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Recurrent mutations at codon 625 of the splicing factor *SF3B1* in uveal melanoma

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Uveal melanoma is the most common primary cancer of the eye and often results in fatal metastasis. Here, we describe mutations occurring exclusively at codon 625 of the *SF3B1* gene, encoding splicing factor 3B subunit 1, in low-grade uveal melanomas with good prognosis. Thus, uveal melanoma is among a small group of cancers associated with *SF3B1* mutations, and these mutations denote a distinct molecular subset of uveal melanomas.

Uveal melanomas can be divided into prognostically distinct subgroups on the basis of their transcriptome signatures. Class 1 tumors rarely metastasize, are less invasive and more differentiated and tend to occur in younger individuals than class 2 tumors[1]. In contrast, class 2 tumors frequently metastasize, and they tend to comprise undifferentiated 'epithelioid' tumor cells lacking a copy of chromosome 3 (ref. 1).

We recently described loss-of-function mutations in *BAP1* (encoding BRCA1-associated protein 1), located on chromosome 3p21.1, in ~40% of uveal melanomas, virtually all of which were aggressive, class 2 tumors[2]. In the present study, we searched for additional mutations in uveal melanoma by exome sequencing of 18 primary tumors, including 7 class 1 and 11 class 2 tumors. Exome data were filtered down to somatic alterations that were predicted to be deleterious (**Supplementary Methods**). Only two genes were found to harbor deleterious somatic variants in at least three tumor samples: *GNAQ* (encoding guanine nucleotide–binding protein Gq subunit α), which is already known to undergo mutation in uveal melanoma[3,4], and *SF3B1* (encoding splicing factor 3B subunit 1). For *SF3B1*, the mutation in all three tumors led to a p.Arg625Cys alteration (hg19 chr. 2: g.198267484G>A), which was confirmed by Sanger sequencing.

We manually examined the entire *SF3B1* coding sequence from the other 15 samples subjected to exome sequencing, but we did not find mutations at any site other than codon 625. *SF3B1* mutations were recently described in myelodysplastic syndrome (MDS) and chronic lymphocytic leukemia (CLL)[5,6], clustering within exons 12 to 15. Thus, we resequenced these exons in a total of 102 primary uveal melanomas and matching blood DNA samples and identified *SF3B1* mutations in 19 tumors (18.6%; **Supplementary Table 1**). This mutation frequency is similar to those found in MDS and CLL[5,6] and is much higher than that recently reported in breast cancer[7]. Notably, all of the *SF3B1* mutations in uveal melanoma occurred at codon 625, comprising 12 p.Arg625His, 5 p.Arg625Cys, 1 p.Arg625Gly and 1 p.Arg625Leu substitutions (**Supplementary Fig. 1**). Codon 625 is one of many sites in *SF3B1* that are mutated in MDS, but it is the only site at which alterations were predicted to be deleterious by the SIFT algorithm[5], which predicts the effect an amino-acid substitution has on protein function. *SF3B1* mutations were not present in matching blood DNA samples, indicating that they were somatic in origin. In each of the tumors with *SF3B1* mutations, wild-type and mutant alleles were present in roughly equal proportions (**Supplementary Fig. 1**). An evaluation of DNA copy number in 30 of the uveal melanomas, including 7 with *SF3B1* mutations, showed no loss of chromosome 2q33.1 where *SF3B1* resides (**Supplementary Fig. 2**), which would have been consistent with a role for *SF3B1* as a classical tumor suppressor. Rather, these findings are more consistent with *SF3B1* mutations acting as dominant-negative, gain-of-function or haploinsufficient alterations.

SF3B1 mutations were associated with favorable prognostic features, such as younger patient age ($P = 0.03$) and fewer undifferentiated epithelioid cells ($P = 0.003$), and they were inversely associated with poor prognostic features, such as the class 2 transcriptome signature ($P = 0.02$), loss of chromosome 3 ($P = 0.001$) and mutation of *BAP1* ($P = 0.002$) (**Table 1**). Individuals with *SF3B1*-mutant tumors trended to have a lower metastasis rate than those with tumors with wild-type *SF3B1* ($P = 0.1$), which was in marked contrast to the high metastasis rate in subjects with *BAP1* mutations ($P < 0.0001$) (**Fig. 1**). Five uveal melanoma samples from distant metastases were available for testing, and none harbored *SF3B1* mutations, further supporting the notion that these mutations might be associated with less aggressive tumors. Taken together, these findings suggest that *SF3B1* mutations are associated with better prognosis in uveal melanoma, which is similar to findings in MDS[5].

SF3B1 is one of the very few genes that are commonly mutated in uveal melanoma, allowing for a more precise molecular taxonomy of this cancer. Activating oncogenic mutations in *GNAQ* or *GNA11* occur in about 85% of primary uveal melanomas and are thought to represent early events because they are found in uveal melanomas of all stages, including premalignant nevi, and they are not associated with prognosis[3,8]. Consistent with this idea, *GNAQ* or *GNA11* mutations were present in most of our *SF3B1*-mutant and *BAP1*-mutant tumors, suggesting that they arise earlier than *SF3B1* and *BAP1* mutations. In contrast, *SF3B1* and *BAP1* mutations were almost mutually exclusive, suggesting that they may represent alternative pathways in tumor progression.

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Table 1 Association of *SF3B1* mutations with clinical, histopathological and genetic features

Variable	*SF3B1* wild type	*SF3B1* mutant	*P*
Subject age			
Mean	63.0	55.3	0.03
Median	65.0	60.0	
Minimum–maximum	24–87	16–76	
Patient sex			
Female	34 (41%)	12 (63%)	0.1
Male	49 (59%)	7 (37%)	
Tumor diameter (mm)			
Mean	16.0	17.1	0.3
Median	16.0	17.2	
Minimum–maximum	3–24	9–24	
Tumor thickness (mm)			
Mean	8.8	8.6	0.8
Median	9.0	8.1	
Minimum–maximum	1–16	2–15	
Ciliary body involvement			
Yes	44 (60%)	9 (47%)	0.4
No	29 (40%)	10 (53%)	
Not available	10	0	
Epithelioid cell type			
Yes	33 (40%)	1 (5%)	0.003
No	49 (60%)	18 (95%)	
Not available	1	0	
Extraocular tumor invasion			
Yes	17 (21%)	5 (26%)	0.8
No	64 (79%)	14 (74%)	
Not available	2	0	
BAP1 status			
Wild type	37 (54%)	16 (94%)	0.002
Mutant	31 (46%)	1 (6%)	
Not available	15	2	
GNAQ status			
Wild type	40 (60%)	10 (53%)	0.6
Mutant	27 (40%)	9 (47%)	
Not available	16	0	
GNA11 status			
Wild type	28 (43%)	12 (67%)	0.1
Mutant	37 (57%)	6 (33%)	
Not available	18	1	
Gene expression class			
Class 1	37 (49%)	14 (82%)	0.02
Class 2	38 (51%)	3 (18%)	
Not available	8	2	
Chromosome 3 status			
Retention of heterozygosity	34 (49%)	14 (93%)	0.001
Loss of heterozygosity	36 (51%)	1 (7%)	
Not available	13	4	

SF3B1 encodes subunit 1 of the splicing factor 3b protein complex, which is a component of the U2 small nuclear ribonucleoprotein complex (snRNP) that participates in the splicing of pre-mRNAs[9]. Splicing factor 3b is also a component of the minor U12-type spliceosome[9]. To explore the effects of mutant *SF3B1* on global RNA expression, we analyzed class 1 tumors, including five with mutant *SF3B1* and six with wild-type *SF3B1*, for differentially expressed transcripts using the Illumina BeadArray platform. This analysis was limited to class 1 tumors because most *SF3B1* mutations occurred in this subtype. Unexpectedly, there were only ten differentially expressed genes between the two groups, and they did not provide insights into the functional consequences of the *SF3B1* mutations (**Supplementary Fig. 3**

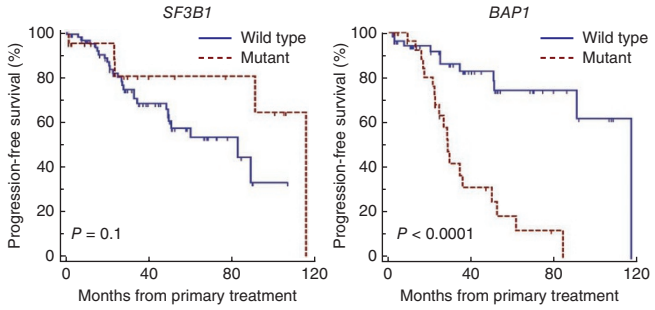

Figure 1 Association of mutation status with clinical outcome in uveal melanoma. Kaplan-Meier survival plots of 102 uveal melanoma cases stratified by *SF3B1* mutation status (left) and *BAP1* mutation status (right).

and **Supplementary Table 2**). Moreover, none of the identified genes were the same as those that were differentially expressed in MDS samples with compared to without *SF3B1* mutations[5,10]. We therefore investigated whether the main consequence of *SF3B1* mutations was intron retention rather than differential expression. We analyzed three class 1 tumors with mutant *SF3B1* and five with wild-type *SF3B1* for alterations in splice donor and splice acceptor retention using RNA sequencing (RNA-seq) (**Supplementary Methods**). However, we found no differences in global splice donor or splice acceptor retention between tumors with mutant and wild-type *SF3B1* (data not shown). Further, we manually analyzed a set of neural crest regulatory transcripts that are aberrantly spliced in *sf3b1*-mutant zebrafish (*SNAI1*, *SOX9*, *TFAP2A*, *SOX10*, *ID2*, *MITF* and *SF3B1*)[11], but we found no splicing abnormalities. Despite the known role of *SF3B1* in RNA splicing, there have not been consistent results linking *SF3B1* mutations to specific splicing errors in MDS or CLL, and the functional consequences of *SF3B1* mutations remain elusive, despite intensive investigation[12]. Recent links between *SF3B1* and chromatin-remodeling complexes[13] raise the question of whether the primary effect of *SF3B1* mutations on tumor progression involves RNA processing at all. Further investigations are under way to elucidate the consequences of *SF3B1* mutations on uveal melanoma progression in order to therapeutically target these effects.

Accession codes. Gene expression microarray and array–comparative genomic hybridization (aCGH) data have been deposited at the Gene Expression Omnibus (GEO) under accessions GSE39717 and GSE42740, respectively. Exome sequences and RNA-seq data are available at the NCBI Sequence Read Archive (SRA) under accessions SRA062369 and SRA062359, respectively).

Note: Supplementary information is available in the online version of the paper.

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AUTHOR CONTRIBUTIONS

J.W.H. participated in the conception and design of the study, provided the samples used in the study, performed biostatistical analysis and drafted the manuscript.

E.D.O.R. performed the analysis of next-generation sequencing data and bioinformatics analysis. H.A. performed Sanger sequencing. M.D.O. performed bioinformatics analysis. L.A.W. managed the tissue bank and clinical database and prepared DNA and RNA samples. A.M.B. participated in the conception and design of the study and analyzed the data. All authors contributed to the final draft of the manuscript.

COMPETING FINANCIAL INTERESTS

The authors declare competing financial interests: details are available in the online version of the paper.

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