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Charting the "Splice" Routes to MDS

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Mutations in components of the 3′ mRNA splicing machinery are found in almost 50% of myelodysplastic syndrome (MDS) cases. In this issue of *Cancer Cell*, Kim and colleagues, Colla and colleagues, and Shirai and colleagues report on the impact of mutated or dysregulated splicing factors to hematopoiesis, mRNA splicing, and MDS pathogenesis.

Among the most unexpected findings of unbiased genome and exome sequencing studies in cancer has been the identification of highly recurrent somatic mutations in genes encoding components of the 3′ pre-mRNA splicing machinery in myelodysplastic syndrome (MDS), chronic lymphocytic leukemia, and various solid tumors (Papaemmanuil et al., 2011; Rossi et al., 2011; Yoshida et al., 2011). The spliceosome is composed of five small nuclear ribonucleoproteins (snRNPs) and over 200 proteins (Wahl et al., 2009). It regulates the alternative splicing of over 95% of multiexonic genes and is essential for normal cellular development. Although intron-exon splice site boundaries are short and degenerate, the precise RNA-RNA, RNA-protein, and protein-protein interactions that are orchestrated by normal spliceosome assembly and function lead to tissue-specific gene expression of particular transcripts.

In MDS, somatic mutations in components of the 3′ pre-mRNA splicing machinery commonly alter specific "hot spot" amino acids and often occur as early pathogenic events. The mutations are invariably heterozygous and are mutually exclusive with other spliceosome mutations. Three reports in this issue of *Cancer Cell* characterize the effects of dysregulation of specific splicing factors on normal hematopoiesis. Kim et. al. (2015) generated a hematopoietic-specific conditional *Srsf2* knock-in mouse with the most common *SRSF2* mutation, P95H (*Srsf2*^{P95H}); Shirai et. al. (2015) made a doxycycline-inducible transgenic mouse model of the most common *U2AF1* mutation, S34F (*U2AF1*^{S34F}); and Colla et. al. (2015) used an inducible telomerase reverse transcriptase-estrogen receptor (*TERT*^{ER}) murine model to demonstrate that telomerase deficiency induces myelodysplasia by dysregulating expression of wild-type *Srsf2* as well as other 3′ spliceosome components.

SRSF2 is a member of the serine/arginine-rich protein family. It promotes exon recognition by binding to exonic splicing enhancer sequences (ESEs) near the 5′ or 3′ splice site through its RNA recognition motif domain (RRM). U2AF1 binds to AG dinucleotides at the 3′ end of the intron and assists in recruitment of the U2 snRNP for spliceosome activation. Telomerase function had not previously been linked to splicing dysregulation in MDS.

An initial question is whether the genetic lesions studied induce the hematologic phenotype of MDS. All of the models have differentiation defects consistent with MDS, each with a distinct hematologic phenotype (Figure 1). *Srsf2*^{P95H} mice develop leukopenia and a macrocytic anemia with bone marrow dysplasia and increased hematopoietic stem cells. *U2AF1*^{S34F} transgenic mice developed leukopenia, without anemia or dysplasia, and increased hematopoietic stem and myeloid progenitor cells. Fourth and fifth generation *TERT*$^{ER/ER}$ mice develop leukopenia with marked lymphopenia, mild anemia, a hypercellular bone marrow with trilineage dysplasia, increased myeloid blasts, and increased hematopoietic stem and myeloid progenitor cells. Whereas neither the *Srsf2*^{P95H} nor the *U2AF1*^{S34F} mice developed acute myeloid leukemia (AML), approximately 5% of the *TERT*$^{ER/ER}$ mice developed AML. In all models, mutant stem cells have a competitive disadvantage when compared to wild-type stem cells in competitive repopulation assays.

A second question is whether the mutations have specific effects on mRNA splicing. Each group of investigators compared the transcriptomes of hematopoietic cells from their murine models to human AML cell lines and/or MDS or AML patient samples and identified changes in exon recognition or decreased spliceosome protein expression. Kim et al. (2015) analyzed RNA sequencing data to identify motifs associated with mutant-specific splicing alterations and found that mutant SRSF2 has altered, rather than a loss of normal, ESE recognition activity. Wild-type SRSF2 recognizes the consensus binding sequences CCNG and GGNG with similar affinity (Daubner et al., 2012), whereas SRSF2^{P95H} appears to preferentially bind CCNG within exons that were differentially spliced. Similarly, Shirai et al. (2015) analyzed RNA sequencing data to identify a splice junction sequence-specific pattern of altered splicing induced by mutant U2AF1. Exons skipped more frequently and alternative splice sites used more often than canonical splice sites by *U2AF1*^{S34F} were enriched for a uracil in the minus 3 position relative to the AG dinucleotide. These findings are consistent with published reports of mutant *U2AF1*-associated splicing abnormalities in lung cancer and myeloid malignancies (Brooks et al., 2014). Colla et al. (2015) found that telomerase dysfunction caused decreased expression of genes involved in 3′-mRNA splice site recognition and that this was associated with abnormal splicing. 40% of the aberrant splicing events in *TERT*$^{ER/ER}$ cells resulted in exon skipping, and 59.5% of aberrant splicing events resulted in exon retention.

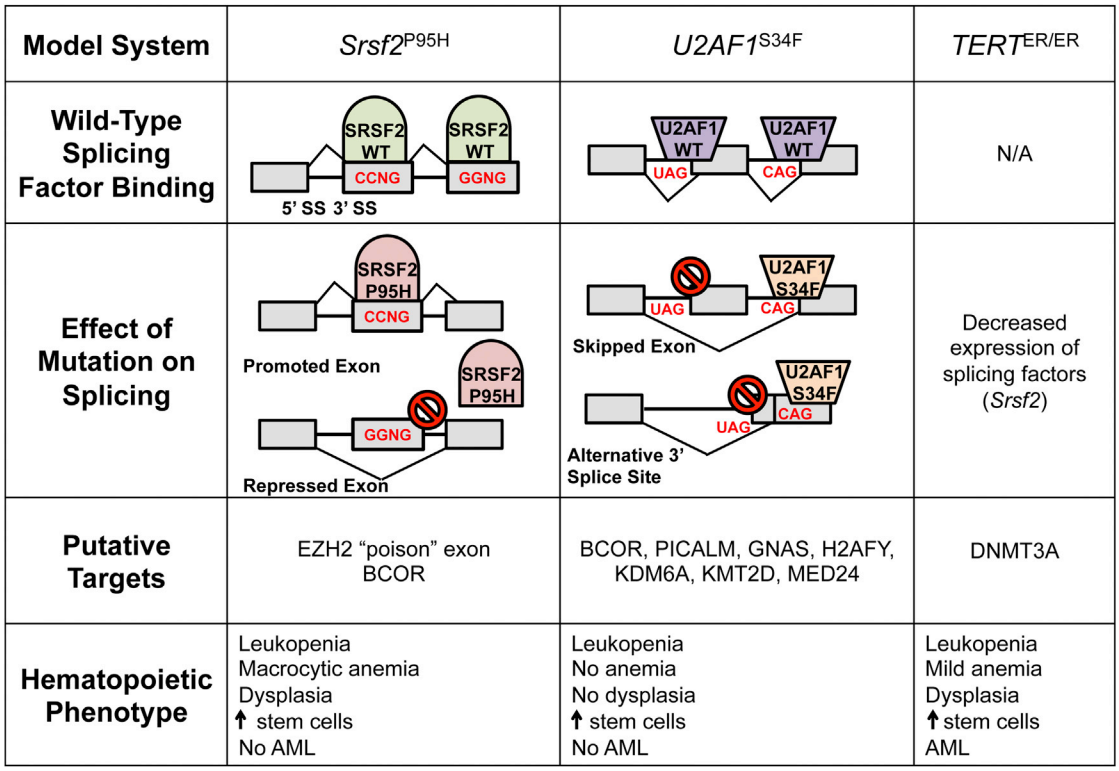

Figure 1. Summary of the Splicing Alterations and Hematopoietic Findings in Three Mouse Models of Altered Splicesosome Protein Function
Mouse models of mutant *Srsf2* and *U2AF1* identified mutant-specific binding preferences and alternative splicing events promoted by the mutant proteins as shown. $TERT^{ER/ER}$ mice have decreased expression of several splicing factors including SRSF2. MDS-associated genes reported to be aberrantly spliced in each model system and the effects of each mutation on hematopoiesis are also indicated. 5′ ss, 5′ splice site; 3′ ss, 3′ splice site.

The biochemical alterations in spliceosome activity were studied in most detail by Kim et al. (2015), who transcriptionally profiled cell lines with expression of wild-type or mutant SRSF2 or cells with shRNA-mediated decreased expression of SRSF2. They found that knock-down of SRSF2 results in preferential skipping of exons with either C- or G-rich variants of the consensus motif, overexpression of wild-type SRSF2 results in inclusion of exons with enrichment of both C- and G-rich consensus motifs, and expression of mutant SRSF2 results in enrichment of CCNG in included exons and enrichment of GGNG in excluded exons. They further showed that these differences are due to changes in SRSF2:RNA interactions using isothermal titration calorimetry to examine the binding affinity of purified RNA ligands to purified RRM from wild-type or P95 mutant SRSF2. Consistent with their motif analysis, P95 mutants have an increased binding affinity for CCNG-containing RNA ligands and a decreased binding affinity for GGNG-containing RNA ligands compared to wild-type SRSF2.

An overarching question is whether the oncogenic impact of mutant splicing factor genes is caused by aberrant splicing of one specific gene in particular, the integrated effects of altered splicing of a large number of genes, or a biological consequence of the mutations that is independent of mRNA splicing. In the case of *SRSF2* mutations, Kim et al. (2015) showed that one functional consequence of mis-splicing in *SRSF2* mutant cells is the inclusion of a "poison" exon in the mRNA of *EZH2*, which encodes a histone H3K27 methyltransferase and is commonly mutated in MDS (Nikoloski et al., 2010). This "poison" exon contains the CCNG motif required for robust splicing by mutant SRSF2 and introduces a premature termination codon in *EZH2* mRNA that results in nonsense-mediated decay of the *EZH2* transcript. They demonstrated that EZH2 protein and H3K27me3 levels are lower in *SRSF2* mutant cells and that *EZH2* loss-of-function mutations are mutually exclusive with *SRSF2* mutations in MDS. They also showed that overexpression of properly spliced *EZH2* cDNA in progenitor cells from $Srsf2^{P95H}$ mice partially rescues the hematopoietic defect induced by mutant SRSF2 in methylcellulose colony assays. Similarly, mutant U2AF1-induced splice isoform changes occur in disease-relevant genes including *BCOR*, *GNAS*, and *PICALM*. Among other alterations in splicing, Colla et al. (2015) found telomerase dysfunction caused aberrant splicing of *Dnmt3a*.

In aggregate, the findings described in these papers are a major advance toward understanding how alterations in the spliceosome alter the transcriptome, hematopoietic differentiation, and hematopoietic stem cell function in MDS. The three studies describe genetic lesions affecting spliceosome function that lead to distinct abnormalities in mRNA splicing and features of MDS. Further studies using these and other models of MDS will help to identify functionally important targets of mis-splicing as well as the genes that cooperate with spliceosome mutations in MDS. Therapeutic agents are

currently in development to target aberrant spliceosome function in MDS and other malignancies, and these models will be invaluable for pre-clinical testing of these drugs.

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Functionally Relevant RNA Helicase Mutations in Familial and Sporadic Myeloid Malignancies

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In this issue of *Cancer Cell*, Polprasert and colleagues identified recurrent mutations in the DEAD/H-box RNA helicase gene *DDX41* in familial and acquired cases of myelodsyplasia and acute myeloid leukemia. These mutations induce defects in RNA splicing and represent a new class of mutations in myeloid malignancies.

The familial myelodysplastic (MDS)/acute myeloid leukemia (AML) syndromes belong to a group of rare inherited disorders that represent a unique resource to study the initial steps toward leukemia progression. Recent findings indicate that AML occurs after a stepwise process of genetic and epigenetic changes, some of which are ancestral and are found in a preleukemic clone that precedes the fully transformed AML cells bearing additional alterations (Pandolfi et al., 2013). In familial MDS/AML, the initial germline mutation can be considered a preleukemic event, required but not sufficient for leukemia initiation. Among the most studied ones are familial AML with mutated *CEBPA*, familial MDS/AML with *GATA2* mutations, and familial platelet disorder with predisposition to AML (FPD/AML) due to *RUNX1* mutations (West et al., 2014). Interestingly, mutations in these transcription factors also play important roles in sporadic AML, showing that, in principle, both germline and acquired mutations of the same genes can deregulate the hematopoietic compartment and lead to AML development. Thus, the study of patients with familial MDS/AML has proven to be extremely valuable for understanding the multi-step leukemogenic process (Antony-Debré et al., 2015), including in sporadic leukemias.

The advent of whole exome sequencing technology is of great interest for the identification of new mutations in familial MDS/AML syndromes, because the genetic origin of many of them remains unknown. A better knowledge of these syndromes would be beneficial not only for research, but also for clinical care, as individuals harboring familial mutations could be identified, e.g., for more frequent follow-up examinations or for exclusion when a bone marrow allograft is considered. In this issue of *Cancer Cell*, Polprasert et al. (2015) identified functionally relevant mutations in the gene coding for the DEAD/H-box RNA helicase DDX41 in both familial and sporadic MDS/AML cases. Initially, they discovered inherited *DDX41* mutations in several MDS/AML pedigrees and showed that additional somatic *DDX41* mutations of the remaining wild-type allele were present at MDS/AML diagnosis in half of the cases (Figure 1A). This is similar to what has been observed in MDS/AML with *CEBPA* or *RUNX1* mutations, where biallelic mutations are found with AML progression (Pabst et al., 2008; Preudhomme et al., 2009). The clonal architecture of leukemic clones revealed that acquired *DDX41* mutations could be a founder event in some patients. Familial DDX41 MDS/AML syndrome was characterized by a long latency, an advanced stage at diagnosis (high risk MDS/AML), normal