Molecular Research in Uveal Melanoma: Ushering in a New Standard of Care

Until recently, there was no hope for individuals stricken by metastatic uveal melanoma. Despite steady progress in diagnostic and therapeutic capabilities over the past century, there has not been a corresponding improvement in survival for patients with this highly aggressive cancer of the eye. This is because patients who eventually succumb to metastatic disease already had undetectable micrometastases when their eye tumor was diagnosed and treated.

By the time the metastatic disease is advanced enough to detect, there are no available anti-cancer agents effective in prolonging survival. The best hope for prolonging survival may be to identify patients likely to harbor micrometastases and begin treating them before they progress to overt metastatic disease (Figure 1).

Developing a State-of-the-Art Molecular Prognostic Test

Although a number of clinical, prognostic, and cytogenetic factors have been used to stratify patients according to metastatic risk, gene expression profiling (GEP) appears to be the most accurate method available.

There are 2 distinct groups of uveal melanomas:

• Class 1 tumors have a low risk of metastasis.
• Class 2 tumors have a high risk.

Researchers in the Department of Ophthalmology and Visual Sciences at Washington University School of Medicine (WUSM), St. Louis, Missouri, have recently found that class-1 tumors can be further subdivided into classes 1A and 1B for even better prognostic accuracy.

Despite continued interest in monosomy 3 and other cytogenetic changes for prognostication, the WUSM research group and at least 2 others have shown in head-to-head comparisons that GEP is more accurate than these other methods. There is a strong association between monosomy 3 and the class-2 GEP, but in about 20% of uveal melanomas, chromosome 3 status and GEP do not agree. In these discordant cases, GEP is almost always correct rather than chromosome 3 status.

The WUSM researchers believe GEP’s superiority is likely due to the fact that it captures a functional snapshot of tumor biology, while chromosomal changes are simply static markers heterogeneously distributed throughout the tumor. Thus, the problem of sampling error due to tumor heterogeneity is much larger for cytogenetics than for GEP, which does not appear to vary significantly within the tumor in most cases.

The WUSM researchers perform GEP on a microfluidics PCR platform, which has a much higher success rate than most available methods for cytogenetic testing on very small samples obtained with 1 pass of a 25- or 27-gauge needle. These advantages prompted WUSM to develop a simple GEP test now in routine clinical use in many centers around the world. This is the only prognostic test in uveal melanoma ever prospectively validated in a multicenter study involving 10 centers and almost 600 patients. The initial report of this multi-institution initiative, called the Collaborative Ocular Oncology Group, will be published shortly.

Discovering Key Mutations in Uveal Melanoma

Although gene expression profiling has been highly successful for prognostication, it cannot tell clinicians which medication to use in high-risk, class-2 patients to delay or prevent overt metastatic disease. A major obstacle to developing better treatments for metastatic uveal melanoma has been a lack of understanding of the genetic mutations that cause uveal melanoma to develop and spread. That situation began to change a few years ago with the discovery of activating mutations in GNAQ, a stimulatory αq G-protein subunit, which result in constitutive activation of the RAF/MEK/ERF and other mitogenic pathways.

Mutations in GNAQ and the closely related GNA11 are present in most uveal melanomas. However, these mutations are found in uveal melanomas of all sizes and types, in both GEP classes in equal propor-
tions, and even in benign nevi. Thus, it seems likely that mutations in these genes are responsible for the early, pre-malignant tumor rather than the subsequent formation of the malignant melanoma, which presumably requires additional mutations.

It is unclear whether therapeutic targeting of such an early event will be effective in advanced metastatic disease. The search for the gene mutation that leads to the actual metastasis of uveal melanomas was aided by the recent development of 2 powerful technologies: exome capture and "next-generation" DNA sequencing (also called "massively parallel" or "deep" sequencing). These techniques allowed WUSM researchers to query the entire genome for mutations in virtually all known genes.

While this approach revealed dozens of mutations, the researchers already had a clue that the gene of interest was on chromosome 3, owing to the association between class 2 and monosomy 3. This work revealed deleterious mutations in a gene called BAP1 (BRCA1-associated protein 1) in almost 85% of class 2, but not in class-1 tumors. Thus, BAP1 may act as a metastasis suppressor gene in which mutational inactivation represents the rate-limiting step in the metastatic process.

**Finding Therapeutic Agents for Metastatic Uveal Melanoma**

Finding the BAP1 mutation has allowed WUSM researchers to focus on the BAP1 pathway as a promising target for therapy of metastatic disease. BAP1 encodes a ubiquitin carboxy-terminal hydrolase that removes the chemical ubiquitin from nucleosomes, which regulate DNA structure, and opposes the action of the polycomb repressive complex-1 (PRC1) that ubiquitinates nucleosomes. This yin-yang relationship allows for tight regulation of specific sets of genes involved in development, DNA damage repair, and cell proliferation.

Although the biochemical details of how BAP1 works are still being elucidated, it was recently reported that PRC1 can be inhibited by histone deacetylase (HDAC) inhibitors such as the commonly used epileptic medication valproic acid. Since BAP1 also opposes PRC1, one might predict that HDAC inhibitors could at least partly substitute for the missing BAP1 in class-2 uveal melanomas. Experiments at WUSM suggest that valproic acid may be effective as an adjuvant agent. Based on these findings, clinical trials are being planned to treat class-2 patients with valproic acid starting at the time of diagnosis of their primary tumor.

**Uveal Melanoma: A Model for Understanding Cancer Genetics**

While uveal melanoma is still a formidable foe, this recalcitrant cancer has begun to yield its secrets. In fact, uveal melanoma has emerged as an important model for understanding cancer genetics in general. Patients at high risk of metastatic disease can now be identified with great precision using state-of-the-art molecular testing in a routine clinical setting.

The very early and possibly initiating gene mutations that lead to the pre-malignant nevi are now known. And at the other end of the spectrum, the gene mutation that accounts for the vast majority of metastatic events has been identified (Figure 2).

Researchers now have unprecedented insight into the metastatic process that will likely lead to new therapies for metastasis in the near future. Very soon, WUSM will conduct clinical trials for high-risk uveal melanoma patients to determine whether adjuvant therapy, initiated at the time the primary eye tumor is diagnosed rather than waiting until metastatic disease is detected, can delay or prevent metastasis. A new standard of care in uveal melanoma is just ahead.

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**References** (bolded references are recommended reading)


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**Financial Disclosures**

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