Genomic Research in Uveal Melanoma

Recent genetic discoveries have provided clues about how to improve prognostic testing of the disease.

BY J. WILLIAM HARBOUR, MD

Uveal melanoma is the most common primary cancer of the eye and is one of the few fatal diseases that ophthalmologists diagnose (Figure 1). Despite dramatic improvements in diagnosis and treatment, the survival of patients with uveal melanoma has not improved over the past half century. The most likely explanation for this is that metastasizing uveal melanomas have spawned undetectable micrometastatic disease by the time of ocular diagnosis, before the primary tumor can be treated. It would not be feasible or effective to institute population-wide surveillance for early detection of melanomas. The most promising strategy for improving survival is to identify which patients with uveal melanoma are most likely to harbor micrometastases at the time of ocular diagnosis rather than waiting for overt metastasis to manifest. This would allow the institution of systemic therapy preemptively to delay or prevent the progression of micrometastic disease to lethal macrometastatic disease. In this strategy, uveal melanoma would be seen as a systemic and chronic disease; the main goal would be to prolong survival rather than cure the disease. Instituting this strategy requires accurate identification of high-risk patients and effective systemic therapy. Genomic research has provided the most promising avenues toward achieving both of these requirements.

CLINICAL AND HISTOPATHOLOGIC FEATURES

Certain clinical and histopathologic features of uveal melanoma are associated with a higher risk for metastasis. These include increased age, larger tumor size, ciliary body involvement, epithelioid cell type, looping extravascular matrix patterns, tumor infiltration by macrophages and lymphocytes, and mitotic rate. Despite the important insights into the biology of uveal melanoma that these features have provided, none of them are sufficiently sensitive or specific to be used as the basis for a clinical prognostic test. Further, these features do not provide molecular clues that could be used to guide the development of targeted therapies.

Figure 1. Montage fundus photograph of a uveal melanoma.

CHROMOSOMAL ABNORMALITIES

In the early 1990s, it was recognized that certain chromosomal alterations within the primary tumor could be used to predict metastasis. The most important of these is monosomy 3 (loss of one copy of chromosome 3), which is closely associated with metastasis. Other chromosomal changes have also been associated with poor prognosis, such as loss of 1p and 8p, and gain of 8q. However, these changes are not as consistent and appear to be secondary in importance, both prognostically and biologically, to monosomy 3. Consequently, analysis of chromosome 3 in clinical samples has increasingly been used as a means of prognostication in individual patients, starting with simple karyotype analysis and advancing to other techniques, such as fluorescence in situ hybridization (Figure 2), comparative chromosomal hybridization (Figure 3), and loss of heterozygosity analysis.

These techniques were clearly superior to clinical and histopathologic variables alone in predicting which patients will develop metastasis; however, techniques for detecting monosomy 3 have several important drawbacks, including false positives and negatives, a high rate of assay failures due to the amount of tissue required,
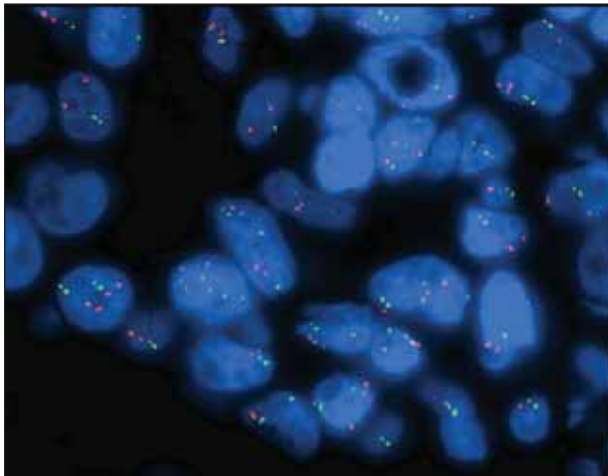
Figure 2. Fluorescence in situ hybridization (FISH) analysis for chromosome 3 in uveal melanoma. The green dots represent probes that recognize a centromeric region on chromosome 3. The red dots represent control probes that recognize a region on chromosome 7. Blue dots represent cell nuclei. Most of the nuclei contain two copies of chromosome 3.

Figure 3. Array-based comparative genomic hybridization scan of the entire genome in a uveal melanoma sample, showing loss (dots below Log2Ratio of 1) on chromosomes 1p, 3 and 8p, and gain (dots above Log2Ratio of 1) on chromosome 8q.

variability and nonstandardization of technique from center to center, and intratumoral heterogeneity. Heterogeneity is perhaps the intrinsic limit to how accurate monosomy 3 analysis can be. A single uveal melanoma can comprise a mixture of cells that contain only one copy of chromosome 3 and others that contain the normal two copies. There is disagreement among ocular oncologists as to what percentage of cells in a tumor must exhibit monosomy 3 before the tumor can be said to be monosomy 3. Sampling a small portion of a tumor can often produce the wrong test result. These shortcomings prompted our group to investigate other approaches to genetic prognostic testing.

GLOBAL GENE EXPRESSION PATTERNS

Thanks to advances in technology approximately 10 years ago, tens of thousands of genes could be monitored simultaneously for microRNA (mRNA) expression. With the advent of software to analyze such massive amounts of data, it quickly became clear that multidimensional analyses could provide powerful new levels of biologic information that were previously unknown. This was particularly true in cancer, where gene expression profiling (GEP) quickly revealed that many forms of cancer that were originally thought to be uniform based on their common tissue sources were composed of multiple subtypes of molecularly distinct cancers. Such was the case with uveal melanoma. In contrast to many other forms of cancer, GEP helped to simplify instead of further complicating our molecular understanding of uveal melanoma. Rather than
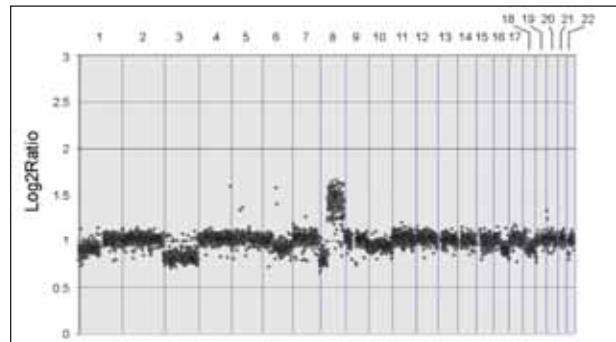
many different molecular subtypes, as one might expect from the multitude of different clinical and histopathologic features, there were only two major uveal melanoma subtypes, which are now called class 1 and class 2 (Figure 4).

Class 1 tumors have a very low risk of metastasis, whereas class 2 tumors have a very high risk. While there was a strong association between GEP class 2 and monosomy 3, multiple groups have now shown that GEP is a more accurate predictor of metastasis. The superior sensitivity and specificity of GEP over monosomy 3 most likely stems from the biologic limitations of monosomy 3 mentioned above. GEP has demonstrated less susceptibility to regional intratumoral heterogeneity. This may be because it is not cell-specific but surveys the entire tumor microenvironment.

The problems of standardization have largely been overcome by further refining GEP to a standardized 15-gene assay that is widely available for routine clinical use. This polymerase chain reaction-based assay requires a much smaller biopsy sample and has a much lower failure rate than available monosomy 3 tests. With these tools, the technology for identifying uveal melanoma patients who are likely to harbor micrometastasis at the time of ocular diagnosis is rapidly maturing. But the question remains, with what therapies are we going to treat high-risk patients? Recent genetic discoveries may provide clues.

GENETIC MUTATIONS

For many years, it has been known that the genetic mutations that cause uveal melanoma would be a difficult mystery to solve. Most cancers are riddled with activating mutations in oncogenes such as Ras, Raf, and Myc, and inactivating mutations in tumor suppressor genes such as Rb, p53, and PTEN. Yet uveal melanomas are peculiarly devoid of such mutations. Two recent discoveries have helped to solve the mystery.
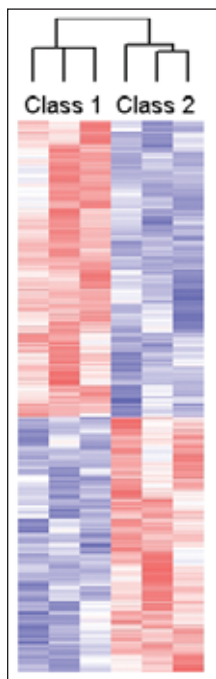
Figure 4. Gene expression profiling of uveal melanoma samples depicted as a heatmap. The columns represent tumor samples, and the rows represent genes. Genes with low expression are depicted in blue, and those with high expression are depicted in red. The genes and tumors are clustered according to their similarity to each other. The class 1 tumors cluster together to the left, and the class 2 tumors cluster together to the right.

Recently, the G·q stimulatory subunit, GNAQ was shown to be mutated in about one half of uveal melanomas. This mutation leads to constitutive activation of the mitogen-activated protein kinase (MAPK) signaling pathway that regulates cell proliferation. We confirmed this and showed that the GNAQ mutation does not correlate with GEP class or other markers of tumor progression, which suggests that this may be an early or even initiating mutation in uveal melanoma. The fact that this mutation occurred very early, coupled with the observation that it resulted in formation of a nevus and not a malignant melanoma, suggested that further mutations were required to produce a uveal melanoma with the capacity to metastasize.

Because class 2 tumors are strongly associated with monosomy 3, the loss of one copy of chromosome 3 may unmask a defective metastasis suppressor gene on the remaining copy. Laboratories around the world have been searching for this putative gene for many years without success. One major hurdle is that chromosome 3 spans almost 200 million base pairs and contains up to 1,500 genes, making a search for a small change in one of these genes a bewildering challenge. Two powerful technologies have recently become available to overcome this problem. Exome capture is a technique for separating the coding genes, where potential mutations reside, from the noncoding regions that make up the bulk of the genome. Massively parallel or next-generation sequencing allows all of these genes to be sequenced simultaneously to identify mutations.

We combined these two techniques to interrogate all of the genes on chromosome 3. We identified one gene, BAP1, located at chromosome 3p21.1, which sustained inactivating mutations in 84% of class 2 tumors but in only one class 1 tumor. We suspect that this tumor may have been in transition to a class 2 tumor. The other class 2 tumors may also contain mutations that have not yet been identified. Further work has provided strong evidence that mutation of this gene may be a critical step in the acquisition of metastatic capacity in uveal melanoma. Consequently, understanding the normal functions of this gene, particularly in the context of metastasis suppression, will lead to novel ideas for treating metastatic disease.

CONCLUSION

After many years of slow, painstaking progress, uveal melanoma is finally yielding its secrets to new scientific technologies. With the pace of progress dramatically accelerating over the past decade, the hope of effective new therapies for uveal melanoma patients, once relegated to some time in the distant future, may soon be a fact of the present. ∎

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Suggested Reading
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