Oncogenic Landscapes of Splicing-Factor Mutant MDS

Rahman MA*

Cold Spring Harbor Laboratory, USA

*Corresponding author: Mohammad Alinoor Rahman, Cold Spring Harbor Laboratory, USA, Tel: +1-516-367-8418; Email: mrahman@cshl.edu

Abstract

Genomic analyses of the myeloid malignancies surprisingly identified recurrent heterozygous somatic mutations in several splicing factors. Among them, SF3B1, SRSF2, U2AF1 and ZRSR2 are most frequently mutated in patients with myelodysplastic syndrome (MDS). Recent studies suggest that mutations in SRSF2 and U2AF1 alter their normal RNA binding and splicing preferences in a sequence-specific manner, whereas mutations in SF3B1 promote selection of cryptic 3' splice sites. In contrast, mutations in ZRSR2 affect splicing of U12-types introns in "minor spliceosome" pathway. Different mutations appear to regulate hundreds of different splicing targets, thereby exclude the possibility of common downstream splicing alterations. Therefore, it is important to focus on common physiological processes contributed to MDS etiology, which are coupled with splicing alterations promoted by different splicing factor mutations. This mini review summarizes the accumulating knowledge about the oncogenic splicing landscapes, underlying mechanisms, and physiological processes affected by mutations in three major factors, SRSF2, SF3B1 and U2AF1.

Keywords: Oncogenic Landscapes; Genetic screenings

Abbreviations: MDS: Myelodysplastic Syndrome; AML: Acute Myeloid Leukemia; PTC: Premature Termination Codon; NMD: Nonsense-Mediated mRNA Decay; ASOs: Antisense Oligonucleotides; TRIDs: Translational Read-Through-Inducing Drugs; ATR: Ataxia Telangiectasia and Rad3.

Myelodysplastic syndrome (MDS) is the most common cause of acquired bone marrow failure in adults, with a frequency of 75/100,000 in USA (≥65 years) [1]. This is a heterogeneous group of clonal hematopoietic neoplasms, with ineffective and dysplastic hematopoiesis, and often progress to acute myeloid leukemia (AML). Genetic screenings identified a set of recurrently mutated genes in MDS, comprising limited number of physiological processes [1]. Recurrent heterozygous somatic mutations in SF3B1, SRSF2, U2AF1, and ZRSR2 represent the most common class of genetic variations and found in ~60% of MDS patients (Figure 1) [1,2].
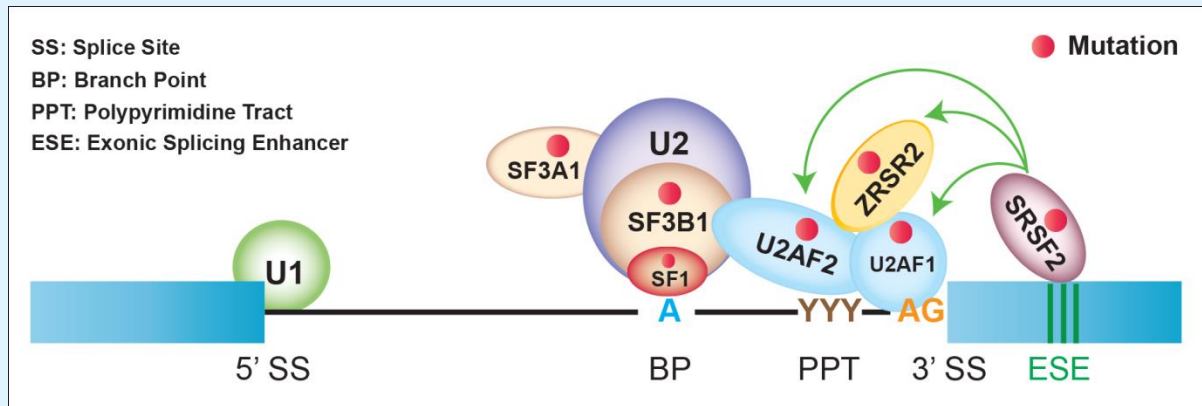
Figure 1: Splicing factor mutations in myelodysplastic syndrome. Schematics showing normal assembly of different splicing factors and their coordination during pre-mRNA splicing. Mutations in different splicing factors are shown by red spot.

SRSF2 is a splicing factor and a member of the serine/arginine (SR)-rich protein family. SRSF2 mutations show poorer survival in MDS and an increased risk of AML transformation [3]. Mutations predominantly occur at the Pro95 codon, which is in close proximity to the RRM domain of SRSF2 [2]. Pro95 mutation changes the RNA-binding preferences of SRSF2 from a G-rich motif [GG(A/T)G] to a C-rich motif [(C/G)C(A/T)G] [4-6]. This subsequently causes genome-wide splicing alterations. Altered splicing could compromise the function of a protein by affecting an important domain. In addition, the resultant mRNA could generate a premature termination codon (PTC), and degraded by nonsense-mediated mRNA decay (NMD). One interesting example is *EZH2*, which encodes Enhancer of zeste homolog 2 protein. EZH2 catalyzes histone methylation and functions in chromatin remodeling, and an important regulator of hematopoiesis. Pro95 mutation in SRSF2 causes the inclusion of a poison exon in *EZH2* transcript, which generates a PTC and subsequently degraded by NMD. Among other important target genes include *BCOR* (also recurrently mutated in MDS and AML), *IKAROS* (associated with stem cell renewal) and *CASP8* (a regulator of apoptosis) [4], *ARMC10* (tumor suppressing factor) and *FYN* (the tyrosine kinase) [6].

SF3B1 is known to promote the stabilization of the U2 snRNP at the branch point during splicing. SF3B1 mutations typically occur in the highly conserved C-terminal domain, between the fourth and eighth HEAT domain repeats [7]. Approximately half of these missense mutations occur at amino acid residue K700. Other nearby hotspots (R625, H662 and K666) are also predicted to have a similar functional impact due to close

proximity [7]. Several studies have shown that SF3B1 mutations promote aberrant splicing by activating cryptic 3' splice site usage [8-11]. As a consequence, many mRNAs give rise to PTC, subsequently degraded by NMD. One well-recognized aberrantly spliced target gene is *ABCB7*, which encodes mitochondrial iron exporter protein. In SF3B1 mutant cells, aberrant usage of 3' splice site causes retention of a 21-bp intronic segment in *ABCB7* transcript, leading to mRNA degradation by NMD [10]. Some other dysregulated genes include *ASXL1*, *CBL*, *ALAS2*, *SLC25A37*, *CRNDE, TMEM14C, UQCC*1 etc. [10,12-15].

U2AF1 is a subunit of the U2 snRNP and functions in recognition of the AG dinucleotide at the 3' splice site (SS). U2AF1 mutations mostly occur at residues S34 or Q157, spanning two separate conserved zinc finger domains [7,16,17]. U2AF1 mutations show shorter survival and increased risk of AML transformation [3,16]. S34 and Q157 mutations differentially affect the recognition of 3' splice site in a sequence-specific manner. S34 mutants promote recognition of 3' SS having a C or A immediately preceding the AG [17-21]. In contrast, Q157 mutants promote recognition of 3'SS bearing a G immediately downstream of AG [19]. In U2AF1 S34 mutant cells, aberrantly spliced and functionally correlated targets are H2AFY (encoding an H2A histone variant) and STRAP (encoding serine/threonine kinase receptor-associated protein) [21]. Some other reported target genes of U2AF1 mutants include *GNAS, BCOR, KDM6A, PICALM, MED24 etc.* [17,19,22-25].

Splicing factor mutations occur in a mutually exclusive manner. Initially it was presumed that the resultant

Rahman MA. Oncogenic Landscapes of Splicing-Factor Mutant MDS. Genom & Gene Ther Int J 2018, 2(1): 000105.

Copyright© Rahman MA.

splicing defects caused by individual mutations might have common downstream splicing consequences. However, it is now evident that different mutations appear to induce distinct splicing defects, suggesting the possibility that another common mechanism might be involved. A recent study reported a chain of events triggered by multiple splicing factor mutations, especially high-risk alleles in SRSF2 and U2AF1, including elevated R-loops, replication stress, and activation of the ataxia telangiectasia and Rad3-related protein (ATR)-Chk1 pathway [26]. Enhanced R-loops result from impaired transcription pause release, which are linked to compromised proliferation of bone marrow derived blood progenitors and the MDS phenotype. In spliceosomal-mutant MDS, several mRNA isoforms promoted by the various splicing-factor mutants harbor a PTC, and are therefore potential targets of NMD [4,8-11]. Therefore, NMD could be a potential mechanism of oncogenesis for several splicing factor mutations in MDS. Although links between alternative splicing and NMD have been proposed, evidence for a specific role of mutant splicing factors in the NMD pathway was lacking. It was reported that over expression of certain individual SR proteins (including SRSF2) enhances NMD [27]. Therefore, mutations in SRSF2 might have a direct regulation in NMD in addition to altering splicing, and it will be interesting to examine. These could be tested for other splicing factor mutations too, especially for SF3B1.

For therapeutic targeting of spliceosomal mutant MDS and related myeloid malignancies, splicing inhibitors show considerable promise in recent preclinical studies. The best-characterized splicing inhibitors can be represented into three major groups: FR901464, pladienolide and herboxidiene [28-30]. This inhibitors target the SF3b complex. One interesting example is E7107, a synthetic derivative of plandienolide D. Secondary leukemic Srsf2$^{P95H/+}$ mice treated with E7107 showed a significantly reduced leukemic burden and longer survival compared to leukemic Srsf2$^{+/+}$ mice [31]. In another study, it was shown that E7107 selectively kills SF3B1^{K700E}-expressing cells [11]. Another interesting example is H3B-8800, a novel orally bioavailable splicing modulator [32]. SF3B1^{K700E}- or SRSF2^{P95H}-knock-in isogenic AML cells treated with H3B-8800 showed preferential growth inhibition compared to wild-type counterparts [32]. Additionally, H3B-8800 inhibited tumor growth in mice xenografted with SF3B1^{K700E}-knock-in K562 cells and reduced the leukemic burden in mice xenografted with CD34$^+$ cells from SRSF2 mutant CMML patients [32]. Another promising strategy is antisense technology to modulate splicing. As for example, antisense oligonucleotides (ASOs) drug developed to correct a splicing defect in the *SMN2* gene in spinal muscular atrophy (SMA) had striking results in phase-3 clinical trials [33-35]. For PTC-harboring transcripts, pharmacological suppression of PTCs by translational read-through-inducing drugs (TRIDs) could be nice strategy [36]. However, the efficacy of TRIDs often compromised by NMD [37]. Suppression of the NMD pathway by inhibiting NMD machinery may exert detrimental consequences by causing general inhibition of the NMD. An alternative approach is gene-specific NMD inhibition. Recently, a promising approach has been reported combining ASOs and TRIDs to effectively restore the expression of full-length protein from a nonsense-mutant allele [38]. More mechanistic analyses on spliceosomal mutant MDS will enable more progress to develop effective therapy with high efficacy and minimum toxicity

References

1. Kennedy JA, Ebert BL (2017) Clinical implications of Genetic mutations in Myelodysplastic syndrome. J Clin Oncol 35(9): 968-974.

2. Yoshida K, Sanada M, Shiraishi Y, Nowak D, Nagata Y, et al. (2011) Frequent pathway mutations of splicing machinery in myelodysplasia. Nature 478(7367): 64-69.

3. Thol F, Kade S, Schlarmann C, Loffeld P, Morgan M, et al. (2012) Frequency and prognostic impact of mutations in SRSF2, U2AF1, and ZRSR2 in patients with myelodysplastic syndromes. Blood 119(15): 3578-3584.

4. Kim E, Ilagan JO, Liang Y, Daubner GM, Lee SC, et al. (2015) SRSF2 Mutations Contribute to Myelodysplasia by Mutant-Specific Effects on Exon Recognition. Cancer Cell 27(5): 617-630.

5. Komeno Y, Huang YJ, Qiu J, Lin L, Xu Y, et al. (2015) SRSF2 Is Essential for Hematopoiesis, and Its Myelodysplastic Syndrome-Related Mutations Dysregulate Alternative Pre-mRNA Splicing. Mol Cell Biol 35(17): 3071-3082.

6. Zhang J, Lieu YK, Ali AM, Penson A, Reggio KS, et al. (2015) Disease-associated mutation in SRSF2 misregulates splicing by altering RNA-binding

Rahman MA. Oncogenic Landscapes of Splicing-Factor Mutant MDS. Genom & Gene Ther Int J 2018, 2(1): 000105.

Copyright© Rahman MA.

affinities. Proc Natl Acad Sci USA 112(34): E4726-E4734.

7. Papaemmanuil E, Gerstung M, Malcovati L, Tauro S, Gundem G, et al. (2013) Clinical and biological implications of driver mutations in myelodysplastic syndromes. Blood 122(22): 3616-3627.

8. Darman RB, Seiler M, Agrawal AA, Lim KH, Peng S, et al. (2015) Cancer-Associated SF3B1 Hotspot Mutations Induce Cryptic 3' Splice Site Selection through Use of a Different Branch Point. Cell Rep 13(5): 1033-1045.

9. DeBoever C, Ghia EM, Shepard PJ, Rassenti L, Barrett CL, et al. (2015) Transcriptome Sequencing Reveals Potential Mechanism of Cryptic 3' Splice Site Selection in SF3B1-mutated Cancers. PLoS Comput Biol 11(3): e1004105.

10. Dolatshad H, Pellagatti A, Liberante FG, Llorian M, Repapi E, et al. (2016) Cryptic splicing events in the iron transporter ABCB7 and other key target genes in SF3B1-mutant myelodysplastic syndromes. Leukemia 30(12): 2322-2331.

11. Obeng EA, Chappell RJ, Seiler M, Chen MC, Campagna DR, et al. (2016) Physiologic Expression of Sf3b1K700E Causes Impaired Erythropoiesis, Aberrant Splicing, and Sensitivity to Therapeutic Spliceosome Modulation. Cancer Cell 30(3): 404-417.

12. Conte S, Katayama S, Vesterlund L, Karimi M, Dimitriou M, et al. (2015) Aberrant splicing of genes involved in haemoglobin synthesis and impaired terminal erythroid maturation in SF3B1 mutated refractory anaemia with ring sideroblasts. Br J Haematol, 171(4): 478-490.

13. Visconte V, Avishai N, Mahfouz R, Tabarroki A, Cowen J, et al. (2015) Distinct iron architecture in SF3B1-mutant myelodysplastic syndrome patients is linked to an SLC25A37 splice variant with a retained intron. Leukemia 29(1): 188-195.

14. Furney SJ, Pedersen M, Gentien D, Dumont AG, Rapinat A, et al. (2013) SF3B1 mutations are associated with alternative splicing in uveal melanoma. Cancer Discov 3(10): 1122-1129.

15. Maguire SL, Leonidou A, Wai P, Marchio C, Ng CK, et al. (2015) SF3B1 mutations constitute a novel therapeutic target in breast cancer. J. Pathol., 235(4): 571-580.

16. Graubert TA, Shen D, Ding L, Okeyo-Owuor T, Lunn CL, et al. (2012) Recurrent mutations in the U2AF1 splicing factor in myelodysplastic syndromes. Nat Genet 44(1): 53-57.

17. Przychodzen B, Jerez A, Guinta K, Sekeres MA, Padgett R, et al. (2013) Patterns of missplicing due to somatic U2AF1 mutations in myeloid neoplasms. Blood 122(6): 999-1006.

18. Brooks AN, Choi PS, De Waal L, Sharifnia T, Imielinski M, et al. (2014) A pan-cancer analysis of transcriptome changes associated with somatic mutations in U2AF1 reveals commonly altered splicing events. PLoS One 9(1): e87361.

19. llagan JO, Ramakrishnan A, Hayes B, Murphy ME, Zebari AS, et al. (2015) U2AF1 mutations alter splice site recognition in hematological malignancies. Genome Res 25(1): 14-26.

20. Okeyo-Owuor T, White BS, Chatrikhi R, Mohan DR, Kim S, et al. (2015) U2AF1 mutations alter sequence specificity of pre-mRNA binding and splicing. Leukemia, 29(4): 909-917.

21. Yip BH, Steeples V, Repapi E, Armstrong RN, Llorian M, et al. (2017) The U2AF1S34Fmutation induces lineage-specific splicing alterations in myelodysplastic syndromes. J Clin Invest 127(6): 2206-2221.

22. Bejar R, Stevenson K, Wahab OA, Galili N, Nilsson BB, et al. (2011) Clinical effect of point mutations in myelodysplastic syndromes. N Engl J Med 364: 2496-2506.

23. Borel C, Dastugue N, Cances Lauwers V, Mozziconacci MJ, Prebet T, et al. (2012) PICALM-MLLT10 acute myeloid leukemia: A French cohort of 18 patients. Leuk Res 36(11): 1365-1369.

24. Chia NY, Chan YS, Feng B, Lu X, Orlov YL, et al. (2010) A genome-wide RNAi screen reveals determinants of human embryonic stem cell identity. Nature 468(7321): 316-320.

25. Gaspar Maia A, Qadeer ZA, Hasson D, Ratnakumar K, Adrian Leu N, et al. (2013) Macro H2A histone variants act as a barrier upon reprogramming towards pluripotency. Nat Commun 4: 1565.

26. Chen L, Chen JY, Huang YJ, Gu Y, Qiu J, et al. (2018) The Augmented R-Loop Is a Unifying Mechanism for

Myelodysplastic Syndromes Induced by High-Risk Splicing Factor Mutations. Mol Cell 69(3): 412-425.e6.

27. Zhang Z, Krainer AR (2004) Involvement of SR proteins in mRNA surveillance. Mol Cell 16(4): 597-607.

28. Sakai T, Asai N, Okuda A, Kawamura N, Mizui Y (2004) Pladienolides, New Substances from Culture of Streptomyces platensis Mer-11107 II. Physico-chemical Properties and Structure Elucidation. J Antibiot (Tokyo) 57(3): 180-187.

29. Nakajima H, Hori Y, Terano H, Okuhara M, Manda T, et al. (1996) New antitumor substances, FR901463, FR901464 and FR901465. II. Activities against experimental tumors in mice and mechanism of action. J Antibiot (Tokyo) 49(12): 1204-1211.

30. Armstrong RN, Steeples V, Singh S, Sanchi A, Boultwood J, et al. (2018) Splicing factor mutations in the myelodysplastic syndromes: target genes and therapeutic approaches. Adv Biol Regul 67: 13-29.

31. Lee SCW, Dvinge H, Kim E, Cho H, Micol JB, et al. (2016) Modulation of splicing catalysis for therapeutic targeting of leukemia with mutations in genes encoding spliceosomal proteins. Nat Med 22(6): 672-678.

32. Buonamici S, Yoshimi A, Thomas M, Seiler M, Chan B, et al. (2016) H3B-8800, an Orally Bioavailable Modulator of the SF3b Complex, Shows Efficacy in Spliceosome-Mutant Myeloid Malignancies. Blood 128: 966.

33. Hua Y, Vickers TA, Baker BF, Bennett CF, Krainer AR (2007) Enhancement of SMN2 exon 7 inclusion by antisense oligonucleotides targeting the exon. PLoS Biol 5(4): e73.

34. Rigo F, Hua Y, Krainer AR, Bennett CF (2012) Antisense-based therapy for the treatment of spinal muscular atrophy. J Cell Biol 199(1): 21-25.

35. Hua Y, Sahashi K, Hung G, Rigo F, Passini MA, et al. (2010) Antisense correction of SMN2 splicing in the CNS rescues necrosis in a type III SMA mouse model. Genes Dev 24(15): 1634-1644.

36. Goldmann T, Overlack N, Moller F, Belakhov V, van Wyk M, et al. (2012) A comparative evaluation of NB30, NB54 and PTC124 in translational read-through efficacy for treatment of an USH1C nonsense mutation. EMBO Mol Med 4(11): 1186-1199.

37. Keeling KM, Wang D, Dai Y, Murugesan S, Chenna B, et al. (2013) Attenuation of Nonsense-Mediated mRNA Decay Enhances In Vivo Nonsense Suppression. PLoS One 8(4): e60478.

38. Nomakuchi TT, Rigo F, Aznarez I, Krainer AR (2016) Antisense oligonucleotide-directed inhibition of nonsense-mediated mRNA decay. Nat Biotechnol 34(2): 164-166.

Rahman MA. Oncogenic Landscapes of Splicing-Factor Mutant MDS. Genom & Gene Ther Int J 2018, 2(1): 000105.

Copyright© Rahman MA.