Splicing-factor alterations in cancers

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ABSTRACT

Tumor-associated alterations in RNA splicing result either from mutations in splicing-regulatory elements or changes in components of the splicing machinery. This review summarizes our current understanding of the role of splicing-factor alterations in human cancers. We describe splicing-factor alterations detected in human tumors and the resulting changes in splicing, highlighting cell-type-specific similarities and differences. We review the mechanisms of splicing-factor regulation in normal and cancer cells. Finally, we summarize recent efforts to develop novel cancer therapies, based on targeting either the oncogenic splicing events or their upstream splicing regulators.

INTRODUCTION

We have known for many years that tumors exhibit abnormal splicing patterns, compared to normal tissues. Changes in splicing occur for genes involved in every step of the transformation process (for review, see David et al. 2010). For example, the increased expression of anti-apoptotic isoforms of genes such as *BCL2L1*, *CASP2*, or *FAS*, has been extensively documented in various tumors types; alternative splicing of *CD44*, *FGFR2, RAC1*, or *MST1R* has been linked with the acquisition of invasive properties; and *VEGFA* splice variants are involved in angiogenesis regulation (Fig. 1). However, in the past few years we have started to appreciate that many of these tumor-associated splicing changes reflect alterations in particular components of the splicing machinery (Fig. 1). The core spliceosome plus associated regulatory factors comprise more than 300 proteins and five small nuclear RNAs (snRNAs), and catalyzes both constitutive and regulated alternative splicing (Hegele et al. 2012). The U1, U2, U4, U5, and U6 snRNAs participate in several key RNA–RNA and RNA–protein interactions during spliceosome assembly and splicing catalysis. These snRNAs associate with seven "Sm" core proteins and additional proteins to form small nuclear ribonucleoprotein particles (snRNPs). Other protein subcomplexes also play key roles, such as the SF3A and B complexes, and the PRP19-associated complexes dubbed NTC and NTR. The architecture of the spliceosome undergoes extensive remodeling in preparation for, during, and after splicing. In addition to the core spliceosome, regulatory proteins are involved in modulating the splicing reaction. These include RNA-binding proteins that function as activators or repressors of splicing by binding specifically to exonic or intronic enhancer or silencer elements, respectively, and they are involved in both constitutive and alternative splicing (for review, see Biamonti et al. 2014). In this review, we discuss the various splicing-factor alterations detected in human tumors, their cell-type specificity, as well as their specific roles in tumor development and progression.

RECURRENT SOMATIC MUTATIONS OF CORE SPLICEOSOME COMPONENTS IN HEMATOLOGICAL MALIGNANCIES

Recently, large-scale sequencing projects identified recurrent somatic mutations in certain components of the spliceosome in several types of hematological malignancies, including myelodysplastic syndromes (MDS), other myeloid neoplasms, and chronic lymphocytic leukemia (CLL) (Table 1; Yoshida et al. 2011; Bejar et al. 2012; Papaemmanuil et al. 2013). These mutations occur most commonly in four genes: *SF3B1* (splicing factor 3b subunit 1), *SRSF2* (serine/arginine-rich splicing factor 2), *U2AF1* (U2 small nuclear RNA auxiliary factor 1), and *ZRSR2* (zinc finger RNA binding motif and serine/arginine rich 2), and almost always as somatic heterozygous missense mutations that are mutually exclusive (Papaemmanuil et al. 2011; Wang et al. 2011; Yoshida et al. 2011). In a very detailed review, Yoshida and Ogawa (2014) discussed the discovery of splicing-factor mutations and their correlation with tumor classification. Here we will focus on the functional differences and similarities between mutant splicing factors in hematological malignancies.

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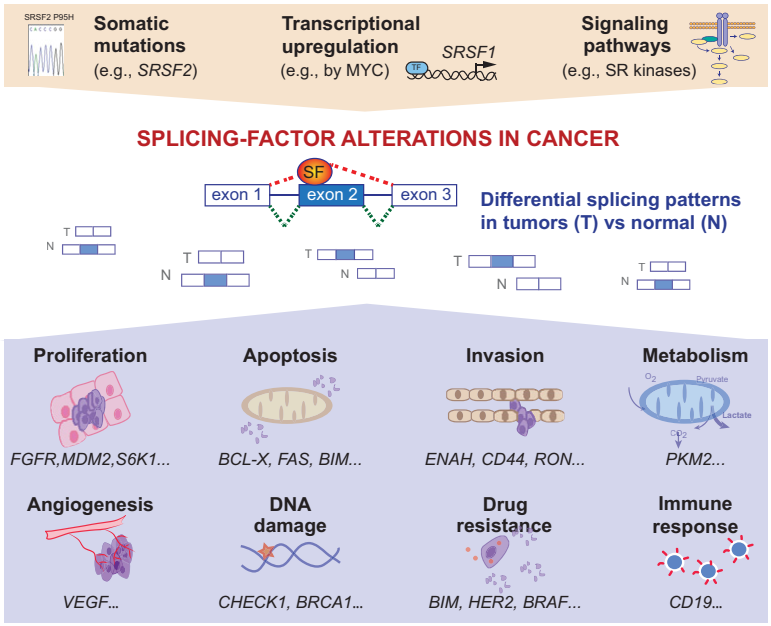

FIGURE 1. Splicing-factor alterations in human tumors. Human tumors exhibit somatic mutations in splicing regulators, or changes in splicing-factor levels in response to cell signaling or transcriptional regulation. These alterations in splicing factors promote differential splicing patterns in tumors compared to normal tissues. Alterations in alternative splicing events lead to the production of pro-tumorigenic isoforms that have been linked to various steps of tumorigenesis, including proliferation, apoptosis, invasion, metabolism, angiogenesis, DNA damage, or even drug resistance and immune response.

SFB3B1—splicing factor 3b subunit 1

SF3B1, the most frequently mutated component of the spliceosome in cancer, is involved in the recognition of the intronic branch point sequence (BPS) during selection of the 3′ splice site (3′SS) (Fig. 2). SF3B1 is a component of the SF3B complex, which associates with the SF3A complex and U2 snRNP to form the 17*S* U2 complex. U2 snRNP binds to BPSs via SF3B14, and to U2AF2 via SF3B1 to stabilize the base-pairing interaction between U2 snRNA and the BPS, leading to the formation of the spliceosomal A complex. *SF3B1* mutations are found in a variety of myeloid malignancies, with extremely high recurrence (48%–57%) in MDS subtypes that show increased ring sideroblasts (RARS/RCMD-RS) (Malcovati et al. 2011; Yoshida et al. 2011; Damm et al. 2012; Patnaik et al. 2012; Visconte et al. 2012), as well in 6%–26% of CLLs (Table 1). *SF3B1* mutations are clustered in several hot spots, including K700, E622, R625, H662, and K666, all of which are located within HEAT (Huntingtin, Elongation factor 3, protein phosphatase 2A, Targets of rapamycin 1) repeats that extend from exon 12 to exon 15 (Fig. 2). In addition, *SF3B1* mutations of residues K700 and K666 have been reported in 1.8% of unselected breast tumors and 4% of luminal breast tumors (Ellis et al. 2012; Maguire et al. 2015), as well as in 3% of pancreatic ductal adenocarcinomas (Biankin et al. 2012), whereas mutations of residues R625 and K666 are found in 15%–29% of uveal melanomas (Furney et al. 2013; Harbour et al. 2013; Martin et al. 2013) and 1% of cutaneous melanomas (Table 1; Kong et al. 2014).

SF3B1 mutations correlate with good prognosis in MDS and melanoma, but with poor prognosis in CLL. The SF3B1 K700 mutant promotes utilization of a different BPS compared to wild-type SF3B1, and recognizes a cryptic 3′SS with a short and weak polypyrimidine tract, located upstream of the canonical 3′SS (Darman et al. 2015). Similarly, *SF3B1* R625 and K666 mutations result in deregulated splicing at a subset of splice sites, mostly involving the use of alternative or cryptic 3′SS (DeBoever et al. 2015; Alsafadi et al. 2016).

SRSF2—serine/arginine-rich splicing factor 2

The second most frequently mutated splicing regulator in MDS is *SRSF2*, a member of the serine–arginine (SR) protein family, which is involved in both constitutive and alternative splicing. SRSF2 bound to an exon plays a role in recruiting U2AF to the upstream 3′SS and U1 snRNP to the downstream 5′SS (Fig. 2). *SRSF2* mutations and insertions/deletions map to a unique hotspot centered on residue P95, within the region linking the N-terminal RNA recognition motif (RRM) and the C-terminal RS domain (Fig. 2). Recurrent *SRSF2* mutations have been found in various hematological malignancies (Table 1), with higher frequencies in patients with MDS (20%–30%) and chronic myelomonocytic leukemia (CMML) (50%) (Yoshida et al. 2011; Papaemmanuil et al. 2013). *SRSF2* mutations correlate with poor prognosis, thus suggesting a major functional difference compared to *SF3B1* mutations. In addition, no recurrent SRSF2 mutations have been reported to date in solid tumors.

Conditional expression from the endogenous murine locus of the $Srsf2^{P95H}$ allele in the bone marrow leads to impaired hematopoietic differentiation in heterozygous animals; in addition, mice heterozygous for the $Srsf2^{P95H}$ allele have distinct hematopoietic defects, compared to animals with heterozygous or homozygous deletion of *Srsf2* (Kim et al. 2015). *SRSF2* mutations are associated with changes in mRNA splicing patterns of key hematopoietic regulators, both in primary murine and patient samples, such as *EZH2* and *BCOR* (Kim et al. 2015). The mutant SRSF2 protein exhibits altered RNA-binding specificity, rather than a loss of RNA-binding activity (Kim et al. 2015). Wild-type SRSF2 recognizes the consensus binding motifs CCNG and GGNG with similar affinity, whereas $SRSF2^{P95H}$ preferentially binds

TABLE 1. Recurrent splicing-factor mutations in human malignancies

Gene name	Mutation hotspot	Tumor type	Frequency	Reference
SF3B1	K700, E622, R625, H662, K666	RARS/RCMD-RS	48%–79%	Malcovati et al. 2011, 2015; Papaemmanuil et al. 2011, 2013; Wang et al. 2011; Yoshida et al. 2011; Bejar et al. 2012; Damm et al. 2012; Makishima et al. 2012; Patnaik et al. 2012; Visconte et al. 2012; Walter et al. 2013; Shirai et al. 2015
		CLL	6%–26%	
		MDS w/o RS	4%–7%	
		CMML	4%–5%	
		De novo AML	3%–7%	
		MDS/sAML	3%–5%	
		MPN	0%–3%	
	K700, K666	Breast tumors	1.8%	Ellis et al. 2012; Maguire et al. 2015
		Luminal breast	4.0%	
	R625	Uveal melanoma	15%–29%	Martin et al. 2013
	R625	Cutaneous melanoma	1%	Kong et al. 2014
	K700	PDAC	3%	Biankin et al. 2012
	E776	Pleural mesothelioma	2%	Bueno et al. 2016
SRSF2	P95	CMML	28%–47%	Yoshida et al. 2011; Bejar et al. 2012; Damm et al. 2012; Makishima et al. 2012; Thol et al. 2012; Papaemmanuil et al. 2013
		MDS w/o RS	11%–15%	
		MDS/sAML	6.5%	
		RARS/RCMD-RS	5.5%	
		JMML	3.7%	
		MPN	1.9%	
		De novo AML	0.7%	
U2AF1	S34, Q157	CMML	8%–17%	Yoshida et al. 2011; Bejar et al. 2012; Damm et al. 2012; Graubert et al. 2012; Makishima et al. 2012; Patnaik et al. 2012; Takita et al. 2012; Thol et al. 2012; Mian et al. 2013; Papaemmanuil et al. 2013; Walter et al. 2013; Stieglitz et al. 2015
		RARS/RCMD-RS	16%	
		MDS w/o RS	5%–12%	
		MDS/sAML	9%	
		MPN	3%–7%	
		JMML	3.7%	
		De novo AML	1.3%–4%	
	S34, Q157	Lung	3%	Imielinski et al. 2012
ZRSR2	Various mutations, no hotspot	MDS w/o RS	1%–11%	Yoshida et al. 2011; Thol et al. 2012; Mian et al. 2013; Papaemmanuil et al. 2013; Walter et al. 2013
		CMML	0.8%–8%	
		MPN	1.9%–7.6%	
		MDS/sAML	1.6%–2.5%	
		RARS/RCMD-RS	1.4%	

AML, acute myeloid leukemia; CLL, chronic lymphocytic leukemia; CMML, chronic myelomonocytic leukemia; JMML, juvenile myelomonocytic leukemia; MDS/sAML, myelodysplastic syndrome with secondary AML; MDS w/o RS, myelodysplastic syndrome without ring sideroblasts; MPN, myeloproliferative neoplasm; PDAC, pancreatic ductal adenocarcinoma; RARS, refractory anemia with ring sideroblasts; RCMD-RS, refractory cytopenia with multilineage dysplasia and ring sideroblasts.

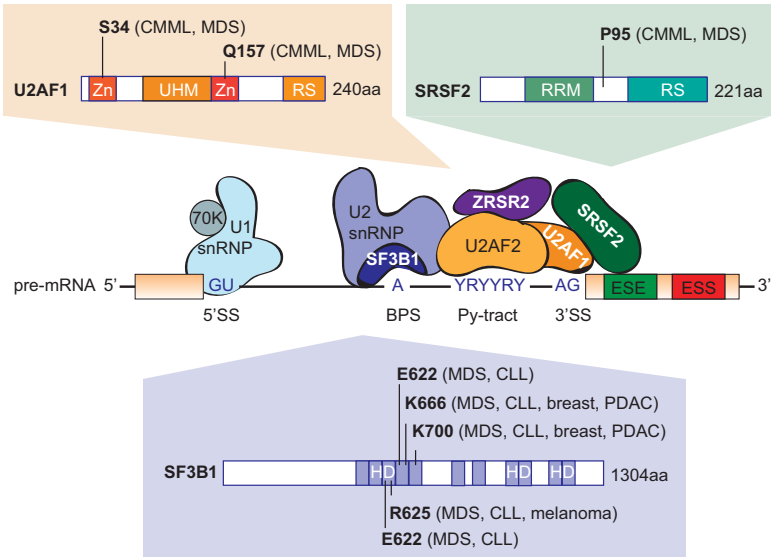

FIGURE 2. Recurrent splicing-factor mutations in human malignancies. Hotspot mutations in the genes coding for splicing factors U2AF1, SRSF2, and SF3B1 detected in myelodysplasia, as well as other tumor types, are indicated. In contrast, ZRSR2 mutations are distributed evenly across the gene (not shown). Domain abbreviations: (Zn) zinc finger domain, (UHM) U2AF homology motif domain, (RS) arginine/serine-rich domain, (RRM) RNA-recognition motif, (HD) heat domain.

CCNG, which is present within exons that are differentially spliced. The altered interaction of mutant SRSF2 with RNA is due to the mutations affecting the conformations of the termini of SRSF2′s RRM domain, as deduced from NMR experiments (Kim et al. 2015).

U2AF1—U2 small nuclear RNA auxiliary factor

U2AF1 mutations are detected in 5%–15% of MDS and 5%–17% of CMML, as well as at lower rates in other hematological malignancies (Table 1). They are also found in 3% of lung tumors (Imielinski et al. 2012). The mutations are clustered in two hotspots centered on residues S34 and Q157, located within the two conserved zinc-finger domains (Fig. 2; Yoshida et al. 2011). U2AF is a heterodimer involved in 3′SS selection, with the small subunit, U2AF1 interacting with the AG dinucleotide, and the large subunit, U2AF2 interacting with the polypyrimidine tract. The MDS mutations in *U2AF1* alter RNA splicing and promote mis-splicing of genes in ways that presumably contribute to abnormal hematopoiesis (Graubert et al. 2012; Quesada et al. 2012; Przychodzen et al. 2013; Brooks et al. 2014; Ilagan et al. 2015; Okeyo-Owuor et al. 2015; Shirai et al. 2015).

ZRSR2—zinc finger RNA binding motif and serine/arginine rich 2

In contrast to the above mutations, *ZRSR2* mutations are distributed evenly across the gene sequence, and most of them disrupt the translational reading frame. This mutation pattern suggests loss of function, rather than gain/change of function, for the *ZRSR2* mutations in MDS. *ZRSR2* mutations are less frequent in MDS or in CMML than *SF3B1*, *U2AF1*, or *SRSF2* mutations (Table 1). ZRSR2 associates with the U2AF heterodimer and plays a role in 3′SS recognition by participating in the formation of the spliceosomal A complex (Fig. 2). shRNA-mediated knockdown (KD) of ZRSR2 does not affect splicing of U2-type introns, but leads to the retention of U12-type introns (Madan et al. 2015), an atypical minor class of introns which are distinct from the canonical U2-dependent introns and represent only ∼0.5% of all human introns (for review, see Turunen et al. 2013).

Other RNA-binding proteins

Finally, alterations in other RNA-binding proteins have been reported at very low frequencies (0.5–1.5%) in hematological malignancies, such as de novo AML, CLL, or MDS, including mutations in *PRPF8*, *SF3A1*, *LUCL7L2*, *SF1*, *U2AF2*, *HNRNPK*, *SRSF6*, *SRSF1*, *SRSF7*, *TRA2*β, *SRRM2*, *DDX1*, *DDX23*, *CELF4* (Makishima et al. 2012; Quesada et al. 2012; The Cancer Genome Atlas Research Network 2013; Walter et al. 2013). The existence of such mutations suggests that alterations in multiple steps of spliceosome assembly and splicing regulation can contribute to hematological malignancies.

ALTERATIONS IN SPLICING-FACTOR LEVELS IN SOLID TUMORS

Interestingly, very few recurrent mutations in splicing regulators have been detected to date in solid tumors, suggesting a fundamental difference in splicing targets and/or regulation in hematological malignancies compared to solid tumors. Mutations in *SF3B1* (located on Chr. 2q33) have been reported in 1.8% of breast tumors, and more specifically in 4% of a subset of luminal breast tumors (Ellis et al. 2012; Maguire et al. 2015), and are significantly associated with the presence of estrogen-receptor (ER)-positive tumor cells, *AKT1* mutations, and distinct copy-number alterations, including frequent chromosomal-segment gains on 16q12–q13 and 16q21–q22, and losses on 1p36–p35, 16q11–q13, and 16q21–q23 (Ellis et al. 2012; Maguire et al. 2015). *SF3B1* mutations are also found at low frequency in pancreatic tumors (Biankin et al. 2012), as well as melanoma (Furney et al. 2013; Harbour et al. 2013; Martin et al. 2013; Kong et al. 2014). *U2AF1* mutations have been detected in 3% of lung tumors

(Imielinski et al. 2012). Interestingly, other splicing-factor mutations recurrent in blood malignancies, i.e., in *SRSF2* or *ZRSR2*, have not yet been detected in solid tumors. However, solid tumors do exhibit frequent alterations in splicing factors; these are mostly changes in levels, occurring through changes in gene copy number and/or changes in gene expression (Fig. 1). Several regulatory splicing factors, such as SRSF1 (Karni et al. 2007; Anczukow et al. 2012), SRSF3 (Jia et al. 2010), SRSF6 (Karni et al. 2007; Cohen-Eliav et al. 2013), HNRNPA2/B1 (Golan-Gerstl et al. 2011), or HNRNPH (Lefave et al. 2011), have oncogenic properties, whereas other factors, including QKI (Zong et al. 2014), RBM5, RBM6, and RBM10 (Bechara et al. 2013), act as tumor suppressors (Table 2). Additional splicing regulators are also involved in tumorigenesis, although their function as oncogenes or tumor suppressors seems less clearly defined and can be different across tissue types (see below) (Table 2).

These RNA-binding proteins elicit changes in alternative splicing in a concentration-dependent manner, and, thus, changes in their levels can alter pre-mRNA splicing of many genes related to cancer, even in the absence of mutations. Alternative splicing changes have been linked to cancer through post-transcriptional regulation of components of many of the cellular processes considered as "hallmarks" of cancer, including cell proliferation, apoptosis, metabolism, invasion, and angiogenesis. However, the biological consequences of these global changes in alternative splicing are only beginning to be unraveled. We will briefly discuss selected examples of oncogenic or tumor suppressor splicing factors that have been found to contribute to cancer. For a detailed review of oncogenic spliced isoforms, see David and Manley (2010).

SRSF1—serine/arginine-rich splicing factor 1

The splicing factor SRSF1 (previously known as SF2/ASF) is a prototypical SR protein involved in both constitutive and alternative splicing, but it has also been implicated in

TABLE 2. Splicing-factor expression changes in human malignancies

Gene name	Alteration type	Tumor type	Reference
SRSF1	AMP, OE	Lung, colon, breast ovary, thyroid, small intestine, kidney	Watermann et al. 2006; Karni et al. 2007; Anczuków et al. 2012, 2015; Das et al. 2012; Iborra et al. 2013
SRSF2	OE	Ovary	Fischer et al. 2004
SRSF3	KD	Liver	Sen et al. 2015
	AMP, OE	Ovary, cervix, lung, breast, stomach, skin, bladder, colon, liver, thyroid, kidney	He et al. 2004, 2011; Jia et al. 2010; Iborra et al. 2013
SRSF5	OE	Breast	Huang et al. 2007
SRSF6	AMP, OE	Lung, colon, breast, skin	Karni et al. 2007; Cohen-Eliav et al. 2013; Jensen et al. 2014
SRSF10	OE	Colorectal	Zhou et al. 2014
TRA2B	OE	Breast, ovary, colon, lung, cervix	Fischer et al. 2004; Watermann et al. 2006; Iborra et al. 2013; Kuwano et al. 2015
HNRNPA1	OE	Breast, lung, colon, glioblastoma	Fielding et al. 1999; Zhou et al. 2001a,b; Karni et al. 2007; Golan-Gerstl et al. 2011
HNRNPA2/B1	AMP, OE	Glioblastoma	Golan-Gerstl et al. 2011
HNRNPH	OE	Glioblastoma	Lefave et al. 2011
HNRNPK	KD	AML	Gallardo et al. 2015
	OE	Breast, esophageal squamous cell, pancreatic, skin, colorectal, oral squamous cell	Mandal et al. 2001; Carpenter et al. 2006; Roychoudhury and Chaudhuri 2007; Zhou et al. 2010
HNRNPM	OE	Breast	Xu et al. 2014
PTB	OE	Breast, ovary, malignant glioblastoma, colorectal and mucosal	Jin et al. 2000; He et al. 2004,2007,2014; Takahashi et al. 2015
ESPR1,2	OE/KD	Breast, oral squamous cell carcinoma	Ishii et al. 2014; Yae et al. 2012
RBFOX2	KD	Ovary, breast	Venables et al. 2009
QKI	KD	Lung adenocarcinoma, squamous cell carcinoma, and large cell carcinoma, oral squamous cell carcinoma, prostate	Lu et al. 2014; Zhao et al. 2014; Zong et al. 2014
RBM5	KD	Lung, prostate	Oh et al. 2002; Rintala-Maki et al. 2007; Zhao et al. 2012
	OE	Breast	
RBM10	OE	Breast	Rintala-Maki et al. 2007
	Mut	Lung	Imielinski et al. 2012

AMP, amplification; KD, knockdown; Mut, mutation; OE, overexpression.

additional functions, such as regulating mRNA transcription, stability and nuclear export, nonsense-mediated mRNA decay (NMD), translation, and protein sumoylation (for review, see Das and Krainer 2014). *SRSF1* is an essential gene and *Srsf1*-null mice are embryonic lethal (Xu et al. 2005). Tissue-specific deletion of *Srsf1* in mouse heart leads to lethality about 6–8 wk after birth, due to heart failure (Xu et al. 2005). These mice have defective Ca^{2+} metabolism, attributed to mis-splicing of the Ca^{2+}/calmodulin-dependent kinase IIδ (CAMKIIδ), which leads to a defective contractile apparatus and cardiomyopathy. SRSF1 was also the first member of the SR protein family to be identified as a proto-oncogene, highlighting the important role of alternative splicing in tumorigenesis (Karni et al. 2007).

SRSF1 is frequently overexpressed in many solid tumors, relative to their respective normal controls, including tumors of the breast (13%), colon (25%), lung (25%), as well as thyroid, small intestine, kidney, and ovarian tumors (Watermann et al. 2006; Karni et al. 2007; Anczukow et al. 2012, 2015; Das et al. 2012; Iborra et al. 2013). Modest SRSF1 overexpression is sufficient to promote transformation of immortal rodent fibroblasts (Karni et al. 2007), as well as human mammary epithelial cells in vitro and in vivo (Fig. 3; Anczukow et al. 2012). SRSF1 up-regulation in some breast cancers is attributable to amplification of its locus as part of the Chr. 17q23 amplicon, which is associated with breast malignancies with poor prognosis (Karni et al. 2007). In addition, SRSF1 is a direct transcriptional target of the MYC oncoprotein, and high levels of SRSF1 are detected in MYC-expressing tumors (Anczukow et al. 2012; Das et al. 2012). *SRSF1* expression is also regulated at the level of splicing, in response to the splicing factor Sam 68 (Valacca et al. 2010). Sam68 activity switches alternative splicing of the *SRSF1* transcript from an NMD-targeted isoform to the major, translatable isoform, thus resulting in an increase in SRSF1 protein levels.

SRSF1 comprises two RRMs and a C-terminal RS domain (Fig. 4). A structure–function dissection of the modular protein domains of SRSF1 showed that SRSF1's role in regulating proliferation and apoptosis during transformation involves splicing targets recognized primarily by RRM1, and does not require SRFS1's activity in NMD or physical interaction with mTOR (Anczukow et al. 2012). SRSF1 overexpression affects specific pre-mRNA splicing events in the *MST1R* (*Ron*) proto-oncogene and the kinases *MKNK2* and *S6K1*, promoting the expression of pro-oncogenic isoforms (Ghigna et al. 2005; Karni et al. 2007; Anczukow et al. 2012). In addition, SRSF1 controls splicing of the tumor suppressor *BIN1* and the apoptotic factor *BCL2L11* (*BIM*), promoting the expression of isoforms that lack pro-apoptotic activity and contribute at least in part to SRSF1's activity in transformation (Fig. 3; Anczukow et al. 2012). In addition, SRSF1 regulates splicing of *BCL2L1, MCL1*, and *CASP2* and *9*, and its KD induces G2 cell-cycle arrest and apoptosis (Li et al. 2005; Moore et al. 2010). Finally, SRSF1-regulated

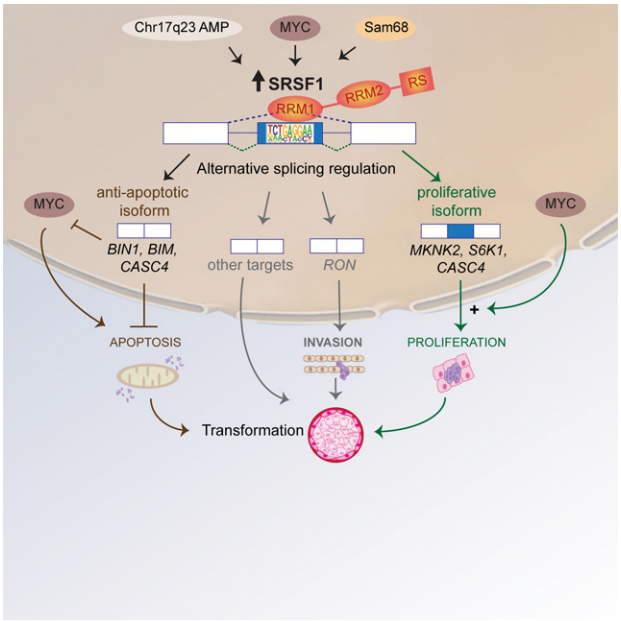

FIGURE 3. Model for SRSF1's roles in transformation in breast cancer. Increased expression of SRFS1 in human tumors results from several distinct types of alterations, such as amplification of the Chr. 17q23 amplicon, transcriptional regulation of *SRFS1* downstream from MYC, or splicing regulation of SRSF1 pre-mRNA by Sam68. Up-regulation of SRSF1 promotes splicing changes in target genes involved in apoptosis, cell motility, proliferation, and other cellular functions. SRSF1 activates splicing by binding to an exonic motif recognized by its RRM1. SRSF1 overexpression promotes expression of anti-apoptotic isoforms unable to interact with pro-apoptotic factors, or that inhibit the action of pro-apoptotic factors, such as MYC or members of the Bcl-2 family. In parallel, SRSF1 overexpression promotes expression of isoforms that stimulate translation and cell proliferation by increasing phosphorylation of translation activators, such as S6 or eIF4E, or by inhibiting translational repressors, such as 4EBP1. In addition, MYC can cooperate with SRSF1 in transformation, and has a synergistic effect in the activation of the eIF4E pathway. By increasing proliferation and decreasing apoptosis, SRSF1 promotes cellular transformation in mammary epithelial cells.

splicing targets were identified by RNA-seq profiling of an organotypic three-dimensional cell culture model that mimics a context relevant to breast cancer, as well as of human breast tumors with SRSF1 overexpression (Anczukow et al. 2015). Overexpression of the SRSF1-regulated, exon-9-included *CASC4* isoform—a target consistently observed in the different data sets—increased proliferation and decreased apoptosis, partially recapitulating SRSF1's oncogenic effects (Anczukow et al. 2015).

Interestingly, SRSF1-mediated oncogenesis appears to be dependent on inactivation of the p53 tumor suppressor pathway, a finding that could potentially be exploited when developing therapies against SRSF1-driven or -dependent tumors. p53 induction is one of the primary responses to *SRSF1* overexpression in primary human and murine fibroblasts, and results in cells entering a state of premature cellular senescence (Fregoso et al. 2013). Briefly, SRSF1 plays a role in the nucleolar stress pathway, by stabilizing the interaction between ribosomal protein RPL5 and the E3 ubiquitin ligase MDM2

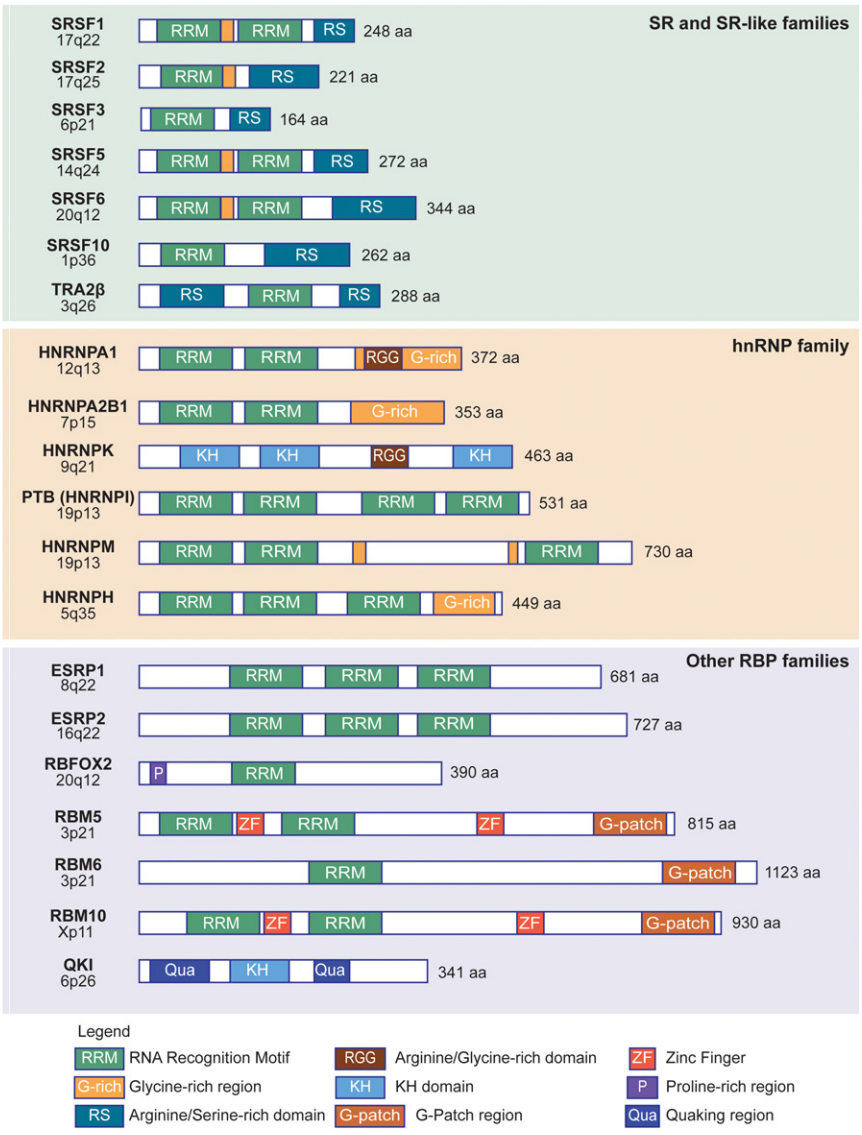

FIGURE 4. Domain structure of splicing factors altered in solid tumors. The chromosomal locus of each splicing-factor-encoding human gene is shown on the *left*. For each RNA-binding protein (RBP) representative of the indicated families, the annotated protein domains or regions are shown in the diagrams (see legend for details), along with the size (in amino acids) of the human protein.

(Fregoso et al. 2013). Sequestration of MDM2 in this complex results in stabilization of the p53 tumor suppressor, which then mediates the stress response. Consistent with the stabilization of p53, increased SRSF1 expression in primary human fibroblasts decreases cellular proliferation and eventually triggers oncogene-induced senescence.

SRSF3—serine/arginine-rich splicing factor 3

Among the other SR proteins, SRSF3 (previously known as SRp20) is also frequently up-regulated in human tumors (Fig. 4). *SRSF3* is an essential gene: *Srsf3* knockout (KO) in mice results in an early lethal phenotype, suggesting a fundamental and nonredundant function for each SR protein in embryonic development (Jumaa et al. 1999). In human tumors, SRSF3 is overexpressed in ovarian, cervical, lung, colon, breast, stomach, skin, bladder, thyroid, liver, and kidney cancer (He et al. 2004, 2011; Jia et al. 2010; Iborra et al. 2013), at least in part due to copy-number changes in Chr. 6p21. Paradoxically, decreased expression of SRSF3 in human hepatocellular carcinoma has also been observed (Sen et al. 2015). Overexpression of SRSF3 promotes transformation on immortal rodent fibroblasts, and KD reduces tumor growth of HeLa cells (Jia et al. 2010). SRSF3 has been shown to regulate splicing of *HIPK2*, a homeodomain-interacting protein, controlling the switch between the full-length isoform and the Δ8 isoform, which induces cell death (Kurokawa et al. 2014). SRSF3 also plays a role in regulating the splicing switch between the *PKM2* and *PKM1* isoforms of pyruvate kinase, the key metabolic enzyme underlying the Warburg effect on cancer cells (Wang et al. 2012a,b). Finally, SRSF3 has been shown to be essential for hepatocyte differentiation and metabolic function in mice, by regulating splicing of target genes involved in glucose and lipid metabolism (Sen et al. 2013). Interestingly, genetic deletion of SRSF3 in hepatocytes causes fibrosis and the development of metastatic hepatocellular carcinoma with aging (Sen et al. 2015).

SRSF6—serine/arginine-rich splicing factor 6

SRSF6 (formerly SRp55) is overexpressed in 50% of lung and colon tumors, as well as in breast tumors, in part due to an amplification at the Chr. 20q12 locus (Fig. 4; Karni et al. 2007; Cohen-Eliav et al. 2013). SRSF6 protein levels are also frequently elevated in basal-cell carcinomas, squamous-cell carcinomas, and malignant melanomas (Jensen et al. 2014). SRSF6 overexpression enhances proliferation and inhibits cell death of mouse lung epithelial cells, whereas SRSF6 KD does not inhibit proliferation, suggesting that SRSF6 is not required for proper growth or survival of these cells, but overexpression may enhance proliferation by gain of function (Cohen-Eliav et al. 2013). SRSF6 is required for both tumor initiation and maintenance of lung cancer and colon cancer cells. In lung and colon, SRSF6 regulates splicing of *INSR* and leads to the

production of the more mitogenic isoform of the insulin receptor (Cohen-Eliav et al. 2013). In human fibroblasts, SRSF6 controls the splicing switch of the kinase MKNK2 and reduces the tumor suppressive isoform *Mnk2a* (Karni et al. 2007). Finally, SRSF6 has a role in normal wound healing that is potentially related to its role in cancer (Jensen et al. 2014). Overexpression of SRSF6 causes skin hyperplasia in mice by deregulating tissue homeostasis, and results in strong up-regulation of *Krt6*, *Krt16*, *Il1b*, and many other wound-healing markers. The effects of SRSF6 on wound healing assayed in vitro depend on controlling splicing of the *Tnc* tenascin-C isoforms (Jensen et al. 2014).

TRA2β—transformer 2β homolog (*Drosophila*)

The SR-like TRA2 proteins are conserved across the animal kingdom, but separate gene paralogs encoding TRA2α and TRA2β proteins evolved early in vertebrate evolution (for review, see Best et al. 2014). KO experiments in mice showed that *Tra2*β is essential for embryonic and brain development (Mende et al. 2010; Roberts et al. 2014). In humans, TRA2β expression changes in several cancers, and this factor has been implicated in the pathology of other diseases, including spinal muscular atrophy, Alzheimer's disease, and frontotemporal dementia with Parkinsonism linked to Chr. 17 (Fig. 4; for review, see Long and Caceres 2009). TRA2β is up-regulated in tumors of the lung, ovary, cervix, colon, and breast (Fischer et al. 2004; Watermann et al. 2006; Iborra et al. 2013). TRA2β up-regulation is associated with invasive breast cancer, and medium to high TRA2β expression is associated with poor prognosis in cervical cancer (Fischer et al. 2004; Watermann et al. 2006; Iborra et al. 2013). Although TRA2β expression is associated with cancer cell survival, its contribution to disease progression is still poorly understood. Up-regulation of TRA2β promotes several splicing switches, including in *CD44*, which were previously associated with tumor progression and metastasis (Watermann et al. 2006). Another example of a TRA2β splicing target is the nuclear autoantigenic sperm protein *NASP*, which is important for cell survival (Grellscheid et al. 2011). TRA2β also regulates splicing of the estrogen receptor α, decreasing the expression of the *ERaΔ7* isoform, which correlates with a better outcome in endometrial cancer (Hirschfeld et al. 2015). Finally, TRA2β regulates turnover of *BCL2* mRNA, in a splicing-independent fashion, by competing with miR-204 for binding to the 3′UTR (Kuwano et al. 2015).

hnRNPA1 and hnRNPA2/B1—heterogeneous nuclear ribonucleoprotein A1 and A2

hnRNPA1 and A2/B1 are structurally related members of the hnRNP protein family, and are likely to play roles in cancer. hnRNPA2/B1 are two spliced isoforms encoded by the same gene, and B1 comprises 12 additional amino acids near the N-terminus (Fig. 4). hnRNPs are a diverse superfamily of abundant RNA-binding proteins expressed in most human tissues (for review, see Chaudhury et al. 2010). The hnRNP A/B family is a subset of hnRNP proteins, with closely related sequences and a conserved modular structure. They regulate alternative splicing, frequently by antagonizing SR proteins, in part through the recognition of exonic splicing silencer elements (Chaudhury et al. 2010). Additional functions of these proteins in mRNA trafficking, and in replication and transcription of cytoplasmic RNA viruses, have also been described. Several studies have reported overexpression of hnRNPA1 or A2/B1 in human tumors, including breast, lung, colon, and glioblastoma (Fielding et al. 1999; Zhou et al. 2001a,b; Karni et al. 2007; Golan-Gerstl et al. 2011). hnRNPA2 is an oncogenic driver in glioblastoma and other brain tumors, and its expression level is correlated with poor prognosis (Golan-Gerstl et al. 2011). In the brain, hnRNPA2 modulates alternative splicing and induces the expression of oncogenic isoforms of the tumor suppressors *BIN1* and *WWOX*, the anti-apoptotic proteins encoded by *CFLAR* and *CASP9*, the insulin receptor gene *IR*, and the *MST1R* proto-oncogene (Golan-Gerstl et al. 2011). Furthermore, hnRNPA1 and A2 modulate alternative splicing of the glycolytic enzyme *PKM2* in cancer cells, suggesting a role in the regulation of tumor metabolism (Clower et al. 2010; David et al. 2010).

hnRNPK—heterogeneous nuclear ribonucleoprotein K

In a subset of AML patients, the Chr. 9q21.32 genomic segment comprising the *HNRNPK* gene is specifically lost, suggesting that a tumor suppressor may reside at this locus (Fig. 4; Kronke et al. 2013). Additionally, AML patients harboring a partial deletion of Chromosome 9 exhibit a significant decrease in hnRNPK protein expression (Gallardo et al. 2015). *Hnrnpk* KO is embryonic lethal in mice, whereas *Hnrnpk* haploinsufficiency results in reduced survival, increased tumor formation, genomic instability, and the development of transplantable hematopoietic neoplasms with myeloproliferation (Gallardo et al. 2015). Reduced hnRNPK expression attenuates p21 activation, down-regulates C/EBP levels, and activates STAT3 signaling (Gallardo et al. 2015). Together, these data suggest that hnRNPK can act as a tumor suppressor in hematological disorders. Conversely, hnRNPK was shown to be overexpressed in a variety of cancers, including breast, esophageal squamous cell, pancreatic, skin, colorectal, and oral squamous cell carcinoma (Mandal et al. 2001; Carpenter et al. 2006; Roychoudhury and Chaudhuri 2007; Zhou et al. 2010). hnRNPK overexpression results in enhanced malignancy and metastasis in vitro, at least in part through alternative splicing of target genes that regulate the extracellular matrix, cell motility, and angiogenesis (Gao et al. 2013). hnRNPK also regulates splicing of the anti-apoptotic *CFLAR* inhibitory protein and the vascular endothelial growth factor *VEGF* in cancer cells (31, 32). Several studies suggested that increased hnRNPK expression

results in a potential oncogenic effect through regulation of c-Myc in solid tumors (Notari et al. 2006; Roychoudhury and Chaudhuri 2007), thus suggesting a fundamental difference between hnRNPK's activity in solid tumors versus hematological malignancies.

PTB—polypyrimidine tract-binding protein

PTB (also known as hnRNPI), is an RNA-binding protein that binds preferentially to pyrimidine-rich sequences (Fig. 4). PTB expression levels are significantly up-regulated in breast, ovarian, glioblastoma, colorectal, and mucosal cancerous tissues compared with the corresponding normal tissues (Jin et al. 2000; He et al. 2004, 2007, 2014; Takahashi et al. 2015). PTB controls alternative splicing of target genes, such as pyruvate kinase *PKM2* (Clower et al. 2010), fibroblast growth factor receptor-1α-exon *FGFR-1* (Jin et al. 2000), and multidrug resistance protein 1 (*MRP1*), which contributes to the drug-resistance phenotype associated with many cancers (He et al. 2004). PTB levels are elevated in cancer cell lines, as well as endometrial tumor tissues, compared to normal tissues, although no correlation was observed between PTB expression and tumor grading (Wang et al. 2008). PTB is also overexpressed in most ovarian tumors, compared with matched normal tissue, and its overexpression is associated with an increased number of expressed *MRP1* spliced isoforms (He et al. 2004, 2007). Furthermore, PTB KD decreases breast and ovarian tumor cell proliferation, anchorage-independent growth, and invasiveness in vitro (He et al. 2007, 2014), and is accompanied by an increased ratio of *PKM1* versus *PKM2* isoform and increased oxygen consumption (He et al. 2014). PTB overexpression also enhances anchorage-dependent growth of immortalized human mammary epithelial cells (HMECs) (He et al. 2014); however, overexpression of PTB alone is not sufficient to transform murine fibroblasts (Wang et al. 2008). Together, these results suggest that PTB plays a role in breast tumorigenesis.

Other hnRNPs

hnRNPM expression is associated with aggressive breast cancers and correlates with increased expression of the CD44 standard (*CD44s*) splice isoform, which comprises exons 1–5 and 16–20, and with the mesenchymal status of breast tumors (Fig. 4; Xu et al. 2014). hnRNPM plays a role in both EMT induction and maintenance, at least in part by potentiating TGFβ signaling and controlling *CD44* isoform switching from the CD44 variable (*CD44v*) exon-8-containing splice isoform to the *CD44s* isoform (Xu et al. 2014).

Splicing factor hnRNPH, which is up-regulated in glioblastoma multiforme, controls splicing of *MADD*, a death-domain adaptor protein, to generate an antagonistic anti-apoptotic isoform that promotes survival and proliferation, and similarly mediates the splicing switch to a ligand-independent, constitutively active variant of the tyrosine kinase receptor *MST1R* (Ron), which promotes migration and invasion (Fig. 4; Lefave et al. 2011).

ESRP1 and 2—epithelial splicing regulator protein 1 and 2

ESRP1 (epithelial splicing regulatory protein 1, also known as RBM35) and ESRP2 (epithelial splicing regulatory protein 2, also known as RBM35B) are two structurally related splicing factors, encoded by genes on Chr. 8 and Chr. 16, respectively, and expressed specifically in epithelial cells (Fig. 4; Warzecha et al. 2009a). The mammalian ESRPs and their orthologs in chicken, *D. melanogaster*, and *C. elegans* comprise three RRMs and display significant phylogenetic sequence conservation within these domains, particularly within the first RRM (Warzecha et al. 2009a). Mice with ablation of *Esrp1* develop cleft lip and palate, whereas tissue-specific ablation in the epidermis revealed ESRP1's requirement for establishing a proper skin barrier (Bebee et al. 2015). Loss of both *Esrp1* and *Esrp2* results in widespread developmental defects (Bebee et al. 2015). ESRPs regulate alternative splicing events associated with epithelial cell phenotypes, and both proteins are down-regulated during the epithelial-mesenchymal transition (EMT) (Warzecha et al. 2009a). ESRP splicing targets are enriched in genes involved in cytoskeletal dynamics, cell motility, cell–cell junctions, and pathways involved in EMT, including *CD44*, *ENAH*, and *FGFR2* isoforms (Warzecha et al. 2009a,b; Shapiro et al. 2011; Dittmar et al. 2012). During oral squamous cell carcinogenesis, ESRPs are up-regulated relative to their levels in normal epithelium, but down-regulated in invasive fronts (Ishii et al. 2014). Importantly, ESRPs are expressed in the lymph nodes, where carcinoma cells metastasize and colonize (Ishii et al. 2014). In head and neck carcinoma cell lines, ESRP1 and ESRP2 suppress cancer cell motility through distinct mechanisms: ESRP1 KD affects the dynamics of the actin cytoskeleton through induction of *RAC1* splicing isoforms, whereas ESRP2 KD attenuates cell-cell adhesion through increased expression of EMT-associated transcription factors (Ishii et al. 2014). In addition, ectopic expression of ESRP1 suppresses the malignant phenotypes of colon and breast cancer cells, suggesting that ESRP1 is a tumor suppressor (Leontieva and Ionov 2009; Horiguchi et al. 2012). In contrast, high levels of ESRP1 expression are associated with lower survival rate in breast cancer patients (Yae et al. 2012). These paradoxical findings may reflect yet unappreciated differences between the tumor subtypes or in the experimental models, which should be further characterized in detail. Thus, ESRP1 is likely to play a dual role during tumor progression, with the potential to act either as a positive or a negative regulator.

RBFOX2—RNA binding protein, Fox-2 homolog

The RBFOX family of splicing regulators includes three mammalian paralogs, RBFOX1 (A2BP1), RBFOX 2

(RBM9), and RBFOX3 (NeuN), which control tissue-specific alternative splicing of exons in brain, muscle, epithelial, and mesenchymal cells, as well as in embryonic stem cells (Kuroyanagi 2009). RBFOX proteins have a single RRM, highly homologous among the three paralogs, and their binding sites are exceptionally highly conserved in sequence (UGCAUG) and position across vertebrate evolution (Sun et al. 2012). Mutations or deletions in *RBFOX1* and *RBFOX3* are found in epilepsy patients (Bhalla et al. 2004; Lal et al. 2013a,b), whereas copy-number variations in *RBFOX1* are associated with autism spectrum disorders and spinocerebellar ataxias (Bill et al. 2013; Weyn-Vanhentenryck et al. 2014). Mouse models with *Rbfox* KO or KD show extensive defects in neuronal and muscle physiology, suggesting that these proteins play key roles in normal development (Gehman et al. 2011, 2012). A role for RBFOX2 in cancer has also been proposed. Its levels are significantly lower in epithelial ovarian cancer than in normal tissue (Venables et al. 2009). In breast cancer, alternative splicing events occurring in cell lines from the claudin-low subtype are enriched in the RBFOX2 binding motif (Fig. 4; Lapuk et al. 2010). RBFOX2-regulated splicing events were also identified in multiple solid tumor types, including breast, colon, and pancreatic tumors (Danan-Gotthold et al. 2015). RBFOX2 was identified as one of the potential drivers of the mesenchymal splicing signature in a mammary cell line, following induction of Twist expression (Shapiro et al. 2011). In this context, the loss of RBFOX2 in mesenchymal cells leads to a partial reversion of the epithelial phenotype, with reduced levels of vimentin, changes in morphology, and restricted migration (Shapiro et al. 2011). Furthermore RBFOX2 regulates events that differ between epithelial and mesenchymal cancer cell lines (Venables et al. 2013). Thus, RBFOX2 may be important to specify the mesenchymal tissue-specific splicing profiles both in normal and in cancer tissues.

RBM5, 6, and 10—RNA binding motif protein 5, 6, and 10

RBM5, RBM6, and RBM10 are highly homologous RNA-binding proteins that share 30–50% amino-acid sequence identity (Fig. 4; Bechara et al. 2013). They comprise two RRMs and an RS-domain, as well as a zing-finger domain, a G-patch domain, and a nuclear localization signal (Bechara et al. 2013). The genes encoding *RBM5* and *RBM6* map to the Chr. 3p21.3 region, which is frequently deleted in heavy smokers, in lung cancer, and in other tissue carcinomas (Angeloni 2007). RBM5 protein levels are down-regulated in 75% of primary lung cancers (Oh et al. 2002), as well as in prostate and breast cancer samples (Zhao et al. 2012). Down-regulation of RBM5 is considered to be one molecular signature associated with metastasis of various solid tumors (Ramaswamy et al. 2003). On the other hand, overexpression of RBM5 in breast cancer samples was also reported (Rintala-Maki et al. 2007), paradoxically suggesting that both up- and down-regulation of RBM5 can play a role in tumor progression. RBM6 and 10 also show altered expression in breast cancer (Rintala-Maki et al. 2007). Overexpression of RBM5 in various cell lines leads to growth arrest, apoptosis, and delayed tumor growth when cells are injected into nude mice (Mourtada-Maarabouni and Williams 2002; Oh et al. 2002, 2006; Mourtada-Maarabouni et al. 2003). RBM5-mediated apoptosis is associated with up-regulation of the pro-apoptotic protein BAX and down-regulation of the anti-apoptotic proteins BCL-2 and BCL-XL (Mourtada-Maarabouni and Williams 2002; Oh et al. 2006; Sutherland et al. 2010). RBM5/6 and RBM10 antagonistically regulate proliferation of breast and lung cancer cells, and display distinct positional effects on alternative splicing regulation (Bechara et al. 2013). The Notch pathway regulator *NUMB* is a key splicing target of RBM5, 6, and 10, which contributes to their role in colony formation in vitro and in tumor xenograft growth in vivo (Bechara et al. 2013). Finally, RBM10 is also among the most frequently mutated genes in lung adenocarcinoma (Imielinski et al. 2012). The *RBM10* mutations identified in patients disrupt *NUMB* splicing regulation and promote proliferation of cancer cell lines (Bechara et al. 2013; Hernandez et al. 2016).

QKI—quaking KH domain containing RNA binding

Quaking KH domain containing RNA binding (QKI) protein belongs to the STAR (signal transduction and activation of RNA) family of RNA-binding proteins (Fig. 4; for review, see Darbelli and Richard 2016). QKI has been implicated in the regulation of myocyte and oligodendrocyte differentiation, as well as in endothelial-cell function (Darbelli and Richard 2016). *QKI* haploinsufficiency is associated with the 6q terminal deletion syndrome, which is characterized by intellectual disabilities (Darbelli and Richard 2016). QKI is also frequently down-regulated in lung adenocarcinoma, squamous cell carcinoma, and large cell carcinoma, compared with normal lung, and its down-regulation is significantly associated with poor prognosis (Zong et al. 2014). QKI expression is also reduced in oral squamous cell carcinoma, compared to normal mucosa (Lu et al. 2014), as well as in prostate cancer samples, at least in part due to the high levels of promoter methylation (Zhao et al. 2014). QKI is highly expressed in benign prostatic hyperplasia, but not in carcinomatous tissue (Zhao et al. 2014). In addition, the decrease in QKI expression closely correlates with the Gleason score, poor differentiation, degree of invasion, lymph-node metastasis, distant metastasis, tumor grading, and poor survival (Zhao et al. 2014). QKI overexpression impairs proliferation and transformation of lung and prostate cancer cells (Zhao et al. 2014; Zong et al. 2014), as well as reduces cancer stem cell sphere formation and stem-cell-associated gene expression in oral cancer cells (Lu et al. 2014). In a tumor-implantation nude-mouse model, QKI expression significantly reduces the tumor-initiation rate, tumor size, and lung-

metastasis rate, whereas QKI KD has the opposite effects (Lu et al. 2014; Zong et al. 2014). QKI expression is also down-regulated in lung, colon, and breast tumors, but is up-regulated in kidney cancer, and QKI-regulated splicing events are altered in the corresponding tumor types (Danan-Gotthold et al. 2015). QKI regulates splicing of *NUMB*, which in turn suppresses cell proliferation and prevents the activation of the Notch signaling pathway (Zong et al. 2014). QKI may also directly regulate SOX2 expression via specific binding to its mRNA 3′UTR in a *cis*-element-dependent way (Lu et al. 2014). Recently, MYB-QKI fusions were identified as a specific and single candidate-driver event in angiocentric gliomas (Bandopadhayay et al. 2016). In vitro and in vivo functional studies demonstrated that MYB-QKI rearrangements promote tumorigenesis through three mechanisms: MYB activation by truncation, enhancer translocation driving aberrant MYB-QKI expression, and hemizygous loss of the QKI tumor suppressor (Bandopadhayay et al. 2016).

REGULATION OF THE SPLICING MACHINERY IN TUMORS

How changes in splicing-factor levels occur in human tumors remains poorly understood. In the absence of genomic alterations at loci encoding splicing regulators, alterations in splicing-factor expression likely result from changes in transcriptional or post-transcriptional regulation, as well as in signaling pathways.

Splicing-factor regulation by the transcription factor MYC

A link between the transcription factor oncoprotein MYC and the splicing machinery has emerged in the past few years. Genes encoding several splicing factors, such as *SRSF1*, *HNRNPA1*, *HNRNPA2*, or *PTB*, were shown to be direct transcriptional targets of MYC (Figs. 1, 3; David et al. 2010; Anczukow et al. 2012; Das et al. 2012). Furthermore, SRSF1 not only contributes to MYC's oncogenic activity (Das et al. 2012), but also cooperates with MYC in transformation, promoting the formation of more aggressive breast tumors (Anczukow et al. 2012). In addition, two recent studies revealed that components of the spliceosome, such as PRMT5 and BUD31, are essential for MYC to function as an oncogene in lymphoma and breast cancer, respectively (Hsu et al. 2015; Koh et al. 2015). As MYC is the most frequently amplified oncogene in human cancers and plays a crucial role in transformation, therapies that exploit the spliceosome would be very attractive. Both studies uncovered an essential role of the splicing machinery in MYC-driven transformation, and identified multiple associated abnormal splicing events, including intron retention (Hsu et al. 2015; Koh et al. 2015). Interestingly, MYC appears to alter splicing by somewhat different mechanisms in lymphomagenesis versus breast cancer. In the former context, MYC hyperactivation affects the levels of specific splicing regulators (Koh et al. 2015), whereas in the latter context, it promotes a global increase in pre-mRNA levels (Hsu et al. 2015), although up-regulation of particular splicing regulators was also previously reported (David et al. 2010; Anczukow et al. 2012; Das et al. 2012). These ostensibly different findings suggest that many of the splicing changes associated with cancer are context-dependent.

Post-transcriptional regulation of SRSFs

SRSFs are essential genes whose expression is tightly regulated at the post-transcriptional level. For example, SRSF1 or SRFS2 regulate splicing of introns in the 3′UTR of their own pre-mRNA; splicing out of these introns introduces an exon–exon junction downstream from the normal stop codon, which targets the mRNA to NMD (Sureau et al. 2001; Lareau et al. 2007a,b). This mechanism is highly conserved and shared by other SR proteins (Sureau et al. 2001; Lareau et al. 2007a,b; Lareau and Brenner 2015). SRSF1 autoregulation can also occur via nuclear retention of alternative SRSF1 transcript variants or regulation of the translational efficiency of other variants (Sun et al. 2010). Autoregulation of SR proteins serves as a negative feedback loop, in which increased SR protein levels promote an increase in unproductive spliced variants of their own transcripts. Furthermore, several miRNAs targeting SRSF1 mRNA have been identified, including mir-7, miR-28, miR-505, miR-10a, and miR-10b (Verduci et al. 2010; Wu et al. 2010; Meseguer et al. 2011). Finally, SRSF1 expression is also regulated at the level of splicing by the splicing factor Sam68 (Valacca et al. 2010). In response to ERK1/2 signaling, Sam68 switches splicing of the *SRSF1* transcript from the NMD-targeted isoforms to the major, translatable isoform, thus resulting in an increase in SRSF1 protein levels. Sam68-mediated SRSF1 overexpression is associated with oncogenic phenotypes, such as increased cell-proliferation, anchorage-independent growth, cell motility and invasion, and epithelial–mesenchymal transition in colon cancer cell lines (Valacca et al. 2010). Thus, SRSF1 transcript levels are fine-tuned by various post-transcriptional mechanisms, though the precise contributions of each step in response to various stimuli remain to be determined.

SR protein regulation by phosphorylation

SR protein localization and activity are regulated by a dynamic cycle of phosphorylation/dephosphorylation, mostly at serine residues within the RS-domain (for a detailed review, see Long and Caceres 2009). For example, phosphorylation of SRSF1's RS domain enhances protein–protein interactions with other RS-domain-containing splicing factors, such as U1–70K (Xiao and Manley 1997), whereas dephosphorylation of SR and SR-related proteins is required for splicing catalysis (Tazi et al. 1993; Cao et al. 1997). The SR protein

kinase family (SRPK) (Gui et al. 1994; Wang et al. 1998), Clk/Sty kinase family (Colwill et al. 1996) and topoisomerase I (Rossi et al. 1996) have been identified as the main regulators of SR protein phosphorylation. A hypo-phosphorylated RS domain is required for the interaction of shuttling SR proteins with the TAP/NFX1 nuclear export receptor (Huang et al. 2004), whereas the RS domain needs to be rephosphorylated before SR proteins can return to the nucleus (Ding et al. 2006). Dephosphorylation also plays an important role in sorting SR proteins in the nucleus, where shuttling SR proteins and nonshuttling SR proteins are recycled via different recycling pathways (Lin et al. 2005). In the cytoplasm, dephosphorylation of the RS domain enhances mRNA binding by SRSF1 and contributes to its stimulatory role in translation (Sanford et al. 2005). The regulation of SR protein phosphorylation has been implicated in the control of cancer-associated splicing events. For example, growth-factor-induced alternative splicing of the fibronectin EDA exon involves phosphorylation of SRSF1 and SRSF7 by AKT kinase (Blaustein et al. 2004). This effect is most likely indirect and occurs via SRPKs. Indeed, AKT was shown to activate SRPKs in response to EGF-signaling, thus leading to enhanced SRPK nuclear translocation and SR protein phosphorylation (Zhou et al. 2012). Multiple components of the AKT pathway function as oncogenes or tumor suppressors, and overexpression of SRPK1 is seen in multiple tumor types (Hayes et al. 2006, 2007; Plasencia et al. 2006; Krishnakumar et al. 2008; Amin et al. 2011); however, how perturbations in phosphorylation processes lead to changes in splicing-factor activity in human tumors remains to be defined.

TARGETING ALTERNATIVE-SPLICING ALTERATIONS FOR CANCER THERAPY

The idea of targeting the spliceosome is not new, and the first general splicing inhibitors were initially identified in the late 1990s, during the course of characterizing certain anti-tumor drugs (for review, see Bonnal et al. 2012). However, improvements in RNA-targeting therapeutics and in particular in antisense-oligonucleotide pharmacology, as well as a better understanding of the mode of action of these molecules, have created novel therapeutic opportunities. Two conceptually different approaches to splicing inhibition are currently being tested.

Blocking splicing-factor activity with small-molecule inhibitors

The first approach is to target general components of the splicing machinery and inhibit splicing at a global level, e.g., using small-molecule inhibitors of the SF3B complex, or of kinases that phosphorylate SR proteins (Bonnal et al. 2012). These drugs inhibit very basic steps in splice-site recognition, lack splicing-target specificity, and can potentially have broad cytotoxic effects. However, several studies have reported that cancer cells are more sensitive to these drugs, compared to normal cells (Bonnal et al. 2012), suggesting that general inhibition of splicing could be a viable anti-tumor strategy. For example, SF3B1 inhibitors, such as spliceostatin A, pladienolide-B, GEX1A, and E1707, partially inhibit splicing in cultured cells and animal models, and show selective action, i.e., greater potency, on tumor cells (for review, see Ohe and Hagiwara 2015). Thus SF3B1 inhibitors are currently being evaluated in the context of splicing-factor mutations in preclinical models of MDS (Lee et al. 2016).

Another way to block splicing-factor activity is to inhibit the regulatory kinases that phosphorylate the SR proteins (Ohe and Hagiwara 2015). For example, inhibition of Cdc2-like kinase 1 (CLK1), dual-specificity-tyrosine-phosphorylation-regulated kinase 1A (DYRK1A), or SR protein-specific kinase (SRPK) affects alternative splicing and induces exon skipping of SR-regulated exons in cell cultures (Ohe and Hagiwara 2015).

Exon-specific splicing modulation

An alternative approach to target splicing alterations is to directly target a tumor-specific splicing event, e.g., by using antisense oligonucleotides that bind to a transcript in a sequence-specific manner to redirect splicing (for review, see Kole et al. 2012). Briefly, antisense oligonucleotides are short single-stranded nucleic-acid molecules with various chemical modifications—compared to RNA or DNA—that bind to complementary sequences on the target pre-mRNA in the nucleus. The target sequences can be, e.g., splice sites, splicing enhancers, or splicing silencers, which are normally recognized by the core splicing machinery or by a particular regulatory splicing factor (activator or repressor). Thus, such antisense compounds can be used either to promote or inhibit a splicing event in a target-specific manner. This approach is expected to have few off-targets effects—provided that unique complementary sequences in the transcriptome are targeted—and with appropriate target selection, it might be more tumor-specific, compared to general splicing inhibitors. However, identifying a key splicing event, or more likely a set of splicing events, required for transformation and tumor maintenance, will require a concerted effort.

Finally, small-molecules that can specifically modulate a single alternative splicing event are currently being investigated as an alternative to antisense-oligonucleotide therapies. For example, several groups identified compounds that enhance *SMN2* exon 7 splicing to promote the production of a full-length SMN protein (Hastings et al. 2009; Naryshkin et al. 2014; Palacino et al. 2015), an approach with therapeutic potential for spinal muscular atrophy. Likewise, small molecules that promote the inclusion of exon 20 in the *IKBKAP* pre-mRNA have been reported, and may have therapeutic utility for familial dysautonomia (Yoshida et al.

2015). However, it is still unknown to what extent this type of strategy may be broadly applicable to other splicing events. These molecules were initially identified using cell-based reporter screens, and while potential mechanisms of action have been proposed, additional work will be required to understand what degree of target specificity is achievable, and to what extent these approaches are generalizable.

In summary, splicing-factor alterations detected in human tumors are a growing area of interest in cancer research, and represent potential targets for personalized cancer therapies. However, the precise roles of splicing factors in normal tissue physiology and the consequences of their dysregulation in disease are still underexplored. A better understanding of the cell-type specificity and functions of cancer-associated splicing factors will be crucial for the development of new therapeutic strategies.

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