pRCC-2, mutations in *FH* have not been detected in sporadic pRCC-2, and sporadic pRCC-2 tumors do not accumulate succinated proteins (Bardella et al., 2011). Thus, how antioxidant genes are upregulated in sporadic pRCC-2 remains unclear.

The contribution of antioxidant genes to renal tumorigenesis in patients with HLRCC requires further exploration. However, there is precedent implicating Keap1 and Nrf2 in tumor development. Somatically acquired loss-of-function *KEAP1* mutations have been found in tumors (Taguchi et al., 2011). Nrf2 is also stabilized in tumors by mutation in either of the two motifs involved in Keap1 binding that are necessary for its degradation (Taguchi et al., 2011). Consistent with the notion that these two proteins function in concert, mutations in *KEAP1* and *NRF2* tend not to be observed together in the same tumor (Taguchi et al., 2011).

Understanding the biology of HLRCC-associated pRCC-2 will hopefully lead to new therapies. Because *FH* is lost in tumor cells, and this truncates the TCA cycle, these tumors may be exquisitely dependent on glycolysis for energy generation. As determined by 2-deoxy-2-(^{18}F)fluoro-D-glucose (FDG) positron emission tomography (PET), FH-deficient tumors take up large amounts of glucose (Yamasaki et al., 2011). Although exploiting this dependency may be challenging (Yamasaki et al., 2011), this may offer an opportunity for therapeutic intervention.

Recently, HMOX1, which is required for heme synthesis and is upregulated in FH-deficient tumors, was proposed as a therapeutic target (Frezza et al., 2011). FH-deficient cells maintain segmental TCA cycle function and NADH generation by using glutamine and disposing of excess carbon through the synthesis of heme and its excretion from the cell as bilirubin (Frezza et al., 2011). Interestingly, inhibition of HMOX1 is synthetically lethal with FH deficiency. However, the selectivity of this approach in patients remains to be determined, particularly because HLRCC patients are heterozygous for *FH*, and mutant FH may be incorporated into FH homotetramers, markedly reducing FH activity in non-neoplastic cells. Nonetheless, this concept deserves to be studied further in primary xenografts and, subsequently, in patients.

REFERENCES

Adam, J., Hatipoglu, E., O'Flaherty, L., Ternette, N., Sahgal, N., Lockstone, H., Baban, D., Nye, E., Stamp, G.W., Wolhuter, K., et al. (2011). Cancer Cell *20*, this issue, 524–537.

Bardella, C., El-Bahrawy, M., Frizzell, N., Adam, J., Ternette, N., Hatipoglu, E., Howarth, K., O'Flaherty, L., Roberts, I., Turner, G., et al. (2011). J. Pathol. *225*, 4–11.

Frezza, C., Zheng, L., Folger, O., Rajagopalan, K.N., MacKenzie, E.D., Jerby, L., Micaroni, M., Chaneton, B., Adam, J., Hedley, A., et al. (2011). Nature *477*, 225–228.

Hayes, J.D., McMahon, M., Chowdhry, S., and Dinkova-Kostova, A.T. (2010). Antioxid. Redox Signal. *13*, 1713–1748.

Ogura, T., Tong, K.I., Mio, K., Maruyama, Y., Kurokawa, H., Sato, C., and Yamamoto, M. (2010). Proc. Natl. Acad. Sci. USA *107*, 2842–2847.

Ooi, A., Wong, J.-C., Petillo, D., Roossien, D., Perrier-Trudova, V., Whitten, D., Wong Hui Min, B., Tan, M.-H., Zhang, Z., Yang, X.J., et al. (2011). Cancer Cell *20*, this issue, 511–523.

Taguchi, K., Motohashi, H., and Yamamoto, M. (2011). Genes Cells *16*, 123–140.

Tomlinson, I.P., Alam, N.A., Rowan, A.J., Barclay, E., Jaeger, E.E., Kelsell, D., Leigh, I., Gorman, P., Lamlum, H., Rahman, S., et al; Multiple Leiomyoma Consortium. (2002). Nat. Genet. *30*, 406–410.

Yamasaki, T., Tran, T.A., Oz, O.K., Raj, G.V., Schwarz, R.E., Deberardinis, R.J., Zhang, X., and Brugarolas, J. (2011). Nat. Rev. Urol. *8*, 165–171.

Zhuang, M., Calabrese, M.F., Liu, J., Waddell, M.B., Nourse, A., Hammel, M., Miller, D.J., Walden, H., Duda, D.M., Seyedin, S.N., et al. (2009). Mol. Cell *36*, 39–50.

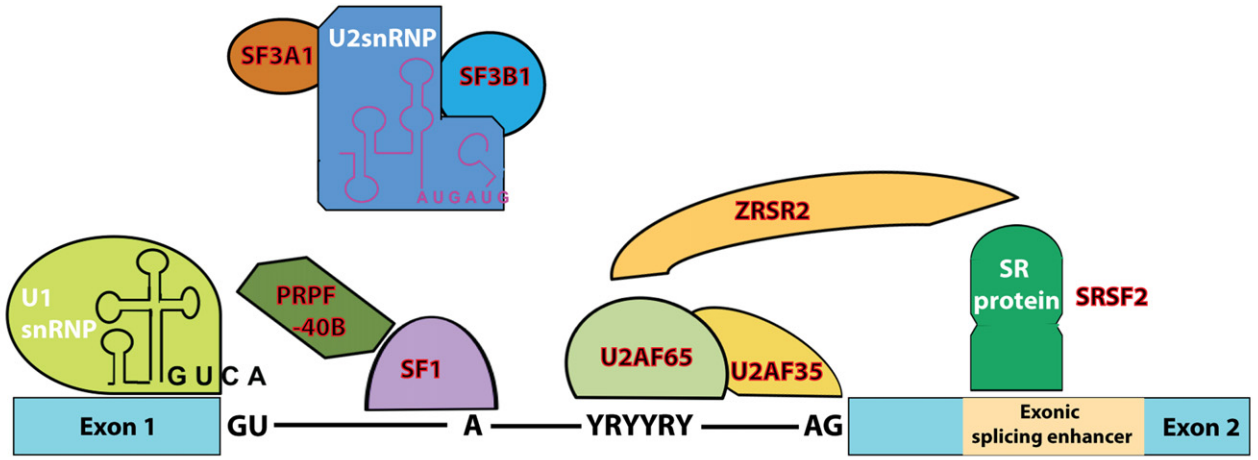
The Spliceosome as an Indicted Conspirator in Myeloid Malignancies

Omar Abdel-Wahab[1] **and Ross Levine**[1,*]
[1]Human Oncology and Pathogenesis Program, Leukemia Service, Memorial Sloan-Kettering Cancer Center, Weill Cornell Medical College, New York, NY 10065, USA
*Correspondence: leviner@mskcc.org
DOI 10.1016/j.ccr.2011.10.004

Reports of whole-exome sequencing in myelodysplastic syndrome (MDS) patients by Yoshida et al. and Papaemmanuil et al. suggest spliceosome mutations have clinical relevance. Identifying the impact of these mutations on MDS pathogenesis holds promise for therapeutic modulation of mRNA splicing.

The myelodysplastic syndromes (MDSs) are a heterogeneous group of myeloid malignancies characterized by clonal hematopoiesis, impaired differentiation, peripheral blood cytopenias, and increased risk of progression to acute myeloid leukemia. Although recent studies have identified recurrent somatic mutations in most patients with MDS, approximately 20% of patients with MDS had no known somatic genetic or cytogenetic abnormalities in the largest studies to date. Two recent studies report the results of whole-exome sequencing in patients with MDS (Papaemmanuil et al., 2011; Yoshida et al.,

A



B

Gene	Mutation Description	Mutational Frequency[1] RARS/ RCMD-RS	MDS without RS	CMML	t-MDS/ sAML	De novo AML	MPN	ALL[2]	NHL[2]	CLL[2]
SF3B1	Predominant heterozygous missense mutations at K700, R625, and H662.	57 - 75.3%	6 - 20%	4.5 - 5%	4.8%	2.6 - 5%	3 - 4%	nd	nd	5%
SRSF2	Recurrent heterozygous missense mutations at P95.	1.5%	11.6%	28.4%	6.5%	0.7%	1.9%	—	—	nd
U2AF35	Recurrent heterozygous missense mutations at S34 and Q157.	—	11.6%	8.0%	9.7%	1.3%	1.9%	—	—	nd
ZRSRS2	Missense, nonsense and frameshift mutations throughout the open-reading-frame.	1.4%	7.7%	8.0%	1.6%	0.7%	1.9%	nd	nd	nd
SF3A1	Missense mutations throughout the open-reading-frame.	—	1.3%	1.1%	1.6%	0.7%	—	nd	nd	nd
PRPF40B	Missense mutations throughout the open-reading-frame.	—	1.9%	—	1.6%	0.7%	1.9%	nd	nd	nd
U2AF65	Missense mutations throughout the open-reading-frame.	—	0.6%	1.1%	—	—	—	nd	nd	nd
SF1	Missense mutations in the proline-rich C-terminal domain.	—	1.3%	—	—	—	1.9%	nd	nd	nd

Figure 1. The Spliceosome and Mutations in Multiple Members of Genes Encoding Spliceosomal Proteins

Five small ribonuclear protein particles (snRNPs) and over 50 accessory proteins are assembled at the exon/intron junction of pre-mRNA to form the spliceosome. The U1 snRNP binds to the 5′ splice site through base pairing between the splice site and the U1 snRNA. The branchpoint, required for the lariat intermediate, is bound by SF1, whereas the polypyrimidine tract is bound by the large subunit of U2AF (U2AF65). The small subunit of U2AF (U2AF35) binds to the AG at the 3′ splice site. The WW domain protein PRPF40B is thought to bind SF1 and serve in early spliceosome assembly, but its functions are not well understood. Following U1 snRNP and U2AF assembly, the U2 snRNP, the U4-6 tri-snRNP, and other splicing factors are assembled sequentially to form the spliceosome. SF3B1 and SF3A1 are components of U2 snRNP, and it is thought that they bind pre-mRNA upstream of the intro branch site in a sequence-independent manner to anchor the U2 snRNP to pre-mRNA. Members of the SR protein family bind to a nearby exonic-splicing enhancer region to directly recruit splicing machinery through physical interactions with U2AF35 and ZRSR2 (a homolog of U2AF35). This interaction is critical in defining exon/intron boundaries. Members of the spliceosomal complex found to be mutated in myeloid malignancies are indicated in red in (A) and described in (B). ALL, acute lymphoblastic leukemia; CLL, chronic lymphocytic leukemia; CMML, chronic myelomonocytic leukemia; MPN, myeloproliferative neoplasms; NHL, non-Hodgkin lymphoma; RARS, refractory anemia with ring sideroblasts; RCMD-RS, refractory-cytopenia with multilineage dysplasia and ring sideroblasts; sAML, secondary AML; t-MDS, therapy-related MDS. In footnote 1, "–" indicates no mutations found, and "nd" indicates sequencing not done. In footnote 2, only regions of recurrent mutations were sequenced in these lymphoid malignancies.

2011). Notably, the most frequent novel recurrent mutations found occurred in genes encoding members of the RNA-splicing machinery (Figure 1).

The paradigm that alterations in splicing contribute to the pathogenesis of human disease and promote tumorigenesis is well described. However, the majority of disease-associated splicing abnormalities discovered previously were in *cis*-acting elements that disrupt splice-site selection at specific loci. By contrast the Papaemmanuil et al. (2011) and Yoshida et al. (2011) reports identified mutations in the *trans*-acting members of the spliceosome necessary for processing pre-mRNA to mature mRNA. The genetic data supporting these mutations as disease alleles are compelling; the majority of the mutations in *SF3B1* and all of the mutations in *U2AF35* and *SRSF2* are recurrent, heterozygous point mutations, suggesting a gain of function conferred by these recurrent mutations (Figure 1). In contrast rarer mutations in *ZRSR2* and *PRPF40B* occurred as missense or nonsense mutations, suggesting that these mutations might result in loss of function. In addition, Yoshida et al. (2011) found that spliceosomal gene mutations are largely mutually exclusive of one another, consistent with a general role of spliceosome mutations in MDS pathogenesis.

In order to understand the spectrum of spliceosomal gene mutations, both groups also sequenced a spectrum of myeloid malignancies in addition to MDS. These data led both groups to note a striking association between *SF3B1* mutations and MDS characterized by the presence of ring sideroblasts (RS). Although rare *SF3B1* mutations have been reported previously in epithelial cancers derived from pancreas (Pleasance et al., 2010), breast (Wood et al., 2007), and ovary (Wood et al., 2007), *SF3B1* mutations occur in the majority of patients with MDS with RS and much less commonly in other hematologic malignancies. Although mutations in the other spliceosomal components were more common in other subtypes of MDS, the mutations appear to be most enriched in myeloid malignancies with some component of dysplasia, including MDS of all subtypes and chronic myelomonocytic leukemia. Papaemmanuil et al. (2011) noted that mutations in *SF3B1* in MDS are associated with longer overall- and leukemia-free patient survival. Given the already-known favorable prognosis of MDS with RS, studies to identify whether the prognostic effect of these mutations is independent of MDS histopathologic findings are needed. Moreover, previous reports noting splicing alterations in hematologic malignancies, such as the report of frequent missplicing of GSK3β in CML (Abrahamsson et al., 2009), will need reevaluation to determine if these cancer-specific splicing alterations result from somatic mutations in the spliceosome.

To understand the biological consequences of spliceosomal mutations in hematopoiesis, the authors overexpressed wild-type and mutant forms of U2AF35 in $Lin^{-}Sca1^{+}c\text{-}Kit^{+}$ hematopoietic cells from wild-type mice. Competitive transplantation with similarly transduced control cells revealed a competitive disadvantage with U2AF35 mutant overexpression. Further work to characterize the effects of these mutations on other aspects of hematopoietic stem cell function including self-renewal, differentiation, and leukemogenesis are needed. Moreover, comparison of the biological effects of expression of recurrent point mutations with downregulation of expression may be very helpful in understanding the biological consequences of spliceosomal component alterations in neoplastic transformation.

The mutations in the spliceosomal complex in different myeloid malignancies suggest that these proteins may have distinct functions at different stages of hematopoietic differentiation. Very little is known about the expression of the various Serine/Arginine-rich (SR) proteins in normal and malignant hematopoiesis or about the function of the spliceosome in normal hematopoietic development. Numerous splicing factors have been targeted for constitutional knockout in mice, but these resulted in largely embryonic or perinatal lethality. Conditional gene targeting in a tissue-specific manner has only been carried out for *Srsf1* (Xu et al., 2005) and *Srsf2* (Ding et al., 2004) so far. Mice with cardiac-specific deletion of *Srsf1* develop severe dilated cardiomyopathy, leading to death by 6–8 weeks of life, whereas cardiac-specific *Srsf2* knockout mice develop a milder cardiomyopathy and have a relatively normal life span. These results suggest that the SR proteins fulfill specialized, nonredundant functions.

Data arguing for a role of spliceosomal components outside of pre-mRNA processing have also come from in vivo modeling. For instance, in vivo analysis of *Sf3b1* knockout mice identified genetic intersection with Polycomb Group protein loss, leading to the identification of multiple physical interactions between SF3B1 and members of the PRC1 complex and the BCL6 corepressive complex (Isono et al., 2005). Further work to analyze the role of disordered PRC1 activity and BCL6 activity in MDS-RS pathogenesis is now warranted.

Yoshida et al. (2011) assessed the effects of expressing U2AF35 in wild-type and mutant forms on gene expression and showed that overexpression of U2AF35 mutants led to a greater frequency of transcripts with unspliced introns and increased expression of members of the nonsense-mediated decay pathway. They concluded that *U2AF35* mutations, and possibly other spliceosomal pathway mutations, function in a dominant-negative manner to inhibit normal splicing, a hypothesis requiring further evaluation. Previous studies have noted overexpression of SR family proteins in epithelial cancers, and overexpression of SR proteins (including SRSF1 and SRSF2) leads to cellular transformation ability in other cellular contexts; as such, future studies will need to dissect differences between the role of mutant and wild-type spliceosome proteins in oncogenic transformation.

Identification of splicing factor mutations in MDS may also provide a possibility for therapeutic intervention. An excellent example comes from investigational therapies for the hereditary disorder Duchenne muscular dystrophy (DMD). DMD most commonly results from mutations in a repetitive domain of *Dystrophin*. Mutations in this domain can be overcome by "skipping" the mutated exon to generate truncated functional dystrophin protein. Amazingly, a strategy of delivering an antisense oligonucleotide to block an enhancer of exon splicing of the mutated exon and result in a stable mRNA transcript and dystrophin gene product has been utilized successfully in early clinical trials (van Deutekom et al., 2007). In addition, compounds that specifically target the SF3A/B subunits of U2 snRNP to result in nuclear export of intron-bearing precursors exist and should be studied further to determine if they interfere with the aberrant splicing due to recurrent mutations in these subunits (Kaida et al., 2007). These data suggest that these two studies have uncovered a novel pathway of importance to myeloid malignancies that may lead to novel therapeutic approaches for patients with MDS.

REFERENCES

Abrahamsson, A.E., Geron, I., Gotlib, J., Dao, K.H., Barroga, C.F., Newton, I.G., Giles, F.J., Durocher, J., Creusot, R.S., Karimi, M., et al. (2009). Proc. Natl. Acad. Sci. USA *106*, 3925–3929.

Ding, J.H., Xu, X., Yang, D., Chu, P.H., Dalton, N.D., Ye, Z., Yeakley, J.M., Cheng, H., Xiao, R.P., Ross, J., et al. (2004). EMBO J. *23*, 885–896.

Isono, K., Mizutani-Koseki, Y., Komori, T., Schmidt-Zachmann, M.S., and Koseki, H. (2005). Genes Dev. *19*, 536–541.

Kaida, D., Motoyoshi, H., Tashiro, E., Nojima, T., Hagiwara, M., Ishigami, K., Watanabe, H., Kitahara, T., Yoshida, T., Nakajima, H., et al. (2007). Nat. Chem. Biol. *3*, 576–583.

Papaemmanuil, E., Cazzola, M., Boultwood, J., Malcovati, L., Vyas, P., Bowen, D., Pellagatti, A., Wainscoat, J.S., Hellstrom-Lindberg, E., Gambacorti-Passerini, C., et al. (2011). N. Engl. J. Med. Published online September 26, 2011. 10.1056/NEJMoa1103283.