotterdam, The Netherlands). Patients with an iris melanoma or suspect iris nevi who underwent biopsy or enucleation between 1992 and 2016 were included. The study conformed to the tenets of the Declaration of Helsinki and was approved by the respective local ethics committees. Informed consent was obtained before treatment. All samples were reviewed by 1 of 2 ophthalmic pathologists (H.S.M. and R.M.V.) to ensure that all tumors were primary iris lesions. Patient charts were reviewed to ascertain diagnosis as primary iris melanoma, clinical, and follow-up data.

Immunohistochemistry

Immunohistochemical staining was performed with a BAP1-antibody (clone sc-28383, 1:50 dilution, Santa Cruz Biotechnology, Dallas, TX) on 4-μm sections of formalin-fixed paraffin-embedded tissue (FFPE). An automated staining system (VENTANA BenchMark ULTRA, Ventana Medical Systems, Tucson, AZ) was used following the protocol as described previously.[20] Only nuclear expression was scored because nuclear expression is prognostic relevant in UM.[20,21] Loss of expression was defined as absent BAP1 expression in the nucleus.

DNA Isolation

DNA was extracted from fresh and FFPE tumor tissue. DNA isolation from fresh material was performed using the QIAmp DNA mini kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. DNA extraction from FFPE tissue was performed using lysis buffer (Promega, Madison, WI) and 5% Chelex (Bio-Rad, Hercules, CA) following the protocol as described previously (Smit KN, Combined mutation and CNV detection by targeted NGS in UM, Modern Pathology, in press). Tumor tissue was confirmed with flanking hematoxylin–eosin slides. DNA samples were stored at −20°C.

Targeted Next-Generation Sequencing

Targeted NGS was performed using the Ion Personal Genome Machine and the Torrent Server (Thermo Fisher Scientific, Waltham, MA) according to the manufacturer's protocol. A panel including amplicons covering *GNAQ, GNA11, BAP1, SF3B1*, and *EIF1AX* was used. Moreover, *NRAS, BRAF, PTEN, c-Kit, TP53*, and *TERT*, genes that harbor mutations in cutaneous melanoma, were included. On chromosome 1, 3, and 8, amplicons that cover highly polymorphic regions were used to identify allelic imbalances (Smit KN, van Poppelen NM, Vaarwater J, et al. Combined mutation and copy number variation detection by targeted next-generation sequencing in uveal melanoma., in press).

Mutation Analysis

Results from Ion Torrent NGS were analyzed using Torrent Suite Software Version 4.4.3 (Thermo Fisher Scientific, Waltham, MA) and Integrative Genomics Viewer Version 2.3.68 (97) (Broad Institute, Cambridge, MA). All data were manually analyzed using Integrative Genomics Viewer for the selected 10 genes by 2 individuals. Mutations that occurred in more than 20% of the reads and with a minimal read count of 50 reads were called. When there was a low DNA concentration or when 1 of the hotspot mutations was present in less than 20% of the total read count, mutations with a percentage between 10% and 20% were called. Intronic, non-coding regions and synonymous mutations were excluded. These results were compared with the mutations from the Variant Call Format files. Mutations were validated using Sanger sequencing following a standardized protocol for FFPE material if material was available.

Copy Number Variation

Allelic imbalances were detected using the highly polymorphic regions on chromosome 3. This data was used to estimate the copy number variation. Furthermore, Nexus Copy Number software (BioDiscovery Incorporated, El Segundo, CA) was used to display copy number variations. Additional single nucleotide polymorphism (SNP) array or fluorescence in situ hybridization (FISH) data were used when available. Single nucleotide polymorphism array and FISH results were obtained as described previously.[22,23] If there was loss of chromosome 3p, this was defined as loss of chromosome 3.

Statistical Analysis

For statistical analysis, IBM SPSS Statistics Version 21 (SPSS for Windows, International Business Machines Corporation, North Castle, NY) was used. Kaplan–Meier analysis with log rank test was used for survival analysis. A P value < 0.05 was considered significant.

Results

Patient Characteristics

Iris Melanomas. Between 1992 and 2016, from 31 patients who were treated for iris melanoma at Erasmus MC, The Rotterdam Eye Hospital and by the Ocular Oncology Service at the Royal Hallamshire Hospital, tissue material was available. From the Royal Hallamshire Hospital Sheffield, 20 patients were included and 11 patients from the Erasmus MC and The Rotterdam Eye