Genetic and clinico-pathologic analysis of metastatic uveal melanoma

Klaus G Griewank¹, Johannes van de Nes², Bastian Schilling¹, Iris Moll¹, Antje Sucker¹, Hojabr Kakavand^{3,4}, Lauren E Haydu^{3,4}, Marina Asher⁵, Lisa Zimmer¹, Uwe Hillen¹, John F Thompson^{3,4}, Richard A Scolyer^{3,4,6}, Dirk Schadendorf¹ and Rajmohan Murali^{5,7}

¹Department of Dermatology, University of Duisburg-Essen, Essen, Germany; ²Institute of Pathology and Neuropathology, University Hospital Essen, University of Duisburg-Essen, Essen, Germany; ³The University of Sydney, Camperdown, NSW, Australia; ⁴Melanoma Institute Australia, North Sydney, NSW, Australia; ⁵Department of Pathology, Memorial Sloan-Kettering Cancer Center, New York, NY, USA; ⁶Tissue Pathology and Diagnostic Oncology, Royal Prince Alfred Hospital, Camperdown, NSW, Australia and ⁷Department of Human Oncology and Pathogenesis Program, Memorial Sloan-Kettering Cancer Center, New York, NY, USA

Uveal melanoma is the most common malignant tumor of the adult eye. Fifty percent of tumors will eventually metastasize, and there are no effective treatments for them. Recent studies of uveal melanoma have identified activating mutations in *GNAQ* and *GNA11*, loss-of-function mutations in the tumor suppressor gene *BAP1*, and recurrent mutations in codon 625 of *SF3B1*. Previous studies have reported the existence of a higher frequency of *GNA11* than *GNAQ* mutations, frequent *BAP1* loss, and rare *SF3B1* mutations in metastatic uveal melanoma. We analyzed a cohort of 30 uveal melanoma metastases for the occurrence of *GNAQ*, *GNA11*, and *SF3B1* mutations, as well as *BAP1* loss, and correlated these parameters with clinical and histopathologic features. Most (92%) tumors were composed of cells with an epithelioid or mixed (<100% spindle cells) morphology. Tumor samples composed exclusively of spindle cells were rare ($n=2$, 8%). Most tumors showed a moderate to marked degree of nuclear pleomorphism ($n=24$, 96%), and contained hyperchromatic, vesicular nuclei with variably conspicuous nucleoli. *GNA11* mutations were considerably more frequent than *GNAQ* mutations (*GNA11*, *GNAQ*, and wild-type in 18 (60%), 6 (20%), and 6 (20%) cases, respectively). *SF3B1* mutation was found in 1 of 26 tumors (4%), whereas loss of *BAP1* expression was present in 13 of 16 tumors (81%). Patients with *GNA11*-mutant tumors had poorer disease-specific survival (60.0 vs 121.4 months, $P=0.03$) and overall survival (50.6 vs 121.4 months, $P=0.03$) than those with tumors lacking *GNA11* mutations. The survival data, combined with the predominance of *GNA11* mutations in metastases, raises the possibility that *GNA11*-mutant tumors may be associated with a higher risk of metastasis and poorer prognosis than *GNAQ*-mutant tumors. Further studies of uveal melanoma are required to investigate the functional and prognostic relevance of oncogenic mutations in *GNA11* and *GNAQ*.

Modern Pathology (2014) 27, 175–183; doi:10.1038/modpathol.2013.138; published online 26 July 2013

Keywords: BAP1; genetics; *GNAQ*; *GNA11*; prognosis; *SF3B1*; uveal melanoma

Approximately 5% of all melanomas occur in the eye, of which most arise in the uveal tract (iris, ciliary

body, or choroid).¹ Uveal melanoma is the most common malignant tumor of the eye, with an incidence of about four cases per million per annum.² Diagnosis often occurs late in the course of disease, and prognosis is generally poor with a 10-year mortality rate of around 40%.³ The most common site of metastasis is the liver.^{4,5} Clinical parameters associated with prognosis include the site of tumor, tumor size, and extraocular invasion.⁶ Histopathologic prognostic factors include cell type (epithelioid or spindle), mitotic/Ki-67 index, extravascular matrix patterns, and vascular invasion.^{6–8}

Advances in genetics have led to a better understanding of the events involved in the development of these tumors. An important finding was the

Correspondence: Dr KG Griewank, MD, Department of Dermatology, University Hospital Essen, University of Duisburg-Essen, Hufelandstrasse 55, Essen 45122, Germany.

E-mail: klaus.griewank@uk-essen.de or Dr R Murali, MBBS, MD, FRCPA, Department of Pathology and Human Oncology and Pathogenesis Program, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10065, USA.

E-mail: MuraliR@mskcc.org

This study was presented in part at the 102nd Annual Meeting of the United States and Canadian Academy of Pathology, held between 2 and 8 March in Baltimore, MD, USA.

Received 11 March 2013; revised 13 June 2013; accepted 13 June 2013; published online 26 July 2013

identification of chromosome 3 loss correlating with metastasis and poor prognosis.^{9,10} Recently, *BAP1*, a gene located in the area frequently lost on chromosome 3, was found to carry inactivating mutations in a large proportion (84%) of metastasizing uveal melanomas.¹¹ *BAP1* is an ubiquitin hydroxyl carboxy-terminal hydrolase. Mutations either affect the active domain of the enzyme or lead to a frameshift or nonsense mutation, resulting in translation of a non-functional protein. Although the functional role of *BAP1* is still not fully understood, studies have implicated a role in chromatin remodeling¹² as well as cell cycle control.¹³

Uveal melanomas lack activating mutations in *BRAF* or *NRAS*,¹⁴ which commonly occur in cutaneous melanoma (approximately 50% and 20%, respectively).¹⁵ Eighty to 90% of uveal melanomas harbor activating mutations in *GNAQ* or *GNA11*^{9,16} in a mutually exclusive pattern. The original report of *GNA11* mutations showed a substantial difference in the distribution of mutations in primary and metastatic tumors. The ratio of *GNA11* to *GNAQ* mutations was 0.7 (52:72) in primary tumors, and 2.6 (13:5) in metastases.¹⁶

Recently, mutations in codon 625 of *SF3B1* on chromosome 2 were found in uveal melanomas. *SF3B1* mutations were almost always mutually exclusive of *BAP1* loss and were associated with a good prognosis. They were not detected in five metastases analyzed.¹⁷ Described as a splice factor, *SF3B1* was assumed to have a role in excising introns from pre-messenger RNA. Harbour *et al*¹⁷ also proposed that the effect of *SF3B1* mutation on tumorigenesis might be linked to an effect on chromatin remodeling. Future studies will be required to delineate the functional role of *SF3B1* mutations.

In this study, we evaluated clinical, pathologic, and genetic features of a cohort of uveal melanoma metastases and analyzed their associations with survival.

Materials and methods

Sample Selection

The databases of Melanoma Institute Australia and the Department of Dermatology, Essen were searched for metastatic ocular melanoma of posterior uveal origin (arising either from the choroid or ciliary body). The study was carried out in accordance with Ethics Committee guidelines at each of the participating institutions.

Histopathology and Immunohistochemistry

Tissue blocks containing tumor were sectioned at thicknesses of 5 μ m (for hematoxylin–eosin staining and immunohistochemistry, IHC) and 10 μ m (for microdissection and DNA isolation). *BAP1* IHC was

performed according to methods that have been described previously.¹⁸ All histologic and IHC sections were reviewed by at least two histopathologists (KGG, JvdN and RM).

DNA Isolation

Ten 10- μ m-thick sections of paraffin-embedded tissue were deparaffinized according to the following protocol: two steps of 10 min xylene, 5 min 100% ethanol, 5 min 95% ethanol, and 5 min 70% ethanol, and then rinsing in water. After drying, tumor tissue was manually macrodissected from the sections. Genomic DNA was isolated using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. DNA from frozen tissue was directly applied to the Qiagen kit for purification.

Sanger Sequencing

The following primers were used: for *GNAQ*, exon 5—5'-TGATCATCGTCATTCAAGAGAA-3' (F), 5'-AGAAACATGATAGAGGTGACATTTT-3' (R), exon 4—5'-TGGTGTGATGGTGTCACTGACATTCTCAT-3' (F), 5'-AGCTGGGAAATAGGTTTCATGGACTCAGT-3' (R); and for *GNA11*, exon 5—5'-CGCTGTGTCC TTTCAGGATG-3' (F), 5'-CCACCTCGTTGTCCGACT-3' (R), exon 4—5'-GTGCTGTGTCCCTGTCTCTG-3' (F), 5'-GGCAAATGAGCCTCTCAGTG-3' (R). Primers for the first 120 bp of exon 14 of *SF3B1* were as follows: 5'-TGTTTACATTTTAGGCTGCTGGT-5' (F), 5'-GCCA GGACTTCTTGCTTTTG-3' (R). The PCR reaction conditions were 10 mM dNTPmix, 1 U Thermoprime Plus DNA Polymerase (Abgene), 1 x Ready Mix PCR Buffer IV with 15 mM MgCl₂ (Thermo Scientific), and 10 pM of each primer. PCR consisted of 35 cycles of 95 °C (45 s), 57 °C (45 s), and 72 °C (45 s) after initial denaturation at 95 °C for 5 min. PCR reaction products were purified with the QIAquick PCR Purification kit (Qiagen) and then used as templates for sequencing in both directions.

Associations of *GNAQ*, *GNA11*, and *BAP1* Status with Clinical and Pathologic Parameters

We investigated associations of mutation status with available clinical and pathologic parameters using χ^2 tests and Fisher's exact tests as appropriate. Univariate Cox regression models were used to analyze associations of mutation status with overall survival from (a) the time of diagnosis of metastasis of uveal melanoma, and (b) the time of diagnosis of primary uveal melanoma to the time of death or last follow-up. Cases in which the specified end points were missing were censored. All statistical analyses were performed using IBM SPSS Statistics software (version 20.0; International Business Machines, Armonk, NY, USA). A *P*-value of ≤ 0.05 was considered statistically significant.

Table 1 Summary of tumors included in the study

Case	Age at primary	Age at metastasis	Sex	Metastasis site	Cell type	GNA11/GNAQ mutation	BAP1 IHC	Follow-up duration (months)	Status at last follow-up
1	61	72	F	NK	NK	WT	NP	149.5	Alive
2	42	47	M	Liver	Epithelioid	<i>GNAQ</i> ex5 c.626A>C	NP	74.4	Alive
3	60	64	F	Liver	NK	<i>GNAQ</i> ex5 c.626A>T	NP	37.3	Alive
4	72	74	M	Skin and soft tissue	Epithelioid	<i>GNA11</i> ex5 c.626A>T	NP	19.3	Dead from UM
5	68	70	M	NK	Epithelioid	<i>GNA11</i> ex5 c.626A>T	NP	27.2	Dead, not UM
6	71	72	M	Skin and soft tissue	Spindle	WT	NP	12.9	Alive
7	70	79	F	Skin and soft tissue	NK	<i>GNA11</i> ex5 c.626A>T	NP	109.1	Alive
8	48	56	M	Skin and soft tissue	Epithelioid	<i>GNAQ</i> ex5 c.626A>C	Negative	107.3	Dead from UM
9	73	73	F	Skin and soft tissue	Mixed	<i>GNA11</i> ex5 c.626A>T	Negative	LTF	NK
10	62	72	M	Liver	Epithelioid	<i>GNAQ</i> ex5 c.626A>C	NP	119.4	Alive
11	61	66	M	Lymph node	Epithelioid	<i>GNAQ</i> ex5 c.626A>C	NP	60.5	Alive
12	46	53	M	NK	Mixed	<i>GNA11</i> ex5 c.626A>T	Positive	95.9	Dead from UM
13	73	75	F	Skin and soft tissue	Epithelioid	<i>GNA11</i> ex5 c.626A>T	Negative	30	Dead, not UM
14	14	22	F	Skin and soft tissue	Spindle	WT	Negative	131.5	Dead, not UM
15	33	34	M	NK	Mixed	<i>GNA11</i> ex5 c.626A>T	Positive	9.8	Dead from UM
16	65	NK	F	NK	NK	<i>GNAQ</i> ex5 c.626A>T	NP	LTF	NK
17	49	53	F	Liver	Mixed	<i>GNA11</i> ex5 c.626A>T	Positive	88.9	Alive
18	52	58	M	Liver	Epithelioid	WT	Negative	65	Dead from UM
19	59	NK	M	Liver	Mixed	WT	NP	76.8	Dead from UM
20	47	51	M	Liver	Epithelioid	<i>GNA11</i> ex5 c.626A>T	Negative	60	Dead from UM
21	57	59	M	Liver	Epithelioid	<i>GNA11</i> ex5 c.626A>T	NP	37.8	Dead from UM
22	56	NK	M	Lung	Epithelioid	<i>GNA11</i> ex5 c.626A>T	NP	83.3	Dead from UM
23	75	75	M	Liver	NK	<i>GNA11</i> ex5 c.626A>T	NP	27.2	Dead from UM
24	54	57	F	Spleen	Epithelioid	<i>GNA11</i> ex4 c.547C>T	Negative	50.6	Dead from UM
25	42	67	M	Mediastinum	Epithelioid	<i>GNA11</i> ex4 c.547C>T	Negative	346.6	Dead from UM
26	51	53	F	Skin and soft tissue	Epithelioid	<i>GNA11</i> ex5 c.626A>T	Negative	38.2	Dead from UM
27	67	77	M	Skin and soft tissue	Mixed	WT	Negative	121.4	Dead from UM
28	58	61	F	Skin and soft tissue	Epithelioid	<i>GNA11</i> ex5 c.626A>T	Negative	43.7	Dead from UM
29	65	71	M	Brain	Epithelioid	<i>GNA11</i> ex5 c.626A>T	Negative	75.1	Dead from UM
30	55	61	F	Skin and soft tissue	Epithelioid	<i>GNA11</i> ex4 c.547C>T	Negative	71.4	Dead from UM

Abbreviations: F, female; LTF, lost to follow-up; M, male; NK, not known; NP, not performed; UM, uveal melanoma.

Results

Thirty-seven metastases from primary uveal melanoma were identified from the institutional databases. Tumor DNA of sufficient quality for genetic analysis was isolated from 30 tumors, which were included in the study (Table 1). The metastases were located in the skin (10, 33%), liver (9, 30%), lung (1, 3%), brain (1, 3%), spleen (1, 3%), lymph node (1, 3%), mediastinum (1, 3%), and unknown sites (6, 20%). Most tumor samples ($n=21$) were paraffin-embedded, and a few ($n=9$) were frozen tissues. In cases with minimal biopsy material ($n=4$), no sectioning for H&E slides or IHC was possible, and only DNA analysis was performed.

Histologic Analysis

Histologic evaluation was performed in 25 tumors for which slides were available or could be prepared. Twenty-three (92%) tumors were composed of cells with an epithelioid or mixed (<100% spindle cells) morphology. Tumor samples composed exclusively of spindle cells were rare ($n=2$, 8%). Most tumors showed a moderate to marked degree of nuclear pleomorphism ($n=24$, 96%), and contained hyperchromatic, vesicular nuclei with variably conspicuous nucleoli. The cytoplasm in most tumors was moderate in amount and amphi-

philic. The following parameters varied widely: degree of pigmentation, number of admixed macrophages, extent of tumor necrosis (Table 1), and tumor mitotic rate (from 0 to 20 per mm²). Lymphovascular and perineural invasion (LVI and PNI, respectively) were rare (Table 2).

GNAQ/GNA11 Mutation Status

Sequencing of *GNAQ* and *GNA11* was successful in all 30 tumors (Tables 1 and 2). Exon 5 of both genes, which contains the hotspot mutation leading to the most frequent alteration of the Q209 amino acid, was amplified and sequenced in all cases (Figure 1). In tumors lacking mutations in exon 5 of both *GNAQ* and *GNA11*, exon 4 of both genes, which harbors the less frequent R183 mutation, was sequenced (Figure 1).

Mutations in *GNA11* were identified in 18 (60%) tumors (1 brain, 7 skin, 1 lung, 5 liver, 1 mediastinum, 2 unknown site, and 1 sequenced from the primary). There were mutations in exon 5 in 15 tumors, all of which were c.626A>T leading to a Q209L amino-acid change. In addition, three c.547C>T mutations in exon 4, resulting in R183C substitutions, were detected. Mutations in *GNAQ* were identified in six (20%) tumors (3 liver, 1 lymph node, 1 skin metastasis, and 1 sequenced from the primary). All mutations, four c.626A>C and two

Table 2 Summary of clinical and pathologic factors

Parameter	N	Level	
Age (years)	30	Diagnosis of primary	Median 58.3 (range 14.4–74.7)
	27	Diagnosis of metastasis	Median 63.5 (range 21.7–78.8)
			N (%)
Sex	30	Female	12 (40%)
		Male	18 (60%)
Status at last follow-up	30	Alive	8 (29%)
		Dead, from UM	17 (61%)
		Dead, not from UM	3 (11%)
		Lost to follow-up	2 (7%)
Mutation status	30	<i>GNAQ</i>	
		Total	6 (20%)
		Exon 5—Q209	6 (20%)
		Exon 4—R183	0
		<i>GNA11</i>	
		Total	18 (60%)
		Exon 5—Q209	15 (50%)
		Exon 4—R183	3 (10%)
Cell type	25	Epitheloid or mixed	23 (92%)
		Spindle	2 (8%)
Pleomorphism	25	Uniform	15 (60%)
		Pleomorphic	10 (40%)
Pigment	25	Absent	6 (24%)
		Focal	9 (36%)
		Moderate	7 (28%)
		Diffuse	3 (12%)
Melanophages	25	Absent	13 (52%)
		Rare	6 (24%)
		Frequent	6 (24%)
TILs ^a	25	Absent	15 (60%)
		Mild	7 (28%)
		Moderate	3 (12%)
LVI	25	Absent	21 (84%)
		Present	4 (16%)
PNI	25	Absent	23 (92%)
		Present	2 (8%)
Loss of BAP1 expression	16		13 (81%)

Abbreviations: LVI, lymphovascular invasion; PNI, perineural invasion; TILs, tumor-infiltrating lymphocytes.

^aTILs grading was based on assessment of distribution and intensity of the infiltrates: mild = mild or moderate and focal TIL infiltrate, or a mild and multifocal TIL infiltrate; moderate = marked and focal TIL infiltrate, moderate or marked and multifocal TIL infiltrate, or mild and diffuse TIL infiltrate.

c.626A>T resulting in Q209P and Q209L amino-acid substitutions, respectively, were found in exon 5.

SF3B1 Mutation Status

In 29 tumors, we sequenced the first 120 base pairs of exon 14 of *SF3B1*, which contains the published hotspot in codon 625. High-quality results allowing confident mutation detection were available in 26

cases. Only one tumor (1/26, 4%) harbored a c.1873C>T mutation leading to an R625C amino-acid change.

BAP1 Expression

BAP1 status was analyzed by immunohistochemistry. This allows the detection of loss of protein expression due to either inactivating mutations or epigenetic silencing mechanisms. BAP1 loss was found in 13/16 (81%) tumors analyzed (Figure 2).

Associations of Mutation Status with Clinical and Pathologic Parameters

There were no statistically significant associations of *GNAQ* or *GNA11* mutation status with: age at either primary diagnosis or metastasis, sex, status (alive or deceased), site of metastasis, cell pleomorphism, pigmentation, melanophages, LVI, PNI, or loss of BAP1 by IHC (Table 3). Cell type showed a significant association ($P = 0.01$), with the only two tumors composed exclusively of spindle cells having neither a *GNAQ* nor *GNA11* mutation. Tumor-infiltrating lymphocytes (TILs) were present in 7/16 (44%) *GNA11*-mutant tumors and 3/4 (75%) *GNAQ*-mutant tumors, but were absent in all 5 tumors wild type for *GNAQ* and *GNA11* ($P = 0.08$).

Median follow-up durations were 68.2 (range 9.8–346.6) months from the diagnosis of primary tumor, and 8.6 (range 0.0–42.3) months from the diagnosis of first distant metastasis. The interval between primary tumor and first distant metastasis ranged between 5.1 and 307.2 months, with a median of 50.8 months.

No statistically significant associations were identified between various clinical, pathologic, or genetic factors and BAP1 expression status (Table 4) or survival following the diagnosis of metastasis (Table 5). However, disease-specific survival and overall survival (measured from the date of diagnosis of the primary tumor) were significantly poorer in (a) patients with *GNA11*-mutant tumors than in those with tumors lacking *GNA11* mutations; and (b) in patients with metastases exhibiting evidence of LVI (Table 5 and Figure 3). Multivariable survival analyses were not performed because of the small number of tumors in subgroups within the study cohort.

Discussion

Traditionally, prediction of prognosis in uveal melanoma patients has relied on evaluation of clinical and pathologic criteria such as tumor size and cell type. More recently, several genetic tests have been shown to aid prognostic prediction. Chromosome 3 loss,¹⁹ BAP1 loss,¹¹ and RNA expression profiling^{20,21} have high predictive values in terms of forecasting the course of disease and the risk of metastasis. *SF3B1* codon 625 mutations also appear to be a genetic marker of good prognosis.¹⁷

Figure 1 Sequencing electropherograms of *GNAQ* and *GNA11*. Wild-type traces are shown in the upper panels, and examples of mutations in metastatic uveal melanoma are illustrated in the lower panels.

Figure 2 Morphology and BAP1 immunohistochemistry in metastatic uveal melanoma. (a and b) Mitotically active tumor composed of epithelioid cells, showing loss of expression of BAP1 ((a) hematoxylin-eosin, $\times 400$; (b) BAP1 IHC, $\times 400$). (c and d) Tumor composed of nests of epithelioid cells, with expression of BAP1 ((c) hematoxylin-eosin, $\times 200$; (d) BAP1 IHC, $\times 200$). (e and f) Tumor composed of a predominance of spindle-shaped cells, showing loss of expression of BAP1 ((e) hematoxylin-eosin, $\times 200$; (f) BAP1 IHC, $\times 200$). Note: the presence of BAP1 expression is indicated by nuclear expression of red chromogen.

Our finding that the majority of uveal melanoma metastases showed loss of BAP1 expression (13/16, 81%) is consistent with prior findings,¹¹ and confirming that BAP1 loss is a marker of bad prognosis. Only one *SF3B1* codon 625 mutation was identified in 26 tumors tested (3.8%), which is much lower than the percentage detected in primary tumors (18.6%) and consistent with the report that found *SF3B1* mutations to be a good prognostic marker.¹⁷ The *SF3B1*-mutant tumor was one of the three tumors that retained expression of BAP1

protein. This suggests that metastasis may still occur in patients whose tumors harbor *SF3B1* codon 625 mutations and retain BAP1 expression. Identifying additional markers that detect those *SF3B1*-mutant tumors that are prone to metastasize would be of great clinical relevance. The presence of *SF3B1* mutation in only one tumor precluded meaningful analyses of clinicopathologic features and survival associated with *SF3B1* mutation.

The majority of metastases were composed of an epithelioid or mixed-cell population, which is in

Table 3 Associations of clinical and pathologic factors with oncogene status

<i>Factor</i>	<i>Level</i>	<i>Total N</i>	<i>WT N (%)</i>	<i>GNA11^{mut}, N (%)</i>	<i>GNAQ^{mut}, N (%)</i>	<i>P-value</i>
Age at primary (years)	<60	14	2 (7)	10 (33)	2 (7)	0.40
	>60	16	4 (13)	8 (27)	4 (13)	
Age at metastasis (years)	<60	11	2 (7)	7 (26)	2 (7)	0.98
	>60 years	16	3 (11)	10 (37)	3 (11)	
	Missing data	3				
Sex	Female	12	2 (7)	8 (27)	2 (7)	0.76
	Male	18	4 (13)	10 (33)	4 (13)	
Status at last follow-up	Alive	8	2 (7)	2 (7)	4 (14)	0.07
	Dead, of UM	17	3 (11)	13 (46)	1 (4)	
	Dead, not of UM	3	1 (4)	2 (7)	0	
	Missing data	2				
Metastasis site	Lymph nodes	1	0	0	1 (4)	0.56
	Skin/soft tissue	11	3 (12)	7 (28)	1 (4)	
	Viscera	13	2 (8)	8 (32)	3 (12)	
	Missing data	5				
Cell type	Spindle	2	2 (8)	0	0	0.01
	Epithelioid/mixed	23	3 (12)	16 (64)	4 (16)	
	Missing data	5				
Pleomorphism	Uniform	15	4 (16)	9 (36)	2 (8)	0.58
	Pleomorphic	10	1 (4)	7 (28)	2 (8)	
	Missing data	5				
Pigment	Absent	6	0	5 (20)	1 (4)	0.52
	Focal	9	1 (4)	6 (24)	2 (8)	
	Moderate	7	3 (12)	3 (12)	1 (4)	
	Diffuse	3	1 (4)	2 (8)	0	
	Missing data	5				
Melanophages	Absent	13	1 (4)	10 (40)	2 (8)	0.25
	Rare	6	2 (8)	4 (16)	0	
	Frequent	6	2 (8)	2 (8)	2 (8)	
	Missing data	5				
TILs	Absent	15	5 (20)	9 (36)	1 (4)	0.08
	Mild	7	0	4 (16)	3 (12)	
	Moderate	3	0	3 (12)	0	
	Missing data	5				
LVI	Absent	21	5 (20)	12 (48)	4 (16)	0.26
	Present	4	0	4 (16)	0	
	Missing data	5				
PNI	Absent	23	5 (20)	14 (56)	4 (16)	0.54
	Present	2	0	2 (8)	0	
	Missing data	5				
BAP1 IHC	Negative	13	3 (19)	9 (56)	1 (6)	0.54
	Positive	3	0	3 (19)	0	
	Missing data	14				

Abbreviations: IHC, immunohistochemistry; LVI, lymphovascular invasion; PNI, perineural invasion; TILs, tumor-infiltrating lymphocytes; UM, uveal melanoma; WT, wild type for *GNAQ* and *GNA11*.

Percentages and *P*-values are based on the total number of cases with non-missing data.

keeping with the known adverse prognostic profile associated with these cell types relative to pure spindle cell tumors.^{22,23} Of potential interest is the finding that metastases composed predominantly of spindle cells and those lacking TILs were wild type for both *GNAQ* and *GNA11*. However, the number of tumors in these groups is very small, and analysis of

larger numbers of tumors will be required to validate these findings.

The presence of LVI was associated with significantly poorer survival (as measured from the date of diagnosis of the primary tumor). This finding may indicate a greater propensity for tumor dissemination, and is consistent with the findings of a recent

Table 4 Associations of clinical and pathologic factors with BAP1 expression by immunohistochemistry

Factor	Level	BAP1-negative, N (%)	BAP1-positive, N (%)	P-value
Age at primary (years)	<57.7	8 (50)	3 (19)	0.51
	>57.7	5 (31)	0	
Age at metastasis (years)	<60	6 (38)	3 (19)	0.21
	>60	7 (44)	0	
Sex	Female	7 (44)	1 (6)	1.00
	Male	6 (38)	2 (13)	
Status at last follow-up	Alive	0	1 (6)	0.10
	Dead of UM	10 (63)	2 (13)	
	Dead not of UM	2 (13)	0	
Mutation	Wild type	3 (19)	0	0.54
	<i>GNA11</i> ^{mut}	9 (56)	3 (19)	
	<i>GNAQ</i> ^{mut}	1 (6)	0	
Metastasis site	Skin/soft tissue	8 (50)	0	0.43
	Viscera	5 (31)	1 (6)	
Cell type	Spindle	1 (6)	0	1.00
	Epithelioid/ mixed	12 (75)	3 (19)	
Pleomorphism	Uniform	9 (56)	2 (13)	1.00
	Pleomorphic	4 (25)	1 (6)	
Pigment	Absent	3 (19)	1 (6)	0.36
	Focal	5 (31)	0	
	Moderate	3 (19)	2 (13)	
	Diffuse	2 (13)	0	
Melanophages	Absent	8 (50)	1 (6)	0.65
	Rare	3 (19)	1 (6)	
	Frequent	2 (13)	1 (6)	
TILs	Absent	6 (38)	3 (19)	0.24
	Mild	4 (25)	0	
	Moderate	3 (19)	0	
LVI	Absent	10 (63)	3 (19)	1.00
	Present	3 (19)	0	
PNI	Absent	13 (81)	2 (13)	0.19
	Present	0	1 (6)	

Abbreviations: IHC, immunohistochemistry; LVI, lymphovascular invasion; PNI, perineural invasion; TILs, tumor-infiltrating lymphocytes; UM, uveal melanoma.

study in which the presence of intravascular tumor was associated with poorer prognosis.²⁴

Although currently not assumed to be of prognostic relevance, the type of driving oncogene involved may have a significant supplemental effect on long-term survival and outcome. Mutations in *GNAQ* and *GNA11* have been shown to be mutually exclusive,¹⁶ with tumors harboring only a single mutation in either gene. We found a strong bias toward *GNA11* mutations in our cohort. Eighteen tumors harbored *GNA11* mutations compared to six with *GNAQ* mutations and six tumors lacking either mutation. The ratio of *GNA11* to *GNAQ* mutations was 3.0. Our finding of the predominance of *GNA11* mutations in

uveal melanoma metastases fits well with the original report of *GNA11* mutations in uveal melanoma.¹⁶ Of the 163 primary tumors analyzed, 52 (32%) had activating mutations in *GNA11* and 72 (45%) had the corresponding mutation in *GNAQ*. On the other hand, in 23 metastases, 13 (57%) had *GNA11* and only 5 (22%) harbored *GNAQ* mutations. Our data confirm the over-representation of *GNA11* mutations in metastases, and raises the possibility of prognostic significance associated with the type of oncogenic mutation. Validation and further exploration of these findings in additional, larger cohorts is warranted.

The oncogenic mutation data suggest that *GNA11*-mutant tumors may have a higher tendency to metastasize than *GNAQ*-mutant tumors. If this is the case, one might expect that the survival of patients with *GNA11*-mutant tumors should be significantly lower than that for patients with *GNAQ*-mutant tumors. Indeed, we found that survival from the diagnosis of the primary tumor was significantly poorer in patients with *GNA11*-mutant tumors compared with patients lacking *GNA11* mutations in their tumors (Table 5 and Figure 3). Since *GNA11* and *GNAQ* mutations are early events in the evolution of uveal melanoma,¹⁶ it is reasonable to presume that there is fidelity between the presence of these mutations in metastases and the primary tumors from which they arose. However, the survival data in a previous study¹⁶ showed that *GNA11*-mutant tumors had a better prognosis than *GNAQ*-mutant tumors. One of the major limitations of the survival analysis in that study is the duration of available follow-up. Initially, the *GNA11*- and *GNAQ*-mutant tumor groups contained 26 and 43 patients, respectively, but the majority of patients were censored early in the follow-up period; there were only 5 and 3 tumors, respectively, at 5 years after diagnosis, and only 2 patients per group remained at 10 years. Given that well-documented long-term follow-up studies have shown that uveal melanoma patients can die of disease 10 or more years after diagnosis,^{3,4,25,26} it is clear that more complete, longer follow-up will be required to establish whether *GNA11*-mutant tumors do indeed have a significantly worse overall survival than *GNAQ*-mutant tumors.

Our results should be interpreted with some caution. The study cohort is small, and is highly selective, namely uveal melanoma metastases, and therefore reflects patients with high-risk disease. There is also some sample bias; the percentage of cutaneous metastases in the cohort is higher than that expected in the general uveal melanoma population, in which hepatic metastases would predominate. This probable selection bias likely reflects the ease of resection and low morbidity associated with biopsying metastases involving superficial cutaneous sites compared with hepatic or other visceral lesions. Nevertheless, the results generate an interesting hypothesis that is worthy of further study: that *GNA11* mutations are associated

Table 5 Associations of clinical and pathologic factors and oncogene mutations with survival

Factor	Level	Survival from date of metastasis				Survival from date of primary tumor			
		Median OS (months)	P-value	Median DSS (months)	P-value	Median OS (months)	P-value	Median DSS (months)	P-value
Age at metastasis (years)	< 60	14.0	0.44	14.0	0.78	71.4	0.83	71.4	0.53
	> 60	6.9		9.8		76.8		76.8	
Sex	Female	18.4	0.11	18.4	0.07	71.4	0.47	71.4	0.30
	Male	7.8		9.4		76.8		76.8	
Metastasis site	Skin/soft tissue	9.8	0.49	9.8	0.67	71.4	0.62	107.3	0.94
	Viscera	15.0		15.0		75.1		75.1	
<i>GNAQ</i> or <i>GNA11</i>	Both WT	2.0	0.34	2.0	0.40	121.4	0.26	121.4	0.24
	Either mutated	9.8		11.8		71.4		75.1	
<i>GNA11</i> mutation	<i>GNA11</i> ^{WT}	14.0	0.16	14.0	0.24	121.4	0.03	121.4	0.03
	<i>GNA11</i> ^{mut}	9.4		9.8		50.6		60.0	
<i>GNAQ</i> mutation	<i>GNAQ</i> ^{WT}	9.4	0.39	9.8	0.49	71.4	0.13	75.1	0.18
	<i>GNAQ</i> ^{mut}	14.0		14.0		107.3		107.3	
Cell type	Spindle	42.3	0.11	NA	0.11	131.5	0.35	NA	0.17
	Epithelioid/mixed	9.4		9.8		71.4		75.1	
Nuclear pleomorphism	Uniform	9.8	0.11	14.0	0.12	76.8	0.52	76.8	0.55
	Pleomorphic	4.8		6.9		71.4		75.1	
TILs	Absent	7.8	0.81	9.8	0.56	64.5	0.63	76.8	0.94
	Mild	9.4		9.4		75.1		75.1	
	Moderate	11.8		11.8		71.4		71.4	
LVI	Absent	7.8	0.98	11.8	0.75	83.3	0.04	95.9	0.007
	Present	9.8		9.8		38.2		38.2	
PNI	Absent	9.8	0.10	11.8	0.48	75.1	0.50	75.1	0.90
	Present	1.3		7.8		27.2		95.9	
BAP1 IHC	Negative	9.4	0.73	9.8	0.85	65.0	1.00	71.4	0.91
	Positive	7.8		7.8		95.9		95.9	

Abbreviations: DSS, disease-specific survival; IHC, immunohistochemistry; LVI, lymphovascular invasion; OS, overall survival; PNI, perineural invasion; TILs, tumor-infiltrating lymphocytes; WT, wild type for *GNAQ* and *GNA11*.

Other parameters including pigmentation, necrosis, and tumor mitotic rate did not show statistically significant associations with survival.

Figure 3 Kaplan–Meier curves showing associations of the presence or absence of *GNA11* mutations with overall survival (left panel) and disease-specific survival (right panel). Significance testing (from which *P*-values were derived) was performed using log-rank tests.

with aggressive disease and poor prognosis in uveal melanoma. It is conceivable that the poor prognostic significance of *GNA11* mutations applies particularly to patients with high-risk disease.

In summary, we found that in metastatic uveal melanoma, *GNA11* mutations were more frequent, and were associated with poorer survival. In addition, loss of BAP1 expression was frequent, and

mutation of *SF3B1* at codon 625 was rare. Larger cohorts, optimally with comparable sets of primary tumors and long-term follow-up, will be needed to validate our findings, which imply more aggressive behavior of tumors harboring *GNA11* mutations. If a prognostic significance of *GNA11* is validated, it may influence management of affected patients. For example, the intensity of follow-up could be stratified according to the oncogene mutation harbored, and potentially treatment strategies should also be adjusted based on the oncogene mutation present.

Acknowledgements

We thank Sabine Prass, Marion Schwamborn, and Nicola Bielefeld for their excellent technical support. Assistance from staff of Melanoma Institute Australia and Royal Prince Alfred Hospital and funding support from the National Health and Medical Research Council (of the Commonwealth Government of Australia) and the Cancer Institute New South Wales is also gratefully acknowledged.

Disclosure/conflict of interest

Dirk Schadendorf is on the advisory board or has received honoraria from Roche, Genetech, Novartis, Amgen, GSK, BMS, Boehringer Ingelheim, and Merck. Lisa Zimmer has honoraria from Roche, Bristol-Meyers Squibb, and Amgen, and travel support from Merck Sharp & Dohme and Bristol-Meyers Squibb. All the other authors declare no conflict of interest.

References

- Shildkrot Y, Wilson MW. Update on posterior uveal melanoma: treatment of the eye and emerging strategies in the prognosis and treatment of metastatic disease. *Curr Opin Ophthalmol* 2009;20:504–510.
- Chang AE, Karnell LH, Menck HR. The National Cancer Data Base report on cutaneous and noncutaneous melanoma: a summary of 84,836 cases from the past decade. The American College of Surgeons Commission on Cancer and the American Cancer Society. *Cancer* 1998;83:1664–1678.
- Kujala E, Tuomaala S, Eskelin S, *et al*. Mortality after uveal and conjunctival melanoma: which tumour is more deadly? *Acta Ophthalmol* 2009;87:149–153.
- Kujala E, Makitie T, Kivela T. Very long-term prognosis of patients with malignant uveal melanoma. *Invest Ophthalmol Vis Sci* 2003;44:4651–4659.
- Singh AD, Borden EC. Metastatic uveal melanoma. *Ophthalmol Clin N Am* 2005;18:143–150; ix.
- Griewank KG, Murali R. Pathology and genetics of uveal melanoma. *Pathology* 2013;45:18–27.
- McLean IW, Foster WD, Zimmerman LE. Uveal melanoma: location, size, cell type, and enucleation as risk factors in metastasis. *Hum Pathol* 1982;13:123–132.
- Mudhar HS, Parsons MA, Sisley K, *et al*. A critical appraisal of the prognostic and predictive factors for uveal malignant melanoma. *Histopathology* 2004;45:1–12.
- Van Raamsdonk CD, Bezrookove V, Green G, *et al*. Frequent somatic mutations of *GNAQ* in uveal melanoma and blue naevi. *Nature* 2009;457:599–602.
- Abdel-Rahman MH, Cebulla CM, Verma V, *et al*. Monosomy 3 status of uveal melanoma metastases is associated with rapidly progressive tumors and short survival. *Exp Eye Res* 2012;100:26–31.
- Harbour JW, Onken MD, Roberson ED, *et al*. Frequent mutation of *BAP1* in metastasizing uveal melanomas. *Science* 2010;330:1410–1413.
- Scheuermann JC, de Ayala Alonso AG, Oktaba K, *et al*. Histone H2A deubiquitinase activity of the Polycomb repressive complex PR-DUB. *Nature* 2010;465:243–247.
- Eletr ZM, Wilkinson KD. An emerging model for *BAP1*'s role in regulating cell cycle progression. *Cell Biochem Biophys* 2011;60:3–11.
- Spendlove HE, Damato BE, Humphreys J, *et al*. *BRAF* mutations are detectable in conjunctival but not uveal melanomas. *Melanoma Res* 2004;14:449–452.
- Sekulic A, Haluska P Jr., Miller AJ, *et al*. Malignant melanoma in the 21st century: the emerging molecular landscape. *Mayo Clin Proc* 2008;83:825–846.
- Van Raamsdonk CD, Griewank KG, Crosby MB, *et al*. Mutations in *GNA11* in uveal melanoma. *N Engl J Med* 2010;363:2191–2199.
- Harbour JW, Roberson ED, Anbunathan H, *et al*. Recurrent mutations at codon 625 of the splicing factor *SF3B1* in uveal melanoma. *Nat Genet* 2013;45:133–135.
- Wiesner T, Murali R, Fried I, *et al*. A distinct subset of atypical Spitz tumors is characterized by *BRAF* mutation and loss of *BAP1* expression. *Am J Surg Pathol* 2012;36:818–830.
- Prescher G, Bornfeld N, Hircche H, *et al*. Prognostic implications of monosomy 3 in uveal melanoma. *Lancet* 1996;347:1222–1225.
- Tschentscher F, Husing J, Holter T, *et al*. Tumor classification based on gene expression profiling shows that uveal melanomas with and without monosomy 3 represent two distinct entities. *Cancer Res* 2003;63:2578–2584.
- Onken MD, Worley LA, Ehlers JP, *et al*. Gene expression profiling in uveal melanoma reveals two molecular classes and predicts metastatic death. *Cancer Res* 2004;64:7205–7209.
- McLean IW, Foster WD, Zimmerman LE, *et al*. Modifications of Callender's classification of uveal melanoma at the Armed Forces Institute of Pathology. *Am J Ophthalmol* 1983;96:502–509.
- Gamel JW, McLean IW, Foster WD, *et al*. Uveal melanomas: correlation of cytologic features with prognosis. *Cancer* 1978;41:1897–1901.
- Ly LV, Odish OF, Wolff-Rouendaal D, *et al*. Intravascular presence of tumor cells as prognostic parameter in uveal melanoma: a 35-year survey. *Invest Ophthalmol Vis Sci* 2010;51:658–665.
- Singh AD, Topham A. Survival rates with uveal melanoma in the United States: 1973–1997. *Ophthalmology* 2003;110:962–965.
- Kivela T, Eskelin S, Kujala E. Metastatic uveal melanoma. *Int Ophthalmol Clin* 2006;46:133–149.