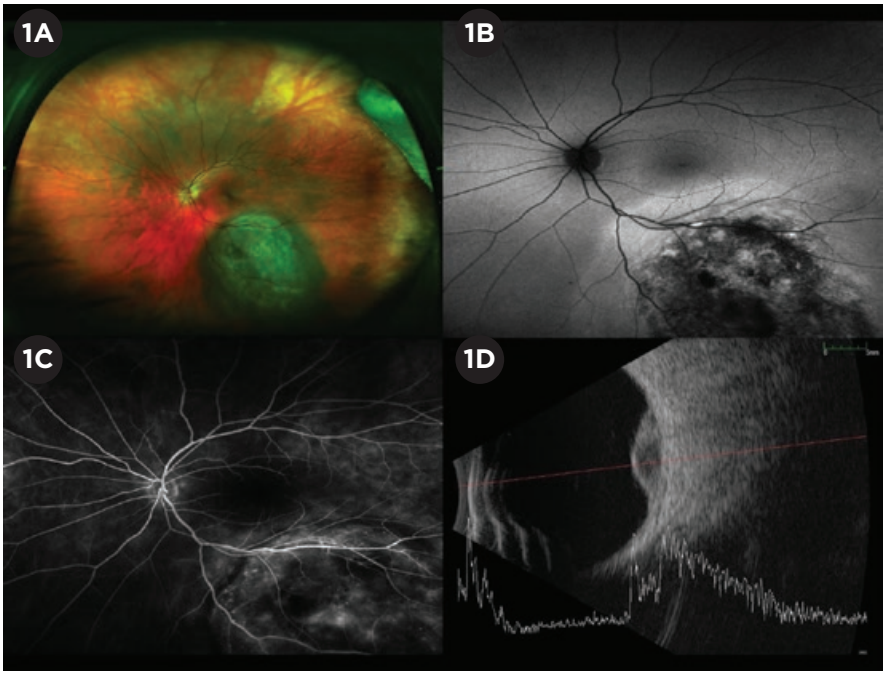
Gene Expression Profiling in Uveal Melanoma

Uveal melanoma (UM) is the most common primary intraocular malignancy in U.S. adults and has an annual incidence of 5.1 persons per million.[1] It can result in significant vision loss, and it metastasizes to distant sites in nearly half of patients.[2,3]

Although much progress has been made in terms of the diagnosis and treatment of UM, a clinician's ability to advise a patient of his/her prognosis has been informed primarily by the identification of certain high-risk clinical features, such as tumor height and largest basal diameter, among others.[3] The development of gene expression profiling for UM has been a significant advancement in this area, as it classifies tumors into highly prognostic subgroups based on the molecular makeup of the tumor at the time of biopsy.[4]



CLINICAL SIGNS. *(1A) Optos wide-field fundus photo of a choroidal melanoma with the posterior margin adjacent to the disc. (1B) Fundus autofluorescence demonstrating lipofuscin overlying the lesion. (1C) Late-frame fluorescein angiography demonstrating leakage, late staining of the lesion, and multiple pinpoint leaks (hot spots). (1D) B-scan ultrasound documenting ultrasonographic hollowness within the tumor.*

Background

Over the years, certain clinical and histopathological features of UM have been found to portend an increased risk for metastasis (Table 1). Until recently, these were the only prognostic indicators available to help predict tumor-related mortality.

These features are not to be confused with those referred to in the mnemonic developed by the Shieldses, which is used to identify choroidal nevi that are at risk of growth. ("To Find Small Ocular Melanoma Using Helpful Hints Daily"—Thickness greater than 2 mm, Fluid, Symptoms, Orange pigment, Margin near disc, Ultrasonographic hollowness, Halo absence, and Drusen absence.[5])

Development of Profiling

Advances in molecular genetic technologies have led to a better understanding of the molecular pathobiology of UM. Using hierarchical cluster analysis of gene expression, Harbour and colleagues found that tumors cluster into 2 groups: Class 1, which are unlikely to metastasize, and Class 2, which have a higher rate of metastasis and disease-related mortality.[6]

Based on these initial findings, the gene expression profile was refined to a 15-gene assay and validated by a prospective study involving 12 independent centers.[7] This study found that

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gene expression profiling was significantly ($P < 0.0001$) more accurate at predicting metastatic risk than was any other prognostic factor.

Further work has found that a certain subset of Class 1 tumors is at increased risk for metastasis. As a result, tumors are now classified as follows: Class 1A tumors, which have a 2% 5-year tumor-related mortality rate; Class 1B, which have a 21% tumor-related mortality rate; and Class 2, which have a 72% 5-year tumor related mortality rate (Table 2).[8]

Technique

The only commercially available gene expression profiling test is the DecisionDx-UM test (Castle Biosciences). Per the manufacturer's website, the preferred method of obtaining tumor tissue for the gene expression profile assay is by fine-needle aspiration biopsy.

Implications in the Clinic

While the 12-center validation study was not published until 2012, gene expression profiling for UM appears to have gained acceptance for use in routine clinical practice. A survey performed in 2012 found that 77% of ocular oncologists surveyed offered gene expression profile testing to most of their patients with UM.[9] Perhaps more importantly, 74% of respondents used the information to change the frequency of metastatic disease surveillance.

Although no prospective randomized trials of routine surveillance in UM have been conducted, there is general agreement that screening should be tailored to each patient according to the estimated risk of UM recurrence. For instance, many practitioners may choose to perform routine liver-specific imaging every 3 to 6 months for high-risk patients and every 6 to 12 months for low-risk patients. The most widely accepted method for risk-stratification in UM is the American Joint Committee on Cancer (AJCC) Cancer Staging Manual TNM classification, which is based on clinical features such as tumor size, ciliary body involvement, and extraocular extension. Gene expression profiling serves as a valuable adjunct to clinical and histopathologic data in classifying patients into these low- and high-risk groups.

Table 1: Clinical and Histologic Features Associated With Risk of Metastasis in Uveal Melanoma

CLINICAL FEATURES	HISTOLOGIC FEATURES
Ciliary body involvement	Closed periodic acid–Schiff-positive loops
Diffuse growth pattern	Degree of pigmentation
Extraocular extensions	Epithelioid cell type
Large tumor basal diameter	High mitotic rate
Older age	Inflammation
Optic nerve involvement	Mean diameter of 10 largest nucleoli
Ring melanoma	Tumor necrosis
Tumor thickness	Vascular invasion

SOURCE: Gill HS, Char DH. *Can J Ophthalmol*. 2012;47(3):246-253.

Table 2: Prognosis Based on Gene Expression Profile Class

Gene Expression Profile Class	Percent Metastasis-Free at 3 Years	Percent Metastasis-Free at 5 Years
Class 1A	98%	98%
Class 1B	93%	79%
Class 2	50%	28%

SOURCE: Castle Biosciences Inc. DecisionDx-UM Summary. www.myuvealmelanoma.com/health-care-professionals/decisiondx-um-summary/. Accessed March 22, 2017.

Limitations of Profiling

It is important to emphasize that gene expression profiling is a prognostic tool and not a diagnostic test. Any tissue sample can be tested and will return a result (even if with a low-confidence rate). The diagnosis of UM should still be made based on clinical examination and can be confirmed only by histopathology.

Clinicians' understanding of the pathobiology of UM is constantly evolving, and gene expression profiling is thus affected—as is evidenced by the recent subdivision of Class 1 tumors into Class 1A and Class 1B. This underscores the fact that gene expression profile class is only one of many features that may help a clinician assess risk of metastatic disease.

Other Cytogenetic Tests

In addition to gene expression profiling, other chromosomal and molecular markers have been used to estimate prognosis in UM (most notably, aberrations in chromosomes 3, 6, and 8). Monosomy 3 has been shown to predict a 3-year survival rate of less than 50%.[10] Chromosomal analysis is the preferred method of cytogenetic screening at many institutions, but it trails gene expression profiling in terms of popularity among all clinicians.[9]

Also, mutations in tumor suppressor gene *BAP1* have been found in 84% of metastasizing UM lesions, although it has been shown that the gene expression profile is more accurate at predicting metastatic disease.[11] Ongoing research

has identified other mutations in UM that may be used in the future not only as prognostic markers but also as potential targets for therapy. In fact, several ongoing clinical trials currently are enrolling patients for targeted, adjuvant therapy of metastatic uveal melanoma (NCT02601378, NCT01551459, NCT01377025, NCT01413191).

Summary

Gene expression profiling is a valuable addition to any ocular oncologist's toolbox. It is a widely available, accurate test, and its results enable us to better advise our patients of their prognosis. Further, as we gain a better understanding of the molecular pathobiology of UM, gene expression profiling will play an important role in identifying patients who may benefit from targeted treatment of metastatic disease in the future.

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See financial disclosure key, page 10.