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# Aberrant RNA Splicing in Cancer

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## Abstract

RNA splicing, the enzymatic process of removing segments of premature RNA to produce mature RNA, is a key mediator of proteome diversity and regulator of gene expression. Increased systematic sequencing of the genome and transcriptome of cancers has identified a variety of means by which RNA splicing is altered in cancer relative to normal cells. These findings, in combination with the discovery of recurrent change-of-function mutations in splicing factors in a variety of cancers, suggest that alterations in splicing are drivers of tumorigenesis. Greater characterization of altered splicing in cancer parallels increasing efforts to pharmacologically perturb splicing and early-phase clinical development of small molecules that disrupt splicing in patients with cancer. Here we review recent studies of global changes in splicing in cancer, splicing regulation of mitogenic pathways critical in cancer transformation, and efforts to therapeutically target splicing in cancer.

### Keywords

RNA; SF3B1; splicing; SRSF2; U2AF1; ZRSR2

## INTRODUCTION

RNA splicing, the process of removing nucleotide sequences from precursor RNA to form mature RNA, is a key regulator of gene expression and proteome diversity. Recent analyses of genetic alterations in cancer and how these alterations relate to the transcriptome and epigenome have uncovered a myriad of means by which splicing is altered in cancer cells. These include mutations in DNA that abolish or generate splicing regulatory sequences in *cis,* mutations in genes encoding RNA splicing regulators, changes in the expression of

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splicing factors by oncogenic processes, and alterations in chromatin state that modify splicing patterns (Figure 1). Splicing is an enzymatic process requiring numerous protein-RNA complexes and posttranslational modifications (PTMs) of splicing proteins, as well as protein-protein, protein-RNA, and RNA-RNA interactions, providing a variety of avenues for pharmacologic perturbation. Here we review patterns of cancer-associated alternative splicing (AS) events, how cancer cells generate novel splicing events to promote disease, and increasing modalities to therapeutically target splicing in cancer.

## RNA SPLICING CATALYSIS AND REGULATION

Splicing is a catalytic process by which introns, noncoding segments of precursor messenger RNAs (pre-mRNAs), and long noncoding RNAs are removed to generate mature RNAs. Splicing is catalyzed by a large RNA-protein complex known as the spliceosome. Recent single-particle cryo-electron microscopy has allowed the characterization of spliceosomes that provide atomic model building. The biochemistry of splicing is covered in several recent reviews (Fica & Nagai 2017; Scheres & Nagai 2017; Shi 2017; Wahl & Luhrmann 2015a,b). Meanwhile, greater analyses of RNA sequencing (RNA-seq) data have elucidated sequences utilized by the spliceosome to differentiate introns from exons and identify branch point sequences. Consensus splice sites (ss) located at the boundaries between the upstream exon and intron (the 5′ ss) and the intron and downstream exon (the 3′ ss), as well as the branch point, are recognized by the spliceosome (Figure 1). The major spliceosome (which recognizes the majority of ss) consists of five small nuclear ribonucleoprotein (snRNP) complexes and more than 150 proteins, while the minor spliceosome (which recognizes a minority of introns after the snRNP complex specialized to recognize these sequences) contains five snRNPs, most of which are distinct from those of the major spliceosome. All ss are either constitutive or alternative, depending on whether they are always (constitutive) or only sometimes (alternative) recognized by the spliceosome.

Although splicing of constitutive and alternative ss is determined by the spliceosome, efficient recruitment of spliceosomal proteins to ss depends on the binding of additional *trans*-acting splicing factors [reviewed recently by Dvinge et al. (2016) and Scotti & Swanson (2016)]. Such regulatory splicing factors bind to motifs associated with the promotion (enhancers) or repression (silencers) of splicing (Figure 1). Enhancer and silencer motifs are recognized by two common families of splicing factors: serine- and arginine-rich splicing factors (SR proteins) (Long & Caceres 2009) and heterogeneous nuclear ribonucleoproteins (hnRNPs) (Krecic & Swanson 1999). In exons, SR proteins bind enhancer sequences to activate splicing, whereas hnRNPs bind silencer sites to inhibit splicing. However, the activities of splicing factors are context dependent (Fu & Ares 2014). In addition to SR proteins and hnRNPs, many other RNA-binding proteins regulate splicing. These include CELF (Dasgupta & Ladd 2012), MBNL (Konieczny et al. 2014), RBFOX, RBM (Sutherland et al. 2005), STAR, NOVA (Ule et al. 2006), epithelial splicing regulatory proteins, TIA1, TIAL1, and others (Fu & Ares 2014). Proteomic studies indicate that the spliceosome consists of more than 170 proteins (Jurica & Moore 2003), while computational studies of exon recognition suggest that hundreds of motifs contribute to the regulation of splicing (Barash et al. 2010). Although the total number of splicing factors is unknown, the above studies suggest that hundreds of proteins regulate splicing.

## GLOBAL ALTERATIONS IN RNA SPLICING IN CANCER

Efforts to analyze the transcriptomes of tumor versus normal tissue, such as The Cancer Genome Atlas, have revealed that many cancers exhibit aberrant splicing (Climente-Gonzalez et al. 2017, Jayasinghe et al. 2018, Jung et al. 2015, Kahles et al. 2018, Supek et al. 2014), including changes in usage of annotated transcript isoforms and increased use of aberrant unannotated splicing events. One recent effort characterized the abundance of annotated transcript isoforms across 4,542 samples from 11 cancer types and identified that splicing changes in cancer impact the same protein-coding domains targeted by somatic mutations (Climente-Gonzalez et al. 2017). Moreover, the number of AS changes in a tumor was inversely correlated with the number of driver mutations, and AS switches displayed some mutual exclusion with driver mutations, suggesting that AS may serve as independent tumorigenic processes. Further efforts revealed that tumors exhibit a ~20% increase in novel splicing events and exon-exon junctions, many of which are specific to cancer type (Kahles et al. 2018).

Aberrant splicing in cancer has also been linked to DNA mutations that abolish ss or generate novel ss. The largest effort yet to characterize mutations altering ss in *cis* utilized whole-exome sequencing of more than 8,000 tumors across 33 cancer types and identified that many mutations that alter ss were previously misannotated as missense or silent mutations (Jayasinghe et al. 2018). Given that critical regulatory splice sequences are far from the consensus 5′ or 3′ ss, it is important to further integrate data from whole-genome sequencing with RNA-seq for a more comprehensive model of how cancer-associated mutations impact splicing in *cis*.

## RECURRENT CHANGE-OF-FUNCTION MUTATIONS IN SPLICEOSOMAL GENES IN CANCER

Recurrent heterozygous change-of-function mutations affecting specific residues (or hot spots) in splicing factors have been described in cancer (Figure 2) (Graubert et al. 2012, Papaemmanuil et al. 2011, L. Wang et al. 2011, Yoshida et al. 2011). SF3B1, a subunit of the U2 snRNP that recognizes the branch point, is the most commonly mutated splicing factor in cancer, occurring frequently in myelodysplastic syndromes (MDS), chronic lymphocytic leukemia (CLL), acute myeloid leukemia (AML), and uveal and mucosal melanoma (Alsafadi et al. 2016, Furney et al. 2013, Harbour et al. 2013, Martin et al. 2013), in addition to many carcinomas (Seiler et al. 2018a). Several *SF3B1*-mutant residues are enriched in specific disease subtypes. For example, mutations at R625 and E902 appear specific to uveal melanoma (UM) and bladder cancer, respectively (Seiler et al. 2018a). *SF3B1* mutational hot spots occur within the HEAT domains [a repeated motif consisting of two alpha helices linked by a short loop found in Huntingtin, elongation factor 3 (EF3), PP2A, and yeast TOR1] and possibly affect protein-protein interactions. Global changes in splicing have been observed in cells harboring *SF3B1* mutations and are also seen in mouse cells upon introduction of the *Sf3b1*$^{K700E}$ mutation (Mupo et al. 2017, Obeng et al. 2016). Cancer-associated *SF3B1* mutations have repeatedly been found to alter 3′ ss via preference of intron-proximal cryptic 3′ ss over normal sites (Alsafadi et al. 2016, Darman et al. 2015,

DeBoever et al. 2015). The aberrant 3′ ss utilized by SF3B1 are associated with shorter, weaker polypyrimidine tracts and are linked to aberrant branch point usage by the mutant SF3B complex. The mechanism governing change in *SF3B1* 3′ ss or the use of aberrant branch points is not fully understood. One possibility is that SF3B1 mutations result in altered interaction with other spliceosomal components required for branch point recognition. To this end, it has been demonstrated that the introduction of SF3B1 mutations in yeast (homolog Hsh155p) alters the physical interaction with Prp5p (DDX46 in humans), an ATP-dependent RNA helicase important in stabilizing the U2 snRNP/pre-mRNA interaction (Carrocci et al. 2017, Tang et al. 2016). Confirmation of physical interactions between mutant SF3B1 and DDX46 in mammalian homologs will be critical. Of note, the change in 3′ ss preference associated with mutant SF3B1 is distinct from splicing changes observed with genetic loss or pharmacologic inhibition of *SF3B1* (Darman et al. 2015, Lee et al. 2016a, Seiler et al. 2018b).

In MDS, *SF3B1* mutations are highly enriched within a low-risk subtype known as MDS with ring sideroblasts (MDS-RS) (Papaemmanuil et al. 2011). Although the exact link between *SF3B1* mutations and MDS-RS is unknown, clinical diagnostic criteria for MDS-RS incorporate mutation status (Arber et al. 2016). In contrast to the favorable outcome of SF3B1 mutations in MDS, *SF3B1* mutations in CLL are associated with adverse outcome and chemoresistance (Quesada et al. 2012, L. Wang et al. 2011). Finally, in the context of UM, *SF3B1* mutations are associated with disomy 3 and intermediate risk (Robertson et al. 2017).

SRSF2, which promotes exon splicing, is found to be mutated in chronic myelomonocytic leukemia (CMML), AML, high-risk MDS, myeloproliferative neoplasms, and a small percentage of patients with disomy 3 UM (Yoshida et al. 2011). SRSF2 normally binds to C- and G-rich exonic splicing enhancers (ESEs) to promote splicing (Daubner et al. 2012). *SRSF2* mutations concentrate on residue P95 (Figure 2) and confer altered RNA-binding preference that favors recognition of C-rich CCNG over G-rich ESEs and leads to altered splicing of hundreds of mRNAs (Kim et al. 2015, Zhang et al. 2015). One key alteration in *SRSF2*-mutant cells is altered splicing of *EZH2* mRNA, encoding a protein that regulates histone methylation that is also affected by loss-of-function mutations in myeloid neoplasms. The aberrant *EZH2* mRNA produced by mutant *SRSF2* is targeted for nonsense-mediated decay, and mutations in *EZH2* and *SRSF2* are significantly mutually exclusive in MDS (Papaemmanuil et al. 2013).

Hot spot mutations in RNA splicing factors occur with mutual exclusivity across the myeloid neoplasms. Initially, this pattern of mutual exclusivity was assumed to indicate redundant effects of these mutations; however, a unifying role for mutations across each mutated splicing factor has been elusive. Mutations across *SF3B1* and *SRSF2* confer distinct effects on RNA splicing. Likewise, mutations in *U2AF1*, required for recognition of the AG-dependent 3′ ss recognized by the major spliceosome, are exclusive to *SF3B1* and *SRSF2* mutations in myeloid neoplasms. *U2AF1* mutations predominantly affect S34 and U157 in the zinc fingers (Figure 2). These mutations alter recognition of the 3′ ss, but mutations at each site are associated with differences in splicing events based on the nucleotide surrounding the 3′ AG dinucleotide (Ilagan et al. 2015).

The seemingly disparate effects of mutations in *SF3B1*, *SRSF2*, and *U2AF1* on splicing led to a search for convergent effects of these mutations in processes unrelated to splicing. To this end, cells bearing mutations in *U2AF1* (Nguyen et al. 2017) as well as *SRSF2* (Chen et al. 2018) have been reported to have augmented the formation of R-loops, three-stranded nucleic acid structures composed of DNA-RNA hybrids. The increased generation of R-loops in *SRSF2*- or *U2AF1*-mutant cells is associated with increased DNA damage and activation of the ATR pathway. Although it is not clear how mutant *U2AF1* augments R-loops, mutant *SRSF2*–induced increased transcriptional pausing appears to increase R-loop generation (Chen et al. 2018). These data provide a potential unifying effect of mutant *U2AF1* and *SRSF2* with important therapeutic implications. It will therefore be important to determine if mutant *SF3B1* similarly impacts R-loop generation.

In addition to an effect on R-loops, one recent report suggested that the *U2AF1* S34F mutation may alter interactions with the cleavage and polyadenylation (CP) machinery, resulting in increased use of a distal CP site and longer 3′ untranslated regions (UTRs) (Park et al. 2016). In particular, altered CP of the mRNA encoding the autophagy protein ATG7 was found to result in decreased ATG7, impaired autophagy, and accumulation of secondary mutations. Future efforts will need to confirm if other mutations in U2AF1 or other splicing factors similarly alter CP usage, 3′ UTR length, or autophagy.

Until recently, recurrent mutations in SF3B1, U2AF1, and SRSF2 were the only splicing factors known to harbor hot spot mutations. However, a recent reanalysis of whole-exome sequencing data from 119 patients with 33 solid tumor types has identified recurrent hot spot mutations in PHF5A (a key U2 snRNP component that interacts with SF3B1) and the hnRNP proteins hn-RNPCL1 and PCBP1 (Figure 2) (Seiler et al. 2018a). Effects of these mutations on splicing and tumorigenesis remain unknown.

## RECURRENT LOSS-OF-FUNCTION MUTATIONS IN SPLICEOSOMAL GENES IN CANCER

In addition to the mutually exclusive mutations in *SF3B1*, *SRSF2*, and *U2AF1* in myeloid malignancies, loss-of-function mutations in *ZRSR2*, essential for 3′ ss recognition in U12-type splicing, were also identified in early reports (Figure 2) (Yoshida et al. 2011). *ZRSR2* mutations are enriched in MDS, a form of AML known as blastic plasmacytoid dendritic cell neoplasms, in a small percentage of T cell acute lymphoblastic leukemias, and in thyroid cancers. *ZRSR2* is affected by nonsense or frameshift mutations, which presumably result in loss of *ZRSR2*. Coincident with the role of ZRSR2 in the minor spliceosome, mutation or suppression of ZRSR2 appears to result in retention of U12-type introns (Madan et al. 2015). Splicing events altered by *ZRSR2* mutations appear to impact expression of MAPK pathway members and E2F transcription factors. Further work defining how *ZRSR2* mutations relate to the mutually exclusive mutations in other splicing factors in MDS may provide novel clues to a shared disease mechanism.

Loss-of-function mutations also prominently affect the splicing factor RBM10, an RNA-binding protein that generally represses splicing (Figure 2). *RBM10* mutations are present in lung and bladder adenocarcinomas as well as fatal nonanaplastic thyroid carcinomas

(Ibrahimpasic et al. 2017, Imielinski et al. 2012, Seiler et al. 2018a). *RBM10* mutations are associated with exon inclusion while *RBM10* loss has been shown to promote the proliferation of mouse and human immortalized cells (Bechara et al. 2013, Hernandez et al. 2016). One critical splicing change regulated by *RBM10* expression is AS of *NUMB*, an inhibitor of NOTCH signaling. *RBM10* loss promotes expression of a form of *NUMB* with the inclusion of exon 9, which promotes NOTCH activity (Bechara et al. 2013, Hernandez et al. 2016). In lung and thyroid cancers, *RBM10* is frequently mutated with commonly mutated kinases (KRAS, BRAF, EGFR, and PI3K), although the biological significance of these concurrent mutations remains unknown. Other splicing factors recurrently affected by loss-of-function mutations are shown in Figure 2. The spectrum and frequency of these mutations across cancer types are best described by Seiler et al. (2018a).

## ABERRANT EXPRESSION OF SPLICING FACTORS AS DRIVERS OF CANCER

Although hot spot mutations have called attention to the concept of splicing factors as potential oncogenes, the expression of splicing factors in tumors changes frequently and may be driven by oncogenic signaling (Goncalves et al. 2017). For example, the transcription factor MYC, commonly amplified in cancers, upregulates the expression of multiple splicing factors and deregulates splicing. MYC's involvement in splicing was first demonstrated in the regulation of pyruvate kinase (PKM). Two mutually exclusive isoforms of PKM exist: PKM2, which is almost universally upregulated in cancer and promotes aerobic glycolysis, and PKM1, which is expressed in most normal adult tissues and promotes oxidative phosphorylation. MYC enhances transcription of specific hnRNPs (hnRNPA1, hnRNPA2, and PTB), which in turn promote the expression of the cancer-associated embryonic PKM2 isoform and aerobic glycolysis in glioma (Clower et al. 2010, David et al. 2010). Interestingly, hnRNPA1 also regulates AS of MYC-associated factor X (MAX) to produce delta MAX, which further promotes MYC-dependent transformation and glycolytic gene expression (Figure 3) (Babic et al. 2013, Roy et al. 2017). MYC also controls expression of hnRNPH, which regulates splicing of ARAF kinase (Rauch et al. 2011), increasing the expression of the long isoform that promotes RAS-induced transformation.

As evidence of the importance of aberrant splicing downstream of MYC (Anczuków et al. 2012, Das et al. 2012, David et al. 2010, Hsu et al. 2015, Koh et al. 2015), several studies have shown that MYC-transformed cells are exquisitely sensitive to perturbations of splicing. Through screening for synthetic lethal genes in MYC-driven human mammary epithelial cells, Hsu et al. (2015) identified several spliceosome components (SF3B1, U2AF1, SNRPF, EFTUD2, and BUD31) as preferentially required in MYC-transformed cells. BUD31 knockdown led to intron retention and cell death in MYC cells, but not in HER2- or EGFR-transformed cells. Consistent with this genetic dependence, pharmacological spliceosome inhibition impaired survival, tumorigenicity, and metastatic proclivity of MYC-dependent breast cancers.

SRSF1, SRSF3, and SRSF6 are also amplified among certain cancer types (Figure 3) and have been proposed as oncoproteins (Cohen-Eliav et al. 2013, Jensen et al. 2014, Jia et al. 2010). Increased expression of *SRSF1* is sufficient to transform human and mouse mammary epithelial cells and regulates splicing of hundreds of transcripts (Anczuków et al. 2012; Karni et al. 2007, 2008). SRSF3 downregulation promoted p53-mediated cellular senescence in part by promoting the expression of p53β, an AS isoform of p53 that enforces p53-mediated senescence (Tang et al. 2013).

## SPLICING REGULATION OF ONCOGENIC SIGNALING PATHWAYS IN CANCER

Alternative and aberrant splicing of numerous members of cancer-associated cell growth and death pathways (MAPK, PI3K-AKT, HIPPO, and apoptosis) have been described. These events either promote the expression of isoform proteins that enhance positive feedback signaling or confer resistance to inhibitors of this pathway (Figure 3). For example, the mRNA encoding KRAS undergoes AS, utilizing one of two mutually exclusive exons to generate the isoforms KRAS4A or KRAS4B (Tsai et al. 2015), which transform cells at different rates and exhibit variable targeting to the plasma membrane, the site of interaction with signaling effectors.

Downstream of KRAS, RAF splice variants have been described. For example, *BRAF* has two annotated variable exons, 8b and 10, generating four distinct isoforms (Papin et al. 1998). The variant that includes exon 10 enhances kinase activity and affinity for downstream kinases MEK1/2, while inclusion of exon 8b has the opposite effect. Aside from these annotated isoforms, several pathological aberrant forms of wild-type and mutant *BRAF* have been described. For example, thyroid carcinomas express BRAF splice variants that lack the N-terminal autoinhibitory domain, resulting in constitutive BRAF activity (Baitei et al. 2009). Similarly, the variant lacking exons 4 to 8 was identified in $BRAF^{V600E}$-mutant melanoma cells, which exhibit acquired resistance to the ATP-competitive BRAF inhibitor vemurafenib (Poulikakos et al. 2011). This variant results in the production of a stable truncated $BRAF^{V600E}$ protein lacking the RAS-binding domain (RBD). In the absence of the RBD, BRAF dimerizes and confers resistance to vemurafenib. Truncated forms of $BRAF^{V600E}$ were identified in several primary melanoma patients with acquired resistance to vemurafenib.

Isoforms of ARAF, MEK1/2, and ERK1/2, as well as members of the PI3K-AKT-mTOR pathway, have been identified and shown to have distinct functions. For example, the tumor suppressor PTEN, which counteracts PI3K activity, is regulated by AS. The splice isoform PTEN-5b acts as a dominant negative to promote the activity of PI3K (Agrawal & Eng 2006). Downstream of AKT, mTOR can undergo AS to generate an active oncogenic form, mTORβ (Panasyuk et al. 2009). SRSF1 promotes the production of short S6K1 isoforms frequently upregulated in tumors, h6A and h6C (Ben-Hur et al. 2013, Karni et al. 2007), which enhance the transformation of cells via activation of the mTOR pathway in the absence of external stimuli (Ben-Hur et al. 2013). S6K1 short isoforms also cause 4E-BP1

inactivation and enhanced translation of oncogenes and antiapoptotic genes (Figure 3) (Ben-Hur et al. 2013).

In addition to AS of MAPK signaling intermediates, the upstream receptor tyrosine kinases activating MAPK signaling are also subject to dysregulation by changes in splicing. For example, the variant of EGFR known as variant EGFRvIII contains an in-frame deletion of exons 2–7 and can be generated by rearrangement or altered pre-mRNA processing (Nishikawa et al. 1994, Sugawa et al. 1990). EGFRvIII lacks part of the ligand-binding domain, is constitutively active, and confers growth advantage to cells (Nishikawa et al. 1994, Weidle et al. 2011). Another EGFR isoform produced by skipping exon 4, de4 EGFR, is also constitutively active and promotes metastases (Figure 3) (H. Wang et al. 2011). Selective expression of EGFR isoforms in several tumors makes them attractive cancer therapy targets (Weidle et al. 2011). Similarly, a variety of truncating mutations resulting in exclusion of exon 14 in MET inhibit degradation of MET, prolonging its oncogenic activity. MET exon 14 alterations have been detected in a variety of cancers and confer sensitivity to MET inhibitors (Frampton et al. 2015).

Activation of RAS pathways increases the expression of PTBP1, which, in turn, shifts AS of transcripts encoding the small GTPase RAC1, NUMB, and PKM, each of which are involved in tumorigenesis (Climente-Gonzalez et al. 2017, Hollander et al. 2016, Israelsen et al. 2013, Takahashi et al. 2015). In addition to transcriptional stimulation of PTBP1 downstream of RAS, ERK phosphorylates splicing factor SAM68 to induce its binding to SRSF1 pre-mRNA (Valacca et al. 2010) and promote retention of an intron required for production of full-length SRSF1, diverting the AS event that would cause SRSF1 degradation (Valacca et al. 2010). In turn, SRSF1 promotes AS of MNK2, producing the oncogenic isoform MNK2B (Figure 3) (Karni et al. 2007, Maimon et al. 2014, Scheper et al. 2003). Lastly, splice variants resulting from increased hnRNPA2 expression induce a positive-feedback loop that promotes MAPK signaling to maintain tumor cells (Shilo et al. 2014).

## STRATEGIES TO TARGET SPLICING IN CANCER

Motivated by altered splicing in a variety of tumor types, compounds that impair splicing catalysis directly or through inhibition of PTMs of splicing factors have been developed (Figure 4). The rationale for these approaches is supported by the observation that cancer cells bearing heterozygous change-of-function mutations in *SF3B1*, *SRSF2*, and *U2AF1* require the wild-type allele for survival and are expressed in a mutually exclusive manner, in part, due to a synthetic lethal interaction between these mutations (Lee et al. 2016a,b). Consistent with this, hematopoietic cells bearing mutations in these factors have been shown to be preferentially sensitive to compounds that bind to the SF3B complex and impede splicing (Lee et al. 2016a, Obeng et al. 2016, Shirai et al. 2017). Furthermore, given that SF3B1 is an essential protein, cancer cells with partial copy number loss of *SF3B1* are preferentially sensitive to inhibition of residual SF3B1 (Paolella et al. 2017). Finally, there is also evidence that MYC-amplified tumors rely on increased splicing activity, rendering them sensitive to inhibition of splicing catalysis.

Among the first compounds known to alter splicing were natural compounds (spliceostatin A, pladienolide B, and herboxidiene) that bind the SF3B complex [reviewed recently by Agrawal et al. (2018) and Lee & Abdel-Wahab (2016)]. These agents informed the development of synthetic analogs E7107 and H3B-8800 (Seiler et al. 2018b). Structures of the SF3B complex bound to pladienolide B or E7107 have shown that these molecules bind in the branch point binding pocket of the SF3B complex and thereby block splicing (Figure 4a) (Cretu et al. 2018, Finci et al. 2018). Moreover, studies of cancer cells with acquired resistance to SF3B inhibitor compounds have identified mutations in *SF3B1* as well as *PHF5A* that confer resistance to these compounds (Teng et al. 2017). The structure of SF3B1 reveals that these key residues are involved in binding compounds as well as branch points, explaining their role in drug resistance.

The SF3B modulator E7107 was previously studied in two phase I clinical trials in patients with advanced carcinomas (Eskens et al. 2013, Hong et al. 2014). However, development of ocular complications via an undefined mechanism halted further development of E7107. H3B-8800 is an orally bioavailable SF3B modulatory complex that similarly interferes with the interaction of the complex with branch points. A phase I dose-escalation study of H3B-8800 in AML, CMML, and MDS has recently opened and is recruiting patients (https://www.clinicaltrials.gov identifier NCT02841540).

Recently, a novel strategy to target splicing has emerged using anticancer sulfonamides, including the agents indisulam, tasisulam, and chloroquinoxaline sulfonamide. Phase I and II clinical trials of sulfonamides were previously conducted in a variety of cancer types (Assi et al. 2018, Dittrich et al. 2003, Talbot et al. 2007). Although target plasma concentrations were achieved and remarkable responses were observed in individual patients, consistent antitumor effects were not identified with single-agent sulfonamides. Recent studies, however, have elucidated the mechanism of action of these agents and provided insight into possible biomarkers for response to sulfonamides. Two publications showed that sulfonamides physically bridge the splicing factor RBM39 (also known as CAPER$\alpha$) to the CUL4-DDB1-DDA1-DCAF15 E3 ubiquitin ligase complex, resulting in polyubiquitination and proteosomal degradation of RBM39 (Figure 4b) (Han et al. 2017, Uehara et al. 2017). Degradation of RBM39, an RNA-binding protein known to associate with the U2AF complex (Loerch et al. 2014, Stepanyuk et al. 2016), causes intron retention and exon skipping. Supporting evidence for on-target effects of sulfonamides for RBM39 includes the fact that mutations within RBM39 confer resistance to these compounds. At the same time, expression of DCAF15 (the substrate-specific receptor component of the Cullin-RING ligase complex responsible for RBM39 degradation) also correlates with sensitivity to sulfonamides. In the absence of DCAF15, RBM39 is not degraded by sulfonamides and cells are resistant to these compounds. In future studies, RBM39 mutational status and DCAF15 expression levels may therefore predict response or resistance to these agents.

In addition to the above approaches targeting proteins involved in RNA splicing, several inhibitors of enzymes that place critical PTMs on splicing proteins have been developed. These include inhibitors of protein arginine methyltransferases (PRMTs), SR protein kinases, CDC-like kinases, and dual-specificity tyrosine phosphorylation–regulated kinases (Figure 4c). At least one such molecule, an inhibitor of PRMT5, is already in phase I clinical

trials in solid tumor patients (identifier NCT02783300). Rationale for the use of PRMT5 inhibitors in cancers sensitive to alterations in splicing comes from work by Koh et al. (2015), which identified several components of splicing machinery as key effectors of MYC in the Eμ-myc mouse model of lymphoma, exposing therapeutic vulnerabilities in MYC-driven cancers where existing therapeutic strategies are limited. Outside of spliceosomal proteins, a genome-wide CRISPR-based screen recently identified that inhibition of DCPS, an mRNA-decapping enzyme, also perturbs splicing and alters RNA degradation (Yamauchi et al. 2018). DCPS deletion or inhibition using RG3039, a DCPS inhibitor, decreased proliferation and induced differentiation of AML cells (Figure 4d). The basis for the specific antileukemic mechanism of DCPS inhibition is unknown. Nonetheless, prior use of RG3039 in clinical trials in spinal muscular atrophy patients will hopefully facilitate use of this compound in cancer patients soon.

## SUMMARY AND FUTURE PERSPECTIVES

In the 40 years since the discovery of splicing and the 10 years since the initial use of RNA-seq, a great deal has been learned about how splicing is altered in cancer. While many individual pathogenic splicing events have been characterized, systematic studies of the functional impact of widespread splicing alterations in cancer have yet to be performed. Determining the functional impact of splicing changes with sufficient resolution at the proteome level would greatly help in this regard. Understanding the true effect of splicing changes on the cancer proteome has the potential to identify novel biomarkers and develop complementary means to therapeutically target altered RNA splicing. Moreover, refined use of RNA-seq and proteomic profiling will help address these outstanding questions and inform the development of a unified theme describing the effects of altered RNA splicing in cancer.

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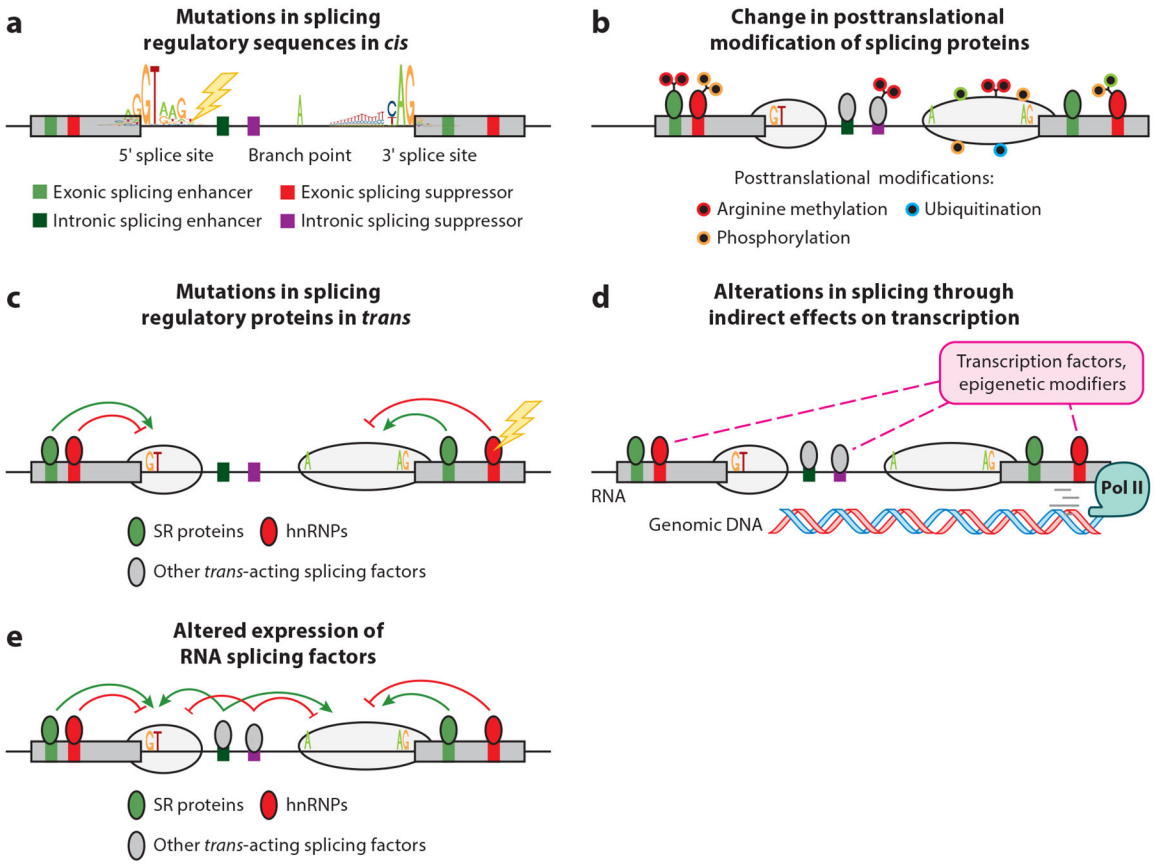
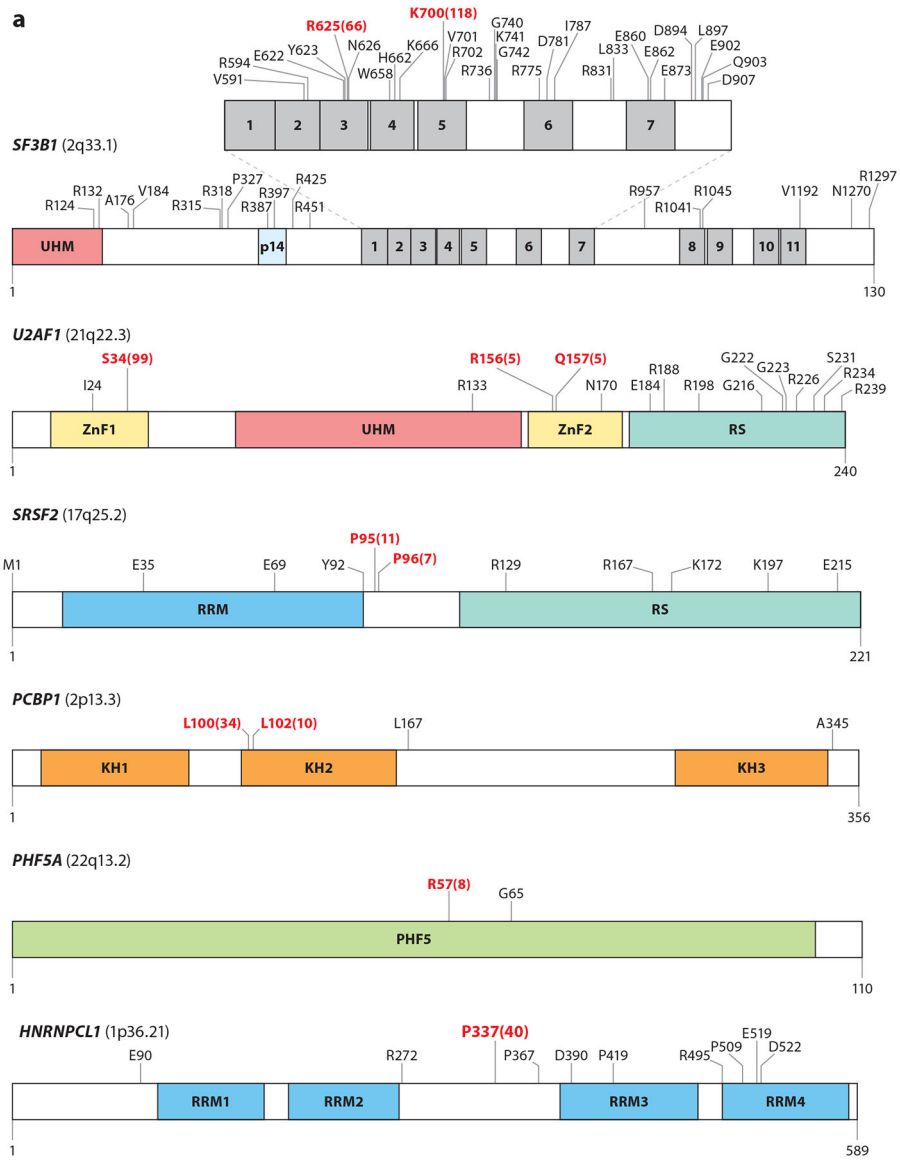



**Figure 1.**

Means by which RNA splicing is altered in cancer. As shown, genetic alterations affecting (*a*) critical sequences required for splicing of genes in *cis* have been described at splice sites, branch points, and splicing enhancers and silencers within exons and introns. In addition, altered (*b*) posttranslational modification of splicing proteins as well as (*c*) change-of-function and loss-of-function mutations in RNA splicing factors themselves occur in a variety of cancers. Given that splicing occurs cotranscriptionally, processes that (*d*) modify the transcription rate of RNA polymerase II (Pol II) may modify splicing. Finally, (*e*) altered expression of RNA splicing factors has been demonstrated to play a pathogenic role in a variety of cancers. Abbreviations: hnRNPs, heterogeneous nuclear ribonucleoproteins; SR proteins, serine- and arginine-rich proteins.



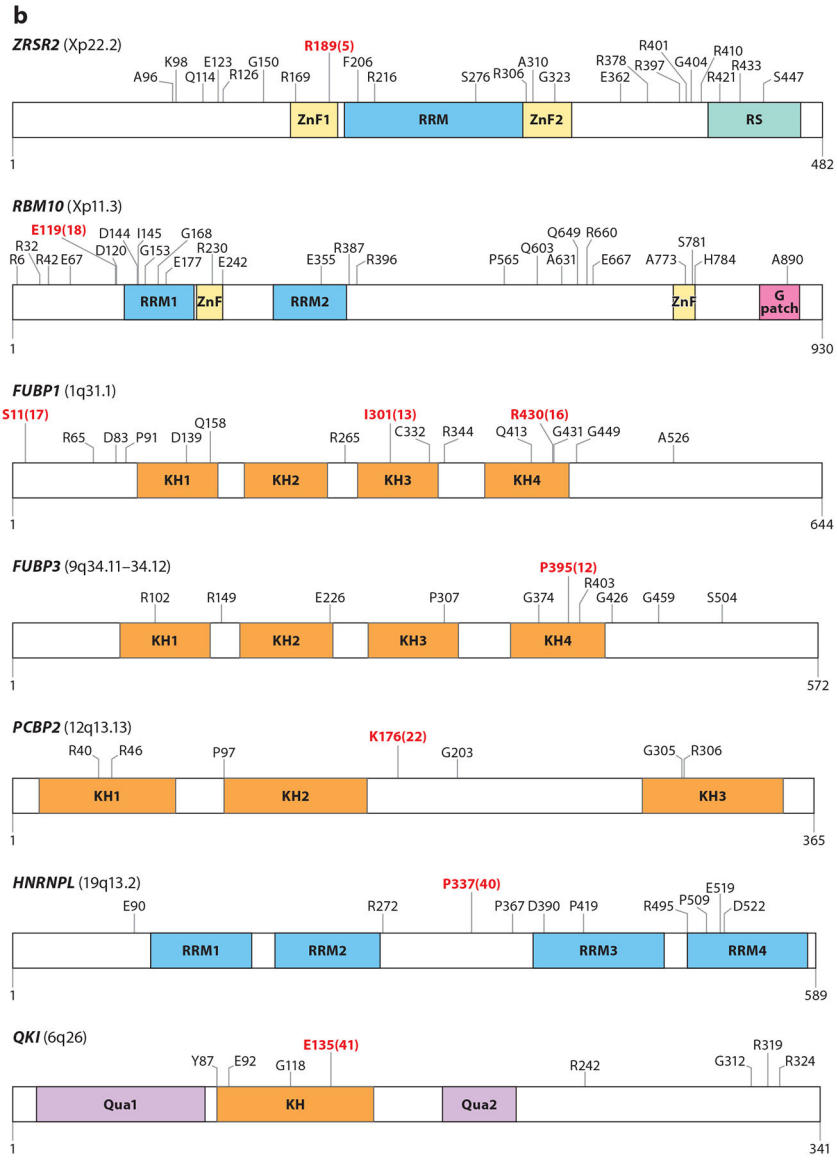



**Figure 2.**

Mutations in RNA splicing factors in cancer. For each protein, mutations occurring in at least three samples are annotated, with the exceptions of *FUBP1* and *RBM10*, where mutations occurring in at least four samples are annotated. Residues in red represent the most frequently reported hot spot mutations, and the total numbers of observed occurrences are displayed in parentheses. Residue and frequency data were mined from cBioPortal (Cerami et al. 2012) and the image was created with DOG 1.0 (Ren et al. 2009). Mutations in proteins in panel *a* are commonly thought to induce change-of-function mutations, whereas mutations in proteins in panel *b* typically result in loss-of-function mutations. Colored regions within each protein diagram represent known domains.

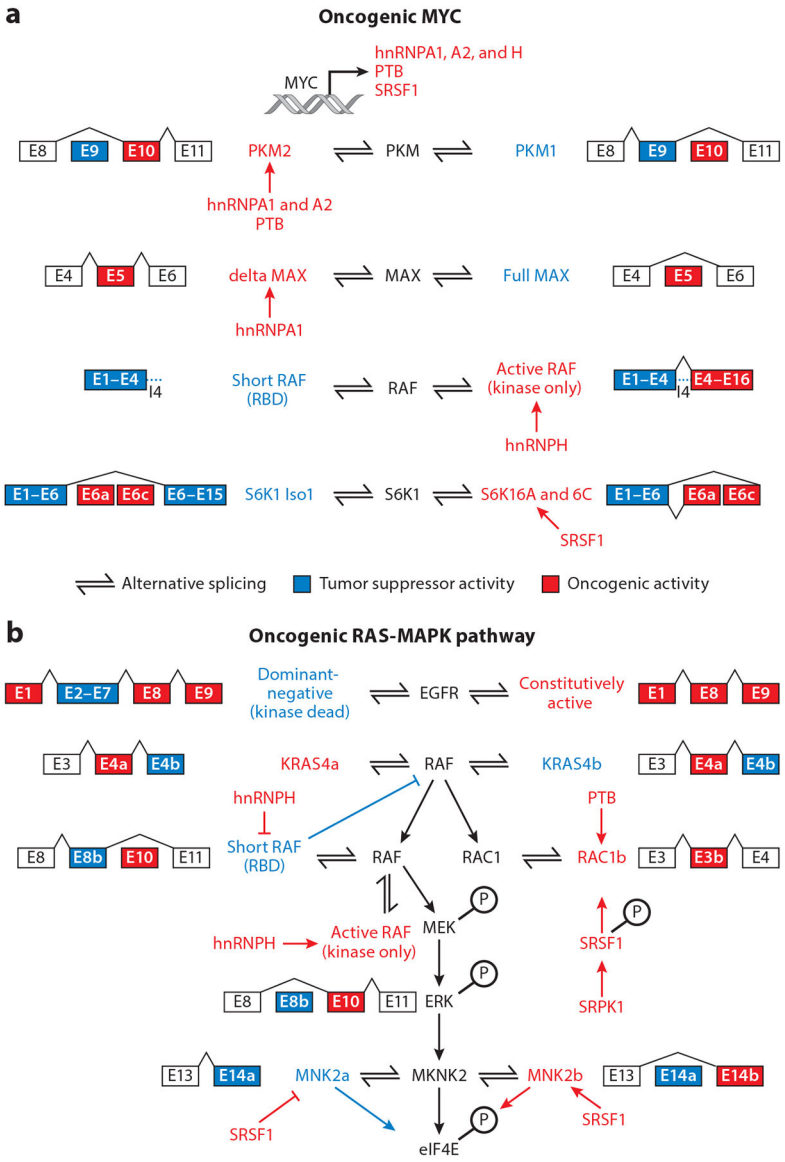



**Figure 3.**

Alternative splicing regulation of the oncogenic MYC and RAS-MAPK pathways. (*a*) MYC increases the expression of splicing regulators PTB, SRSF1, and hnRNPA1, A2, and H, which in turn change the expression of isoforms of PKM, RAF, MAX, and S6K1. hnRNPA1, A2, and PTB promote the expression of PKM2, a variant of PKM that promotes aerobic glycolysis. hnRNPA1 has also been found to promote the expression of delta MAX, an isoform of MAX that further promotes MYC-dependent transformation and glycolytic gene expression. hnRNPH, under a MYC oncogenic background, promotes the expression of active oncogenic RAF while repressing the short RAF containing only the RBD, which inhibits RAS. The splicing factor SRSF1 can affect the splicing of S6K1, inducing oncogenic short isoforms of this kinase (h6A and h6C), which bind mTOR and enhance 4E-BP1 phosphorylation and cap-dependent translation.(*b*) Multiple splicing factor regulators change the expression of oncogenic isoforms of proteins involved in the RAS-MAPK

pathway. Receptor tyrosine kinases, such as EGFR, are alternatively spliced to generate truncated isoforms, which act in a dominant-negative manner, or constitutively active isoforms (EGFRvIII), which are active regardless of ligand binding. RAS can be alternatively spliced to generate RAS4A, an isoform commonly found in cancers. RAS activates RAF, which can be alternatively spliced to generate a short isoform with RBDs that inhibit RAS or constitutively active isoforms containing only the kinase domain. hnRNPH inhibits the production of dominant-negative RAF isoforms. RAF phosphorylates MEK, which in turn phosphorylates ERK. ERK can phosphorylate MNK2, which is alternatively spliced and regulated by SRSF1. SRSF1 upregulates a pro-oncogenic MNK2B isoform and reduces the MNK2A isoform. Figure adapted from Siegfried et al. (2013). Abbreviations: E, exon; hnRNP, heterogeneous nuclear ribonucleoprotein; RBD, RAS-binding domain.

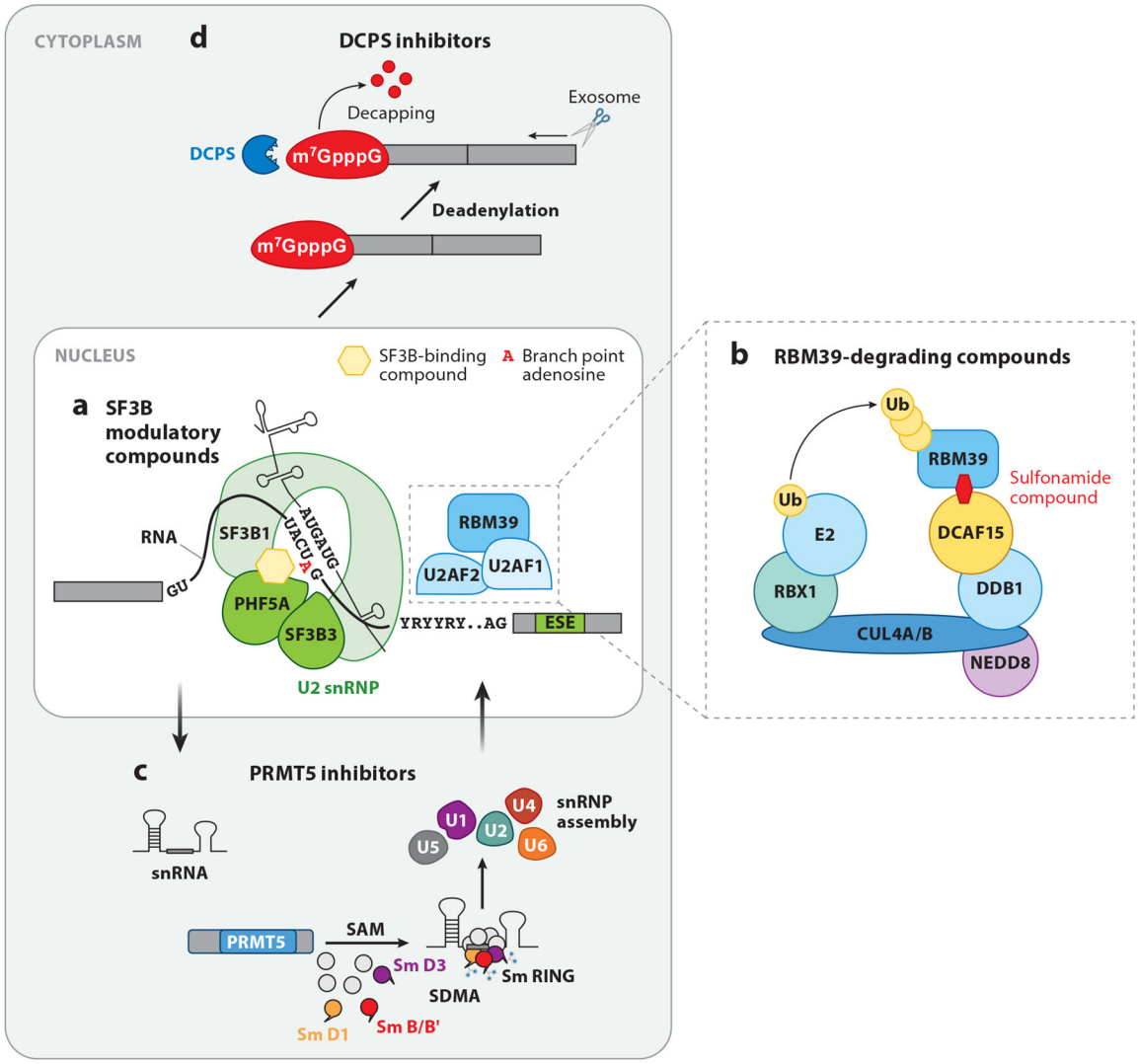



**Figure 4.**

Means to pharmacologically target splicing in cancer. (*a*) SF3B modulatory compounds bind to the SF3B complex and block its interaction with the branch point adenosine, a process essential for RNA splicing. (*b*) Recently, a series of molecules known as anticancer sulfonamides were described (Han et al. 2017, Uehara et al. 2017) that bridge the cellular CUL4-DDB1-DDA1 E3 ubiquitin ligase complex to RBM39 via the adaptor protein DCAF15, resulting in polyubiquitination and subsequent proteosomal degradation of RBM39. (*c*) Arginine methylation of a variety of splicing proteins by protein arginine methyltransferases (PRMTs) is required for normal spliceosome assembly and function. Symmetric dimethylation of arginines (SDMA) on Sm proteins is required for assembly of the small nuclear ribonucleoprotein (snRNP) complexes. (*d*) It has recently been shown that small-molecule inhibitors of the scavenger messenger (mRNA)-decapping enzyme DCPS [required for removal of the N7-methylated guanine cap ($m^7GpppG$) on mRNAs] result in a change in dose-dependent effects on splicing (Yamauchi et al. 2018).