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Genomic, Prognostic, and Cell-Signaling Advances in Uveal Melanoma

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Abstract

Uveal melanoma (UM) is the second-most common form of melanoma and the most common primary intraocular malignancy. Up to one-half of patients are at risk for fatal metastatic disease. The metastatic potential of an individual tumor can be accurately determined by analysis of a fine-needle aspirate with gene expression profiling assay that is available for routine clinical use through a commercial Clinical Laboratory Improvement Amendments (CLIA)-certified laboratory. The test renders one of two results—class 1 (low metastatic risk) or class 2 (high metastatic risk)—and has been extensively validated in multiple centers. Until recently, the genetic mutations and signaling aberrations in UM were largely unknown. With the advent of new genomic sequencing technologies, however, the molecular landscape of UM is rapidly emerging. Mutations in the G_q alpha subunits *GNAQ* and *GNA11* are mutually exclusive and represent early or initiating events that constitutively activate the MAPK pathway. Mutations in BRCA1-associated protein-1 (*BAP1*) and splicing factor 3B subunit 1 (*SF3B1*) also appear to be largely mutually exclusive, and they occur later in tumor progression. *BAP1* mutations are strongly associated with metastasis, whereas *SF3B1* mutations are associated with a more favorable outcome. *BAP1* mutations can arise in the germ line, leading to a newly described *BAP1* familial cancer syndrome. These discoveries have led to new clinical trials to assess several classes of compounds, including MEK, protein kinase C, and histone deacetylase inhibitors, in the adjuvant setting for high-risk patients identified as class 2, as well as in the setting of advanced disseminated disease.

Uveal melanoma (UM) is the most common primary malignancy of the eye, with an incidence of about 1,200–1,500 new cases per year in the United States, and it accounts for approximately 5% of all melanomas.^{1,2} UMs can arise anywhere in the uveal tract, comprising the iris, ciliary body, and choroid, and they often involve more than one of these

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structures. Approximately 5% of UMs are confined to the iris and exhibit less aggressive behavior. UMs metastasize by hematogenous dissemination, and the most common sites are liver (93%), lung (24%), and bone (16%).³

MOLECULAR CLASSIFICATION OF UVEAL MELANOMA

Clinicopathologic staging systems like the American Joint Committee on Cancer (AJCC) tumor, node, metastasis staging system⁴ provide a uniform vocabulary for communication among health care professionals, and they allow patients to be organized into prognostically comparable groups based on retrospective analysis. In the case of UM, however, the complexity of such systems is not well suited for prospective, personalized clinical decision making in individual patients, such as whether a given patient should be offered adjuvant therapy.

It has been known for several decades that certain chromosomal copy number alterations can be used as prognostic markers in UM, the most accurate being loss of chromosome 3 (monosomy 3), which is associated with poor outcome.⁵ Because monosomy 3 is associated with high false-positive and -negative rates, the inclusion of other chromosomal gains and losses and clinicopathologic information has been advocated to improve prognostic accuracy.⁶ Unfortunately, this approach results in the same limitations as the tumor, node, metastasis staging system classification: namely, it generates multiple sub-groups that are not optimal for everyday clinical decision making. Further, chromosome-based tests suffer from a susceptibility to sampling error resulting from intratumoral genetic heterogeneity, limited clinical validation, lack of standardized testing platforms, and high technical failure rates.⁷

To overcome these limitations and provide clinicians with a practical, simple, straightforward and highly accurate prognostic tool, we developed a test based on gene expression profiling (GEP) using a highly sensitive microfluidics polymerase chain reaction (PCR) platform that can be used for analysis of minute tumor samples from fine-needle aspiration biopsies.⁸ The commercially available form of the test is known as DecisionDx-UM and is performed in a College of American Pathologists (CAP)/CLIA laboratory as a standalone test that requires no other data input for excellent prognostic accuracy. The test simultaneously measures the expression of 15 carefully selected genes from primary UM samples containing as few as a few dozen tumor cells. Low metastatic risk is reported as class 1, and high metastatic risk as class 2, which allows patients to be individualized into risk categories that allow appropriate intervention.⁹ For example, high-risk patients can be offered intensive metastatic surveillance and adjuvant therapy while low-risk patients can be spared these interventions. DecisionDx-UM is the only prognostic test in UM that has been prospectively validated in multiple centers⁹ and that meets the highest level “1” rating for cancer biomarkers according to the National Comprehensive Cancer Network (NCCN) task force and the Tumor Marker Utility Grading System.^{10,11} This test is now used in most North American ocular oncology centers as part of the standard of care. Biologically, the GEP of class 1 tumors closely resembles that of normal uveal melanocytes and low-grade, differentiated uveal melanocytic tumors, whereas the GEP of class 2 resembles that of primitive neural/ectodermal cells.^{12,13}

SIGNALING PATHWAY ABERRATIONS

The PI3K/AKT pathway is constitutively activated in a majority of UMs, and phosphorylated AKT correlates with poor prognosis.¹⁴ This may result, at least in part, from loss of PTEN activity. In one study, loss of heterozygosity at the PTEN locus was found in 76% of UMs, and mutations within the PTEN coding region were found in 11% of tumors.¹⁵ PTEN inactivation was also found to be associated with increased aneuploidy and decreased survival in UM.^{15,16}

The mitogen-activated protein kinase (MAPK) pathway is also activated in most UMs, suggesting the presence of upstream activating mutations.^{17,18} However, mutations in known upstream activators such as KIT and the RAS and RAF family members are extremely rare in UM.^{18–22} A systemic interrogation of 21 other candidate oncogenes in the MAPK pathway identified no mutations in UM.²³

More recently, mutually exclusive mutations in two closely related G-coupled protein receptor Gq alpha subunits Gαq and Gα11 were found to occur in almost 85% of UMs.²⁴ These mutations lead to constitutive activation of the MAPK pathway.²⁵ *GNAQ/11* mutations are found in benign uveal nevi and in the vast majority of UMs regardless of cytogenetic status or GEP class,^{23,26,27} suggesting that these mutations are early or perhaps initiating events and are not sufficient for full malignant transformation.²⁵

BAP1

It has been known for many years that loss of one copy of chromosome 3 in UM is associated with metastasis and poor prognosis,⁵ which led to speculation that one or more tumor suppressor genes may reside on this chromosome that are mutated in UM.²⁸ In 2010, we identified such a gene using exome sequencing.²⁹ We found that BRCA1-associated protein 1 (*BAP1*), located at chromosome 3p21.1, was mutated in approximately 85% of class 2 UMs, but such mutations were rare in low-grade class 1 UMs, suggesting that *BAP1* may function as a metastasis suppressor in this cancer. *BAP1* encodes a deubiquitinating enzyme with several substrates, including BRCA1, histone H2A, host cell factor-1 (HCF-1) and O-linked N-acetylglucosamine transferase (OGT).^{30,31} The precise molecular explanation for why loss of *BAP1* leads to metastasis in UM remains unclear.

BAP1 FAMILIAL CANCER SYNDROME

Familial UM is generally regarded as rare, so we were surprised to find that one patient with UM in our original study carried a germ-line *BAP1* mutation that was reduced to homozygosity in tumor cells by loss of the other chromosome 3.²⁹ Subsequently, there have been many groups reporting families with germ-line *BAP1* mutations in association with UM and many other cancers, including mesothelioma, cutaneous melanoma, renal cell carcinoma, and others.^{32–36} Thus, although familial UM is uncommon, it is not as rare as once believed and may represent 2% to 3% of new patients with UM.

SF3B1 MUTATIONS

We searched for additional mutations in UM by exome sequencing and identified novel mutations in splicing factor 3B subunit 1 (*SF3B1*). Among 102 primary tumors, 19 (18.6%) contained mutations in *SF3B1*, similar to the frequency in myelodysplastic syndrome and chronic lymphocytic leukemia,^{37,38} and higher than that in breast cancer.³⁹ Interestingly, the mutations always involved an amino acid substitution at arginine-625, and all were somatic in origin. The molecular effect of the mutations appeared to be dominant-negative, gain-of-function or haploinsufficiency, but this remains to be firmly established. *SF3B1* mutations were largely mutually exclusive with *BAP1* mutations and were associated with favorable prognosis. *SF3B1* encodes a splicing factor subunit, but the cancer-promoting effect of these mutations remains unclear.

CONCLUSION

With the recent genetic discoveries in UM discussed herein, the genetic landscape of this cancer is rapidly coming into focus and is providing an unprecedented opportunity for individualized patient care and targeted therapy. Molecular GEP-based classification of UM allows patients to be stratified according to metastatic risk into class 1 (low risk) and class 2 (high risk) for purposes of individualized patient care and inclusion in clinical trials. *GNAQ/11* mutations have stimulated interest in MEK and protein kinase C inhibitors in UM.^{40,41} *BAP1* mutations have suggested a utility for histone deacetylase (HDAC) inhibitors to reverse the biochemical effects of *BAP1* loss by reversing histone H2A hyperubiquitination.⁴² With attention now focused on these mutations, not only in UM but in other cancers as well, it is anticipated that new classes of therapeutic compounds that target these pathways will soon emerge.

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KEY POINTS

- Uveal melanomas can be divided into low and high risk for metastasis based on a validated test based on gene expression profile.
- Mutually exclusive mutations in *GNAQ* and *GNA11* represent early or initiating events in uveal melanoma.
- Mutations in *BAP1* and *SF3B1* represent later events associated with poor and good outcome, respectively.
- Targeted therapies to counteract the effects of these mutations are becoming increasingly available.