Molecular Profiling of Uveal Melanoma Patients

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Abstract

Background: Although uveal melanoma represents only 5% of all melanomas, it is the most common primary intraocular malignancy of the adult eye. Approximately 50% of patients will develop metastases which are resistant to medical interventions. There is a great need for improved therapy as the prognosis is poor for advanced-stage disease. Our study was undertaken to investigate the presence of novel therapeutic targets.

Methods: We analyzed 49 uveal melanoma patients with immunohistochemistry for 23 markers including cKIT, PDGFR, cMET, PTEN and IGF1R. Furthermore, microarray analysis was performed on 29 samples using the Illumina platform. We also investigated FISH amplification of EGFR and mutational analysis of cKIT, BRAF, KRAS and NRAS on a smaller patient subset.

Results: Overexpression of KIT at the protein and RNA level was 74% (28 out of 38) and 45% (13 out of 29), respectively. Expression of cKIT did not correlate with gain-of-function cKIT mutations in any of the 34 samples tested. In our study, MET was overexpressed in 15 out of 17 cases at the RNA level and IGF1R was high in 4 out of 6 patients indicating poor prognosis. PTEN expression by IHC was present in 90% (36 out of 40) patients, indicating the PI3K pathway is not activated in the majority of uveal melanoma patients. BRAF was wild-type in all 42 patients tested. Similarly, no KRAS or NRAS mutations were detected. Protein and RNA expression of PDGFR were low in our patients. MGMT was lost in 16 out of 40 patients at the protein level and 10 out of 29 patients at the RNA level. EGFR expression, copy number and protein levels were low in the patients tested.

Conclusions: Our data on cKIT suggests that it is a promising target in uveal melanoma. Low expression of MGMT in about a third of our patients may indicate the likelihood of favorable response to alkylating agents like dacarbazine or temozolomide. There are currently several clinical trials investigating various cKIT inhibitors, as well as temozolomide in advanced uveal melanoma patients. Our findings highlight the importance of molecular profiling uveal melanoma patients.

Background

Uveal melanoma, which arises from neuroectodermal melanocytes, is the most common intraocular malignancy of the adult eye and differs considerably from cutaneous melanoma in its etiology, histology, and genetic features. This disease arises from either the choroid, ciliary body, or iris. In the United States, the incidence of this disease is 0.51 per 100,000 people. Hence, studies of this disease are limited and, when available, involve small cohorts.

Although relative, age-adjusted five-year survival rates of uveal melanoma are 81.1%, a dire need exits to identify those patients who do not have clinically apparent metastatic disease when first diagnosed but will eventually progress. And, for those individuals who present with metastasis – usually in the liver – a need exists to offer better treatment than what is conventionally performed – historically, metastatic uveal melanoma tends to be resistant to chemotherapy.

The purpose of this study, then, is to evaluate a series of metastatic uveal melanoma patients profiled at our facility and look for biomarkers that have prognostic or (potentially) predictive utility in hopes of identifying higher-risk populations and better targeting therapy for those with metastatic, aggressive disease.

Methods

A series of 49 uveal melanoma patients were profiled using the Caris Target Now platform between January 1, 2010 and December 31, 2011.

For immunohistochemistry (IHC), sections were prepared from formalin-fixed, paraffin-embedded (FFPE) tissue, and biomarkers predictive of chemotherapeutic response were analyzed using a Dako or Ventana platform. Included in this evaluation were AR (AR27), BCRP (6D171), c-kit (polyclonal), cMET (8F11), COX-2 (SP-21), EGFR (2-18C9), ERCC1 (8F1), HER2 (4B5), MGMT (MT23.3), MRP1 (33A6), PDGFR (polyclonal), PGP (C494), PR (1E2), PTEN (6H2.1), RRM1 (polyclonal), SPARCm (1222511), SPARCp (polyclonal), TLE3 (polyclonal), TOPO1 (1D6), TOPO2A (3F6), and TS (TS106). Results were evaluated by board-certfied pathologists and categorized into above threshold, below threshold, or negative based on defined, evidence-based cut-offs.

Microarray (DASL process, Illumina) was performed whenever possible. Results of gene overexpression, underexpression, and "no change" (no difference in expression) were based on a tissue-specific normal control. Results of "not performed" and "not informative" were included when analyzing microarray results.

Fluorescent in-situ hybridization (FISH) and DNA direct sequencing were also performed, sometimes based on physician request.

Results

IHC results from 49 patients profiled at our facility are shown in **Table 1** (below). Several results are worth mentioning. The biomarker, c-kit, is overexpressed in 74% (28 out of 38) of the specimens profiled. IGFR1R, considered a biomarker for poor prognosis when elevated, was above threshold in four out of six patients. Meanwhile, PTEN levels were considered adequate (i.e. not negative) in 90% of this cohort. The aforementioned result is potentially of clinical significance, since agents like everolimus are of potential benefit when PTEN is lost. Finally, MGMT – an agent associated with clinical benefit to temozolomide or dacarbazine when negative - was negative in 32.5% of patients. See **Figure 1** (above, right) for examples of IHC stains performed.

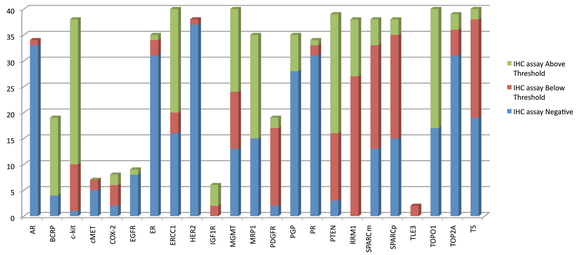

Table 1 – IHC results in uveal melanoma. Immunohistochemical (IHC) protein expression of twenty-three biomarkers. The X-axis indicates each, individual biomarker., while the Y-axis corresponds to the number of tests performed.

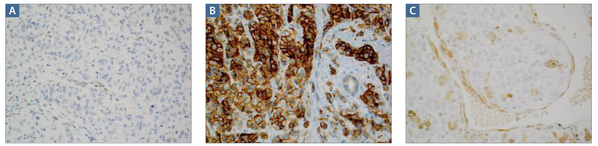

Figure 1 – Representative IHC stains in uveal melanoma. **(A)** An MGMT by IHC negative result. **(B)** C-kit by IHC considered "above threshold". **(C)** PTEN by IHC negative result.

Microarray was also utilized to interrogate the tumor. In all, 88 biomarkers were analyzed by this methodology in 29 tumors. **Table 2** and **Table 3** (shown below) are graphical illustrations showing the twenty biomarkers with the highest relative overexpression and the twenty biomarkers with the highest relative underexpression, sorted by percentage (from highest to lowest). Overexpressed biomarkers worth mentioning in **Table 2** include MET (88.2%), KIT (44.8%), ERCC1 (41.4%), GART (41.4%), SPARC (41.4%), PDGFRB (34.5%), and RRM2B (34.5%). Some of these results are associated with potential benefit to agents like sunitinib (KIT), pemetrexed (GART), and nab-paclitaxel (SPARC), while others are associated with a lack of clinical benefit to agents like cisplatin/carboplatin (ERCC1). By contrast, biomarkers worth mentioning in **Table 3** include EGFR (55.2%), TOP2A (51.7%), PDGFRA (48.3%), RRM1 (48.3%), MGMT (34.5%), and TYMS (34.5%). Some of these results are associated with benefit to fluorouracil (TYMS) and temozolomide/ dacarbazine (MGMT).

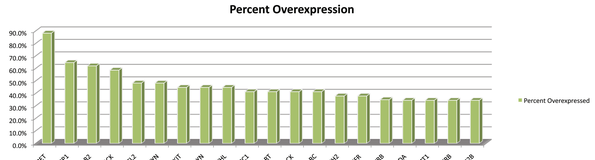

Table 2 – Microarray overexpression results in uveal melanoma, top overexpressors , ranked by percentage

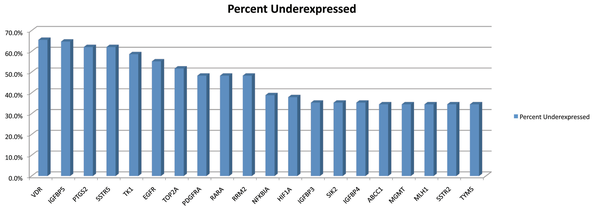

Table 3 – Microarray underexpression results in uveal melanoma, top underexpressors, ranked by percentage

FISH Assay	Total (n)	Result	
		Normal*	Amplified or Positive
ALK	1	1	0
cMYC	1	1	0
EGFR	20	20	0
HER2	3	3	0
TOP2A	1	1	0

Table 4 – FISH results in uveal melanoma.
*Results in Table 4 show that ALK was not re-arranged and no amplification of cMYC, EGFR, HER2 or TOP2A was observed in any case studied.

Sequencing	Total (n)	Result		
		Wild Type	Mutated	Cancelled/ Indeterminate
c-kit	34	29	0	5
EGFR	1	1	0	0
BRAF	42	37	0	5
KRAS	14	12	0	2
NRAS	1	1	0	0

Table 5 – Sequencing results in uveal melanoma.
Direct sequencing shows no mutation in the biomarkers analyzed.

Fluorescent in-situ hybridization (FISH) was also performed, either by reflexing from PTEN or HER2 IHC results or by physician request. Results in **Table 4** show that ALK, cMYC, EGFR, HER2, and TOP2A showed no amplification. Note how EGFR by FISH results indicate a lack of clinical benefit to anti-EGFR-targeted therapy, in keeping with EGFR by microarray.

Sequencing was also utilized to interrogate biomarkers. In our cohort, the biomarkers EGFR, BRAF, KRAS, and NRAS showed no mutations – see **Table 5**. Interestingly, despite overexpression of c-kit by IHC and KIT by microarray, no mutations were detected in c-kit. BRAF and NRAS showed no mutations, which is in contrast to cutaneous melanoma.

Conclusions

- Results achieved in our laboratory are consistent with what has been reported in the medical literature. For instance, c-kit IHC overexpression (74% protein overexpression in this group) with no corresponding c-kit mutation by direct sequencing has been reported. Also, in contrast to cutaneous melanoma, BRAF and NRAS mutations are non-existent, consistent with pubished studies.
- Many of the results contained herein reinforce what is known about uveal melanoma. Once the disease has spread, control with chemotherapy becomes difficult. However, biomarkers utilized at our facility could provide valuable targets to the clinician deciding whether to utilize a certain agent, especially in scenarios where metastasis has occurred outside the liver, to sites like lung or skin.
- Future studies should correlate accepted clinical practice to these biomarkers. For instance, MET scores can be compared to response to cMET inhibitors like (pending approval) ARQ197. Also, prognostic markers could be compared to histopathologic and cytologic features to better predict the future course of disease.
- Mutations in GNAQ and GNA11 are being investigated as potential targets in uveal melanoma and are currently performed in our laboratory.

References

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