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Personalized Prognosis of Uveal Melanoma Based on Cytogenetic Profile in 1059 Patients over an 8-Year Period

The 2017 Harry S. Gradle Lecture

Carol L. Shields, MD,[1] Emil Anthony T. Say, MD,[1] Murat Hasanreisoglu, MD,[1] Jarin Saktanasate, MD,[1] Brendan M. Lawson, BS,[1] Jeffrey E. Landy, BA,[1] Anjali U. Badami, BS,[1] Meera D. Sivalingam, BS,[1] Alexander J. Hauschild,[1] Robert J. House, BA,[1] Zachary E. Daitch, BS,[1] Arman Mashayekhi, MD,[1] Jerry A. Shields, MD,[1] Arupa Ganguly, PhD[2]

Purpose: To determine the personalized rate of uveal melanoma–related metastasis on the basis of individual tumor cytogenetic profile.

Design: Retrospective case series.

Participants: A total of 1059 patients with uveal melanoma.

Methods: Fine-needle aspiration biopsy (FNAB) for DNA amplification and whole genome array–based assay were performed for analysis of chromosomes 3, 6, and 8.

Main Outcome Measures: Melanoma-related metastasis.

Results: The mean patient age was 57 years, and most were white (1026/1059, 97%). The melanoma involved the choroid (938/1059, 89%), ciliary body (85/1059, 8%), or iris (36/1059, 3%), with 19% being macular in location. The mean largest basal diameter was 11 mm (median, 12 mm; range, 3–24 mm), and mean thickness was 5 mm (median, 4 mm; range, 1–20 mm). On the basis of individual chromosomal mutations, risk for metastasis was increased for chromosome 3 partial monosomy (hazard ratio [HR], 2.84; $P = 0.001$), 3 complete monosomy (HR, 6.7, $P < 0.001$), 6q loss (HR, 3.1, $P = 0.003$), 8p loss (HR, 21.5, $P < 0.001$), and 8q gain (HR, 9.8, $P < 0.001$). Kaplan–Meier estimate for melanoma-related metastasis in 1, 3, 5, and 7 years for 3 partial monosomy was 1%, 5%, 14%, and 17%; for 3 complete monosomy was 3%, 19%, 28%, and 37%; for 6q loss was 8%, 23%, 49%, and 49%; for 8p loss was 8%, 29%, not estimable (NE), and NE; and for 8q gain was 6%, 21%, 35%, 48%, respectively. On the basis of personalized cytogenetic profiles, Kaplan–Meier estimates (1, 3, and 5 years) for melanoma-related metastasis for 3, 6, and 8 disomy (1%, 1%, 4% [HR, 1]) were low compared with the higher-risk combinations of 3 complete monosomy, 6p gain, and 8q gain (0%, 29%, 29% [HR, 10.6, $P = 0.02$]); 3 complete monosomy, 6 disomy, 8q gain, and 8p gain (14%, 14%, NE [HR, 18.3, $P = 0.02$]); 3 complete monosomy, 6 disomy, and 8q gain (8%, 27%, 39% [HR, 19.5, $P < 0.001$]); and 3 complete monosomy, 6 disomy, 8q gain, and 8p loss (3%, 28%, NE [HR, 31.6, $P < 0.001$]), respectively.

Conclusions: Risk for melanoma-related metastasis strongly correlates with personalized cytogenetic profiles, with 5-year Kaplan–Meier estimates ranging from 4% with chromosomes 3, 6, and 8 disomy up to 39% for 3 complete monosomy, 6 disomy, and 8q gain. *Ophthalmology 2017;■:1–9 © 2017 by the American Academy of Ophthalmology*

Uveal melanoma is a dangerous intraocular malignancy with several documented predictors of patient prognosis, including patient age, tumor size and location, clinical category and stage by the American Joint Committee on Cancer classification 7th edition, presence of ocular melanocytosis, histopathologic cell type and invasiveness, and cytogenetic findings.[1–11] Cytogenetic testing for uveal melanoma is a fundamental predictor of patient prognosis, and therapeutic strategy often is based on testing results.[3,12–15] This testing has evolved over the past 2 decades from single chromosome 3 evaluation to an array of relevant analyses for chromosomes 3, 6, and 8 or gene expression profiling.[16–38] Initial studies were performed on enucleated eyes with solid tissue analysis, and later it became evident that the testing could be performed reliably on fine-needle aspiration biopsy (FNAB) specimens, achieving adequate sample in up to 96% of cases.[25,39–42] The latter testing is particularly important because most eyes with uveal melanoma currently are treated conservatively with globe salvage using plaque radiotherapy or proton beam radiotherapy.

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The understanding of prognostication based on tumor cytogenetic results was initially performed retrospectively using enucleated eyes harboring uveal melanoma in patients with known outcomes and then backwards correlating the cytogenetic results with patient survival. More recently, testing for chromosomal abnormalities known to affect prognosis on chromosomes 3, 6, and 8 with comparison of outcomes against normal disomy 3, 6, and 8 has been the standard. There are a myriad of combinations for these 3 chromosomes with abnormalities, including partial or complete monosomy 3, 6p gain/loss, 6q gain/loss, 8p gain/loss, and 8q gain/loss, leading to 52 combinations of individualized genetic signatures and each associated with potentially different outcomes. By using FNAB, we offer cytogenetic analysis for all patients with uveal melanoma. We recently correlated clinical features of uveal melanoma with cytogenetic profile (phenotype–genotype correlation) demonstrating significant association of high-risk profile with ciliary body location, greater tumor thickness and basal dimension, older patient age, and presence of ocular melanocytosis specifically correlated with chromosome 3 or 8 abnormalities.[42] In the current article, we focus on the 52 personalized, individual tumor cytogenetic signatures in 1059 patients and correlate each with risk for metastasis at 5 to 7 years.

Methods

The clinical records of all patients with the diagnosis of uveal melanoma from the Ocular Oncology Service at Wills Eye Hospital, Thomas Jefferson University, Philadelphia, Pennsylvania, between January 1, 2006, and May 1, 2014, and managed with FNAB yielding DNA for genetic testing of chromosomes 3, 6, or 8 at initial treatment were retrospectively reviewed. This analysis adhered to the tenets of the Declaration of Helsinki and was approved by the Institutional Review Board of Wills Eye Hospital.

Data were gathered regarding demographic, clinical, and cytogenetic features, as well as melanoma-related metastasis. The demographic information included patient age, race (white, African-American, Asian, Hispanic, Middle-Eastern), gender (male or female), affected eye (right or left), ocular melanocytosis, and presenting Snellen visual acuity. The tumor-related clinical features included anatomic epicenter (geographic center of the mass), location (iris, ciliary body, choroid), quadrant (superior, inferior, nasal, temporal, macula), anterior margin (macula, macula-equator, equator-ora, ciliary body, iris), posterior margin (macula, macula-equator, equator-ora, ciliary body, iris), distance to the optic disc (millimeters), distance to the foveola (millimeters), largest tumor basal diameter (millimeters), tumor thickness (millimeters by ultrasonography), and tumor treatment (plaque radiotherapy, resection, enucleation).

Fine-Needle Aspiration Biopsy and DNA Analysis

The procedure of single-pass FNAB is described in the literature.[42] The DNA Microkit (Qiagen, Valencia, CA) was used to isolate genomic DNA from the cytologic specimen using the manufacturer's listed protocol. The Affymetrix Human 100K, SNP-5.0, and SNP-6.0 genotyping arrays (Affymetrix, Santa Clara, CA) were used to identify whole genome copy number variation. Cell files generated with GCOS software (Affymetrix) were assessed using the Partek Genomic Suite v6.5 (Partek Inc., St. Louis, MO) and analyzed with the Copy Number Analysis workflow selection that provides copy number from allele intensity using the HapMap controls. The option for segmentation analysis was used to define the specific region of DNA amplification and deletion as listed in the software manual. For each case, the specific abnormality in chromosome 3 (disomy, complete monosomy, partial monosomy), 6 (6p gain, 6p loss, 6q gain, 6q loss), and 8 (8p gain, 8p loss, 8q gain, 8q loss) was recorded.

Statistical Analysis

Data were tabulated in Microsoft Excel 2011 (Microsoft Corp., Redmond, WA) and summarized as total number and as percentages of the entire population. Each patient was monitored for systemic metastasis by his or her general oncologist with liver function tests and physical evaluation every 4 to 6 months and liver magnetic resonance and chest x-ray every 6 to 12 months, depending on the oncologist's preference. Kaplan–Meier analysis was performed to estimate the cumulative probability of metastasis at 1, 3, 5, and 7 years of follow-up within each individual chromosome profile. Cox proportional hazard model was performed to estimate the risk ratio for uveal melanoma metastasis for each chromosomal abnormality compared with its counterpart disomy. The hazard ratios (HRs) accompanied by their 95% confidence intervals are presented. Kaplan–Meier estimates and risk ratios for uveal melanoma metastasis are provided for each multichromosome copy number aberration (CNA) signature.

Results

There were 1103 cases sampled by FNAB for cytogenetic analysis, and sufficient DNA for analysis was obtained in 1059 cases (96%). Among the 1059 samples obtained, 507 (507/1103, 46%) had only chromosome 3 analyzed, but all others (606/1103, 54%) had chromosomes 3, 6, and 8 analyzed.

Patient demographics are presented in Table 1. The mean patient age was 57 years, and most were white (1026/1059, 97%). Ocular melanocytosis was noted in 47 eyes (47/1059, 4%), and mean presenting Snellen visual acuity was 20/40.

Tumor features are presented in Table 2. The melanoma location was in the choroid (938/1059, 89%), ciliary body (85/1059, 8%), and iris (36/1059, 3%). The melanoma was

Table 1. Uveal Melanoma Prognosis Based on Cytogenetics in 1059 Cases: Patient Demographics

	Number (%), N = 1059
Mean age (median, range), yrs	57 (58, 10–95)
Gender	
Male	517 (49)
Female	542 (51)
Race	
White	1026 (97)
African-American	2 (<1)
Hispanic	26 (2)
Asian	2 (<1)
Others	3 (<1)
Affected eye	
Right	546 (52)
Left	513 (48)
Ocular melanocytosis	47 (4)
Mean visual acuity (median), Snellen	20/40 (20/30, 20/20–NLP)

NLP = no light perception.