SF3B1 mutations are associated with alternative splicing in uveal melanoma

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The authors declare no potential conflicts of interest.
ABSTRACT

Uveal melanoma, the most common eye malignancy causes severe visual morbidity and is fatal in about 50% of patients. Primary uveal melanoma can be cured by surgery or radiotherapy, but the metastatic disease is treatment refractory. To understand comprehensively uveal melanoma genetics, we performed SNP arrays and whole genome sequencing on 12 primary uveal melanomas. We observed only ~2000 predicted somatic single nucleotide variants per tumor and low levels of aneuploidy. We did not observe an ultraviolet radiation DNA-damage signature, but identified SF3B1 mutations in three samples and a further 15 mutations in an extension cohort of 105 samples. SF3B1 mutations were associated with good prognosis and were rarely coincident with BAP1 mutations. SF3B1 encodes a component of the spliceosome and RNA sequencing revealed that SF3B1 mutations were associated with differential alternative splicing of protein coding genes including ABCC5 and UQCC, and of the long non-coding RNA (lncRNA) CRNDE.

SIGNIFICANCE

Our data show that despite its dismal prognosis, uveal melanoma is a relatively simple genetic disease characterized by recurrent chromosomal losses and gains and a low mutational burden. We show that SF3B1 is recurrently mutated in uveal melanoma and the mutations are associated with aberrant alternative splicing.
INTRODUCTION

Uveal melanoma arises in the iris, ciliary body and choroid. Light skin complexion, fair hair, blue eyes and the presence of cutaneous nevi are risk factors in uveal melanoma (1), but the UVR-associated increase in cutaneous melanoma that occurred in countries such as Australia over the past 4 decades has not been accompanied by parallel increases in uveal melanoma (2-4). Thus the role of UVR in uveal melanoma etiology is unclear.

Class 1 uveal melanomas present a low risk of metastasis, whereas class 2 tumors are highly metastatic and are characterized by monosomy of chromosome 3 and gain of 8q. Mutually exclusive mutations in GNAQ or GNA11, the principal driver oncogenes in uveal melanoma, occur in ~85% of cases (5, 6), and inactivating mutations in the tumor suppressor BAP1 occur in ~85% of metastatic tumors and are associated with disease dissemination (7). Recently, exome sequencing of uveal melanomas has identified recurrent mutations in EIF1AX and SF3B1 (8, 9), predominantly in low grade tumors. Somatic mutations in SF3B1, which encodes a component of the spliceosome, also occur in hematological, breast and pancreatic cancers (10-13). Mutant SF3B1 is associated with differential gene splicing in chronic lymphocytic leukemia (14), but aberrant splicing in SF3B1 mutant uveal melanoma has not been reported (9).

To gain insight into uveal melanoma genetics, we performed SNP array analysis, whole genome sequencing, and RNA sequencing (RNA-seq) on 12 frozen primary uveal melanoma samples. Despite its dismal prognosis, we find that uveal melanoma has a remarkably low mutation burden, and we did not observe a UVR DNA-damage signature. However, we found recurrent mutations in SF3B1 that were associated with differential alternative splicing of both coding and non-coding genes that may play a role in the etiology of this disease.
RESULTS

Our discovery cohort comprised 12 primary uveal melanoma T3-T4 tumors that represented different histological types (1 epithelioid cell, 3 spindle cell and 8 mixed cases) and were treated by primary enucleation (Supplementary Table 1). One case was metastatic at diagnosis and 6 patients subsequently developed metastases. SNP array analysis was performed using Illumina HumanOmni2.5 SNP arrays and whole genome sequencing was performed on the Illumina HiSeq 2000 platform. The whole genome sequence coverage was >30\(\times\) (Supplementary Table 2) and the data were aligned to the reference genome and duplicate reads excluded. The whole genomes were compared to their matched normal DNA to identify chromosomal translocations, short insertions/deletions (indels) and somatic single nucleotide variants (SNVs).

The SNP arrays revealed low levels of aneuploidy in 11 of the tumors and tetraploidy in tumor #7 (Supplementary Fig. S1; Supplementary Table 3). The diploid tumors presented recurrent chromosome 3 monosomy (9 tumors), losses of 1p (5 tumors), 6q (3 tumors) and 8p (5 tumors), and gains in 6p (4 tumors) and 8q (7 tumors; Supplementary Figs. S1, S2). These aberrations are characteristic of uveal melanoma, but we also observed loss of 16q in three tumors and gain of chromosome 11 in two (Supplementary Figs. S1, S2). The presence of two identical copies of chromosome 3 in tumor #7 suggested that chromosome 3 monosomy preceded acquisition of the tetraploid state. Tumor #7 also displayed trisomy for 1q and monosomy for 8p.

Commensurate with the SNP array data, whole genome sequencing also revealed a low level of chromosomal aberrations. We predicted 2-59 inter-chromosomal translocations, 0-7 intra-chromosomal translocations, no inversions, 2-25 large deletions and 0-5 large insertions (Supplementary Fig. S2; Supplementary Table 4). Thus, the frequency of structural variations in uveal melanoma is only ~40% of that reported in cutaneous melanoma and only ~20% of that reported in acral melanoma (Fig 1A)(15, 16). It is also only ~10% of that we recently found in mucosal melanoma (Fig. 1A) (17).

The whole genome sequencing also revealed a very low number of SNVs. We predicted only 1629-2604 (median 2112) somatic SNVs and 47-
178 (median 67) somatic short indels (Table 1). The SNV mutation rate of <1 per Mb (Table 1) is markedly lower than is seen in most other types of cancer (Fig. 1B) and significantly lower than is seen in cutaneous (~30,000 mutations/genome), mucosal (~8000 mutations/genome), or acral (~5,000 mutations/genome) melanoma (15-17).

UVR-induced DNA damage is characterized by C>T transitions at the 3’ end of pyrimidine dinucleotides (18), a “signature” that accounts for 80-90% of mutations in cutaneous melanoma, and up to 60% of the mutations in acral melanoma (15, 16). Although C to T (G to A) transitions were the commonest mutation in uveal melanoma, they accounted for only ~35% of the lesions (Fig. 1C) and were not enriched at the 3’ position of pyrimidine dimers (Fig. 1D). Thus, UVR induced DNA damage does not appear to play a role in uveal melanomagenesis.

In line with the generally low level of mutations, we observed very few coding region mutations, predicting only 4-19 non-synonymous SNVs per tumor; of which 92% of those tested were validated by Sanger sequencing (Supplementary Table 5). We also predicted only 0-2 coding region indels per tumor (Table 1). The only recurrent mutations were A>T, p.209L mutations in GNA11 (7 tumors) and T>A/T>G, p.209L mutations in GNAQ (3 tumors; Supplementary Table 6). We did, however, observe a small number of non-recurrent mutations in individual genes with possible functional significance, including C>T p.P107L in GNA15 that was coincident with A>T, p.209L GNA11 in tumor #10, C>T p.G8R in EIF1AX in tumor #8, and BAP1 mutations in 7 tumors (Supplementary Table 6).

Critically, we also observed non-recurrent mutations in SF3B1 in three tumors (T>G, p.K666T: tumor #6; T>C, p.K700E: tumor #11; C>T, p.R625H: tumor #12) and although there were no chromosome losses or gains in the region encoding SF3B1 (2q33.1) these data suggested a role for SF3B1 in uveal melanoma. We screened SF3B1 in 105 additional consecutive archival primary uveal melanomas and detected 15 additional mutations (8 p.R625H, 4 p.R625C, 1 p.R625P, 1 p.R625L, 1 p.K666T; Supplementary Table 7). Our overall mutation rate of 15% (18/119) is similar to the rate (18.6%) recently reported for SF3B1 mutations in uveal melanoma by whole exome sequencing (9), but note that in addition to the R625 codon mutations
reported therein, we also observed K666 and K700 codon mutations (Supplementary Fig. S3). The SF3B1 mutations are inversely associated with chromosome 3 monosomy and notably, they are associated with improved progression-free and cancer survival (Supplementary Table 7, Supplementary Fig. S4).

SF3B1 encodes subunit 1 of splicing factor 3b, a component of the spliceosome, so to evaluate the effects of SF3B1 mutations on uveal melanoma transcripts, we hybridized three SF3B1 mutated tumors and three SF3B1 wild-type tumors to Affymetrix Human Transcriptome Arrays (HTA2), which contain both exon and exon-exon junction probes. 325 genes were predicted to be differentially expressed, with 46 genes up-regulated and 279 genes down-regulated in the SF3B1 mutant compared to the SF3B1 wild-type tumors (Supplementary Table 8). Gene Ontology (GO) and pathway analysis of the differentially expressed genes did not predict any significant GO term(s), and did not predict KEGG or REACTOME pathway enrichment. However, splicing level analysis predicted 130 genes that contained at least one differentially regulated exon and/or splicing pattern (Supplementary Table 9). Manual inspection of the predicted events by the GenoSplice EASANA® visualization interface revealed eight high-confidence or very high-confidence events, including alternative terminal exons (four events), alternative 3’ acceptor splice sites (two events), alternative cassette exons (one event), and intron retention (one event; Table 2). Critically, six of these events (GUSBP11, UQCC, ANKHD1, ADAM12, CRNDE and ABCC5) were also identified when we analyzed the RNA-seq data from Harbour et al. (Table 2).

Next, we compared the RNA-seq data from our three SF3B1 mutant to our nine SF3B1 wildtype tumors. For this we used DEXSeq, a Bioconductor package that uses generalized linear models to detect differential exon usage (19), and also MATS, which uses a Bayesian statistical framework to identify alternative splicing (20). 47 genes were predicted to be differentially spliced in these two populations by at least one algorithm (Supplementary Tables 10 & 11). Strikingly, when we compared our analysis of our HTA2 and the Harbour et al. data with our RNA-seq analyses, three alternative splicing events, CRNDE, ABCC5 and UQCC, were identified by all three analyses (Table 2). These data suggest that CRNDE, ABCC5 and UQCC are strong candidates
for alternative splicing in SF3B1 mutant tumors, hence we examined the sequencing profiles for three genes in our RNA-seq data. We normalized the number of mapped bases for these genes and compared nucleotide coverage at each base in SF3B1 mutant and wild-type tumors (Fig. 2).

The profiles for UQCC, revealed clear evidence of alternative terminal exon use in the SF3B1 wild-type and SF3B1 mutant tumors (Fig. 2A). For CRNDE we observed near uniform representation of all bases of exon 4 in the SF3B1 wild-type tumors, but an enrichment of the reads at the 3’ end of this exon in the SF3B1 mutant tumors (Fig. 2B). Finally, in ABCC5, we observed clear evidence of differential splicing of intron 8 (Fig. 2C). Critically, we detected all three of these splicing events when we analysed the RNA-seq data from Harbour et al (Supplementary Figure S5)(9) providing independent confirmation of these alternative splicing events.

Finally, to further validate our findings, we assessed alternative splicing of GUSBP11, UQCC, ANKHD1, GAS8, F8, ADAM12, CRNDE and ABCC5 – the eight genes that provided the strongest evidence of splicing – by qRT-PCR in 74 independent uveal melanomas comprising 58 SF3B1 wild-type and 16 SF3B1 mutant tumors. This analysis confirmed that all eight genes were alternatively spliced in SF3B1 mutant compared to SF3B1 wild-type tumors (Fig. 2D, Supplementary Fig. S6).
DISCUSSION

We describe here the first whole genome sequencing of uveal melanoma, and our data reveal that this is a comparatively simple genetic disease characterized by recurrent chromosomal gains and losses and a relatively low number of SNVs and structural variants. The tumor genomes display a homogenous SNV burden, both in terms of number (1629-2604) and mutation class, and notably, they do not display a canonical UVR-induced DNA damage signature at pyrimidine dinucleotides. The absence of this signature negates an obvious influence of UVR in the etiology of this disease.

We confirm that GNAQ and GNA11 are the most commonly mutated driver oncogenes and that BAP1 is the most commonly mutated tumor suppressor. In addition, we confirm SF3B1 as recurrently mutated in 15% of cases, with a mutation hotspot at codon R625. SF3B1 mutations have been reported in hematological, breast and pancreatic cancers (10-13). Intriguingly, in those cancers codon K700 mutations predominate whereas in uveal melanoma R625 codon mutations predominate. This suggests that either the gene mutations have distinct etiology, so different hotspots are targeted in each disease, or that the diverse biology of the diseases favors selection of discrete mutations. Notably, in common with previous studies (8, 9), we confirm that in uveal melanoma SF3B1 mutations are associated with better prognosis. Thus, in uveal melanoma and myelodysplastic syndrome SF3B1 mutations are associated with improved outcome, whereas in chronic lymphocytic leukemia (CLL), SF3B1 mutations are associated with poorer prognosis (21).

SF3B1 encodes subunit 1 of splicing factor 3b, a component of the spliceosome, a large intracellular machine that processes precursor mRNA into mature transcripts. Specifically, splicing factor 3b anchors precursor mRNA onto the spliceosome to define the splicing site. Previous studies show that spliceosomal component mutations can alter splicing within a gene, can cause intron retention or can cause aberrant alternative splicing, affecting protein isoform balance and thereby cell proliferation and differentiation (22, 23). In CLL, SF3B1 mutations are associated with alternative splicing at the 3’ ends of genes to generate truncated variants of the vitamin C transporter SLC23A2, the T-cell regulator TC1RG1, and the forkhead transcription factor FOXP1. We show that in uveal melanoma SF3B1 mutations are also associated with
alternative splicing (Fig. 2D). We show that in common with CLL, SF3B1 mutations in uveal melanoma are associated with alternative splicing of the 3' end of transcripts, for example in UQCC, which encodes the ubiquinol-cytochrome c reductase complex chaperone, a protein implicated in bone development and stature. We also see differential splicing of ABCC5, a multidrug resistance-associated protein that is implicated in breast cancer metastasis (24) and colorectal cancer (25). Interestingly, in uveal melanoma, we observe evidence of intron retention in ABCC5 in the SF3B1 wild type samples compared to the mutant samples, suggesting that this gene is more efficiently spliced in the SF3B1 mutant than wild-type tumors.

More intriguing, we show that SF3B1 mutations are associated with cryptic alternative splicing of exon 4 of CRNDE. This long non-coding RNA (IncRNA) exists in several alternatively spliced forms and is upregulated in both solid tumors and leukemias (26, 27). CRNDE in general, and exon 4 in particular is alternatively spliced in colorectal cancer and the alternative forms are thought to regulate gene expression by regulating chromatin-modifying enzymes (27, 28). We show that in uveal melanoma, mutations in SF3B1 are associated with cryptic alternative splicing within exon 4 of CRNDE, and considering the comparatively simple genetics of uveal melanoma, it will be important to determine how alternative splicing of this non-coding gene affects cellular function.

In conclusion, we show that despite its appalling prognosis, uveal melanoma is a relatively simple genetic disease characterized by recurrent chromosomal losses and gains, and a low mutational burden. We confirm that GNAQ/GNA11 are the most commonly mutated oncogenes and BAP1 the most commonly mutated tumor suppressor. We identify SF3B1 mutations in ~15% of cases and show these are associated with better prognosis, which will guide clinical management of this disease. Intriguingly, we show that SF3B1 mutations are associated with diverse alternative splicing events, including alternative terminal exon usage, intron retention and cryptic splicing within exons of both protein coding and non-coding genes. Future studies will focus on how these events affect uveal melanoma biology.
METHODS

Patient Cohorts

Discovery set
Twelve patients with uveal melanoma were included in the WGS study (Supplementary table 1) from whom tumour and matched blood samples were obtained. This study was approved by the ethics committee of Institut Curie and informed consent was obtained from all subjects.

Validation set
Consecutive patients diagnosed at the Institut Curie between January 2006 and December 2008 who underwent primary enucleation and with sufficient material at the Biobank were included in the validation series with exclusion of patients with metastasis at diagnosis. Patient characteristics are reported in Supplementary table 6. The follow-up for this analysis ended on December 2012, with a median time of 38 months. During this period, 57 patients (53%) developed metastatic disease and 50 patients (47%) died. Metastatic melanoma was the cause of the death in 43 patients (40%). DNA was extracted from frozen materials or FFPE sections. SF3B1, GNAQ, GNA11 and BAP1 were sequenced by Sanger methods. Oligonucleotide primer sequences are available upon request.

DNA and RNA extraction and sequence analysis
Tumor DNA and RNA were provided by the Biological Resource Center of the Institut Curie. The DNA was extracted from frozen tumor or formalin-fixed paraffin-embedded samples using a standard phenol/chloroform procedure. The total RNA was isolated from frozen tumor samples using TRIZol reagent and cDNA synthesis was performed with MuLV Reverse Transcriptase in accordance with the manufacturers' instructions (Invitrogen, Cergy-Pontoise, France), with quality assessments performed on an Agilent 2100 bioanalyzer. For Sanger sequencing, gDNA was amplified by PCR and the products were sequenced using dye-terminator chemistry as previously described (16). Primer sequences are available upon request. Sequences were visualized
using Sequencher software.

**Whole genome sequencing and analysis**

Extracted DNA samples were sequenced on three lanes of Illumina Hiseq2000 sequencers to produce paired-end reads of 100bp. FASTQ files from each lane were aligned to the human reference genome (GRCh37). Data for each sample were merged and duplicate reads were marked using Picard (http://picard.sourceforge.net/). Somatic variants were identified by comparing matched tumor and normal genomes (Supplementary Methods).

**SNP array analysis**

Genome-wide genotyping for tumor and blood DNA samples was conducted on Illumina HumanOmni2.5 SNP arrays. Raw data files were processed using GenomeStudio and somatic alterations were identified (Supplementary Methods).

**RNA extraction and Array hybridization**

Total RNA was isolated from frozen biopsy using a miRNeasy mini kit (Qiagen) and quality assessment was conducted using RNA 6000 Nano labchip (Bioanalyzer, Agilent) and by a Nanodrop spectrophotometer (Thermo). Total RNA RIN values were between 7.7 and 9 (average: 8.65). Affymetrix Human Transcriptome Array 2.0 ST arrays were hybridized according to Affymetrix recommendations using the Ambion WT protocol (Life technologies, France) and Affymetrix labeling and hybridization kits. 100ng of total RNA were processed in parallel with an external MAQC A RNA to control robustness of data. Labeled DNA mean yield was 7.19 μg (min: 6.27μg; max: 7.57μg). Affymetrix GeneChip® Human Transcriptome 2.0 ST microarrays (HTA2) were hybridized with 4.7 μg of labeled DNA. Raw data, transcript data and exon data were controlled with Expression console (Affymetrix) at the Institut Curie microarray core facility. The benefit of this array is to highlight spliced RNA isoforms using both exon and exon-exon junction probes that can measure excluded or included exons/regions.

**Microarray data analysis**
Affymetrix HTA2 dataset analysis was performed by GenoSplice technology (www.genosplice.com and Supplementary Methods). We performed an unpaired Student’s t-test to compare gene intensities between SF3B1 wild-type and SF3B1 mutated tumors. Genes were considered significantly differentially expressed when fold-change was ≥1.5 and P-value ≤0.05 (unadjusted P-value). Analysis at the splicing level was first performed taking into account only exon probes ('EXON analysis'; see Supplementary Methods). Results were considered statistically significant for unadjusted P-values ≤ 0.05 and fold-changes ≥ 1.5 for SPLICING PATTERN analysis and unadjusted P-values ≤ 0.05 and fold-changes ≥ 2.0 for EXON analysis. After bioinformatics analysis of microarray data, a manual inspection using the GenoSplice EASANA® interface was conducted to select high-confident events.

**RNA-seq analysis**

RNA from the 12 tumor samples was sequenced (Supplementary Methods). Reads were aligned using Tophat (29). Differential splicing analysis between the mutant (n=3) and wild-type SF3B1 (n=9) samples was conducted using DEXseq (19) and events with an FDR <0.1 were regarded as significant. Differential splicing analysis was also conducted by MATS (20), using the mapped read bamfiles as input and events with an FDR <0.1 were regarded as significant. The RNA-seq data from the Habour et al. study (9) were downloaded from the NCBI Sequence Read Archive (http://www.ncbi.nlm.nih.gov/sra?term=SRA062359) and analysed using the same methodology with the exception that read lengths were trimmed to 99bp. One of the samples described as wild-type SF3B1 was predicted to have an SF3B1 R625C mutation (Supplementary Figure S7) and was designated SF3B1 mutant for the differential splicing analysis.

**Splice variant analysis in the validation series**

The validation set was used to measure the predicted splice variant of 8 genes using specific probes (Supplementary Table 12). 1.3ng of cDNA were analyzed in duplicate to quantify spliced and unspliced forms by real time PCR. 45 cycles of QPCR were conducted on 384 well plates using QuantiTect
SYBR Green reagents (Qiagen, Courtaboeuf, France) onto the ABI 9700HT device. To perform the splice variant analysis, 3 steps were achieved per gene of interest. First, CT values were averaged per sample, then a ratio of spliced form was calculated per sample using the formula: $2^{\Delta CT_{\text{form1 mRNA}} - \Delta CT_{\text{form2 mRNA}}}$. Finally, for each splicing event, a Mann-Whitney U test was applied between SF3B1 mutated and wildtype cases.

**Statistical methods**

Cancer-specific survival (CSS) was calculated from the date of diagnosis to death from uveal melanoma or last follow-up. Event-free survival (EFS) was calculated from the surgical resection to development of metastasis or last follow-up. Survival curves were constructed using the Kaplan–Meier method and the difference between groups was compared with the log-rank test. $\chi^2$ and Fisher's exact tests were used to determine association between variables. P-values less than 0.05 (two-sided) were considered statistically significant.

**Data access**

Whole genome, RNA-seq and SNP array data have been submitted to the European Genome-phenome Archive (EGA) under study accession number EGAS00001000472.

**ACKNOWLEDGMENTS**

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REFERENCES

### Table 1. Summary of whole genome sequencing mutations for uveal melanoma.

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Table 2. Alternative gene splicing associated with SF3B1 mutations in uveal melanoma.
TABLE LEGENDS

Table 1. Summary of whole genome sequencing mutations for uveal melanoma. For each tumor (#1-12), the table shows the total number of predicted SNVs (WGS SNVs), the mutation rate per Mb, the number of coding region SNVs, the total number of predicted indels (WGS indels), and the number of coding region indels.

Table 2. Alternative gene splicing associated with SF3B1 mutations in uveal melanoma. For each gene indicated, the table presents the prediction of alternative splicing by the GenoSplice EASANA visualization interface in our HTA2 data, and the Harbour et al. (9) RNA-Seq data. Also shown is the prediction of these events by DEXSeq and/or MATS in our RNA-Seq data and validation of the events by PCR.
FIGURE LEGENDS

Figure 1. Somatic mutations in uveal melanoma.

(A) Comparison of predicted somatic structural variation in uveal, cutaneous, acral and mucosal melanoma subtypes.

(B) Comparison of non-synonymous point mutation rates identified from whole genome and exome sequencing studies in various solid tumors (Details and references in Supplementary Methods).

(C) Proportion of predicted somatic single nucleotide variants in uveal melanoma genomes by class of mutation.

(D) Frequency of bases ±1 bp of C>T/G>A mutations in the uveal melanoma genomes.

Figure 2. Alternative splicing in SF3B1 mutant uveal melanoma.

(A - C) Plots showing normalized RNA-seq reads for UQCC (A), CRNDE (B) and ABCC5 (C) in SF3B1 wildtype (orange) and SF3B1 mutant (mauve) tumors. Above the graphs we show representations of the splicing events. Exons are represented as boxes, with major splicing events indicated by the solid lines/solid boxes and minor splicing by the dotted lines/hashed boxes. Te: terminal exon; e3, e4, e5, e6: exons 3, 4, 5, 6; i5: intron 5.

(D) Heat map of the 8 differentially spliced genes validated by RT-QPCR in a cohort of 74 independent uveal melanoma samples (16 SF3B1 mutant, 58 SF3B1 wild-type). The brackets present the splicing form that was measured, together with exons number involved in each case (e: exon, i: intron, ae: alternative exon). Primer sequences used are presented in Supplementary Table 12 and p-values (Mann-Whitney U test) are indicated without adjustment for the 8 tests. Primary data is shown in Supplementary Fig. S6. Genes that are alternatively spliced are shown in blue; non-spliced genes are shown in red; unexpressed genes are shown in yellow. For each sample, the status of the SF3B1 gene is indicated.
Figure 1

(A) Bar chart showing the distribution of different types of melanoma: Uveal, Cutaneous, Acanal, and Mucosal.

(B) Bar chart illustrating the frequency of various cancer types: Retinoblastoma, Uveal melanoma, Neuroblastoma, Breast cancer, Ovarian carcinoma, Prostate cancer, Endometrial cancer, Head/neck squamous, Colon cancer, and Cutaneous melanoma.

(C) Graph depicting the proportion of mutations at different positions: A>C, A>G, A>T, C>A, C>G, and C>T.

(D) Graph showing the distribution of mutations at specific positions T1, T2, T3, T4, T5, T6, T7, T8, T9, T10, T11, and T12.
Figure 2

A  
SF3B1 wild-type

SF3B1 mutant

UQCC  
\[ e_6 \]

CRNDE  
\[ e_3 \, e_4 \, e_5 \]

ABCC5  
\[ e_5 \, i_5 \, e_6 \]

B  
CRNDE  
\[ e_3 \, e_4 \, e_5 \]

C  
ABCC5  
\[ e_5 \, i_5 \, e_6 \]

D  
SF3B1 wildtype tumors  
SF3B1 mutant tumors

- Altered splicing
- Normal splicing
- Not expressed

Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

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SF3B1 mutations are associated with alternative splicing in uveal melanoma

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