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Somatic *SF3B1* Mutations in Myelodysplastic Syndrome with Ring Sideroblasts and Chronic Lymphocytic Leukaemia

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Abstract

SF3B1 is the largest subunit of the SF3B complex and part of the U2 small nuclear ribosomal protein. It functions as an important part of spliceosomal assembly, converting pre-mRNA to mRNA ready for ribosomal translation. Mutations of *SF3B1* are commonly seen in myelodysplastic syndromes with ring sideroblasts (MDS-RS and MDS/MPN-RS-T). These mutations are typically heterozygous missense substitutions, of which, 55% involve K700E. MDS-RS and MDS/MPN-RS-T usually carry a more favourable prognosis than other subtypes of MDS. *SF3B1* itself does not influence survival in these conditions, but does correlate with increase thrombotic risk. Mutated *SF3B1* is present in 9-15% of CLL cases and on its own correlates with improved responsiveness to Ibrutinib, but is associated with additional adverse genetic abnormalities including *TP53* and *ATM* mutations, which traditionally confer adverse outcomes.

Introduction

In humans, genes are expressed as precursor messenger RNA (pre-mRNA), which in turn are converted to messenger RNA (mRNA) by splicing. Splicing removes non-coding introns and can also remove alternate exons from mRNA, leaving coding exons, which are ligated together ready for translation in the ribosome. Pre-mRNA splicing is catalyzed by the spliceosome, a series of small nuclear ribonuclear proteins (snRNPs), which act in a step-wise fashion to remove introns.[1]

Spliceosome Assembly

Each snRNP consists of snRNA and a variable number of specific proteins. SnRNAs comprise a group of highly abundant, non-polyadenylated, non-coding transcripts that function in the nucleoplasm.[2] In humans, there are 2 spliceosome systems; the major U2-dependent and minor U12-dependent systems, which catalyze removal of U2-type introns and U12-type introns, respectively. These systems recognize different classes of splice sites and differ in snRNA composition.[3]

Spliceosome assembly occurs anew on each pre-mRNA and results from the ordered interaction of snRNPs and other splicing factors.[1] The first step of spliceosome assembly within the major U2-dependent system is formation of the E complex, in which, U1 snRNP is recruited to the 5'splice site (5'ss) and non-snRNP factors such as SF3B1, U2AF and U2AF1 interact with the branch point site (BPS), polypyridamine tract (PPT) and 3'splice site (3'ss), respectively. Formation of complex E enhances U2 snRNP recruitment to the BPS, leading to formation of complex A. The pre-assembled tri-snRNP U4/U6•U5 is then recruited to form complex B*. Complex B* then carries out the first catalytic step of splicing, generating complex C, which contains the free exon 1 and the intron-exon 2 lariat intermediate. Complex C catalyzes the second step of splicing, in which, the spliceosome dissociates, is remodeled, and the released U2, U5 and U6 snRNPs can then take part in additional rounds of splicing. Ultimately, the 5'ss and 3'ss exons are ligated together, forming mRNA, and the branch site is discarded.[1],[4],[5] The two-step splicing process is illustrated in *Figure 1*.[4]

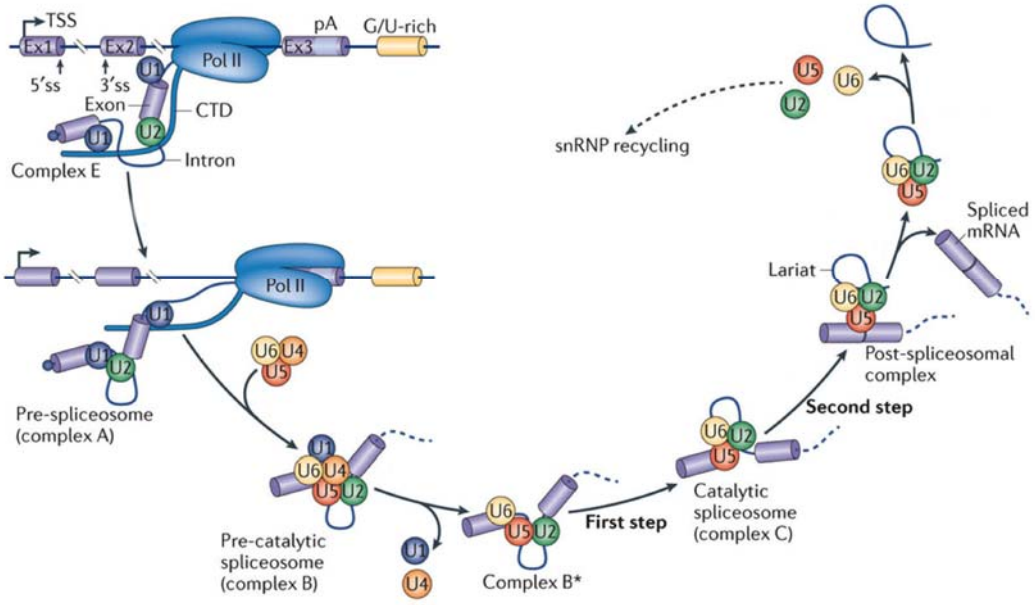

Figure 1: Spliceosomal assembly and pre-mRNA splicing (Adapted from *Matera & Wang, 2014*). [4]

Role of SF3B1 within the spliceosome

Spliceosome Factor 3B (SF3B) is a heptameric protein complex that is essential for pre-mRNA splicing. It comprises subunits SF3B1, SF3B130, SF3B145, SF3B49, SF3B14b, p14/SF3b14a, and SF3B10.[6] SF3B, in combination with SF3A and a 12S RNA unit.[7] SF3B1 is the largest subunit of the SF3B protein complex,[6] and forms the site of U2 snRNP-BPS recognition and selection, which is a key step in the early stages of spliceosome assembly.[8] The SF3B protein complex contacts the pre-mRNA at or near the BPS, through interaction of SF3B1 with p14[9] and with C-terminal RNA recognition motif of U2AF65 bound at the PPT.[9],[10]

Structure of SF3B1 protein

In the literature, SF3B1 has also been variously termed MDS, PRP10, Hsh155, PRPF10, SAP155 and SF3B155. SF3B1 comprises a N-Terminal Domain (NTD) and a Carboxyl-terminal HEAT repeat domain (HD). The HD (amino acids 431-1304) is organized into 22 non-identical tandem HEAT repeats that form helical rod-like structures marking out an S-shaped path.[3],[8], The NTD (amino acids 1-430) has a molecular mass of around 47kDa and appears to act as a scaffold with an elongated structure to maximize its interaction surface for binding many factors simultaneously, including U2AF65, SPF45, PUF60, p14, NIPP1 and cyclin E.[11]

Location of *SF3B1* gene

SF3B1 is encoded by the *SF3B1* gene located on chromosome 2 at position 2q33.1 with the molecular location of base pairs 197,391,974 to 197,435,093.[12] The full length of *SF3B1* is 27 exons, corresponding to a 146kDa protein of 1304 amino acids.[8],[13]

The Role of SF3B1 in the Pathophysiology of Ring Sideroblasts

Ring sideroblasts (RS) are defined as erythroblasts in which there are a minimum of 5 siderotic granules covering at least a third of the nuclear circumference.[14] Ring sideroblasts result from abnormal accumulation of heavy-ferritin in the mitochondria of erythroblasts and has been shown in patients with both X-linked congenital sideroblastic anaemia (*ALAS* gene) and myelodysplastic syndromes with ring sideroblasts, but not in healthy controls. Gene expression analysis has shown upregulated *ALAS* (heme biosynthesis enzyme) and downregulated *ABCB7* (involved in iron transport from mitochondria to cytoplasm) in myeloid cell lines from patients with mutated *SF3B1*. *PPOX*, which encodes an enzyme of heme biosynthesis, is another target of mutant SF3B1-associated misrecognition of 3′ splice sites that introduces a frameshift.[15,16]

Primitive CD34+, CD45- lymphomyeloid haematopoietic stem cells appear to represent the origin and propagating cells of the *SF3B1*-mutated clone in MDS-RS.[17] $SF3B1^{K700E}$ cells have defects in the splicing and cytoplasmic export of tRNA synthetases and RNA metabolism-related factors. Better understanding of pathophysiology of MDS in the context of *SF3B1* mutations may provide an opportunity for future development of therapies.[18]

SF3B1 Mutations in Myelodysplastic Syndromes with Ring Sideroblasts

In 2011, *SF3B1* mutations were first described in patients with Myelodysplastic Syndromes (MDS).[19,20] MDS are a group of clonal haematopoietic stem cell diseases characterized by cytopenias, dysplasia in one or more major myeloid lineages, ineffective haematopoiesis, recurrent genetic abnormalities and increased risk of developing acute myeloid leukaemia (AML).[21] The high frequency of spliceosome mutations in MDS suggests a common impact on the initial steps of pre-mRNA splicing, including 3'ss recognition and branch point usage during pre-mRNA processing, thereby inducing abnormal RNA splicing and resulting in abnormal haematopoiesis.[19,22]

Papaemmanuil *et al., 2011*, identified *SF3B1* mutations in 20% (72/354) of patients with MDS, with a higher frequency (65%) in those whose disease was characterized by the presence of ring sideroblasts.[20] A similar study found *SF3B1* mutations in 82.6% of patients with refractory anaemia with RS (re-classified by the World Health Organization in 2016 as MDS with single-lineage dysplasia and ring sideroblasts[23]) and in 76% of those with multilineage dysplasia and RS.[19] This association has also been shown in other studies.[24,25]

A number of *SF3B1* mutations have been described in MDS patients and nearly all are heterozygous missense substitutions within regions coding for the Huntingtin, elongation factor 3, protein phosphatase 2A, and yeast PI3-kinase TORI (HEAT) domains of SF3B1.[5,20] There are a number of mutational hotspots clustered around the 5th to 8th C-terminal domain HEAT repeats between exon 12 and exon 15 (*Figure 2*).[26] The commonest of these is c.2098A>G leading to a substitution of K→E at position 700, accounting for 58% of *SF3B1* mutations in MDS.[24] Other amino acid hotspots for substitution mutations occur with lower frequency, including E622D, Y623C, R625H (also R625G, C or L), N626D, H662Q or D, K666E (also K666N, R or T), and I704V (also I704N).

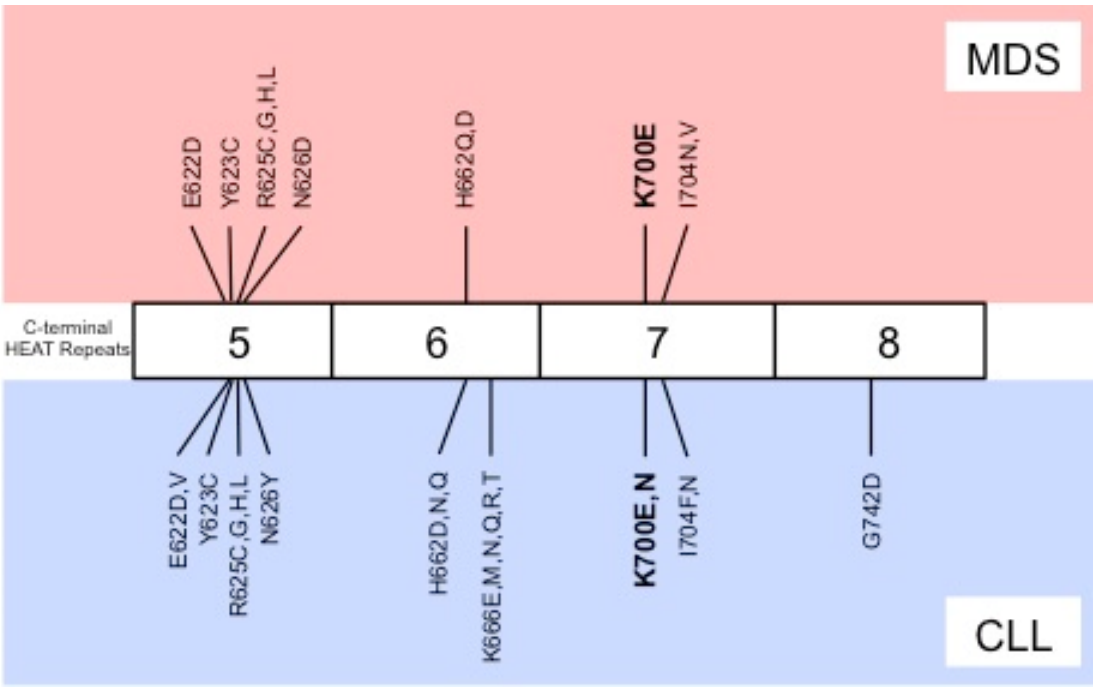

Figure 2: Mutations of *SF3B1* in MDS and CLL are largely localized to its C-terminal domain 5th to 8th HEAT repeats (exons 12 to 15).

In MDS, mutated *SF3B1* correlates with presence of ring sideroblasts, normal or elevated platelet counts, increased bone marrow cellularity, red cell transfusion dependency[27] and increased incidence of thrombotic events.[23,28] It negatively correlates with presence of multilineage dysplasia and high-risk karyotype.[27] In one large study, *SF3B1* mutations had a positive predictive value for formation of RS of 97.7% (95% confidence interval 93.5-99.5%). The proportion of patients with a WHO RS-subtype was significantly higher in those with a mutant allele burden of ≥25%, than those with <25%.[27] In MDS-RS with mutated *SF3B1*, the percentage of RS does not affect prognosis.[27] Therefore, in the 2016 classification, the WHO has reduced the percentage of RS required for a diagnosis of MDS-RS from 15% to 5%, where there is an identified *SF3B1* mutation.[23]

MDS-RS carries a more favourable prognosis than other forms of MDS, with lower risk of transformation to acute myeloid leukaemia, and better overall survival.[24] The prognostic significance of *SF3B1* mutation in MDS remains controversial. Some studies have suggested improved outcomes in patients with MDS-RS harbouring the

SF3B1 mutation[24,29]. However, recent large meta-analyses have found that, by multivariate analysis, *SF3B1* mutations in MDS are not independently prognostically significant.[30,31]

Patients with MDS/MPN-RS-T have features of MDS-RS together with a persistent thrombocytosis (platelets >450x10^9/L) and large atypical megakaryocytes similar to those seen in myeloproliferative neoplasms.[21,23] The diagnosis requires ≥15% RS irrespective of *SF3B1* mutation status. In one study, 90.7% of cases harboured the *SF3B1* mutation and 79% of cases harboured >1 genetic abnormality. *JAK2 V617F* mutation was seen in 57% of cases, with a number of other mutations of activated signalling, epigenetic modifiers, and transcription, also found including *MPL*, *TET2, ASXL1*, *and ETV6*.[32] *SF3B1* mutation reduces thrombosis free survival in patients with MDS/MPN-RS-T, but with no effect on overall survival. The effect on thrombosis risk in this group requires further study.[33]

***SF3B1* Mutations in other Myeloid Neoplasms**

Chronic myelomonocytic leukaemia (CMML) is a clonal haematopoietic malignancy characterized by persistent monocytosis, combining myeloid cell proliferation with myeloid cell dysplasia and ineffective haematopoiesis.[34] It is classified by the WHO in 2016 as a myelodysplastic/myeloproliferative neoplasm (MDS/MPN) overlap syndrome.[23] Six percent of CMML cases express mutated *SF3B1*, with K700E again being the most common mutation, followed by H662Q and K666N. Again, there is a strong correlation with presence of bone marrow RS but no impact on overall survival (median 17 months).[35,36]

Data is more limited on the prevalence of *SF3B1* mutations in patients with myeloproliferative neoplasms (MPN). A recent study found that 10% of patients with *BCR-ABL*1-negative MPN possess *SF3B1* mutations, concurrent with their underlying driver mutation (*JAK2*, *CALR*, *MPL*, or triple-negative). Its presence in this group was associated with the RS phenotype (40% of *SF3B1*-mutated patients), did not appear to increase rates of dysplastic change and was more commonly found in the primary myelofibrosis subtype.[37]

SF3B1 mutations are seen in 2-6% of adult de novo AML cases, but in 15% of those with AML inv(3)(q1q26.2). K700E, K666N, K666Q, T663I, R625C mutations have all been reported.[38,39]

***SF3B1* Mutations in Chronic Lymphocytic Leukaemia (CLL)**

The prevalence of *SF3B1* mutations in CLL ranges from 9-15%.[40,41] *Wang et al,* demonstrated that 50% of mutations involve the K700E missense amino acid change and whilst many of the others are heterozygous mutations localized within the C-terminal PP2A-repeat regions 5 to 8.[40] This finding was later supported by a much larger study of 1160 CLL patients, where 104/1160 (9%) harbored the somatic *SF3B1* mutation in exons 13-16, with 44% of these being K700E and a median mutation allele burden of 35%. Other mutations such as K666E and G742D occur and are clustered in selected HEAT repeats of the SF3B1 protein. [41]

In a murine model, *SF3B1* K700E mutation induced a phenotype of cellular senescence and defective cell proliferation, but on its own was insufficient to induce CLL. However, when combined with *ATM* mutation, lead to intron retention, genomic instability and ultimately CLL developed in 9% of subjects. This suggests that *SF3B1* induces a senescent B-cell in waiting, which when exposed to further genomic aberrations, overcomes cancer checkpoints to induce a CLL clone.[42]

Karyotype not only influences prognosis in CLL but now helps guide therapeutic options, in particular del(17p) or *TP53* mutations, which confer adverse prognosis.[43] Eighteen percent of CLL cases with an *SF3B1* mutation also carry a *TP53* mutation. *SF3B1* mutations as the sole abnormality in CLL are strongly associated with male gender, advanced clinical stage at diagnosis, and faster rate of disease progression leading to time to first treatment. The type of *SF3B1* mutation does not appear to influence this.[44] *SF3B1* mutations are most frequently associated with a normal karyotype in CLL.[41] *SF3B1* mutation is associated with down-regulation of B-cell receptor (BCR). This appears to increase sensitivity of *SF3B1*-mutated CLL to Ibrutinib, a Bruton's tyrosine kinase inhibitor.[42]

However, 20% of patients will have a co-existent del 11q22.3 (the locus for the *ATM* gene),[41] which reduces time to first treatment compared to wild-type patients.[44] The presence of del 11q22.3 as the sole abnormality confers shorter treatment-free survival in those receiving conventional chemotherapy,[45] but novel therapies, such as ibrutinib, have significantly improved progression-free survival in this group also.[46]

Conclusion

SF3B1, as the largest component part of the SF3B protein complex, plays an important role in spliceosome assembly, in particular, BPS recognition. *SF3B1* mutations in MDS are strongly correlated with the presence of ring sideroblasts, but it appears that it is the RS phenotype, rather than the presence of the *SF3B1* mutation itself, that is responsible for its favourable outlook. It is much less prevalent within myeloid neoplasms, which lack RS, such as CMML, MPN and AML. Within CLL, however, mutant *SF3B1* is commonly found and is associated with advanced disease at presentation as well as shorter time to first treatment. It is often present concurrent with other unfavourable genetic abnormalities, such as *TP53* mutation or del 11q22.3. Other malignancies such as uveal melanoma and breast cancer also commonly exhibit mutated *SF3B1*[47–50].

Take Home Messages

- Splicing removes non-coding introns from pre-mRNA, leaving coding exons ready for mRNA translation in the ribosome.
- SF3B1 plays an important role in spliceosome assembly, particularly branch point site recognition and selection.
- Missense substitutions involving *SF3B1* are associated with MDS-RS, which carries a favourable prognosis.

- The most common mutation of *SF3B1* is a missense substitution of K→E at position 700.
- In CLL, *SF3B1* mutation is associated with male gender and faster time to first treatment.
- It is commonly co-mutated with important prognostically unfavourable genes such as *TP53* or *ATM*.

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