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. 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. 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 melanoma1,3,4, 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 CLL5,6 and is much higher than that recently reported in breast cancer7. 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 algorithm5, 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 MDS5.

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 prognosis5,6. 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            |        |
| Extracocular 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            |        |
| GNA11 status                      |                |              |        |
| Wild type                        | 40 (60%)       | 10 (53%)     | 0.6    |
| Mutant                           | 27 (40%)       | 9 (47%)      |        |
| Not available                     | 16             | 0            |        |
| 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. Splicing factor 3b is also a component of the minor U12-type spliceosome. 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 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. 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), 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. Recent links between SF3B1 and chromatin-remodeling complexes 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 accession 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.

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).
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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