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Misregulation of pre-mRNA alternative splicing in cancer

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Abstract

Alternative splicing of mRNA precursors enables one gene to produce multiple protein isoforms with differing functions. Under normal conditions, this mechanism is tightly regulated in order for the human genome to generate proteomic diversity sufficient for the functional requirements of complex tissues. When deregulated, however, cancer cells take advantage of this mechanism to produce aberrant proteins with added, deleted, or altered functional domains that contribute to tumorigenesis. Here we discuss aspects of alternative splicing misregulation in cancer, focusing on splicing events affected by deregulation of regulatory splicing factors and also recent studies identifying mutated components of the splicing machinery.

Introduction

The vast majority of protein-coding genes in humans contain multiple exons. Splicing of mRNA precursors (pre-mRNA), the removal of introns and the joining of flanking exons, is a fundamental step in the production of the encoded protein. Although the splicing of individual exons must be precise, the selection of exons to be included in the final mRNA allows a certain degree of plasticity. Alternative use of exons, or alternative splicing (AS), enables a single gene to produce multiple mRNA variants. More than 90% of human genes produce transcripts that are alternatively spliced (1, 2), and 60% of the splice variants encode distinct protein isoforms (3). Protein isoforms of a given gene can have different or even opposing functions (4, 5). Thus, AS is considered to be a major mechanism for generating proteomic diversity (6).

Regulation of AS is tightly controlled during normal tissue differentiation (7, 8). Misregulation of AS can lead to production of aberrant protein isoforms, which may contribute to diseases including cancer. Genome-wide studies have revealed more than 15,000 tumor-associated splice variants in a wide variety of cancers (9–11). Computational analysis of tumor-associated splice variants indicates that AS occurs with genes involved in almost every aspect of cancer cell biology, including proliferation, differentiation, cell cycle control, metabolism, apoptosis, motility, invasion, and angiogenesis (9). In a functional screen of selected splice variants, it was found that 10% (4 out of 41 tested) AS events specific to breast and/or ovarian cancers contribute to cancer cell survival (12). Although the functional significance of cancer-specific AS events is still largely unexplored, the link between aberrant AS and cancer has been established (4, 13, 14).

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Aberrant AS events often reflect abnormalities in splicing regulation. Pre-mRNA splicing is generally regulated by cis-acting splicing sequences in primary transcripts and trans-acting splicing factors that bind these RNA sequences (15). Alterations in protein levels and activity of regulatory splicing factors, mutations in cis-acting splicing sequences, and mutations in the core components of the splicing machinery itself may result in aberrant AS in cancer and contribute to many cancer phenotypes. Here we discuss recent studies on the misregulation of AS in cancer. For more insights into the importance and mechanisms of AS regulation in health and disease, the reader is referred to several excellent reviews (4, 5, 14, 15).

AS Patterns in Cancer

AS patterns in cancer cells reflect those found in normal cells. Global analysis of more than 15,000 cancer-specific splice variants in 27 types of cancers shows that the average number of cancer-specific splice variants per gene is smaller than that of tissue-specific splice variants in 35 normal tissues (1.51 vs. 1.99)(9). This is expected, because tissue-specific splice variants are required for generating the necessary proteomic complexities of human tissues and the splice variants have undergone extensive natural selection during the course of evolution (16, 17). Cancer-specific splice variants, which may bestow survival advantages to cancer cells, often result in rapid death of the human subject that harbors the cancer, and therefore are selected against rather than selected for at the organismal level. Regardless, cancer-specific AS includes all of the five main AS patterns observed in normal tissues: cassette exons, alternative 5′ splice sites (ss), alternative 3′ ss, intron retention, and mutually exclusive exons (Figure 1), suggesting that cancer cells and differentiated cells use fundamentally similar splicing mechanisms. To illustrate the AS patterns that cancer cells use to gain survival advantage, we describe below an exemplary set of functionally important AS events (Figure 1).

Cassette exons—skipping of one exon

The *RON* gene encodes a tyrosine kinase receptor for macrophage-stimulating protein (MSP). Under normal conditions, RON is involved in cell mobility and invasion in response to MSP binding (18, 19). A splice isoform, ΔRON, which lacks exon 11, is overexpressed in a number of cancers (20). Skipping of exon 11 results in the deletion of an extracellular domain that affects the proteolytic maturation of the protein. The truncated ΔRON is constitutively active (even in the absence of its ligand) and promotes cancer invasiveness (21).

Cassette exons—skipping of multiple exons

BRAF is a proto-oncogene encoding the serine/threonine-protein kinase BRAF, which regulates the MAPK/ERK signaling pathway. An often fatal mutation (V600E) was found in more than half of the patients with malignant melanomas (22). Effective treatment can involve the use of BRAF inhibitors such as PLX4032, to which BRAF(V600E) is sensitive. However, skipping of exons 4–8 during splicing of *BRAF(V600E)* transcripts results in an in-frame deletion of the N-terminal RAS-binding domain. The truncated enzyme is insensitive to the inhibitors and therefore confers melanoma cells resistance to the drugs (23).

Cassette exons—exon inclusion

SYK, or spleen tyrosine kinase, functions as a tumor suppressor in breast cancer (24), but acts as an oncogene in T-cell lymphomas (25), chronic leukemias (26) and head and neck carcinomas (27). This paradox had not been explained until recently when Prinos *et al.* found that *SYK* expresses two distinct splice isoforms, a longer SYK(L) and shorter SYK(S)

isoform (12). SYK(L), which includes exon 9 and is found in many cancers, promotes cell survival and tumor malignancy. Switching SYK(L) to SYK(S), which lacks exon 9, induces apoptosis of ovarian cancer cells, while a switch in the opposite direction, which can be induced by epidermal growth factor, leads to cancer cell growth.

Alternative 5’ ss

AS of *BCL-X* pre-mRNA is the best-known example of this pattern. BCL-X belongs to the BCL-2 protein family, whose members form hetero- or homodimers that act as anti- or pro-apoptotic regulators both in health and in disease (28). *BCL-X* produces two splice isoforms, BCL-X_L and BCL-X_S, through the alternative use of two competing 5′ ss in exon 2 (29). The longer isoform BCL-X_L has anti-apoptotic effects and is overexpressed in various cancer types (30–32). In contrast, the shorter isoform BCL-X_S is pro-apoptotic and is down-regulated in cancer (33).

Alternative 3’ ss

VEGF (vascular endothelial growth factor) is a mitogen that stimulates angiogenesis required for tumor growth (34, 35). Pre-mRNA of *VEGF* contains eight exons, with two competing 3′ ss in exon 8. Alternative use of the 3′ ss leads to production of two families of VEGF isoforms (36). Selection of the proximal 3′ ss produces one family of isoforms called VEGFxxx, where xxx indicates the number of amino acids on the protein. When the distal 3′ ss is used, VEGF produces the other isoform family VEGFxxxb. These two isoform families have opposing functions: VEGFxxx isoforms are pro-angiogenic, and are overexpressed in a number of tumors, whereas VEGFxxxb isoforms are anti-angiogenic and downregulated in tumors (36). It is believed that the opposing functions are caused by the distinct C-termini produced by alternative use of the 3′ ss. The C-terminus of VEGFxxxb (for example VEGF165b) fails to bind its receptor, neurophilin 1, which is required for full activation of the VEGF-signaling transduction (37).

Intron retention

STAT2 (signal transducer and activator of transcription 2) is a transcription factor and a main component of the JAK (Janus kinase)/STAT signaling pathway (38). Upon interferon (IFN) stimulation, STAT2 dimerizes with STAT1 and the heterodimer translocates to the nucleus and activates transcription of IFN responsive genes. Through this pathway, IFN induces apoptosis of cancer cells (38). IFN is used as a treatment for many cancers, and is most effective on hematological malignancies (39). However, cancer cells frequently develop resistance to IFN. Du *et al.* discovered that IFN resistant cells produce a STAT2 splice variant containing intron 19 (40). This retained intron introduces a stop codon before the Src homology 2 domain, leading to disruption of STAT dimerization.

Mutually exclusive exons

PKM (pyruvate kinase M) is a metabolic enzyme that catalyzes the last step of glycolysis, and AS of *PKM* pre-mRNA is critical for tumor metabolism. Tumor cells have long been known for their use of massive amounts of glucose and production of large quantities of lactate, even in the presence of oxygen (aerobic glycolysis or the Warburg effect) (41). Aerobic glycolysis produces ATP less efficiently, but it is believed to promote accumulation of glycolytic intermediates that are channeled to biosynthesis pathways for making new tumor cells. It is now clear that the switch between aerobic glycolysis and oxidative phosphorylation is at least partly achieved by AS of *PKM* pre-mRNA. *PKM* has two mutually exclusive exons: exon 9 (E9) and exon 10 (E10). AS of these exons results in production of two isoforms: the adult isoform PKM1, which includes E9 but not E10, and the embryonic isoform PKM2, which includes E10 but not E9 (42). PKM2 is ubiquitously

expressed in tumors, while PKM1 is expressed in differentiated tissues, such as muscle and brain (42–44). Replacing PKM2 with PKM1 in tumor cells reduced lactate production and increased oxidative phosphorylation. When the cells were injected into nude mice, tumor growth was greatly inhibited (44).

Complex splicing patterns

MDM2 (Mouse double minute 2 homolog) is a negative regulator of the p53 tumor suppressor (45). The *MDM2* gene has 12 exons, and AS of its pre-mRNA involves skipping of one or more exons and use of several cryptic splice sites, leading to production of at least 40 splice variants in various tumors and normal tissues (46). Full-length MDM2 binds to p53 and acts as an ubiquitin ligase and facilitates proteasomal degradation of p53 (47, 48). The functions of most MDM2 splice isoforms are unclear. At least four of the splice isoforms (MDM2-A, -B, -C, and -D) in human cancers lack part of the p53 binding domain, and therefore are unable to bind to and degrade p53 (49). Interestingly, the most frequently expressed tumor isoform, MDM2-B, binds to full-length MDM2 and sequesters it, leading to accumulation of p53. However, the increased p53 activity contrasts with the transforming ability of MDM2-B (49), consistent with a more complex view of MDM2 function (50).

As described above, and in many other instances not discussed here, aberrant AS in cancer enables individual genes to produce distinct protein isoforms with deleted, added, or altered domains. This in turn brings about different or even opposing functions that contribute to a variety of cancer cell activities such as growth, apoptosis, invasiveness, drug resistance, angiogenesis, and metabolism.

Misregulation of AS in Cancer by Regulatory Splicing Factors

Splicing regulation is essentially the process of selecting splice sites in pre-mRNA transcripts. This process is generally directed by cis-acting regulatory sequences and trans-acting RNA binding proteins (RBPs) (15). Well-studied RBPs include two families: serine/arginine-rich (SR) proteins and heterogeneous nuclear ribonucleoproteins (hnRNPs) (15, 51–55). SR proteins bind to exonic splicing enhancers (ESEs) and intronic splicing enhancers (ISEs) and usually promote exon inclusion. In contrast, hnRNPs bind to exonic splicing silencers (ESSs) and intronic splicing silencers (ISSs) and in most cases lead to exon skipping (15, 51–55). Thus, one major mechanism of AS misregulation is through alterations in the levels and activity of RBPs. Below we describe a few RBPs that have been implicated in misregulation of AS in cancer.

SRSF1 (formerly known as ASF/SF2) is perhaps the best-known SR protein, and is involved in both constitutive and regulated splicing as well as other cellular processes. It is upregulated in various human tumors and its induced overexpression leads to transformation of mammary epithelial cells and immortal rodent fibroblasts, suggesting that it may be a proto-oncogene (56, 57). SRSF1 affects AS of many target pre-mRNAs, some of which are known to contribute to tumorigenesis. It binds to an ESE in exon 12 of *RON* and promotes skipping of exon 11 to produce ΔRON, which enhances cancer invasiveness (20). Overexpression of SRSF1 leads to inclusion of exon 12a of *BIN1* (a tumor suppressor gene), and the resulting isoform loses tumor-suppressor activity due to its inability to interact with MYC (56). In addition, SRSF1 promotes the production of isoform 2 of S6K1 through AS, and overexpression of this isoform is able to transform NIH3T3 cells (56). SRSF1 also modulates AS of *MNK2* pre-mRNA: overexpression of SRSF1 results in production of the MNK2b isoform, promoting MAPK-independent phosphorylation of the eukaryotic initiation factor eIF4E, which enhances cap-dependent translation and may contribute to oncogenic transformation (56). It has also been shown that SRSF1 can interact directly with mTOR to facilitate phosphorylation of the translation inhibitor 4E-BP, leading to 4E-BP

release from eIF4E and activation of translation (58). Recently, Anczukow *et al.* found that SRSF1 stimulates production of isoform BIM γ1, which lacks exons 2 and 3 of BIM (a pro-apoptotic BCL-2 family member), and concomitantly downregulates production of BIN1+13, a BIN1 isoform that includes exon 13 (57). Expression of BIM γ1 increased acinar size and decreased apoptosis whereas expression of isoform BIN1+13 did the opposite. Therefore, it was proposed that BIM γ1 upregulation and BIN1+13 downregulation combined contributes to SRSF1-induced tumorigenesis (57). Recently, it was shown that SRSF1 is regulated by MYC: MYC directly binds to two non-canonical E-boxes in the *SRSF1* promoter and activates its transcription. Knockdown of MYC downregulates SRSF1 expression in lung cancer cell lines (59).

SRSF3 (formerly SRp20) is another SR protein that has been implicated in misregulation of AS in cancer. It is overexpressed in human ovarian, lung, breast, stomach, skin, bladder, colon, liver, thyroid, and kidney cancers (57, 60). Overexpression of SRSF3 leads to transformation of rodent fibroblasts, suggesting that it is a proto-oncogene (60). Knockdown of SRSF3 results in apoptosis of a variety of cancer cells (57, 60, 61). It was shown that knockdown of SRSF3 led to skipping of exon 8 of homeodomain-interacting protein kinase-2 (*HIPK2*), an antioncogene that induces tumor cell apoptosis. Full-length HIPK2 binds to an E3 ubiquitin ligase (Siah-1) and is constantly degraded, whereas the isoform HIPK2 Δe8, which lacks 27 amino acids, loses the ability to bind Siah-1 and therefore is resistant to protein degradation. HIPK2 Δe8 still retains antioncogenic activity and therefore induces apoptosis (61). Tang *et al.* provided evidence that SRSF3 also regulates AS of p53. Binding of SRSF3 to exon i9 of p53 inhibits production of isoform p53β. Downregulation of SRSF3 induces p53β production and promotes cellular senescence (62). Finally, Wang *et al.* identified an SRSF3 binding site in exon 10 of *PKM* pre-mRNA and showed that knockdown of SRSF3 resulted in an ~20% switch from PKM2 to PKM1 and reduced lactate production (63).

hnRNP A1 and hnRNP A2 are two structurally and functionally related hnRNPs that likely play a role in cancer. Both proteins are overexpressed in a wide variety of cancers (42, 64, 65). RNAi-mediated knockdown of hnRNP A1 and A2 (A1/A2) together results in apoptosis in cancer cells, but not in normal cells, suggesting that the two proteins are important for cancer cell growth (64). Golan-Gerstl *et al.* showed that overexpression of hnRNP A2 in NIH3T3 cells induces skipping of *RON* exon 11 and production of ΔRON. Knockdown of RON inhibited the hnRNP A2-mediated transformation (65). A recent genome-wide analysis of AS events using high-throughput sequencing of RNA isolated by crosslinking immunoprecipitation (HITS-CLIP) showed that hnRNP A1 and A2 each potentially regulates more than 2,000 AS events (66). One important finding is that A1/A2 share many (one third) common targets, one of which is *PKM*. As mentioned above, deregulation of *PKM* AS is known to be critical for glucose metabolism in tumor cells. David *et al.* showed that A1/A2 together with another hnRNP protein, PTB, bind to sequences flanking exon 9 of *PKM* pre-mRNA and repress E9 inclusion and promote E10 inclusion (42). Expression levels of A1/A2 and PTB were found to correlate perfectly with the ratios of PKM2/PKM1 in a number of normal brain and glioma samples. Knockdown of A1/A2 and PTB, or of c-Myc, which drives their expression, results in switching from PKM2 to PKM1 (42). Consistent with these findings, another study showed that A1/A2 and PTB knockdown leads to a decrease of lactate production in a glioblastoma (GBM) cell line (43). Chen *et al.* further demonstrated how mechanistically the protein levels of A1/A2 and PTB determine the outcome of *PKM* AS: at high levels, A1/A2 and PTB predominantly bind to sites in and around exon 9 to repress E9 inclusion, but when their levels are reduced, their binding shifts to sites flanking exon 10, preventing E10 inclusion (67).

PTB (also known as hnRNP I) is an hnRNP that binds polypyrimidine-rich intronic elements and in most cases represses the inclusion of the regulated exon (68). It has been shown that PTB is upregulated in ovarian cancer and gliomas (42, 69, 70). Knockdown of PTB suppresses ovarian tumor cell growth and invasiveness *in vitro* (70). However, overexpression of PTB in immortalized or normal cells does not enhance proliferation, anchorage-independent growth, or invasion (71), suggesting that PTB may play a necessary, but not transforming, role in tumorigenesis. PTB is known to regulate several AS events that are relevant to cancer. Binding of PTB to an ISS element in the *FGFR-1* transcript leads to skipping of the α exon and production of isoform FGFR-1β (72). This truncated receptor has higher affinity for FGF-1 (73), and might facilitate malignant progression of astrocytic tumors (74). PTB also regulates AS of USP5, a deubiquitinating enzyme whose knockdown can lead to accumulation of p53 (75). Two USP5 isoforms can be generated by use of alternative 5′ ss in exon 15. In GBM, high levels of PTB inhibit the proximal 5′ ss and use of the distal 5′ ss produces USP5 isoform 2. Switching isoform 2 to isoform 1 using antisense oligonucleotides inhibited growth and migration of two GBM cell lines (69). Genome-wide studies of PTB-regulated AS events in HeLa cells using HITS-CLIP showed that PTB not only represses but also activates exon inclusion, depending on whether its binding sites are located within and upstream of or downstream of the regulated exons (68, 76). The functional significance of these PTB-regulated AS events has not been examined, although one of the PTB targets is an ISS element upstream of exon 9 of *PKM* (68). As discussed earlier, PTB binds to this ISS and, together with hnRNP A1/A2, regulates *PKM* AS (42). The fact that tumor cells overexpress and recruit three different hnRNP proteins to regulate *PKM* AS reinforces the importance of producing PKM2 in tumor cells.

hnRNP H has recently been implicated in oncogenesis through the misregulation of AS of both *IG20/MADD* and *RON* pre-mRNAs (77). hnRNP H is upregulated in gliomas and binds to an ESS in exon 16 of *IG20/MADD*, leading to skipping of exon 16 and production of the MADD isoform, which is necessary and sufficient for cell survival (77). RNAi-mediated knockdown of hnRNP H reverses AS, producing the exon 16-containing IG20 isoform, and results in cell death of both U373 glioma and HeLa cells, possibly through IG20-triggered caspase 8 activation (78). In addition, hnRNP H binds to a similar ESS in exon 11 of *RON* and leads to skipping of exon 11 and production of ΔRON, which promotes cell invasiveness (77).

In the above examples, misregulation of AS occurs in the absence of genetic mutations and, in many cases, without changes in the overall levels of the alternatively spliced transcripts. Switching from one isoform to another is regulated by the levels and activity of RBPs, either individually or in combination. A recent proteomic study revealed as many as 860 RBPs in human (79). However, only a couple of dozens are well studied. A genome-wide analysis shows that each of six tested RBPs binds multiple sites and more than half of all AS events are regulated by multiple RBPs (66). It remains a difficult challenge to determine how these hundreds of RBPs cooperate and coordinately regulate the tens of thousands of normal AS events that are required for tissue differentiation (16, 17, 80). Any misregulation in this process may generate aberrant AS that leads to serious consequences, such as cancer.

Mutations in the Core Splicing Machinery and Cancer

The above studies all describe how changes in the intracellular levels of splicing regulatory proteins can contribute to cancer. What was lacking, however, were any examples of mutations in genes encoding splicing proteins that either cause or contribute to neoplastic transformation. A number of recent studies, though, have identified mutations affecting components of the core splicing machinery that play critical roles in neoplasia. Given the

importance of these findings to our appreciation of the role of splicing in cancer, we discuss these studies in some detail.

In 2011, Yoshida *et al.* reported recurrent somatic mutations in the genes encoding components of the RNA splicing machinery in Myelodysplastic syndromes (MDS), a diverse group of myeloid neoplasms characterized by an abnormality in myeloid blood cell production and propensity of progression into acute myeloid leukemia (81). The most frequently mutated genes encode splicing factors SF3B1, U2AF1, SRSF2, and ZRSR2. Mutational frequencies for SF3B1 are particularly high in refractory anemia with ring sideroblasts (RARS) and RARS with thrombocytosis (RARS-T), ranging from 64–83% (81–83). *SF3B1* is also frequently mutated in Chronic Lymphocytic Leukemia (CLL) (84–86), as well as in uveal melanoma (87, 88). All *SF3B1* mutations are heterozygous, and none are nonsense mutations or introduce a frameshift (Figure 2A). The mechanism through which the splicing factor mutations misregulate RNA splicing and subsequently lead to disease is still unknown. Here we offer our perspectives.

SF3B1, U2AF1, SRSF2, and ZRSR2 are all involved in the selection of splice sites at the 3′ end of introns. Mutations in these genes most likely reflect defects in 3′ ss recognition during RNA splicing. As shown in Figure 2, defects in 3′ ss recognition (but with normal 5′ ss recognition) can result in two 5′ ss competing for one 3′ ss, an AS pattern that resembles alternative 5′ ss. A frequent outcome of alternative 5′ ss is the selection of the 5′ ss proximal to the downstream 3′ ss (89). As a result, the final mRNA product often has retained introns (Figure 2B). In support of this speculation, Yoshida *et al.* showed that expression of mutant U2AF1 results in large scale (~5%) intron retention in HeLa cells (81). Because introns are rich with stop codons, retained introns frequently introduce into the mRNA premature termination codons (PTCs) that activate nonsense-mediated mRNA decay (NMD), which was observed in the HeLa cells expressing mutant U2AF1 (81). It must be noted that not every intron is retained by 5%, but instead some introns are retained while others are not. For example, intron 59 of *BIRC6* pre-mRNA is mostly retained, but no retention is found in intron 58 or intron 60 of the same gene. This ″all-or-nothing″ (technically ″more-or-less″) splicing pattern implies that there is intron sequence specificity for mutant U2AF1. Because most of the mRNAs with retained introns are rapidly degraded by NMD, it is difficult to identify sequence conservation in those degraded introns. Therefore, NMD inhibitors may be useful to help identify the targets of the mutated splicing factors.

Several studies have begun to examine the role of SF3B1 in MDS. Visconte *et al.* showed that knockdown of SF3B1 in K562 cells resulted in retention of introns (90). However, SF3B1 knockdown did not produce ring sideroblasts (RS), possibly because K562 cells are not able to differentiate along the erythroid lineage. Indeed, in healthy human bone marrow cells, RS formation was induced by meayamycin, an SF3B1 inhibitor (90). However, this SF3B1 “haploinsufficiency” hypothesis cannot explain the absence of nonsense and frameshift mutations in *SF3B1*, which suggests that the mutated protein likely maintains structural integrity, but with altered function. RNA sequencing analysis of samples from one healthy donor and two patients with *SF3B1* mutations revealed that 130 genes were differentially expressed, of which 94% (an unusually high percentage) had lower expression in patients (90). One explanation for this is that some introns in these genes are retained and the mRNA is rapidly degraded through NMD. Nevertheless, none of these downregulated genes is involved in mitochondrial function or related to the RS phenotype. This may reflect the choice of control. Visconte *et al.* used total bone marrow cells from a healthy donor as a control, which contains a mixture of all types of blood cells with unknown cell ratios, making a complex gene expression profile. When purified CD34+ cells were used as a control, Papaemmanuil *et al.* found that key genes in the mitochondrial pathways are downregulated in MDS patients with *SF3B1* mutations (83). In particular, the mitochondrial

gene *ABCB7* is consistently downregulated in RARS patients, suggesting that it may be a key mediator of ineffective erythropoiesis of RARS (91). Indeed, Nikpour *et al.* recently demonstrated that reduced expression of ABCB7 in normal bone marrow markedly reduced erythroid differentiation and growth with accumulation of mitochondrial ferritin, a phenotype similar to that observed in intermediate RARS erythroblasts (92). It remains to be determined how *SF3B1* mutations result in downregulation of ABCB7, although it is possible that intron retention followed by NMD contributes.

The link between the splicing gene mutations and clonal expansion of hematopoietic stem cells remains unclear. Expression of mutant U2AF1 leads to death, rather than promoting growth, of both HeLa cells and TF-1 cells *in vitro* (81). This unexpected result might reflect the fact that the outcome of splicing defects may depend on certain cellular contexts, as knockdown of SF3B1 in K562 does not induce the RS phenotype, while inhibition of SF3B1 in bone marrow cells does (90). Another unexpected result is that mutant U2AF1 impairs the reconstitution capability, rather than promoting clonal expansion, of mouse CD34+ cells (81). A possible explanation for this stems from the perhaps unexpected differences between mouse and human AS. Recent studies showed that mouse AS is drastically different from human AS (16, 17). Even though human and mouse splicing factors are almost identical (SF3B1 and SRSF2 are 100%, U2AF1 96% and ZRSR2 82% identical between human and mouse), only one quarter of human AS events were observed in mouse (16). Therefore, using mouse models to study human diseases that reflect changes in AS, or aberrant splicing more generally, may be misleading.

Several other lines of evidence also suggest that the effects of spliceosomal gene mutations may be dependent of cellular contexts. For example, MDS patients with *SF3B1* mutations generally have a favorable prognosis while *SF3B1* mutations in CLL correlate with poor overall survival and resistance to chemotherapy (85, 86, 93–95). Unlike in adult MDS, spliceosomal mutations are rare in pediatric MDS and juvenile myelomonocytic leukemia (96). In unveal melanoma *SF3B1* mutations are frequent (87, 88), but none of the 85 cutaneous melanomas has an *SF3B1* mutation (97). This cellular-context dependency of effects provides an opportunity for developing anti-tumor drugs: tumor cells and normal cells are known to have different cellular contexts; therefore, modulating the activities of spliceosomal proteins will likely yield different, even opposing, effects. Indeed, spliceosome modulators such as sudemycins, pladienolide B, FR901464 and its derivative spliceostatin A (SSA), have potent toxicity to tumor cell lines, but display little toxicity to normal cells (98–100). It has been shown that FR901464 and SSA bind to SF3b complex and promote retention of intron 1 of p27, a cyclin-dependent kinase (CDK) inhibitor. Translation of the intron-1 containing pre-mRNA leads to production of a C-terminal truncated protein isoform p27*, which is resistant to proteasomal degradation and inhibits CDK2 kinase activity, thereby inhibiting cell growth (101). SSA treatment also leads to intron retention in *VEGF* and results in reduction of VEGF levels (possibly by NMD), inhibiting cancer cell angiogenesis (102). Although the exact mechanism of selective tumor cytotoxicity remains to be fully explored, one explanation is that growth of cancer cells often relies on oncogenic protein isoforms (arising from AS), which are lacking in normal cells.

Conclusions

It has become clear that aberrant pre-mRNA AS is a major contributor to cancer phenotypes. With the rapid advances in high-throughput RNA sequencing technologies, more cancer-specific AS events will likely be discovered. However, our understanding of the misregulation of AS in cancer lags far behind. The past decades have implicated only a handful of RBPs in this process. It remains a challenge to study systematically how the likely hundreds of RBPs (as well as components of the core splicing machinery)

coordinately regulate tens of thousands of AS events in normal tissues and how they misregulate AS in cancer. Nevertheless, therapeutic intervention targeting either the cancer-specific AS events themselves or the splicing factors that misregulate them is promising. Given that cancer cells utilize AS mechanism to gain survival advantages, it also will be important in the future to explore AS regulation in still greater depth to find ways to combat cancer.

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Significance

An increasing body of evidence indicates that aberrant splicing of mRNA precursors leads to production of aberrant proteins that contribute to tumorigenesis. Recent studies show that alterations in cellular concentrations of regulatory splicing factors and mutations in components of the core splicing machinery provide major mechanisms of misregulation of mRNA splicing in cancer. A better understanding of this misregulation will potentially reveal a group of novel drug targets for therapeutic intervention.

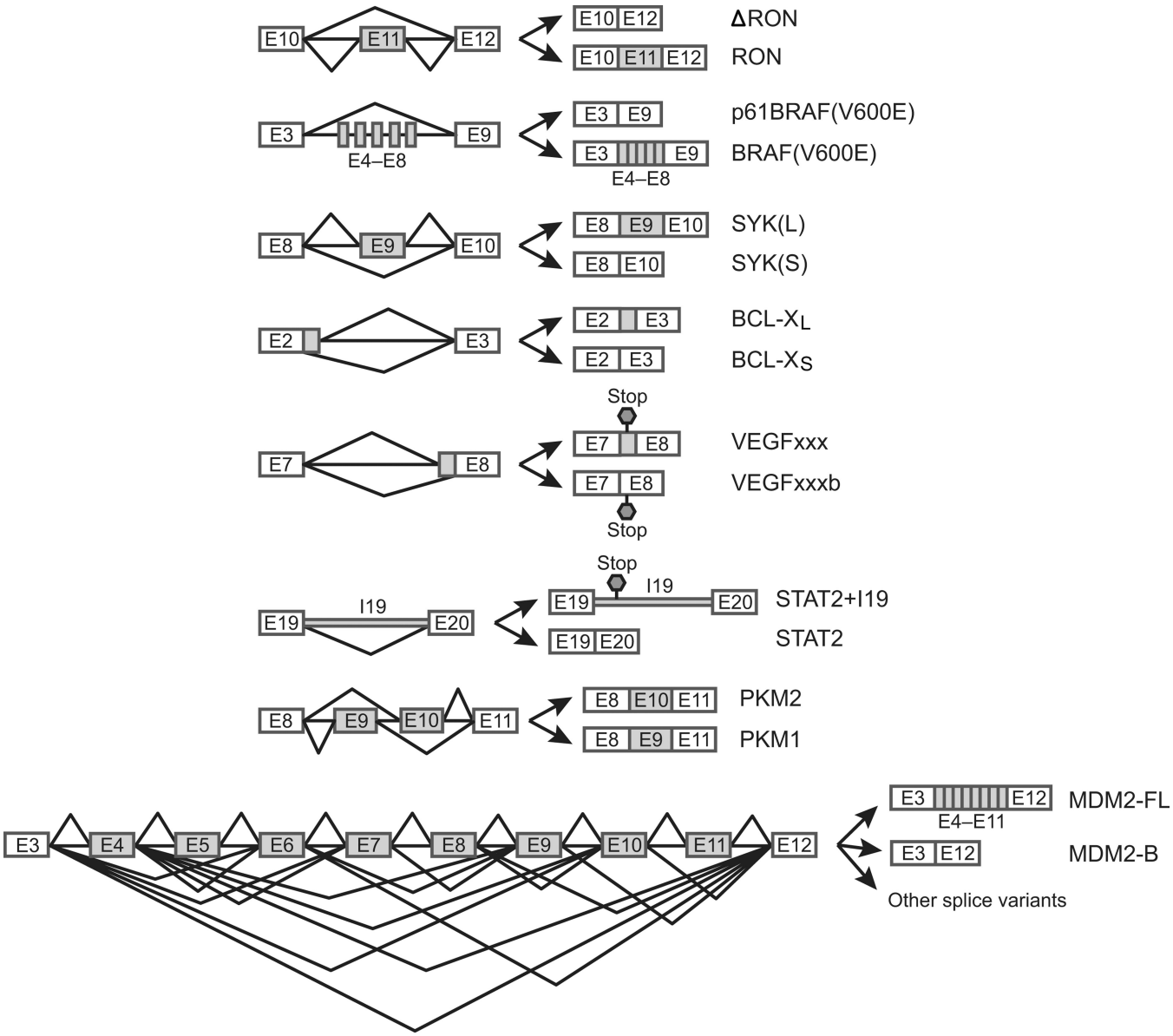
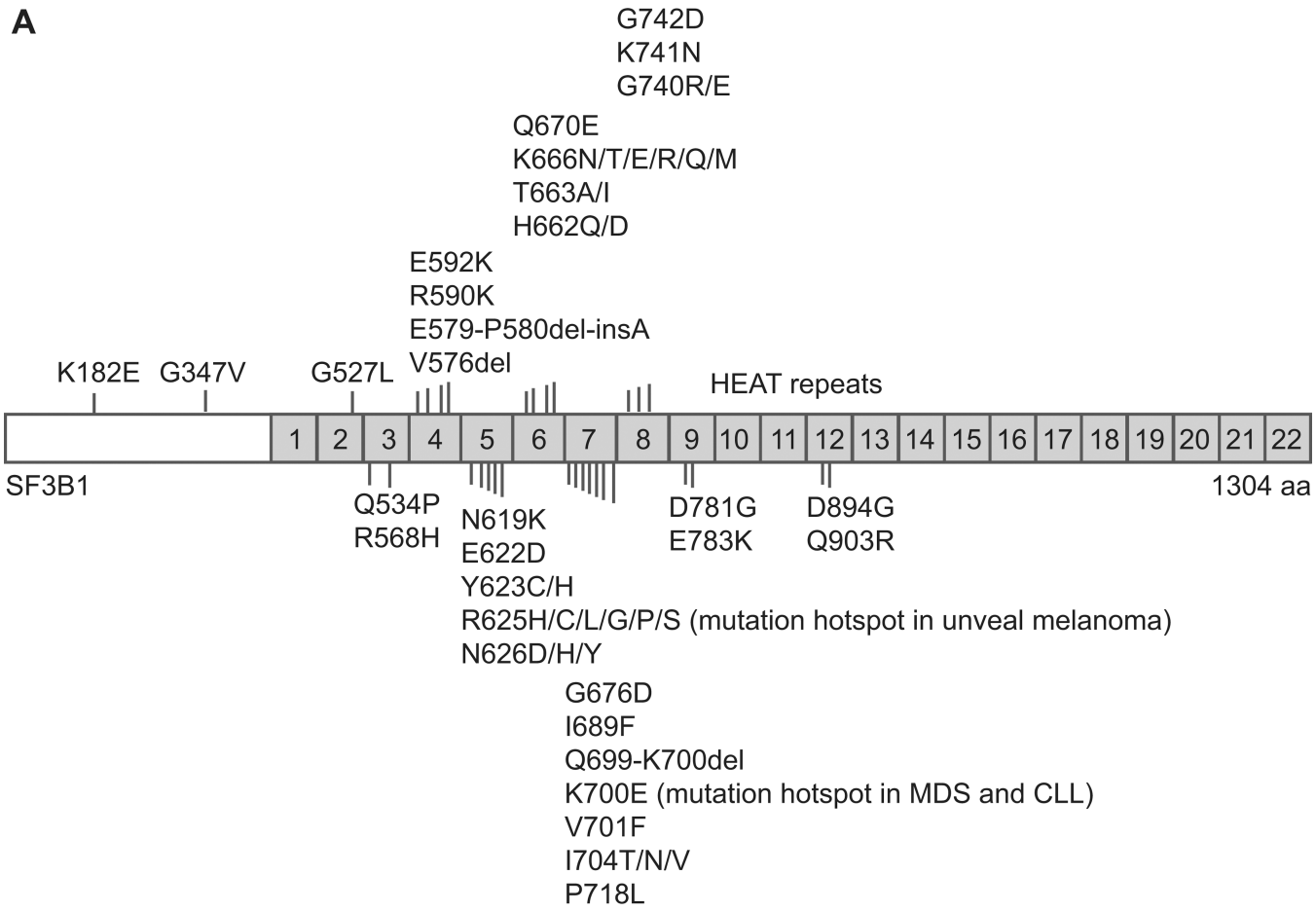


Figure 1.

Examples of AS patterns in cancer. The main AS patterns in cancer cells include cassette exons (skipping of one exon, skipping of multiple exons, and exon inclusion), alternative 5' ss, alternative 3' ss, intron retention, and mutually exclusive exons. Specific examples discussed in the text are shown.



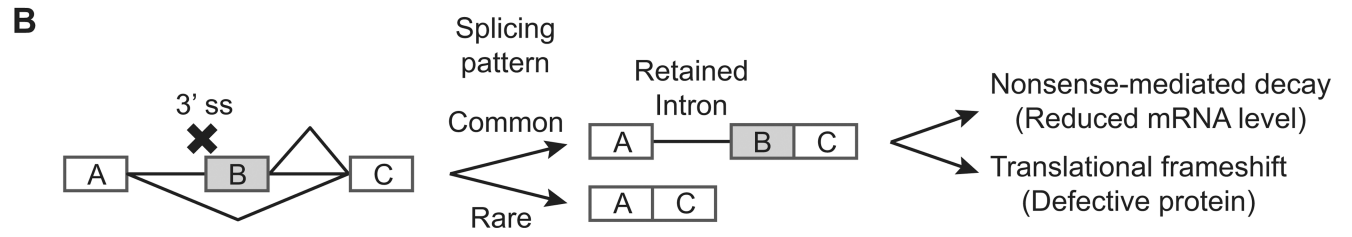


Figure 2.

Splicing factor mutations and aberrant splicing patterns. **A**, distribution of mutations in *SF3B1*. **B**, aberrant splicing with defects in 3' ss recognition. Outcomes of defects in 3' splice recognition or utilization are diagrammed.