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

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Abstract

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

Uveal melanomas can be divided into prognostically significant subgroups based on their transcriptomic signature. Class 1 tumors rarely metastasize, and they tend be less invasive, more differentiated and 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[1].

We recently described loss of function mutations in *BAP1* (BRCA1-associated protein 1), located at chromosome 3p21.1, in ~40% of uveal melanomas, virtually all of which were the 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 seven class 1 and eleven 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: guanine nucleotide-binding protein G(q) subunit alpha (*GNAQ*), which is already known to undergo mutation in uveal melanoma[3,4], and splicing factor 3B subunit 1 (*SF3B1*). In the case of *SF3B1*, the mutation in all three tumors led to a p.R625C alteration (hg19 chr2:198267484G>A) which was confirmed by Sanger sequencing.

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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 bioinformatic 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.

Supplementary Information: Supplementary File
• Supplementary Methods, Supplementary Tables 1-2, Supplementary Figures 1-3

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We manually examined the entire *SF3B1* coding sequence from the other 15 exome samples, but we did not find mutations at any sites other than R625. *SF3B1* mutations were recently described in myelodysplastic syndrome (MDS) and chronic lymphocytic leukemia (CLL), clustering within exons 12 to 15[5,6]. Thus, we re-sequenced these exons in a total of 102 primary uveal melanomas and matching blood DNA samples and identified *SF3B1* mutations in 19 (18.6%) tumors (Supplementary Table 1). This frequency is similar to that in MDS and CLL[5,6], and much higher than that recently reported in breast cancer[7]. Strikingly, all of the mutations in uveal melanoma occurred atarginine-625, including twelve R625H, five R625C, one R625G and one R625L substitutions (Supplementary Fig. 1). Interestingly, R625 is one of many sites in *SF3B1* that are mutated in MDS, but it is the only site that was identified as 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, wildtype 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 seven with *SF3B1* mutations, revealed no loss of chromosome 2q33.1 where *SF3B1* resides (Supplementary Fig. 2),which would have been consistent with a classical tumor suppressor. Rather, these findings are more consistent with *SF3B1* mutations functioning as dominant-negative, gain-of-function or haploinsufficient alteration.

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 transcriptomic signature (P = 0.02), loss of chromosome 3 (P = 0.001) and mutation of *BAP1* (P=0.002) (Table 1). Patients with *SF3B1*-mutant tumors trended toward a lower metastatic rate than those with *SF3B1*-wildtype tumors (P=0.1), which was in striking contrast to the high metastatic rate in patients 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 indicate that *SF3B1* mutations are associated with better prognosis in uveal melanoma, which is similar to findings in MDS[5].

SF3B1 is one of 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 pre-malignant nevi, and they are not associated with prognosis[3,8]. Consistent with this idea, *GNAQ/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.

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 five *SF3B1*-mutant and six *SF3B1*-wildtype class 1 tumors for differentially expressed transcripts using the Illumina Bead Array platform. This analysis was limited to class 1 tumors because most *SF3B1* mutations occurred in this subset. Surprisingly, there were only 10 differentially expressed genes, and they provided no insights into the functional significance of the *SF3B1* mutations (Supplementary Table 2 and Supplementary Fig. 3). Moreover, none of these genes were the same as those that were differentially expressed in MDS[5,10]. We therefore investigated if the main consequence of *SF3B1* mutations was intron

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retention rather than differential expression. Three *SF3B1*-mutant and five *SF3B1*-wildtype class 1 tumors were analyzed for alterations in splice donor and splice acceptor retention using RNA-Seq (Supplementary Methods). However, no differences in global splice donor or acceptor retention were found between *SF3B1*-mutant and –wildtype tumors (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 no splicing abnormalities were found. 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 remodelling complexes[13] raise the question of whether the primary effect of *SF3B1* mutations on tumor progression involve RNA processing at all. Further investigations are under way to elucidate the effects of *SF3B1* mutations on uveal melanoma progression in order to therapeutically target these effects.

Data access

Gene expression micro array and array CGH data have been deposited at the Gene Expression Omnibus (GEO) (accession numbers GSE39717 and GSE42740, respectively). Exome sequences and RNA-seq data are available at the NCBI Sequence Read Archive (accession numbers SRA062369 and SRA062359, respectively).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Harbour JW. Pigment Cell Melanoma Res. 2012; 25:171–81. [PubMed: 22268848]
2. Harbour JW, et al. Science. 2010; 330:1410–3. [PubMed: 21051595]
3. Onken MD, et al. Invest Ophthalmol Vis Sci. 2008; 49:5230–4. [PubMed: 18719078]
4. Van Raamsdonk CD, et al. Nature. 2009; 457:599–602. [PubMed: 19078957]
5. Papaemmanuil E, et al. N Engl J Med. 2011; 365:1384–95. [PubMed: 21995386]
6. Wang L, et al. N Engl J Med. 2011; 365:2497–506. [PubMed: 22150006]
7. Ellis MJ, et al. Nature. 2012; 486:353–60. [PubMed: 22722193]
8. Van Raamsdonk CD, et al. N Engl J Med. 2010; 363:2191–9. [PubMed: 21083380]
9. Golas MM, Sander B, Will CL, Luhrmann R, Stark H. Science. 2003; 300:980–4. [PubMed: 12738865]
10. Visconte V, et al. Blood. 2012; 120:3173–86. [PubMed: 22826563]
11. An M, Henion PD. Int J Dev Biol. 2012; 56:223–37. [PubMed: 22562198]
12. Visconte V, Makishima H, Maciejewski JP, Tiu RV. Leukemia. 2012
13. Isono K, Mizutani-Koseki Y, Komori T, Schmidt-Zachmann MS, Koseki H. Genes Dev. 2005; 19:536–41. [PubMed: 15741318]

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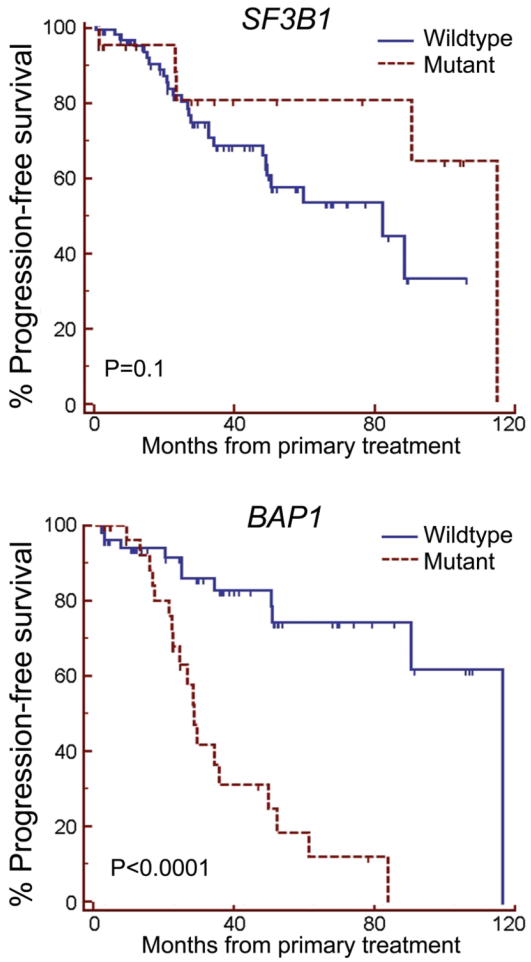

Figure 1.

SF3B1 mutations in uveal melanoma. **(a**) Sanger sequence traces of representative tumor samples harboring wild type or mutant *SF3B1* alleles at codon 625. **(b)** Kaplan-Meier survival plots of 102 uveal melanoma patients stratified by *SF3B1* mutation status (top) and *BAP1* mutation status (bottom).

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Table 1
Associations between *SF3B1* mutation and clinical, histopathologic and genetic features

Variable	*SF3B1* wildtype	*SF3B1* mutant	P-value
Patient 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			
Wildtype	37 (54%)	16 (94%)	0.002
Mutant	31 (46%)	1 (6%)	
Not available	15	2	
GNAQ status			
Wildtype	40 (60%)	10 (53%)	0.6
Mutant	27 (40%)	9 (47%)	
Not available	16	0	

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Variable	*SF3B1* wildtype	*SF3B1* mutant	P-value
GNA11 status			
Wildtype	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	