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Rare *SF3B1* R625 mutations in cutaneous melanoma

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Abstract

RNA splicing is the cellular process that has only recently been found to be an important target for various cancers. Among the spliceosome genes that are involved in cancers, *SF3B1* is most frequently mutated. Recurrent mutation in codon 625 has been found in uveal melanoma, but this mutation has not been identified in cutaneous melanoma. We used whole-exome sequencing to explore the mutational landscape of 295 melanoma samples, 231 of which are cutaneous melanoma. Out of these cutaneous melanoma samples, we found 2 samples with R625 mutation in *SF3B1* gene. The results were validated by Sanger sequencing. We conclude that *SF3B1* R625 mutation does occur in cutaneous melanoma, although with a low frequency (~1%).

Keywords

Melanoma; *SF3B1*; Cancer genetics; Whole-exome sequencing

Introduction

Recent high throughput sequencing of cancer genomes has led to new discoveries of mutations in cellular processes that were not previously known to play a causal role in cancer. One such processes is RNA-splicing (for review see [1]). Among the recently discovered spliceosome genes that are involved in cancers, *SF3B1* is the most frequently mutated. *SF3B1* mutations are found with high frequency in myelodysplastic syndromes (MDS) and chronic myelogenous leukemia (CLL); it is also mutated in solid tumors such as lung adenocarcinomas, breast cancer, and pancreatic cancer. In uveal melanoma, recurrent mutations at codon 625 of *SF3B1* have been identified [2–4].

SF3B1 encodes subunit one of the splicing factor 3b protein complex, which is part of the U2 small nuclear RNAs (snRNP) that binds pre-mRNA upstream of the intron's branch site.

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SF3B1 mutations usually occur within the 22 HEAT repeats in the C-terminal region. Codon R625 has the highest mutation frequency in uveal melanoma [2–4], whereas the mutation hotspot is at K700 for MDS and CLL.

While multiple groups identified *SF3B1* R625 mutations in uveal melanoma [2–4], none identified this mutation in cutaneous melanoma [5]. We have sequenced the whole exome of a cohort of 295 melanoma samples, 231 of which are cutaneous melanoma [6]. We report here that although the frequency is low, *SF3B1* R625 mutation does occur in cutaneous melanoma.

Material and methods

Sample selection

For details of sample selection, see reference [6].

Exome sequencing and data analysis

For details of exome sequencing and data analysis, see reference [6].

Sanger sequencing

The following primers were used to amplify the region of the *SF3B1* R625 for validation by Sanger sequencing: F1: 5′-CCTCGTGGTCATTGAACCGC -3′; B1: 5′-ACTTCTAAGATGTGGCAAGATGGC -3′

Results

Our cohort contains all samples described in an earlier publication [6] with additional samples (Table 1). The whole-exome sequencing of 295 melanoma samples identified 5 with mutations in *SF3B1* R625 (Table 2). Out of these five samples, two (YUBOO and G2306T) are uveal melanoma, two (YUPAO and YUGAFFE) are cutaneous melanoma, and one sample for which the location of the primary lesion is unknown (YUKAY). Three samples have p.R625H mutation, while the other two have p. R625C mutation (amino acids are numbered based on GenBank accession NM_012433.2). The alignments of reads from whole-exome sequencing around the mutations convincingly show that the mutations are authentic, even though the mutation frequency is low. The whole-exome sequencing results were validated by Sanger sequencing, as shown in Figure 1. The mutation peaks in both samples are weak when compared with the reference peaks. These mutation peaks, however, correlate with the mutation frequencies detected in the whole-exome sequencing. For the cutaneous sample YUPAO (shown in the top of Figure 1), there are total 251 reads mapped to this position, with the number of reads for each base as: A=0, C=211 (84%, 80+, 131−), G=0, T=40 (16%, 15+, 25−). The “+” and “−” signs indicate the numbers of reads in forward and reverse strands respectively. *SF3B1* is on the reverse strand of hg19 reference genome and the bases shown here are the original counts on hg19 forward strand. For the uveal sample YUBOO (shown in the bottom of Figure 1), there are total 137 reads, with the number of reads for each base as: A=0, C=112(82%, 39+, 73−), G=0, T=25 (18%, 9+, 16−). The other samples with mutations in *SF3B1* R625 show similar mutation frequencies. The

low mutation frequencies might reflect the presence of stroma in the samples. The matching normal DNAs available for four of the five samples were all wild-type, indicating the somatic origin *SF3B1* R625.

One melanoma sample in this cohort has BRAF V600 mutation (YUGAFFE) and none has NRAS mutation (Table 2).

Other *SF3B1* non-synonymous somatic mutations were also present as listed in Table 3 (amino acids are numbered based on GenBank accession NM_012433.2).

Discussion

The report of mutations in codon 625 of *SF3B1* in uveal melanoma [2–4] prompted further investigation regarding the presence of this mutation in cutaneous melanoma [5]. The published study examined 85 cutaneous melanoma samples using direct Sanger sequencing [5]. The cohort included 22 superficial spreading, 24 acral-lentiginous, 36 nodular, and 3 lentigo-maligna melanomas. The mutation was not detected in 81 samples (four samples failed or were ambiguous in the sequencing). In our larger cohort, we found five samples with *SF3B1* R625 mutations, two of which are cutaneous melanoma and one of unknown origin, showing that the mutation does occur in this type of melanoma, although at low frequency.

We also noticed that most of our samples that have *SF3B1* R625 mutations are metastatic melanoma. This is in contrast to previous finding that *SF3B1* R625 mutations are rare in metastatic tumors and are associated with better prognosis [2, 4, 7]. Harbour *et al* did not detect any *SF3B1* mutation in their five distant metastatic uveal melanoma [2], and Griewank *et a*l found one out of 26 metastatic uveal melanoma samples [7]. The difference might be due to sample bias, since there are more metastatic than primary tumors in our cohort. The other possibility is that the *SF3B1* mutations might play different roles in uveal and cutaneous melanomas. *SF3B1* mutations have been found to be associated with different prognosis in different type of cancers. The *SF3B1* mutations in CLL are associated with poorer prognosis [8], while in MDS the mutations are associated with better prognosis [9].

Different cancers also have different predominate mutations in *SF3B1*. In hematological, breast and pancreatic cancers codon K700 mutations predominate, whereas in uveal melanoma the R625 codon mutations predominate. We did not detect any K700 mutation in our cohort. It seems that *SF3B1* plays different roles in the biology of different cancers. Although now it is clear that *SF3B1*, as well as other genes involved in RNA splicing, plays causal role in cancer, the exact effect of the mutations on protein functions and splicing patterns are still needed to be fully elucidated by further experiments.

We detected mutations in *BAP1* in both sun-exposed and uveal melanoma [6]. Another gene, *EIF1AX*, which encodes eukaryotic translation initiation factor 1A (eIF1A), was also recently found to be frequently mutated in uveal melanoma [3]. Interestingly, in our cohort we also found *EIF1AX* mutations in both uveal and cutaneous melanomas. We found 6 mutations in 25 uveal melanomas (~24%) and 5 mutations in 231 cutaneous melanomas (~2%). As discovered previously in uveal melanomas [3], the nonsynonymous *EIF1AX*

mutations are clustered around the N terminus of the protein for both cutaneous and uveal melanomas in our cohort (data not shown). It would be interesting to see if interactions between the different cellular machinery encoded by these two genes play a role in uveal and cutaneous melanoma biology.

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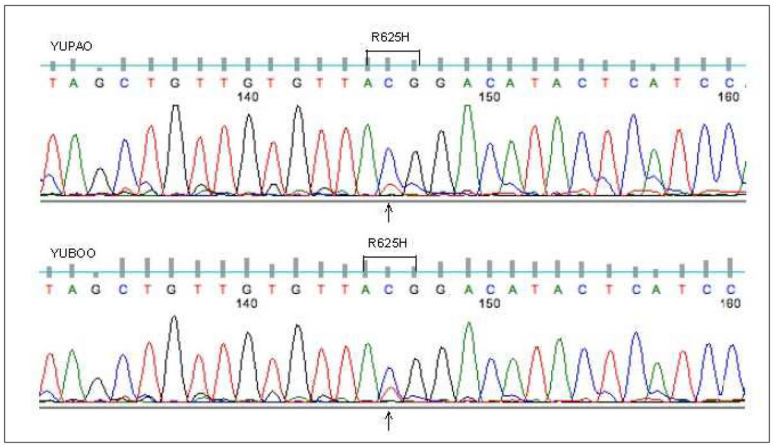

Figure 1.

Sequencing chromatograms for two samples showing the *SF3B1* mutation at R625 position. On the top is cutaneous sample YUPAO and on the bottom is uveal sample YUBOO.

Table 1

Samples types and their metastatic status

Tumor type	Primary	Metastasis	Total
Cutaneous	66	165	231
Mucosal	3	7	10
Ocular	20	5	25
Unknown	0	29	29
Total	89	206	295

Table 2

Samples with *SF3B1* R625 mutation

Sample name	Chromosome position (chr2, hg19/GRCh37)	Nucleotide change	Amino acid change	BRAF V600 mutation	NRAS Q61 mutation	Tumor type/origin/tumor location	Primary or metastatic
YUBOO	198267483	C > T	R625H	-	-	uveal/uvea/head/neck	metastatic
YUKAY	198267483	C > T	R625H	-	-	unknown/unknown/trunk	metastatic
YUPAO	198267483	C > T	R625H	-	-	acral/acral/extremity	metastatic
YUGAFFE	198267484	G > A	R625C	V600K[a]	-	cutaneous/head/neck/lymph node	metastatic
G2306T	198267484	G > A	R625C	-	-	uveal/uvea/uvea	primary

[a] double mutations in the same codon with chr7:140453136 A>T and chr7:140453137 C>T

Table 3

Additional *SF3B1* non-synonymous somatic mutations.

position	Nucleotide change	Amino acid change	Sample names	Sample type/origin/tumor location	Primary or metastatic
198273258	G > A	R318*-R	YUKLAB	cutaneous/unknown/trunk	metastatic
198267432	G > A	P642L-P	YULAN	cutaneous/head/neck/lymph node	metastatic
198267360	T > G	K666K-T	YUGISMO	cutaneous/trunk/trunk	metastatic
198266795	C > T	A713A-T	YUPAT	cutaneous/trunk/lung	metastatic
198266497	G > C	P780P-R	G2310T	uveal/uvea/uvea	primary
198262755	G > A	R1074C-R	YUSEN	cutaneous/head/neck/trunk	metastatic
198260927	G > T	A1131A-D	YUEGO	cutaneous/head/neck/trunk	metastatic
198257761	T > C	M1231M-V	YUKNOLL	unknown/unknown/liver	metastatic
198257176	G > A	H1256H-Y	YUBEL	unknown/unknown/lymph node	metastatic