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A Molecular Revolution in Uveal Melanoma

Implications for Patient Care and Targeted Therapy

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Uveal melanoma is the most common primary intraocular malignancy and has a strong propensity for fatal metastasis. Recent advances in the molecular genetics of uveal melanoma are revolutionizing our understanding of this cancer and the care of patients. The development of a new molecular classification of uveal melanoma based on a widely available 15-gene expression profile now allows patients at high risk of metastasis to be identified early so that individualized management can be offered. The recent discovery of major driver mutations in uveal melanoma provide a rational basis for development of new targeted therapies. Taken together, these advances are transforming our understanding and management of uveal melanoma with the ultimate goal of improving patient outcomes. *Ophthalmology 2014;121:1281-1288 © 2014 by the American Academy of Ophthalmology.*

Uveal melanoma (UM) is diagnosed in approximately 1200 to 1500 individuals per year in the United States, and up to half of these are at risk of fatal metastasis.[1] Despite many advances in the diagnosis and treatment of uveal melanoma in recent decades, there has not been a corresponding demonstrable improvement in survival rates.[2] This is likely because of this cancer's proclivity for early micrometastasis before treating the primary tumor, followed by a prolonged latency period before manifesting clinically as metastatic disease. Until recently, progress in developing effective treatments for metastatic uveal melanoma has been hampered by a limited understanding of the pathobiology of this cancer. Within the past few years, however, this picture has begun to change rapidly. Discoveries driven by powerful new molecular genetic technologies now allow us to construct a detailed molecular landscape of uveal melanoma as a foundation for improved patient care and drug discovery. For the first time, there is now realistic hope of improving patient survival through molecular prognostic testing to individualize patient care and mutational profiling to individualize the choice of targeted therapeutic agents.

Molecular Classification of Uveal Melanoma

A voluminous literature has been produced on the clinical, histopathologic, and chromosomal features of uveal melanoma and their association with patient outcomes.[1] Nevertheless, these empirical correlations fail to provide a mechanistic understanding of the fundamental tumor biology of uveal melanoma. The first breakthrough in this regard came with experiments using high-density microarrays to study gene expression profiles of primary uveal melanomas. This work showed that uveal melanomas exist in 1 of 2 basic molecular forms that are associated strongly with metastatic proclivity: class 1 tumors have a low risk of metastasis and class 2 tumors have a high risk of metastasis.[3,4] Using sophisticated bioinformatic analyses, the genes that are upregulated and downregulated in class 1 versus class 2 uveal melanomas were compared with gene lists associated with other biological conditions. Such studies showed that the genes expressed in class 1 tumors are similar to those in normal uveal melanocytes and cells committed to the neural crest lineage, whereas the genes expressed in class 2 tumors resembled those in less committed, primitive stem-like cells.[5,6] Thus, an important feature that distinguishes uveal melanomas that metastasize from those that do not is that the former exhibit loss of melanocytic differentiation and acquisition of primitive stem-like cells. In retrospect, histopathologic risk factors for metastasis such as epithelioid cell type and vasculogenic mimicry patterns probably reflect this de-differentiated stem-like state.[5–7]

Not only did gene expression profiling provide new insights into the pathobiology of uveal melanoma, it proved to

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be a more accurate prognostic tool than chromosomal analysis, as confirmed by multiple independent groups.[8–10] Consequently, the gene expression profile has been refined to a 15-gene assay performed on a microfluidics polymerase chain reaction platform that allows accurate and reproducible molecular classification from minute samples obtained from small-gauge needle biopsies.[11] The predictive accuracy of this 15-gene assay was validated by a prospective study involving 12 independent centers,[12] which sets it apart from any other prognostic method used for uveal melanoma. The accuracy of this test allows patients to be stratified into low- and high-risk categories for individualized metastatic surveillance and entry into clinical trials. Additionally, the 15-gene assay is simpler for the surgeon to use, requires far fewer tumor cells and less aggressive biopsy methods, is less prone to sampling error resulting from intratumoral heterogeneity, and is easier to deploy for individualized patient care than chromosomal analysis.[13–15]

Driver Mutations in Uveal Melanoma

Another major advance in recent years has been the discovery of key driver mutations that underlie tumor initiation, progression, and metastasis.

GNAQ and *GNA11*

Oncogenic mutations that activate growth-stimulating signaling pathways, such as those in *RAS* (retrovirus-associated sequence) family members, *BRAF* (v-raf murine sarcoma viral oncogene homolog B1), and *KIT* (v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog), are common in cancer. Yet, the discovery of such mutations in uveal melanoma remained elusive for many years despite intensive investigation.[16] The first breakthrough came with the discovery of mutations in the *GNAQ* (guanine nucleotide-binding protein subunit α-Q) gene in approximately half of all uveal melanomas,[17,18] followed shortly thereafter by the discovery of mutations in the closely related *GNA11* (guanine nucleotide-binding protein subunit α-11) gene in many other uveal melanomas (Fig 1A).[19] It seems that *GNAQ* and *GNA11* mutations are mutually exclusive and are present in approximately 85% of uveal melanomas. These mutations are somatic (i.e., not inherited) and heterozygous (i.e., only present on 1 allele). *GNAQ* and *GNA11* encode closely related Gq α subunits of the heterotrimeric Gq protein that activates phospholipase C, leading to a series of downstream signaling effects, one of which is activation of the mitogen-activated protein kinase growth signaling pathway (Fig 2). Activation of Gq normally is terminated by a GTPase activity intrinsic to the G-α subunit. However, the mutations that occur in uveal melanoma at amino acid residues glutamine-209 and arginine-183 disable the GTPase activity and prevent inactivation of the protein, leading to constitutive activation of the mitogen-activated protein kinase pathway and other growth pathways.

Because *GNAQ* and *GNA11* mutations are found in uveal melanomas of all stages, including benign nevi, they are thought to be early or initiating events in tumorigenesis (Fig 3).[17] Metastasis has been reported to be more common in tumors with mutations in *GNA11*, leading to the speculation that mutations in *GNA11* may be more virulent than those in *GNAQ*.[19] However, *GNA11* mutations also are more common in tumors involving the ciliary body, which is an independent risk factor for metastasis, and in tumors with mutations in the metastasis suppressor *BAP1* (breast cancer 1, early onset [BRCA1]-associated protein 1; see below), so it remains possible or even likely that the association between *GNA11* and metastasis is not causal. Taken together, existing evidence suggests that mutations in *GNAQ* and *GNA11* represent early events in uveal melanoma tumorigenesis, but they are not sufficient for development of a metastatic tumor.

BAP1

Because *GNAQ* and *GNA11* mutations do not correlate with patient outcome and do not account for the class 2 signature or metastasis, the search continued for gene mutations associated with metastasis in class 2 tumors. Using next-generation DNA sequencing techniques, we discovered mutations in *BAP1* in the vast majority of class 2 metastasizing uveal melanomas, but rarely in the less aggressive class 1 tumors (Fig 1B).[20] Consistent with its presumed function as a tumor suppressor gene, *BAP1* undergoes mutational inactivation of 1 copy and deletion of the other copy through loss of the entire chromosome. Interestingly, the *BAP1* gene is located at chromosome 3p21, so this discovery likely explains, at least in part, the long-known association between loss of chromosome 3 and metastasis in uveal melanoma.

BAP1 encodes an enzyme that removes single ubiquitin moieties from various substrates, including histone H2A, HCF-1 (host cell factor 1), BRCA1, and others.[21] Ubiquitin often is added to proteins to target them for degradation, but *BAP1* does not seem to be involved in that pathway. Rather, *BAP1* seems to regulate critical cellular functions such as gene expression, cell cycle, and cell identity (Fig 2).[22,23] The precise reason why loss of *BAP1* promotes uveal melanoma progression and metastasis remains unclear and is the subject of intense investigation.

SF3B1

In our ongoing research using next-generation DNA sequencing in uveal melanoma, we discovered frequent driver mutations in another gene called *SF3B1* (splicing factor 3B, subunit 1; Fig 1C).[24] The *SF3B1* mutations reported so far are quite distinct in several respects: (1) they are single nucleotide point mutations predominantly occurring at a single amino acid (arginine-625), (2) they involve only 1 allele (heterozygous), (3) they are almost mutually exclusive with *BAP1* mutations but occur with equal frequency in *GNAQ* versus *GNA11* mutations, and (4) they are associated with class 1 tumors and better prognosis. Mutations in *SF3B1* alter the splicing of certain

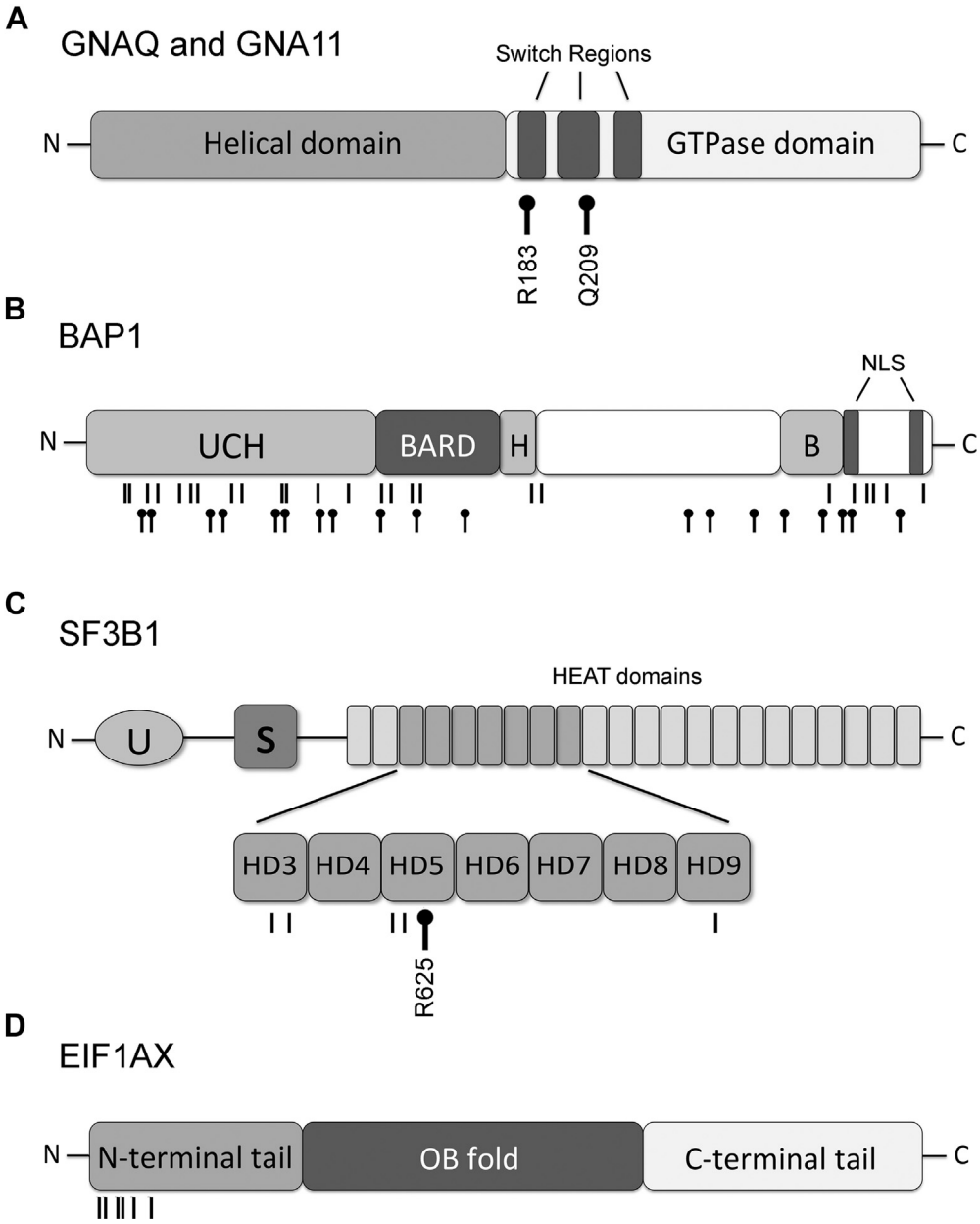

Figure 1. Protein domain structure and mutation profile of key driver mutations in uveal melanoma. **A**, *GNAQ* and *GNA11* encode closely related Gq α subunits that consist of helical and catalytic GTPase domains. *GNAQ* and *GNA11* mutations are mutually exclusive, occur at 2 amino acid residues (Q209 and R183), lead to constitutive protein activation, and chronically stimulate downstream growth-signaling pathways such as the mitogen-activated protein kinase (MAPK) pathway. **B**, *BAP1* consists of a ubiquitin carboxyl-terminal hydrolase (UCH) catalytic domain, BARD binding domain, HCF1 binding motif (H), BRCA1 binding domain (B), and nuclear localization sequences (NLS). Reported nontruncating mutations (bars) and truncating mutations (pins) are indicated. **C**, *SF3B1* consists of a U2AF2 interaction motif (U), SF3B14 interaction motif (S), and 17 nonredundant HEAT domains (HD). The most common mutation in uveal melanoma occurs at R625 in HEAT domain 5 (large pin). Less common mutations are indicated (small bars). **D**, *EIFAX1* consists of an N-terminal tail, oligonucleotide binding (OB) fold, and C terminal tail. Mutations identified so far in uveal melanoma have been substitutions or deletions of 1 to 2 amino acids near the amino terminus (bars). BARD = BRCA1-Associated RING Domain Protein 1; C = carboxy terminus; HEAT = Huntingtin, elongation factor 3, protein phosphatase 2A, and TOR1; N = amino terminus.

mRNA transcripts,[25] but it remains unclear how this promotes tumorigenesis.

Other Driver Mutations

Powerful genomic sequencing technologies are becoming less expensive and more widely available, so it is likely that more mutations will be reported in uveal melanoma. Recently, mutations in *EIF1AX* (eukaryotic translation initiation factor 1A, X-linked), located on the X chromosome, were described in a subset of uveal melanomas (Fig 1D).[25] *EIF1AX* mutations reported to date are nontruncating and heterozygous (present in only 1 copy of

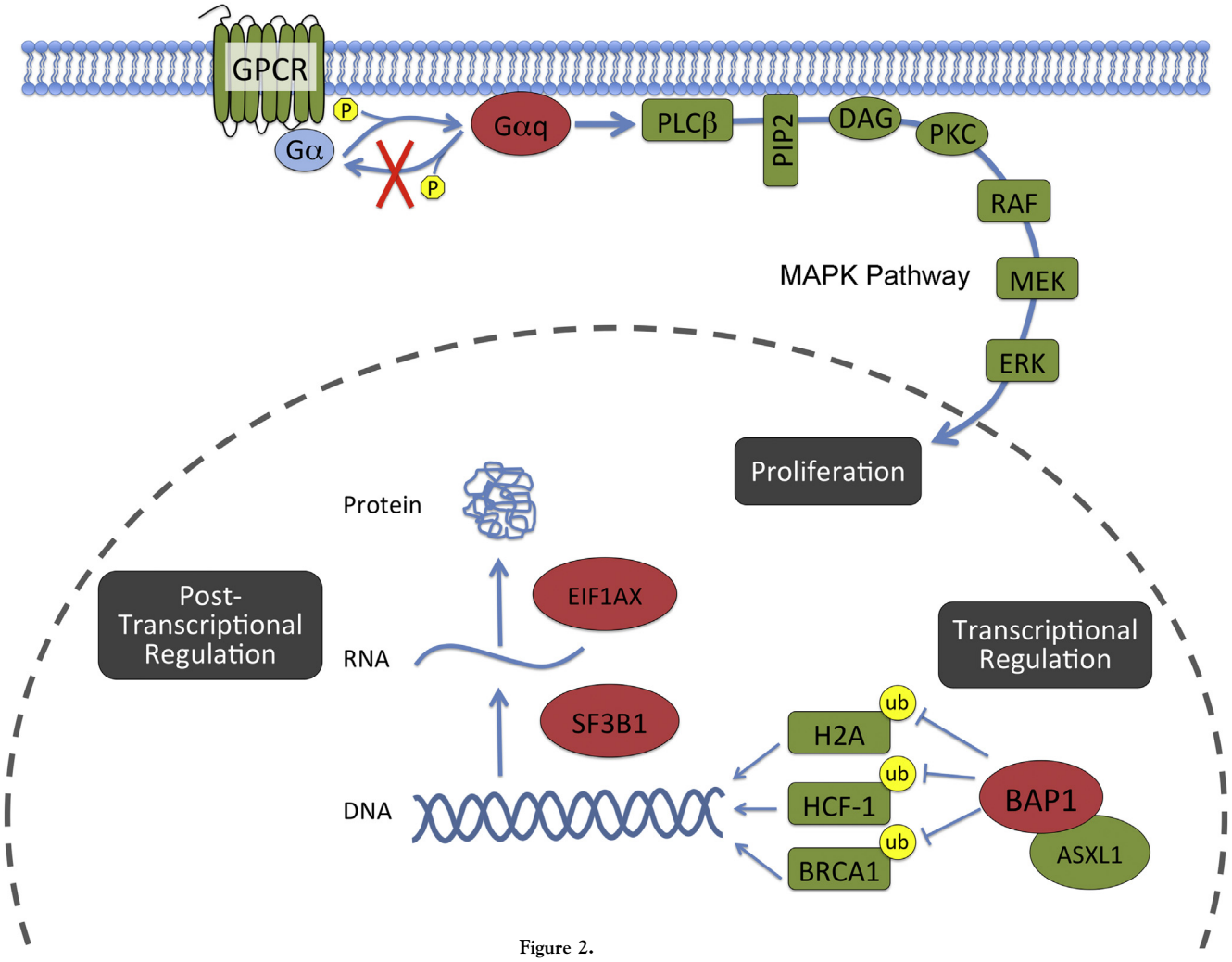

Figure 2.

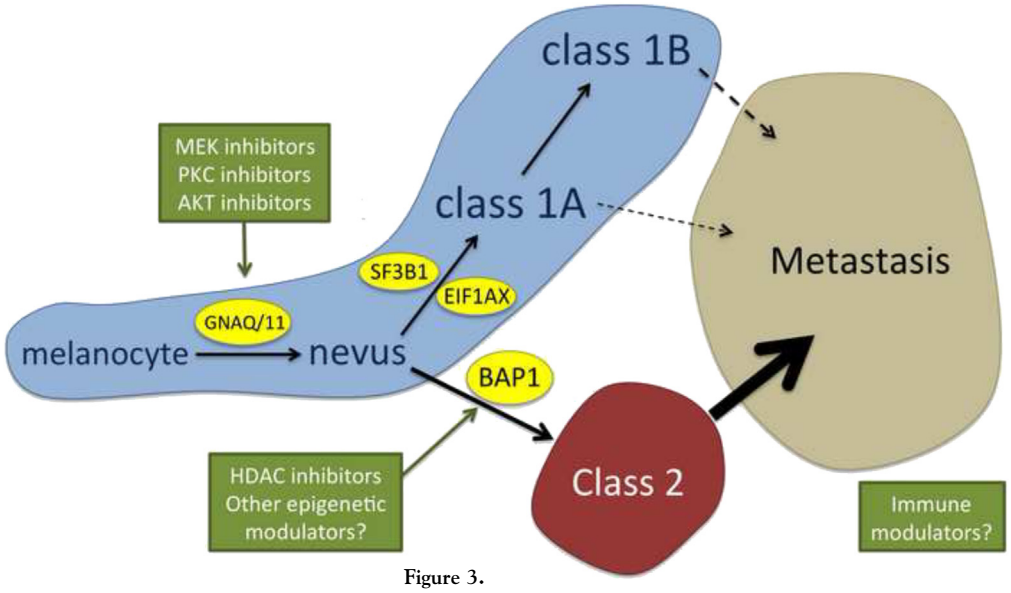

Figure 3.

the gene), which are characteristics usually associated with dominantly acting oncogenes. In uveal melanomas harboring *EIF1AX* mutations, however, only mutant mRNA transcripts were expressed, suggesting that the wild-type copy of *EIF1AX* is epigenetically inactivated, in which case *EIF1AX* mutations might behave in a recessive fashion. Like *SF3B1*, *EIF1AX* mutations seem to be associated with class 1 uveal melanomas and with better prognosis. Further, *EIF1AX* mutations seem to be mutually exclusive with *SF3B1* mutations, which have important implications in the construction of a molecular landscape model (below).

As increasingly rare mutations of unclear significance are identified, it will become challenging to distinguish truly pathogenic mutations from silent polymorphisms and so-called passenger mutations that are swept along during tumor evolution without providing any selective advantage.[26] Our focus will remain on the common driver mutations that have a clear mechanistic role in tumor progression. Enough of these key mutations have been discovered that now we can construct a provisional molecular landscape for uveal melanoma.

Molecular Landscape of Uveal Melanoma

Gene expression profiling not only offers a highly accurate molecular prognostic classification, it also provides a rational framework for understanding how the major driver mutations relate to one another with respect to tumor evolution and patient prognosis. Based on transcriptomic and phenotypic features, we can surmise that class 1 uveal melanomas are more closely related to normal uveal melanocytes than are class 2 tumors.[6] Furthermore, a few uveal melanomas have been discovered that were in transition from class 1 to class 2 (J.W.H., unpublished data, 2013), suggesting that uveal melanomas may progress from premalignant nevus to class 1 melanoma to class 2 melanoma (Fig 3).

Mutations in *GNAQ* and *GNA11* are mutually exclusive and seem to represent early or initiating events because they are found in premalignant nevi and in uveal melanoma of all stages.[17–19] In contrast, *BAP1* mutations occur almost exclusively in class 2 tumors and are associated strongly with metastasis, a phenotype that presumably arises later in tumor evolution.[20] Thus, we can place *GNAQ* and *GNA11* mutations early and *BAP1* mutations later in this schema based on a gene expression profile. *SF3B1* mutations occur mostly in class 1 tumors and largely are mutually exclusive with *BAP1* mutations.[24] Similarly, *EIF1AX* mutations seem to occur primarily in class 1 tumors, in a mutually exclusive fashion with *SF3B1* and *BAP1* mutations.[25] When 2 or more mutations occur in a mutually exclusive fashion, this may indicate that the genes have an overlapping function in a common pathway, such that mutation of 1 gene relieves the selective pressure to mutate the other.[26] Such events may represent nodal points of evolutionary divergence and, as such, allow us to construct a provisional model of the uveal melanoma molecular landscape that provides valuable insights into tumor biology and targeted therapy (Fig 3).

Clinical Implications

Targeted Molecular Therapy

Contemporary treatment methods for primary (intraocular) uveal melanoma are highly effective in achieving local tumor control, but effective therapies for metastatic uveal melanoma have remained elusive.[27] In most types of cancer, traditional nonspecific chemotherapeutic agents rapidly are being replaced by targeted therapies designed to counteract specific oncogenic mutations. For example, small molecule inhibitors of a mutant form of the BRAF oncoprotein have shown efficacy in metastatic cutaneous melanoma.[28] Until recently, however, this mutation-driven approach to cancer therapy has been hampered in uveal melanoma by a lack of known mutations. Fortunately, this

Figure 2. Diagram showing cellular functions of proteins commonly mutated in uveal melanoma. Mutations in *GNAQ* and *GNA11* disable GTPase activity (indicated by X), resulting in constitutive activation of downstream signaling pathways, including the mitogen-activated protein kinase (MAPK) pathway. *BAP1* encodes an enzyme that removes monoubiquitin moieties (ub) from specific substrates, including histone H2A, HCF1, and BRCA1. Interaction of BAP1 with ASXL family members is required for this enzymatic activity. These interactions affect chromatin structure and transcription. The SF3B1 protein is an integral component of both the major U2 and minor U12 spliceosomes. *SF3B1* mutations in uveal melanoma alter the splicing of specific RNA transcripts. *EIF1AX* encodes an essential translation initiation factor required for the formation of proteins from mRNA. Mutations in *EIF1AX* have not been studied extensively but would be expected to alter the production of proteins that may promote tumor progression. DAG = diacylglycerol; ERK = extracellular signal-regulated kinases; Gα = G α subunit; Gαq = G-α-q subunit; GPCR = G protein coupled receptor; MEK = mitogen-activated protein kinase/extracellular signal-regulated kinase kinase; PKC = protein kinase C; PIP2 = phosphatidylinositol biphosphate; PLCβ = phosphoinositide-specific phospholipase C beta; RAF = rapidly accelerated fibrosarcoma oncoprotein.

Figure 3. Diagram showing the molecular classification and key genetic events in uveal melanoma. The gene expression profile-based molecular classification provides a context for understanding the key events in uveal melanoma progression. Mutations in *GNAQ* and *GNA11* are likely to be early events that cause increased proliferation, leading to a low-grade, premalignant melanocytic tumor with a class 1 gene expression profile. The vast majority of nevi become dormant and progress no further as a result of innate tumor suppression mechanisms. Rarely, such a tumor acquires additional mutations that allow it to progress to a uveal melanoma. If progression involves mutation of either *SF3B1* or *EIF1AX*, the tumor retains the class 1 gene expression profile and has a low metastatic potential. However, if it acquires a mutation in BAP1 and loses the remaining copy of BAP1 through loss of chromosome 3, it acquires the class 2 gene expression profile and a high potential for metastasis. Boxes indicate targeted therapeutic compounds that are under investigation in uveal melanoma. AKT = protein kinase B; HDAC = histone deacetylase; MEK = mitogen-activated protein kinase/extracellular signal-regulated kinase kinase; PKC = protein kinase C.

Table 1. Clinical Trials for Uveal Melanoma Based on Targeted Therapy of Driver Mutations

ClinicalTrials.gov Identifier	Compound Being Tested	Molecular Target	Mutation Rationale
NCT01430416 NCT01801358	AEB071	PKC	GNAQ/11
NCT01551459 NCT01005472	Sunitinib	MEK	GNAQ/11
NCT01252251 NCT01587352 NCT00121225	Everolimus Vorinostat	mTOR HDAC	GNAQ/11 BAP1
NCT01377025 NCT01893099 NCT00329641	Sorafenib	RAF-kinases	GNAQ/11
NCT01801358	MEK162	MEK	GNAQ/11
NCT01143402	Selumetinib	MEK	GNAQ/11
NCT01835145	Cabozantinib	MET, KIT	GNAQ/11
NCT00104884	Romidepsin	HDAC	BAP1

BAP1 = breast cancer 1, early onset (BRCA1)-associated protein 1; GNAQ = guanine nucleotide-binding protein subunit α-Q; GNA11 = guanine nucleotide-binding protein subunit α-1; HDAC = histone deacetylase; KIT = v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog; MEK = mitogen-activated protein kinase/extracellular signal-regulated kinase kinase; MET = met proto-oncogene (hepatocyte growth factor receptor); mTOR = mammalian target of rapamycin; PKC = protein kinase C; RAF= rapidly accelerated fibrosarcoma oncoprotein.

situation is changing rapidly with the recent discovery of high-frequency mutations described in this article. Several clinical trials are underway using agents that specifically target these driver mutations in uveal melanoma (Table 1).

Direct inhibition of the mutant G-α-q and G-α-11 proteins has not been possible to date, because of the molecular nature of these mutations, but several proteins that are activated downstream of *GNAQ* and *GNA11* mutations have been targeted successfully. For example, the mitogen-activated protein kinase/extracellular signal-regulated kinase kinase (MEK) is activated in *GNAQ*- and *GNA11*-mutant tumors,[29,30] and inhibition of MEK can slow the growth of uveal melanomas in vitro and in vivo.[31,32] Indeed, a recent phase 2 clinical trial showed that the MEK inhibitor selumetinib extended progression-free survival by nearly 9 weeks compared with the traditional chemotherapeutic agent temozolomide, making it the first effective drug for metastatic uveal melanoma.[33] Other downstream effectors of *GNAQ* and *GNA11* mutations also have shown promise as therapeutic targets, including protein kinase C and members of the protein kinase B/mammalian target of rapamycin pathway.[34–36]

The strong association between *BAP1* mutations and metastasis suggests that pharmacologic targeting of *BAP1* mutations may be of therapeutic value. Because of the recessive nature of *BAP1* mutations, they will be difficult to target directly, but it may be possible to counteract the effects of *BAP1* mutations indirectly by understanding its biochemical and cellular functions. For example, *BAP1* encodes an enzyme that removes single ubiquitin moieties from histone H2A by which it regulates gene expression.[23] Consequently, *BAP1* inactivation leads to abnormally high histone H2A ubiquitination.[37] We showed that histone deacetylase (HDAC) inhibitors reverse this histone hyper-ubiquitination, which shifts class 2 uveal melanoma cells to a class 1 profile and reverts the tumor cells to a less aggressive, differentiated phenotype.[37] Further, we showed that *BAP1* loss in uveal melanoma cells causes them to revert to a primitive, stem-like state that can be reversed with HDAC inhibitors.[38] Because the effect of HDAC inhibitors in uveal melanoma seems to be cytostatic rather than cytotoxic, these compounds are more likely to be valuable in the adjuvant setting, with the goal of delaying or preventing the development of metastatic disease in high-risk patients. Although *BAP1* mutations are associated strongly with the class 2 profile, *BAP1* mutations can be distributed heterogeneously throughout the tumor and thus are prone to sampling error. Therefore, gene expression profiling is still preferred as a tool for prognostication, stratifying patients for clinical trial entry, and as a surrogate for *BAP1* mutations directly in the tumor.

BAP1 Familial Cancer Syndrome and Patient Screening

Traditionally, familial uveal melanoma has been considered to be so rare as to merit publication.[39] Therefore, we were surprised to find that one of the uveal melanoma patients in our original description of *BAP1* mutations carried a germline *BAP1* mutation.[20] Subsequently, we found germline *BAP1* mutations in 2 additional unrelated uveal melanoma patients, both of whom had first-degree relatives with uveal melanoma and other cancers (J.W.H., unpublished data, 2013). Prompted by our report, investigators have discovered *BAP1* mutations in a subset of other cancer types, including mesothelioma, renal cell carcinoma, atypical intradermal nevi, and cutaneous melanoma, leading to the discovery of a newly described *BAP1* familial cancer syndrome.[21,40–43] This cancer syndrome is autosomal dominant with incomplete penetrance for any given cancer type.

Physicians involved in the care of patients with uveal melanoma should be aware of this cancer syndrome and the indications for *BAP1* testing and genetic counseling. A germline *BAP1* mutation should be suspected when a patient is diagnosed with uveal melanoma at an early age (younger than 30 years) or when one of more of the following is present in the patient or a first-degree relative: (1) multiple atypical cutaneous nevi, (2) cutaneous melanoma, (3) mesothelioma, (4) renal cell carcinoma, or (5) 2 or more primary cancers of any type. High-risk patients and family members should be offered *BAP1* sequencing of DNA from a blood sample. Because of the technical challenges of sequencing a large gene such as *BAP1* and the complexities of genetic counseling surrounding this syndrome, we strongly recommend that testing be performed by a Clinical Laboratory Improvement Amendments (CLIA)—certified reference laboratory that uses a validated testing protocol staffed by experienced cancer genetics counseling experts, such as the University of Miami Clinical Molecular Genetics Diagnostic Laboratory (medgen.med.miami.edu/cmgdl; accessed on November 23, 2013).

In conclusion, the application of advanced molecular biology and genetic techniques have revolutionized our classification, understanding, and management of uveal melanoma. We now have highly accurate clinical prognostic testing that allows high-risk patients to be identified and offered more intensive metastatic surveillance. Earlier detection of metastasis allows earlier intervention with locoregional targeted therapies such as transhepatic arterial chemoembolization, which can forestall fulminant liver progression in some patients with liver metastasis.[44,45] Molecular prognostic testing also allows high-risk patients to be entered into clinical trials to assess the efficacy of adjuvant therapy, with the goal of delaying or preventing the outgrowth of micrometastatic disease. Based on the discovery of the driver mutations described herein, we now have rational agents to test in the adjuvant setting, including small molecule inhibitors of MEK, mammalian target of rapamycin, protein kinase C, and HDACs (Fig 3), as well as promising immune checkpoint inhibitors such as ipilimumab.[46] High-risk class 2 patients, as well as patients with established metastatic disease, should be educated about the potential benefits of participating in clinical trials. Even when trials show no therapeutic benefit, they can be extremely important by providing surrogate end point data and other insights to narrow the search for effective therapies further.

At the Bascom Palmer Ocular Oncology Service, patients with uveal melanoma are offered gene expression profile-based molecular prognostic testing performed from a needle biopsy sample obtained at the time of primary tumor treatment. Patients who are found to have a high-risk class 2 tumor are offered increased metastatic surveillance and are informed of ongoing clinical trials of adjuvant therapy. Patients with established metastatic disease are offered personalized therapeutic options and participation in appropriate clinical trials. Patients at high risk for harboring a germline *BAP1* mutation are offered *BAP1* sequencing and genetic counseling. To provide the best possible patient care, ocular oncologists must remain informed and knowledgeable of this rapidly changing field.

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