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Review article

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RNA splicing dysregulation and the hallmarks of cancer

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Abstract

Dysregulated RNA splicing is a molecular feature that characterizes almost all tumour types. Cancer-associated splicing alterations arise from both recurrent mutations and altered expression of *trans*-acting factors governing splicing catalysis and regulation. Cancer-associated splicing dysregulation can promote tumorigenesis via diverse mechanisms, contributing to increased cell proliferation, decreased apoptosis, enhanced migration and metastatic potential, resistance to chemotherapy and evasion of immune surveillance. Recent studies have identified specific cancer-associated isoforms that play critical roles in cancer cell transformation and growth and demonstrated the therapeutic benefits of correcting or otherwise antagonizing such cancer-associated mRNA isoforms. Clinical-grade small molecules that modulate or inhibit RNA splicing have similarly been developed as promising anticancer therapeutics. Here, we review splicing alterations characteristic of cancer cell transcriptomes, dysregulated splicing's contributions to tumour initiation and progression, and existing and emerging approaches for targeting splicing for cancer therapy. Finally, we discuss the outstanding questions and challenges that must be addressed to translate these findings into the clinic.

Sections

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Introduction

RNA splicing is a fundamental step in the expression of most human genes. In addition to its essential role in removing introns from pre-mRNA to produce mature mRNAs, splicing also influences other steps in gene expression, including nuclear export, mRNA translation and mRNA quality control via nonsense-mediated decay (NMD)[1]. Almost all multi-exon human genes undergo alternative splicing, wherein a single gene generates multiple distinct mature mRNAs to expand the cell's protein-coding repertoire[2]. High-throughput sequencing studies have revealed that alternative splicing both regulates and is regulated by many biological processes and phenomena, ranging from neural development to epithelial-to-mesenchymal transition (EMT) or T cell activation[3,4].

Alternative splicing plays a similarly important role in many tumours. Most tumours exhibit widespread splicing abnormalities relative to peritumoral healthy tissues, including frequent retention of normally excised introns, inappropriate expression of isoforms normally restricted to other cell types or developmental stages, and splicing errors that disable tumour suppressors or promote oncogene expression[5–7]. Aberrant splicing in tumours can arise from diverse causes, including altered expression of key splicing regulatory proteins or RNAs, which themselves can function as proto-oncoproteins or tumour suppressors; *cis*-acting somatic mutations that alter splicing of the genes bearing those lesions; and *trans*-acting somatic mutations that cause gain-of-function or loss-of-function alterations affecting splicing regulators, driving pervasive splicing changes across the transcriptome[6,7]. Each of these mechanisms can cause pro-tumorigenic splicing changes, with the last — recurrent mutations in the genes encoding specific splicing factors that typically appear as initiating or early events during tumour formation — providing a particularly clear genetic illustration of the fundamental role that splicing dysregulation plays in tumorigenesis. In recent years, a better understanding of individual spliced isoforms that impact cancer cell transformation has led to the development of novel approaches to target these individual events[8]. Molecular inhibitors of oncogenic splicing factors or splicing machinery components are currently being developed as anticancer therapeutics[9]. RNA splicing dysregulation plays pervasive and causative roles in tumorigenesis, frequently via disruption of the molecular and cellular processes termed 'cancer hallmarks' as proposed by Weinberg and Hanahan[10,11].

In this Review, we outline both the basic biology and the cancer relevance of RNA splicing. We discuss splicing regulatory alterations that are implicated in tumour initiation, as well as individual splicing events associated with tumour initiation, progression and drug resistance. We describe how splicing dysregulation could be therapeutically targeted with small molecules and the technical challenges and outstanding questions that need to be addressed to translate our fast-improving understanding of splicing's critical role in tumorigenesis into the clinic.

Splicing catalysis and regulation

RNA splicing is a highly regulated process performed by the spliceosome — a very large complex consisting of both RNA and protein components — along with additional regulatory splicing factor proteins that fine-tune its activity. The spliceosome recognizes core regulatory sequences in the pre-mRNA including the 5′ and 3′ splice sites (5′SS and 3′SS) that mark intron–exon boundaries, the branch point site (BPS) and the polypyrimidine tract[12] (Fig. 1a). Two spliceosomal complexes carry out splicing reactions, the U2-type (major spliceosome) or the U12-type (minor spliceosome). They differ mainly in a subset of RNA components used during their respective splicing reactions and in the splice site sequences they recognize[12]. The major U2-type spliceosome, which preferentially recognizes GT-AG splice sites and is responsible for the removal of ~99% of introns, contains more than 300 components — including small nuclear RNA (snRNA) molecules that interact with 'Sm' core proteins and additional proteins to form small nuclear ribonucleoprotein (snRNP) particles[12]. The Sm proteins associate with each other to form a ring-shaped complex that binds to U1, U2, U4 and U5 snRNAs. The minor or U12-type spliceosome, which recognizes both AT-AC and GT-AG sites, is involved in the removal of fewer than 1% of introns and regulates a distinct set of splicing events and utilizes different spliceosomal snRNA and protein components, including ZRSR2 (ref. [13]). The U12-type spliceosome has distinct 5′SS and BPS sequence contexts that guide recognition of these introns. The U2-specific snRNPs are U1, U2, U4 and U6, whereas the U12-type snRNPs are U11, U12, U4atac and U6atac (ref. [12]).

The detailed compositions and structures of the spliceosomal complexes have been reviewed extensively[12]. Several spliceosomal components are altered in human tumours, including via recurrent hot-spot mutations in components of the 'Early' or E complex and pre-spliceosome A complex (Fig. 1a), and will be discussed further below.

Except for the dinucleotides adjacent to the 5′SS and 3′SS, the core regulatory sequences recognized by the spliceosome are rather degenerate in humans and allow for a huge diversity in their sequences[14]. This provides an additional layer of regulation that depends on both *cis*-acting regulatory sequences and *trans*-acting splicing factor proteins that can strengthen or weaken the spliceosome's recognition of the splice sites[14]. Together, these *cis*-acting sequences and *trans*-acting splicing factors regulate alternative splicing, allowing a single gene to encode multiple different RNA isoforms that can be translated into different, and frequently functionally distinct, protein isoforms (Fig. 1b). Alternatively spliced isoforms can differ in their coding potential, stability, localization, translation efficiency and other molecular features. For example, alternative exons are enriched in gene regions that encode protein–protein interaction surfaces[15]. It is currently estimated that each human protein-coding gene encodes an average of 7.4 RNA isoforms; however, much more extreme examples of alternative splicing have been described[16].

Regulatory, *trans*-acting splicing factors that modulate alternative splicing are a class of RNA-binding proteins (RBPs) that recognize and bind *cis*-regulatory elements on the pre-mRNA, namely exonic or intronic splicing enhancer (ESE or ISE) or exonic or intronic splicing silencer (ESS or ISS) sequences, and promote or repress inclusion of that exon into mature mRNA, respectively (Fig. 1c). The serine/arginine-rich (SR) proteins and heterogeneous nuclear ribonucleoproteins (hnRNPs) are two well-known splicing factor families that regulate alternative splicing in a concentration-dependent manner by binding regulatory elements in the pre-mRNA[17,18]. SR proteins contain an RNA recognition motif (RRM) domain that binds RNA and an arginine/serine-rich (RS) domain that mediates protein–protein and protein–RNA interactions. hnRNPs typically contain one or multiple RRMs, along with a glycine-rich and/or arginine/glycine-rich region, and/or the K homology (KH) domain[18]. hnRNPs play diverse roles in alternative splicing, mRNA transport and translation, and often function as antagonists to SR protein-regulated alternative splicing events[18]. The distinct RNA-binding motifs of SR proteins and hnRNPs suggest that these splicing factors can work antagonistically or cooperatively, and the intricate interplay of these regulatory splicing factors is only beginning to be unravelled.

Splicing alterations in tumour initiation

Mutations or expression changes affecting components of the splicing machinery or splicing factors can play critical roles in cancer initiation and progression (Fig. 2). By inducing splicing changes affecting many downstream genes, these alterations have the potential to disrupt a network of gene products and cancer pathways. Several key examples are highlighted in the following sections.

Recurrent mutations in splicing factors

Recurrent somatic mutations in *SF3B1*, *SRSF2*, *U2AF1* and *ZRSR2* occur frequently in haematological malignancies, including in myelodysplastic syndromes (MDS), chronic myelomonocytic leukaemia (CMML), acute myeloid leukaemia (AML) and chronic lymphocytic leukaemia (CLL)[19,20] (Fig. 2a,b). These mutations are frequently termed 'spliceosomal mutations'. *SF3B1* and *U2AF1* are also recurrently mutated in diverse solid tumour types[7,21–23]. Mutations in *SF3B1*, *SRSF2* and *U2AF1* almost always occur as heterozygous missense point mutations affecting specific residues in both haematological malignancies and solid tumours, whereas mutations in the X-linked gene *ZRSR2* frequently disrupt its open reading frame and preferentially occur in males. Detailed functional studies have revealed that recurrent *SF3B1*, *SRSF2* and *U2AF1* mutations cause gain or alteration of function, whereas *ZRSR2* mutations cause loss of function, consistent with the spectra of mutations observed in patients. Spliceosomal mutations are almost always mutually exclusive as they elicit redundant and/or synthetically lethal effects due to their cumulative impact on alternative splicing and haematopoiesis[24], although there are rare exceptions to this rule[25].

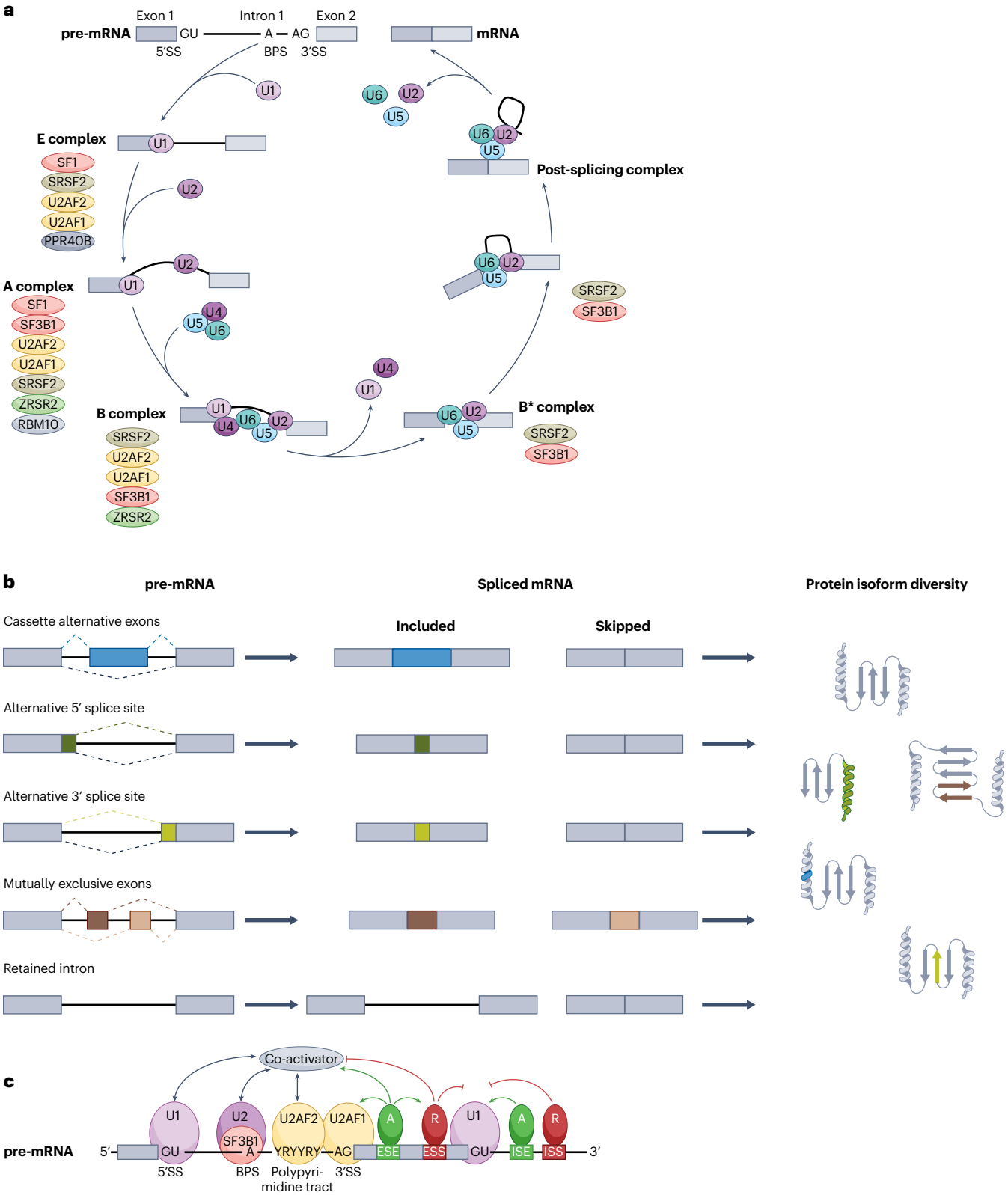
SF3B1 is the most frequently mutated spliceosomal component in cancer, with recurrent somatic mutations detected in ~30% of all patients with MDS, including 83% of cases of MDS-subtype refractory anaemia with ring sideroblasts and 76% of cases of MDS-subtype refractory cytopenia with multilineage dysplasia and ring sideroblasts[19,26]. *SF3B1* mutations are also detected in other cancers, including 15% of CLL, 3% of pancreatic cancer, 1.8% of breast adenocarcinomas, 1% of cutaneous melanomas and 20% of uveal melanomas (UVMs)[21,22] (Fig. 2a). SF3B1 is a core component of the U2 snRNP that is involved in BPS recognition and spliceosomal complex A assembly (Fig. 1). *SF3B1* mutations near-universally occur as heterozygous, missense mutations that affect multiple hot-spot residues within the carboxy-terminal HEAT domains (HDs) (Fig. 2b). These mutations induce altered BPS recognition with consequent changes in 3′SS recognition, resulting in widespread splicing alterations including cryptic 3′SS usage, differential cassette exon inclusion and reduced intron retention[27,28]. The prognostic implications of an *SF3B1* mutation depend upon the specific mutation and indication. For example, *SF3B1*^{K700E} is associated with comparatively good prognosis in MDS with ring sideroblasts[19,20], whereas *SF3B1*^{K666N} is associated with disease progression[29]. In CLL, *SF3B1*^{G742D} correlates with poor prognosis[26]. Although how mutant SF3B1 promotes disease phenotypes and tumorigenesis is still under active investigation, numerous cellular pathways have been implicated. For example, *SF3B1* mutations cause aberrant inclusion of a poison exon (an exon that contains an in-frame premature termination codon (PTC)) in bromodomain containing 9 (*BRD9*) across tumour types to promote cell transformation[30], induce *MAP3K7* mis-splicing to promote hyperactive nuclear factor-κB (NF-κB) signalling and disrupt erythropoiesis[24,31], and disrupt splicing of genes involved in haem biosynthesis to cause ring sideroblast formation[32].

Recurrent mutations affecting the SR protein SRSF2 have been observed in 10% of all patients with MDS and related disorders, including 31–47% for CMML and 11% for AML[20,33], and less commonly in solid tumours[34] (Fig. 2a). *SRSF2* mutations are linked with poor clinical outcomes in MDS and increased progression to AML[20]. Required for both constitutive and alternative splicing, SRSF2 mediates exon inclusion and recognition of the 5′SS and 3′SS by interacting with U1 and U2 snRNPs (Fig. 1). Heterozygous mutations immediately adjacent to SRSF2's RRM domain, which predominantly occur as missense mutations and universally affect the P95 residue (Fig. 2b), alter its RNA-binding preference. Mutant SRSF2 favours recognition of C-rich sequences (CCNG motif) and has reduced affinity for G-rich sequences (GGNG motif), whereas wild type SRSF2 recognizes both[35,36]. This alters the efficiency of SRSF2-mediated exon inclusion and results in mis-splicing. For example, mutant SRSF2's altered binding preference results in downregulation of EZH2, a histone methyltransferase implicated in MDS pathogenesis, due to increased inclusion of a poison exon[35]. Notably, *EZH2* loss-of-function mutations in CMML are mutually exclusive with *SRSF2* mutations. *SRSF2* mutations frequently co-occur with specific additional somatic mutations, such as isocitrate dehydrogenase 2 (*IDH2*) mutations, which functionally collaborate with *SRSF2* mutations to promote leukaemia, in part via increased intron retention in *INTS3* that arises from direct effects of mutant SRSF2 as well as IDH2 (ref. [33]).

U2AF1 is mutated in 5–15% of MDS, 5–17% of CMML and 3% of lung adenocarcinomas[20,23,37,38] (Fig. 2a). The U2AF1–U2AF2 heterodimer recognizes the 3′SS (U2AF1 binds to the AG dinucleotide and U2AF2 to the polypyrimidine tract) and is critical for U2 snRNP binding (Fig. 1). *U2AF1* is subject to recurrent mutations affecting two hot spots, S34 and R156/Q157, within U2AF1's two zinc finger domains (Fig. 2b). Mutations at the two hot spots cause different alterations in RNA binding affinity and 3′SS recognition to induce largely distinct splicing patterns[38,39]. The means by which *U2AF1* mutations cause disease are not fully understood, with dysregulated pathways including DNA damage response, RNA localization and transport, the cell cycle, epigenetic regulation, innate immunity, stress granule formation and pre-mRNA splicing[40,41].

ZRSR2, an X-linked gene, is mutated in 1–11% of MDS without ring sideroblasts, in 0.8–8% of CMML and at lower rates among other haematological cancers (Fig. 2a), with most mutations occurring in male patients[20,42,43]. In contrast to the hot-spot alterations described above, *ZRSR2* mutations are distributed across the gene (Fig. 2b), preferentially disrupt the open reading frame or key functional residues to cause loss of function and can co-occur with *SF3B1*, *SRSF2* or *U2AF1* mutations[20]. ZRSR2 heterodimerizes with ZRSR1 and is reportedly involved in recognition of 3′SS for both U2-type and U12-type introns (Fig. 1). *ZRSR2* loss results in improper retention of U12-type introns, with few direct effects on U2-type introns[44], and promotes a clonal advantage, in part, by causing intron retention in *LZTR1*, which encodes a regulator of RAS-related GTPases[45,46].

Mouse models have provided insight into the initiating roles of recurrent spliceosomal mutations for myeloid malignancies by specifically inducing these lesions in the haematopoietic compartment. *Sf3b1*$^{K700E/+}$ knock-in mice exhibit macrocytic anaemia, erythroid dysplasia and long-term haematopoietic stem cell expansion[47]; *Srsf2*$^{P95H/+}$ knock-in mice exhibit impaired haematopoiesis, myeloid and erythroid dysplasia, and haematopoietic stem cell expansion[35]; *U2af1*^{S34F}-expressing transgenic mice exhibit altered haematopoiesis[48], whereas *U2af1*$^{S34F/+}$ knock-in mice exhibit multilineage cytopenia, macrocytic anaemia and low-grade dysplasias[49]; and, finally, *Zrsf2*-knockout mice exhibit modest dysplasia and increased haematopoietic stem cell self-renewal[45]. One important factor to keep in mind when interpreting


a
Exon 1
Intron 1
Exon 2
pre-mRNA
GU
A
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5′SS
BPS
3′SS
mRNA
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U6
U2
U5
E complex
SF1
SRSF2
U2AF2
U2AF1
PPR4OB
Post-splicing complex
A complex
SF1
SF3B1
U2AF2
U2AF1
SRSF2
ZRSR2
RBM10
U4
B complex
SRSF2
U2AF2
U2AF1
SF3B1
ZRSR2
B* complex
b
pre-mRNA
Spliced mRNA
Protein isoform diversity
Cassette alternative exons
Included
Skipped
Alternative 5′ splice site
Alternative 3′ splice site
Mutually exclusive exons
Retained intron
c
Co-activator
pre-mRNA
5′
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Fig. 1 | Principles of constitutive and alternative splicing. a, Stepwise assembly of spliceosomal complexes on a pre-mRNA molecule and catalysis of the splicing reaction to generate mature spliced mRNA. During the first step of the splicing reaction, the ATP-independent binding of U1 small nuclear ribonucleoprotein (snRNP) to the 5′ splice site (5′SS) initiates the assembly of the E complex, whereas SF1 and U2AF2 bind, respectively, to the branch point site (BPS) and polypyrimidine tract. In the second step, the ATP-dependent interaction of U2 snRNP with the BPS, stabilized by U2AF2–U2AF1 and SF3a–SF3b complexes, leads to A complex formation and SF1 displacement from the BPS. Recruitment of the U4/U6/U5 tri-snRNP complex marks the formation of the catalytically inactive B complex. The active B* complex is formed following major conformational changes, including release of U1 and U4, and the first catalytic step generates the C complex and results in lariat formation. The C complex performs the second catalytic step, which results in joining of the two exons. The spliceosome then disassembles releasing the mRNA and the lariat bound by U2/U5/U6. Spliceosomal core factors that exhibit alterations in human tumours are coloured next to each complex. **b**, Alternative splicing patterns are classified into cassette alternative exon splicing, alternative 5′SS and 3′ splice site (3′SS) usage, mutually exclusive exons and intron retention. These splicing patterns lead to distinct spliced mRNA isoforms that can be translated into protein isoforms with distinct sequences and functions. **c**, *trans*-Acting regulatory splicing factors act as splicing activator (A) or repressor (R) and promote or inhibit spliceosome assembly by binding exonic or intronic splicing enhancer (ESE or ISE) or exonic or intronic splicing silencer (ESS or ISS) *cis*-acting regulatory sequences.

results from such mouse models is the imperfect conservation of alternative splicing between human and mouse. The particularly high conservation of U12-type versus U2-type introns may explain why *Zrsr2* loss leads to a competitive advantage in mouse models, as is expected given its enrichment in human disease, whereas mouse models of other spliceosomal mutations do not[45].

Genetic evidence similarly indicates that spliceosomal mutations are commonly initiating events in the pathogenesis of myeloid malignancies. Clonality studies of MDS with *SF3B1* mutations indicate that these lesions are initiating events that occur in human haematopoietic stem cells and persist in their myeloid progeny[50]. A recent longitudinal study revealed differences in clonal expansion driven by distinct somatic mutations during ageing of the human haematopoietic system and clonal haematopoiesis. Spliceosomal mutations drove expansion later in life, exhibited some of the fastest expansion dynamics and were strongly associated with transformation to overt malignancy, whereas clones with mutations in epigenetic regulators preferentially expanded early in life and displayed slower growth with old age[51]. Spliceosomal mutations are frequently expressed at allelic ratios that indicate a presence in the dominant clone in many solid tumours, suggesting that they may be early or even initiating events in those malignancies as well. However, further genetic studies in primary patient samples and functional studies in animal models are necessary to reach firm conclusions about the timing of their acquisition.

Genes encoding other spliceosomal components are also mutated in both haematological and solid malignancies (Fig. 2). For example, *RBM10* is recurrently mutated in lung, thyroid and other cancers, resulting in disrupted splicing and pro-tumorigenic effects[52,53]. *SF3A1*, *PRPF8*, *SF1*, *HNRNPK*, *U2AF2*, *SRSF6*, *SRSF1*, *SRSF7*, *TRA2B* and *SRRM2* mutations have also been reported, although at relatively low rates[54]. A recent study suggested that >100 genes encoding spliceosomal components contain putative driver mutations across multiple cancer types[55]. The functional roles of such low-frequency splicing factor mutations in cancer are unclear, although they could potentially be important given the pleiotropic role of splicing in gene expression.

Finally, mutations affecting proteins that are not canonically involved in splicing regulation can have potent effects on splicing. For example, mutations in *IDH2* alter alternative splicing as discussed above[33], whereas hot-spot missense mutations in *TP53* are associated with dysregulated alternative splicing in pancreatic cancer[56].

Splicing factor expression alterations

Splicing factor levels and activity are tightly controlled epigenetically, transcriptionally, post-transcriptionally via alternative splicing coupled with NMD, translationally and post-translationally, including via phosphorylation by specific kinases[17,18]. Changes to any of these regulatory pathways can lead to altered splicing factor expression and consequent altered alternative splicing of the splicing factors' downstream targets. Whereas recurrent splicing factor mutations are common in haematological malignancies, altered splicing factor levels and copy number changes are particularly prominent in solid tumours[6] (Fig. 2a). Splicing factors regulate alternative splicing of downstream mRNA targets in a concentration-dependent manner; therefore, changes in splicing factor levels alone can induce alternative splicing deregulation in tumours[17,18]. Causal links have been identified between splicing factor misregulation and multiple cancer types. Of note, several splicing factors that are upregulated in breast tumours exhibit oncogenic functions and are sufficient to promote tumour initiation in breast cancer models[57–60]. Splicing factors can also serve as tumour suppressors, and therefore splicing factor downregulation can contribute to tumour development[61].

An archetypal example of pro-tumorigenic altered splicing factor expression is the upregulation of the SR protein SRSF1 in breast, lung, colon and bladder tumours[57,60,62]. This can arise, in part, from amplification of Chr.17q23 but is also observed in tumours with amplifications of the gene encoding the transcription factor MYC[57,60,63] (Fig. 2a). SRSF1 overexpression enhances alternative splicing of isoforms associated with decreased cell death (for example, *BIN1*, *BIM* (also known as *BCL2L11*), *MCL1* and *CASC4*), increased cell proliferation (for example, *RON*, *MKNK2*, *S6K1*, *CASC4* and *PRRC2C*) and resistance to DNA damage (for example, *PTPMT1* and *DBF4B*), resulting in cell transformation in vivo and in vitro[57,58,62–64]. SRSF1 can act synergistically with MYC, often resulting in higher tumour grade and shorter survival in patients with breast and lung cancers, in part by potentiating the activation of eukaryotic translation initiation factor 4E (eIF4E), a translational regulator of cell growth signalling pathways[57,63]. Further, SRSF1 can activate mTOR complex 1 (mTORC1) growth signalling and promote translation initiation, in part, via interactions with the phosphatase PP2A and mTOR and by enhancing phosphorylation of eIF4E binding protein 1 (4E-BP1)[65,66].

Another SR protein family member, *SRSF3*, is overexpressed in lung, breast, ovarian, stomach, bladder, colon, bone, liver, brain and oral tumours, in part due to copy number[6] (Fig. 2a). Decreased expression of SRSF3 is also observed, for example in hepatocellular carcinoma[67], suggesting a complex role in tumorigenesis. Targets of SRSF3 play roles in cellular metabolism, growth, cytoskeletal organization and alternative splicing[67–69]. For example, overexpression of SRSF3 regulates the switch between the two isoforms of pyruvate kinase (PKM), a key metabolic enzyme underlying the Warburg effect on cancer cells[69], promoting splicing of the *PKM2* isoform and decreasing *PKM1* (ref. [69]). SRSF3 also regulates splicing of *HIPK2*, a serine/threonine protein kinase involved in transcription regulation and apoptosis. SRSF3

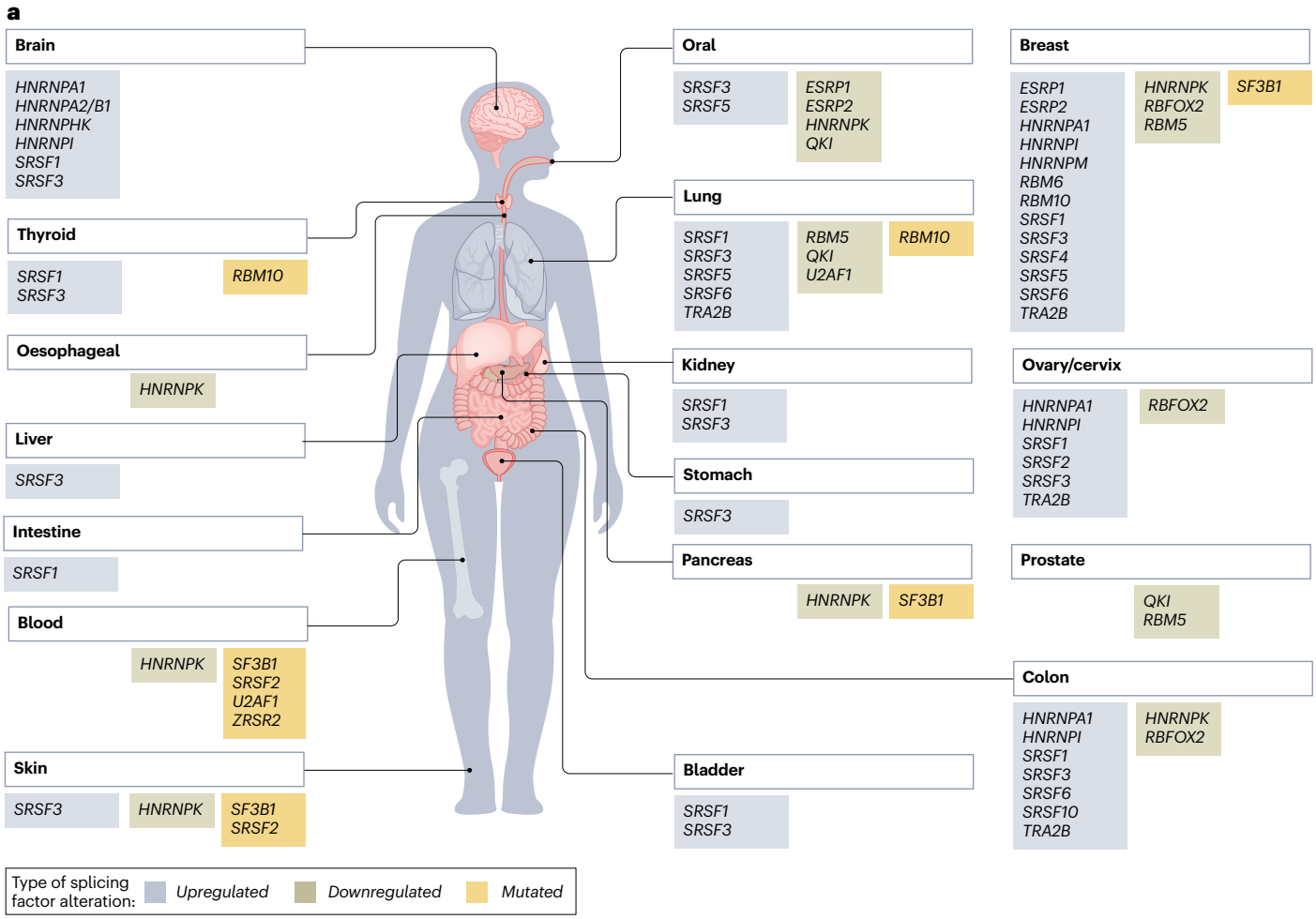


Fig. 2 | Recurrent splicing factor alterations in cancer. a, Examples of splicing factors frequently upregulated, downregulated or mutated in human primary tumours shown per tumour type. **b**, Recurrent hot-spot mutations in components from the spliceosomal A complex detected in human malignancies. Positions of recurrent mutations are indicated along with the protein structures and domains. *ZRSR2* mutations primarily affect U12-type introns, but as ZRSR2 has been biochemically implicated in U2-type splicing as well, it is illustrated in association with a U2-type intron above[264]. 3′SS, 3′ splice site; 5′SS, 5′ splice site; BPS, branch point site; BRCA, breast carcinoma; CLL, chronic lymphocytic leukaemia; CMML, chronic myelomonocytic leukaemia; HD, HEAT domain; MDS, myelodysplastic syndromes; PDAC, pancreatic adenocarcinoma; RRM, RNA recognition motif; RS, arginine/serine-rich domain; snRNP, small nuclear ribonucleoprotein; UHM, U2AF homology motif domain; UVM, uveal melanoma; Zn, zinc finger domain.

knockdown promotes *HIPK2* exon 8 skipping, leading to expression of an isoform associated with cell death[70]. SRSF3 also controls alternative splicing of target genes involved in glucose and lipid metabolism, and its conditional knockout in mouse hepatocytes causes fibrosis and the development of metastatic hepatocellular carcinoma with ageing[67]. Finally, high SRSF3 levels in tumours and cell lines are associated with the splicing of isoforms 1 and 2 of *ILF3* (ref. [71]), a double-stranded RBP implicated in cell proliferation regulation[71].

Additional splicing factors that are frequently upregulated in cancers include other members of the SR protein family, for example SRSF4, SRSF6 or SR-like TRA2β; members of the hnRNP protein family, for example hnRNPA1, hnRNPA2/B1, hnRNPM or PTB (also known as hnRNPI); and other splicing factors, for example ESPR1, ESPR2, RBM5, RBM6 and RBM10 (refs. [72–79]) (Fig. 2a).

Conversely, several splicing factors are downregulated in human tumours, including hnRNPK, ESRP1, ESRP2, RBFOX2, RBM5 or QKI (Fig. 2a). Decreased levels of QKI, a KH domain-containing RBP, are detected in several tumour types, including lung, oral and prostate cancers, and are associated with poor prognosis[80,81]. QKI regulates alternative splicing of *NUMB*, which encodes a membrane-associated inhibitor of Notch, leading to an isoform that decreases cell proliferation and prevents Notch signalling[81]. QKI also regulates the expression of *SOX2* (which encodes a transcription factor) by binding a *cis*-element in its 3′ untranslated region[80]. In addition, gene fusions of *QKI* with *MYB* have been described in angiocentric gliomas, a subtype of paediatric low-grade brain tumours, and shown to promote transformation in vitro and in vivo[82].

In addition to SRSF3 discussed above, other splicing factors (for example, ESRPs, other SR proteins and RBM proteins) can similarly be either upregulated or downregulated depending on the tumour type, suggesting context-dependent functions as both oncoproteins and tumour suppressors and complex roles in regulating tissue-specific splicing. For example, ESRP1 exhibits tumour-suppressive functions, and its downregulation during EMT regulates a specific set of EMT-associated splicing switches and promotes a more aggressive EMT-phenotype in vitro[83–85]. By contrast, it also exhibits oncogenic activity; high levels of ESRP1 been associated with poor prognosis in prostate tumours[72] and oestrogen receptor-positive breast tumours[73] and lead to increased lung metastasis in animal models of breast cancer[86]. Adding to the complexity, in oral tumours, ESRP1 – which is expressed at low levels in normal epithelium – becomes upregulated in pre-cancerous lesions, carcinoma in situ and advanced lesions but then is downregulated in invasive tumour fronts[76]. Another example of a splicing factor with dual functions is RBM5, which is often considered to be a tumour suppressor[77,87,88] and is downregulated in lung and prostate cancers[87,89], but is upregulated in primary breast tumours[78].

Aberrantly spliced RNA isoforms

Tumours often exhibit a more complex splicing repertoire than do normal tissues (Box 1), and tumorigenicity may be associated with cancer-specific alternative splicing events that arise during the transformation process. In some cases, *cis*-acting mutations can disrupt splicing to promote tumorigenesis. Such *cis*-acting mutations frequently cause *MET* exon 14 skipping in lung cancer[90], and other *cis*-acting mutations can similarly disrupt gene expression by inducing retention of specific introns[91].

Cancer-specific alternative splicing frequently arises independently of the presence of such *cis*-acting mutations or recurrent mutations affecting splicing factors[5]. Such alternative splicing switches impact thousands of genes and are often specific to a given tumour type[5], or even subtype, likely because baseline splicing profiles differ between normal tissues. Nonetheless, numerous alternative splicing isoforms are frequently dysregulated across multiple tumour types, suggesting shared splicing regulatory networks across tissue types.

These dysregulated isoforms often impact the so-called 'hallmarks of cancer', a series of biological capabilities acquired during the development of human tumours that are frequently used as an organizing principle for rationalizing cancer complexity. Cancer-associated alternative splicing isoforms can provide a proliferative advantage, improve cell migration and metastasis, enable escape from cell death, rewire cell metabolism or cell signalling, promote an abetting microenvironment, alter immune response or enable drug resistance (Fig. 3). Such cancer-associated alternative splicing switches can arise from changes in splicing factor levels or activity, *cis*-acting mutations affecting specific splice sites or exons, or other means. Functional studies in model systems have demonstrated that alterations in a single isoform can impact tumour growth but are often not sufficient to fully recapitulate splicing factor-mediated transformation[57–59,92,93], suggesting that the combination of multiple alternative splicing isoform switches is likely required to promote the different steps of tumorigenesis[5].

Differential splicing in tumours can lead to the expression of isoforms that increase proliferative potential (Fig. 3). For example, splicing of the *RPS6KB1* gene encoding the protein S6K1, a substrate of mTOR that controls translation and cell growth, has been associated with sustained cell proliferation and tumour growth. The *RPS6KB1-1* isoform produces a full-length protein, whereas the PTC-containing *RPS6KB1-2* isoform, created by the inclusion of three cassette exons 6a, 6b and 6c, generates a shorter isoform that lacks a portion of the kinase domain and differentially activates downstream mTORC1 signalling[94]. This splicing switch is regulated by SRSF1 (ref. [60]). *RPS6KB1-2* is highly expressed in breast and lung cancer cell lines and primary tumours, and its knockdown decreased cancer cell proliferation and tumour growth, whereas, conversely, knockdown of *RPS6KB1-1* induced transformation[94–96].

Splicing of the *PKM* gene can lead to deregulated cell metabolism (Fig. 3). Inclusion of either of the two mutually exclusive exons, exon 9 or exon 10, produces the constitutively active PKM1 or the cancer-associated PKM2 isoform, respectively[69,97,98]. These isoforms differ by 22 amino acids, and whereas both perform the same catalytic function, PKM2 can switch between the active and inactive states[99]. High PKM2 levels in human solid tumours correlate with shorter patient survival, advanced stage and poor prognosis[99]. *PKM2* splicing is regulated either by repressing inclusion of exon 9 via binding of PTBP1, hnRNPA1 or hnRNPA2, or by promoting exon 10 inclusion via binding of SRSF3 (refs. [97,98,100]).

To survive, cancer cells need to acquire the ability to resist cell death. Multiple genes that control cell death are regulated at the splicing level, giving rise to distinct isoforms that either exhibit anti-apoptotic or pro-apoptotic functions, including BCL-2 family members, such as *BCL2L1*, *BIM* or *MCL1* (Fig. 3). *BCL2L1* generates two isoforms, *BCLxL* and *BCLxS*, which respectively suppress and promote apoptosis[101–104]. This splicing switch relies on the usage of an alternative 5′SS in exon 2 and is regulated by SAM68, RBM4, PTBP1, RBM25, SRSF1, hnRNPF, hnRNPH, hnRNPK and SRSF9 (refs. [66,105–112]).

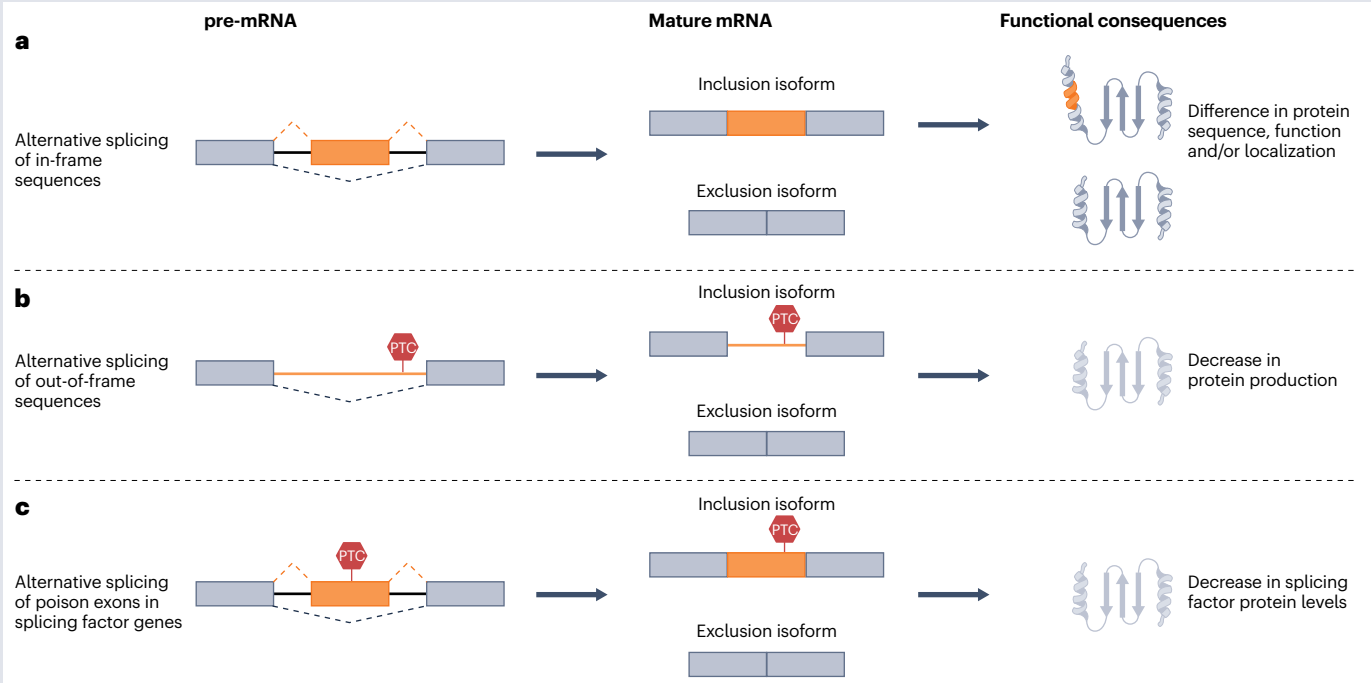
Genomic instability is one of the hallmarks of tumours, and one of the proteins that senses single-strand DNA breaks and activates the DNA damage response is the serine/threonine checkpoint kinase CHK1 (Fig. 3). Skipping of *CHK1* exon 3 produces the shorter isoform *CHK1-S*

Box 1

Common splicing patterns detected in tumours

Tumour-associated alterations in splicing patterns can lead to a wide variety of functional consequences that impact cancer hallmarks. Although every alternative splicing event will be unique, a few broader categories have emerged. First, inclusion or skipping of in-frame sequences as a consequence of cassette exon splicing or alternative splice site selection can lead to the addition or deletion of amino acid-encoding nucleotides, impacting protein structure, function and/or localization (see the figure, panel **a**). On the other hand, inclusion or skipping of out-of-frame sequences will introduce premature termination codons (PTCs), which will typically trigger nonsense-mediated decay (NMD) and prevent production of a corresponding protein isoform (see the figure, panel **b**). Those PTC-containing transcripts in tumours can arise from intron retention, due to both transcriptome-wide intron retention[265] and focal retention due to *cis*-acting mutations[91], as well as other alternative splicing events.

A special subclass of out-of-frame alternative splicing events that trigger NMD are 'poison exons', which correspond to cassette exons that when included introduce a PTC in the transcript (see the figure, panel **c**). Poison exons are particularly common in genes encoding splicing factors and frequently endogenously regulate splicing factor protein levels[215,216]. Their altered splicing can cause overexpression of oncogenic splicing factors and downregulation of tumour-suppressive splicing factors across tumour types. Interestingly, many of the alternative splicing events detected in tumours correspond to isoforms initially expressed during embryonic development and then switched when adult cells differentiate[30,35]. This reversion to embryonic patterns has been postulated to enable cancer cell proliferation and phenotypic plasticity.



that uses an alternative downstream initiation start site compared with the full-length isoform[113]. The resulting protein lacks the ATP-binding amino-terminal domain and represses full-length CHK1. High levels of CHK1-S are detected in ovarian, testicular and liver cancer tissues[113,114].

Nearly all cancer cells upregulate telomerase to re-elongate or maintain telomeres. Splicing of the reverse transcriptase component of telomerase, TERT, can generate at least 22 distinct isoforms, which differ in their activity; many of these lack telomerase activity and have a dominant negative effect[115] (Fig. 3). A splicing switch to favour the full-length TERT, which has telomerase activity, occurs in cancer cells, and is regulated by splicing factors hnRNPK, hnRNPD, SRSF11, hnRNPH2, hnRNPL, NOVA1 and PTBP1 (refs. [116–120]).

An example of tumour suppressor evasion involves the transcription factor KLF6, which regulates cell proliferation, differentiation and survival, and is often inactivated in tumours by mutation or deletions (Fig. 3). Alternative splicing of *KLF6* can produce an oncogenic isoform *KLF6-SV1*, as opposed to the full-length tumour suppressor isoform. *KLF6-SV1* uses an alternative 5′SS that causes a frameshift and produces a protein with 21 novel amino acids but lacking all three of the zinc finger domains[121,122]. *KLF6* splicing is regulated by SRSF1, TGFβ1 and

RAS signalling[123,124]. Increased KLF6-SV1 levels are detected in prostate, lung, ovarian, brain, breast, pancreatic and liver tumours, and correlate with poor survival[122,123,125]. KLF6-SV1 knockdown increases apoptosis and prevents tumour growth, whereas its overexpression promotes cancer cell proliferation, survival or invasion in vitro and in vivo[122,123,125].

Splicing alterations and tumour progression

Many alternative splicing isoforms have been linked with increased cell invasion, angiogenesis and metastatic dissemination (Fig. 3). Several genes encoding proteins that regulate cell adhesion and migration express distinct spliced isoforms during cell invasion or EMT. These include alternative splicing of *CD44*, *RAC1*, *RON* (also known as *MST1R*) or *MENA* (also known as *ENAH*) that generate isoforms enabling cell invasion and metastatic dissemination. For example, *MENA*, a regulator of actin nucleation and polymerization that modulates cell morphology and motility, generates three main isoforms that play different roles in tumour progression. Inclusion of exon INV or 11a produces, respectively, isoforms *MENA-INV* or *MENA11a* which are expressed in breast and lung tumours but not in normal tissues[126–130], whereas skipping of exon 6 produces *MENAΔv6* (ref. [129]). These splicing events are regulated by many splicing factors, including ESPR1 and ESPR2 (ref. [131]). Isoform ratios are altered during tumour progression, with increased *MENA-INV* and *MENAΔv6* and decreased *MENA11a* associated with tumour grade and metastasis[126–130,132,133].

Splicing switches can also impact angiogenesis and promote tumour growth and dissemination to distant organs (Fig. 3). Alternative splicing of *VEGFA*, a growth factor that promotes proliferation and migration of endothelial cells, leads to protein isoforms with differential functions in angiogenesis. Inclusion of variable exons 6a, 6b, 7a or 7b produces pro-angiogenic $VEGFA_{xxx}$ isoforms, whereas inclusion of variable exon 8b, instead of exon 8a, produces anti-angiogenic $VEGFA_{xxx}b$ isoforms[134,135]. Both isoforms exhibit similar binding affinity to their receptor in vitro; however, $VEGFA_{xxx}b$ is unable to stimulate VEGF signalling and, thus, inhibits angiogenesis[136]. Splicing of $VEGFA_{xxx}b$ is promoted by SRSF6, whereas SRSF1 and SRSF5 shift the balance towards $VEGFA_{xxx}$ isoforms[137]. Expression of anti-angiogenic $VEGF_{xxx}b$ often decreases as tumours progress[136,138–141], and its overexpression can reduce tumour growth in mice[140,142].

Moreover, alternative splicing has been linked with changes in the tumour microenvironment through effects on both stromal and immune components (Fig. 3). Several extracellular matrix components undergo alternative splicing switches during tumour progression[143]. These include splicing of fibronectin and its receptor, α5β1 integrin, both of which have been linked to radiation resistance[144–146]. Inclusion of the fibronectin *ED-A* exon leads to an isoform expressed during embryonic development and in malignant cells, and which differs in its integrin binding domain compared with the pro-angiogenic fibronectin isoform that includes exon *ED-B*[144–146]. Similarly, tumour-specific isoforms of tenascin-C (*TNC*) or osteopontin (*SPP1*) have been linked with disease progression[147,148]. Furthermore, changes in extracellular matrix stiffness and composition can lead to differential splicing[58,149], for example, through differential phosphorylation and activation of splicing factors[150].

Finally, alternative splicing also impacts multiple regulatory steps in immune cell development and function[151] (Fig. 3). For example, alternative splicing of *CD45* is a key step during activation of T cells, whereas *CD44* alternative splicing is involved in lymphocyte activation[151]. Alternative splicing regulates multiple genes that mediate Toll-like receptor (TLR) signalling and controls the production of positive regulators of TLR signalling, including IRAK1, CD14 and IKKβ, as well as the negative regulators sTLR4 and RAB7B (refs. [152–154]). Similarly, soluble isoforms of interleukin receptors, such as IL-4R, IL-5R and IL-6R, are generated by alternative splicing in immune cells[151]. However, it remains unclear how cell compositional changes in the immune repertoire of tumours impact splicing patterns detected in bulk tissue RNA sequencing (RNA-seq).

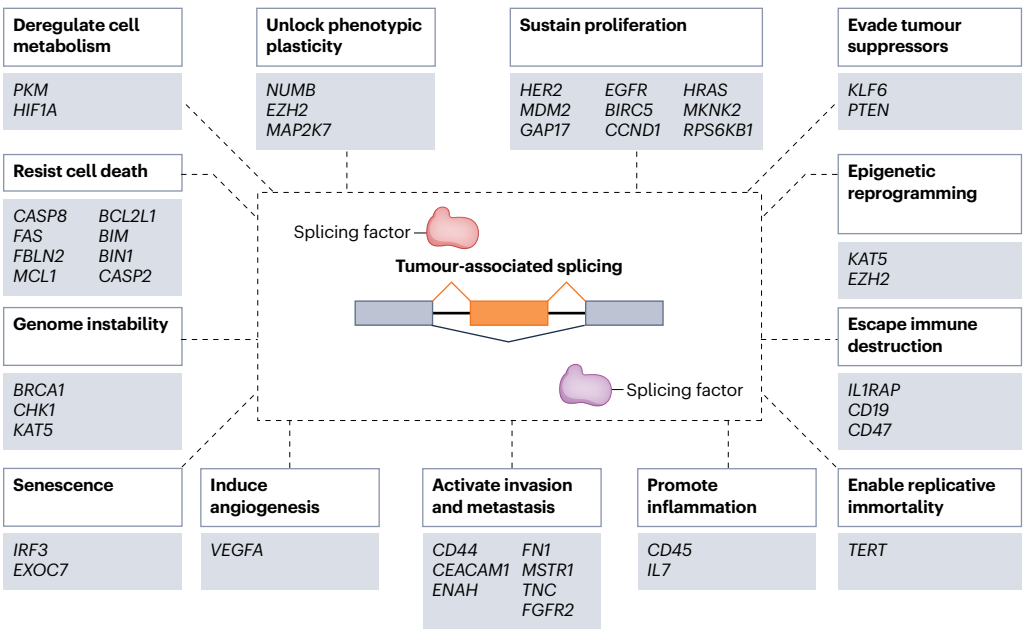


Fig. 3 | Splicing hallmarks of cancer. Examples of spliced isoforms implicated in the regulation of critical cellular processes defined as cancer hallmarks by Weinberg and Hanahan[10,11]. Note that the cancer hallmark 'Polymorphic microbiomes' is not included here.

Splicing alterations and response to therapy

Resistance to targeted therapies

Alterations in alternative splicing can lead to resistance to targeted therapy via effects on the target or signal transduction pathway (Fig. 4). Treatment with vemurafenib, a BRAF-V600E inhibitor, selects for resistant cells expressing an alternative splicing *BRAF* isoform that does not encode the RAS-binding domain that normally regulates BRAF dimerization and activation[155]. Similarly, the *BRCA1Δ11q* isoform, a variant lacking the majority of exon 11, promotes resistance to poly(ADP-ribose) polymerase (PARP) inhibition and cisplatin[156]. In addition, *BRCA1* wild type colon cancer cells that are resistant to PARP inhibition express *BARD1β* (ref. [157]), an oncogenic spliced isoform of the BRCA1 interaction partner BARD1 required for BRCA1 tumour suppressor activities. Expression of *BARD1β* correlated with impaired homologous recombination and its exogenous expression increased resistance to PARP inhibitors. Likewise, splicing of the BH3-only pro-apoptotic protein BIM, which is regulated by SRSF1, has been linked with response and resistance to tyrosine kinase inhibitors[57,158]. Finally, alternative splicing of *HER2* (also known as *ERBB2*), including skipping of exon 16 — which encodes Δ16HER2, a constitutively active protein that lacks 16 amino acids in the extracellular domain — decreases sensitivity to the HER2-targeting antibody trastuzumab[159,160].

Drugs that inhibit hormone receptor signalling are often used as frontline treatments for prostate tumours expressing androgen receptor (AR) or breast cancers expressing oestrogen receptor-α (ERα). Patients often develop resistance to these therapies, and splicing alterations can contribute to drug sensitivity (Fig. 4). For example, expression of AR isoforms that activate AR signalling despite lacking the ligand-binding domain where hormones and anti-androgen antagonists act (for example, AR-V7 and AR-v567es) is associated with anti-androgen resistance and metastasis[161–163]. Similarly, breast cancers expressing ERα36, an isoform lacking the constitutive activation function (AF-1) domain and part of the hormone-dependent activation function (AF-2) domain, do not respond well to tamoxifen treatment compared with patients whose tumours express other ERα isoforms[164].

Resistance to immunotherapy

A breakthrough in the treatment of B cell acute lymphoblastic leukaemia has been the development of immunotherapeutics directed against CD19, including CD19-directed chimeric antigen receptor (CAR) T cells. Yet relapses occur in 50% of patients due to immune rejection and T cell exhaustion or loss of the targeted epitope[165]. Epitope loss can be driven by alternative splicing of *CD19*, generating spliced isoforms that lack exon 2 and are not recognized by CAR T cells, leading to resistance[166] (Fig. 4). Another example of alternative splicing-driven acquired resistance to CAR T cell therapies is alternative splicing of *CD22* (ref. [167]). Skipping of exons 5 and 6 leads to resistance to CAR T cells targeting

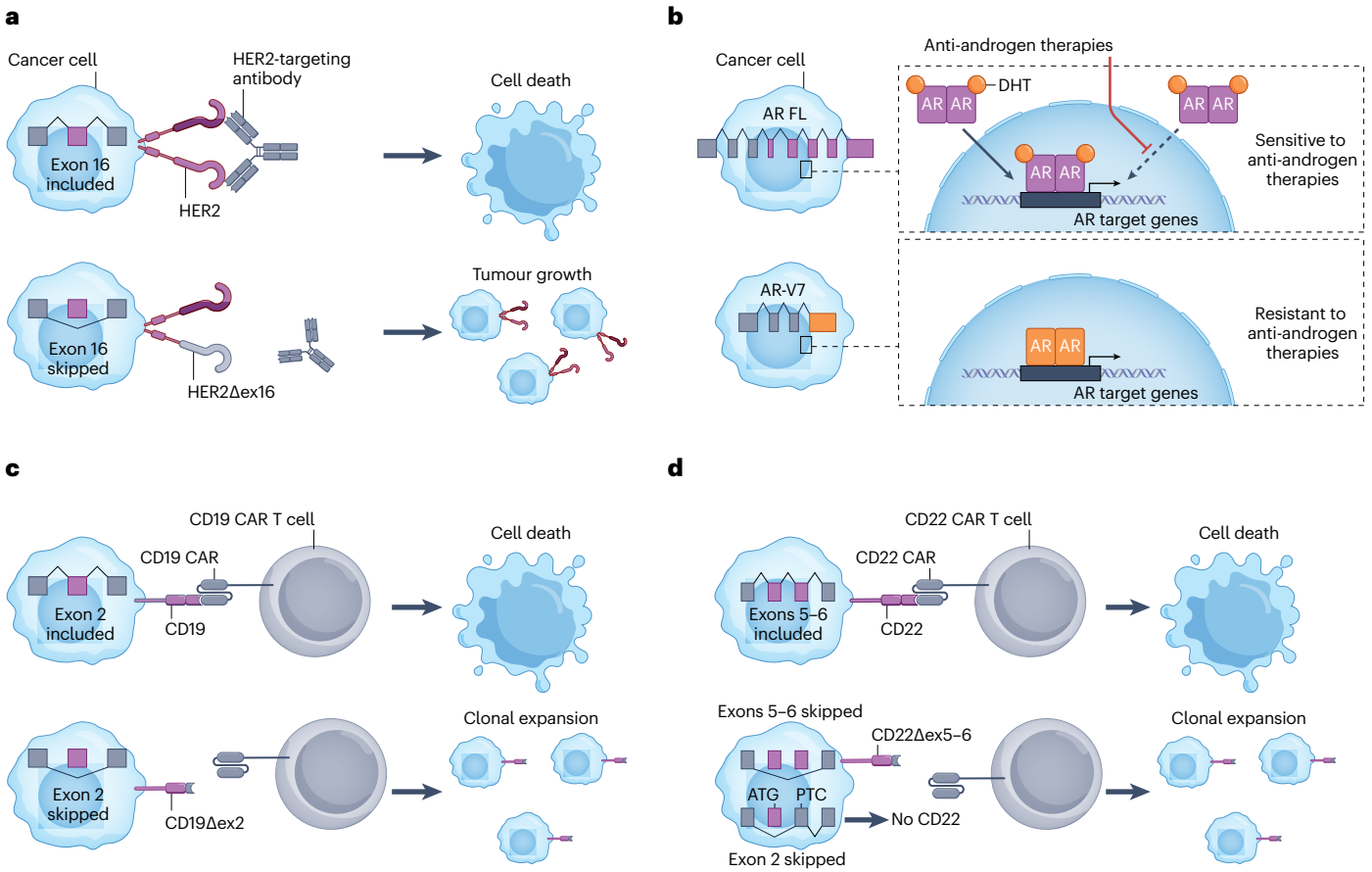


Fig. 4 | Splicing-driven alterations in drug responses. a–d, Examples of alternatively spliced isoforms associated with altered response or resistance to targeted therapies, including isoforms that confer resistance to therapies targeting HER2 (panel **a**) or the androgen receptor (AR) (panel **b**), as well as to chimeric antigen receptor (CAR) T cells (panels **c**,**d**). FL, full length; PTC, premature termination codon. DHT, dihydrotestosterone.

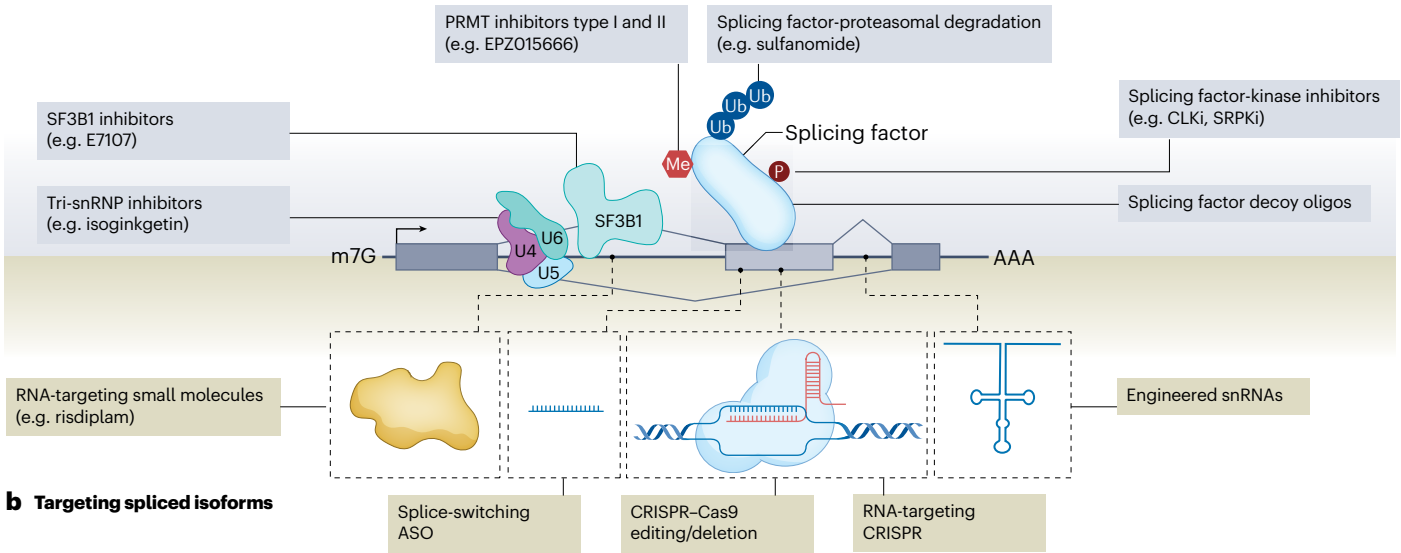


Fig. 5 | Therapeutic approaches to target splicing in cancer. Current strategies either target the splicing machinery itself or the aberrantly spliced isoforms expressed in tumour cells. **a**, Approaches targeting the spliceosome and splicing factors include broad-spectrum inhibition or modulation as well as splicing factor-specific inhibition both directly or through inhibition of upstream regulators of post-translational modifications (for example, targeting methylation (Me), phosphorylation (P) or ubiquitination (Ub) processes). **b**, Modulation of specific isoforms can be achieved using small molecules, splice-switching antisense oligonucleotides (ASOs), DNA-targeting or RNA-targeting Cas with CRISPR-based approaches, or engineered small nuclear RNAs (snRNAs). CLK, CDC-like kinase; snRNP, small nuclear ribonucleoprotein; SRPK, serine-rich protein kinase.

the third immunoglobulin-like domain of CD22, whereas skipping of the start codon-containing exon 2 prevents CD22 protein production, thereby decreasing the levels of protein available for epitope presentation[167].

Targeting splicing for cancer therapy

Given splicing's critical role in tumorigenesis, there is intense interest in targeting alternative splicing for cancer therapy. Various approaches, ranging from inhibiting key spliceosomal proteins or regulatory splicing factors to modulating specific alternative splicing events, are under preclinical and clinical development. The following discusses these approaches, starting from broad-spectrum splicing modulation to specific isoform-level approaches and ending with a discussion of novel approaches that have shown potential preclinically (Fig. 5).

Broad-spectrum splicing modulation

Targeting the core spliceosome. One approach for targeting splicing for cancer therapy is to inhibit the spliceosome itself. SF3B1 is a spliceosome component critical for BPS and 3′SS selection (Fig. 1), and limiting its function disrupts splicing at very early stages in spliceosome assembly. Multiple natural products and derivative molecules that target SF3B1 have been identified or developed, including FR901464 and its derivatives (for example, spliceostatin A, meayamycin and thailanstatins); sudemycin E; pladienolide B and FD-895, and their derivatives (for example, E7107 and H3B-8800); and herboxidiene[168–173] (Fig. 5). Mechanistically, SF3B1 inhibition prevents BPS recognition and leads to widespread disruption of both constitutive splicing and alternative splicing, including in transcripts involved with cell proliferation and death[174]. Interestingly, only a subset of introns and alternative splicing events are affected by SF3B1 inhibition, indicating that some splice sites are more sensitive than others to spliceosomal inhibitors[47,174]. Cancer cells bearing recurrent mutations in spliceosomal genes are particularly sensitive to SF3B1 inhibitors compared with wild type cells[47,175]; however, no compounds that selectively target only mutant SF3B1 have been developed. Several SF3B1 inhibitors have been taken into clinical trials. E7107 entered into phase I trials for solid tumours and resulted in dose-related alternative splicing changes in patient cells but did not demonstrate broad efficacy and was associated with ocular toxicities that led to study discontinuation[176,177]. H3B-8800 has also undergone phase I clinical trials as a treatment for myeloid neoplasms. Although no complete or partial responses were observed, a decreased need for blood transfusions was observed in some patients, with minor adverse events[178]. Given the critical role of the SF3b complex in normal splicing, it is unclear whether there will be a sufficient therapeutic index for compounds that inhibit wild type SF3B1 function in a clinical setting.

Another broad-spectrum spliceosome inhibitor is isoginkgetin, which prevents recruitment of the U4/U5/U6 tri-snRNP and leads to stalling at the pre-spliceosomal A complex[179]. In preclinical models, isoginkgetin treatment influences numerous cancer-relevant pathways including cell death[180], invasion[181] and immune response[182].

Targeting alternative splicing factors. The development of inhibitors targeting specific RBPs and splicing factors has been challenging, in part due to the lack of catalytically active sites that are readily targetable by most classical small-molecule inhibitor approaches. One notable exception is the serendipitous discovery that several aryl sulfonamides, which have anticancer activity via previously unknown mechanisms of

action, act as molecular glues that cause degradation of the RBP RBM39 via recruitment to the CUL4–DCAF15 ubiquitin ligase complex. These compounds (for example, E7820, indisulam, tasisulam and chloroquinoxaline sulfonamide) induce highly specific degradation of RBM39 and its paralog RBM23 (refs. [183–185]) (Fig. 5). RBM39 is a regulatory splicing factor that works with U2AF65 and SF3B1 in the initial stages of spliceosome assembly and splice site recognition[186–188], and additionally coordinates the action of other regulatory splicing factors, including SR proteins[189]. RBM39 knockdown broadly impacts alternative splicing events, and RBM39-regulated alternative splicing events have a 20% overlap with those regulated by U2AF65 (refs. [190,191]). Clinical trials of aryl sulfonamides have been undertaken[191], including a phase III trial comparing tasisulam with paclitaxel for metastatic melanoma that was halted due to myeloid toxicity and lack of evidence that tasisulam was superior to the standard of care[192]. However, those trials were conducted prior to the discovery of the mechanism of action of these compounds, and so target engagement and consequent splicing alterations have not yet been measured in clinical trials.

Given that overexpression and underexpression of specific splicing factors are common and can promote tumorigenesis, developing means to correct splicing factor expression could be therapeutically valuable. No such general-purpose ways of targeting individual splicing factors currently exist, but future efforts to develop them could include identification of molecular glues for splicing factors beyond RBM39; promoting or suppressing inclusion of poison exons within splicing factors via antisense oligonucleotides (ASOs) or small molecules to suppress or enhance splicing factor protein levels, respectively; and targeting upstream regulators of splicing factor activity or expression that are more readily druggable than are many splicing factors themselves.

Targeting upstream regulatory proteins. Splicing factors are subject to extensive post-translational modifications that provide opportunities for therapeutic interventions. For example, spliceosomal proteins and splicing factors are subject to extensive arginine methylation, such that both type I (PRMT1, PRMT3, PRMT4, PRMT6 and PRMT8) and type II (PRMT5) protein arginine methyltransferases are critical for regulation of both constitutive and alternative splicing through their methylation of Sm proteins and regulatory splicing factors[193,194]. *PRMT5* itself is a direct target of the MYC oncogene, providing a link between MYC-driven tumours and alternative splicing[93]. Many small molecules that inhibit type I or type II PRMTs have been identified (Fig. 5). Both type I and type II PRMT inhibitors exhibit promising preclinical activity, such as antitumour activity against lymphoma and leukaemias with spliceosomal mutations in cell lines and mouse models[195], and several are currently in early clinical trials.

Many splicing factors, particularly SR proteins, are heavily phosphorylated. These phosphorylation events alter splicing factor activity and localization, and are ultimately required for their splicing activity. Inhibition of the kinases that regulate these phosphorylation events may therefore be a viable strategy to diminish the activity of oncogenic SR proteins (Fig. 5). Serine-rich protein kinase 1 (SRPK1) inhibitors lead to decreased phosphorylation of multiple SR proteins and have anti-angiogenic effects through SRSF1-mediated alternative splicing of *VEGF*[196–198]. Another compound, TG003, influences SR protein phosphorylation by inhibiting CDC-like kinase 1 (CLK1)[199], and exhibits anticancer effects in prostate and gastric cancer models[200,201]. Other inhibitors targeting CLK1, CLK2 and CLK4 impair the viability of colorectal cancer cells in vitro by impacting the interaction of SRSF10 with these kinases[202]. Inhibitors of dual-specificity tyrosine-regulated kinases (DYRKs) can similarly modulate splicing factor phosphorylation and activity. Most of these kinase inhibitors impact the activity of multiple SR proteins, and it remains to be determined whether greater selectivity is required to limit toxicity in patients with cancer[202]. Phosphorylation of other splicing factors is important for their activity as well. For example, CDK11 phosphorylates SF3B1, and inhibition of CDK11 via the compound OTS964 impairs splicing catalysis and causes intron retention[203].

In sum, multiple approaches that induce broad-spectrum splicing modulation and/or inhibition show preclinical promise and are currently being tested in the clinic. However, as all existing approaches affect splicing in both healthy and malignant cells, careful assessment of potential toxicity and therapeutic indices is critical. Given this current limitation, the future development of compounds that selectively target or otherwise antagonize the neomorphic activities of mutant spliceosomal proteins has the potential to yield substantial therapeutic benefit with favourable side effect profiles.

Targeted splicing correction

Small molecules targeting individual isoforms. As many disease-related splicing factors are not currently druggable with small molecules, targeting key downstream mis-spliced RNAs instead may offer a promising therapeutic approach. However, only a few compounds that work by targeting a specific RNA transcript have shown clinical utility to date[204]. Risdiplam is the first US Food and Drug Administration (FDA)-approved small molecule for the treatment of spinal muscular atrophy that works by targeting the RNA transcript[204,205]. Risdiplam promotes exon 7 inclusion by selectively binding a splicing enhancer in exon 7 and the intron downstream of the 5′SS in the *SMN2* pre-mRNA[206]. The past 5 years have seen an increase in similar efforts to identify small molecules that target specific cancer-relevant RNAs. Small-molecule ligands that target RNA can be rationally designed by taking into account the preferred binding sites or RNA structure for each small molecule, which can be identified from sequence information and in vitro studies[207,208]. Small molecules can be used to induce targeted degradation of RNAs, direct cleavage or splicing modulation through steric hindrance[207,208]. However, development of such approaches is much more advanced in genetic diseases than in oncology.

Splicing modulation with oligonucleotides. RNA-based therapeutics offer the potential for extraordinary specificity for virtually any pre-mRNA sequence for the purpose of altering pre-mRNA splicing. Splice-switching ASOs are short, chemically modified RNA oligos that are designed to bind a reverse complementary sequence in a target pre-mRNA, thereby preventing its interaction with the splicing machinery (Fig. 5). Splice-switching ASOs can be designed to specifically target a 5′SS or 3′SS, thus blocking its usage; a splicing enhancer sequence, thus preventing binding of a splicing factor activator and promoting exon skipping; a splicing silencer sequence, thus preventing binding of a splicing factor repressor and promoting exon inclusion; or a cryptic splice site that arises due to a mutation, thus restoring the wild type splice site[209]. Chemical modifications to the phosphate backbone and/or the ribose ring have generated highly stable ASOs with high substrate specificity, low toxicity, low immunogenicity and reduced ribonuclease H degradation rate[210]. Delivery of ASOs to a target tissue remains a substantial challenge to their widespread therapeutic usage, except for delivery to the liver, for which GalNAc conjugation is very effective[211,212]. Current splice-switching FDA-approved ASOs are delivered directly to their target location or systemically[213], but delivery to some

tissues, including tumours, remains challenging. Novel approaches to delivery involve packaging formulations that enhance cellular uptake or targeted approaches such as aptamer or antibody conjugation that direct the ASO to specific tissues or cell types[213]. A further important challenge to utilizing ASOs in oncology is the importance of delivery to most or all tumour cells for efficacy, at least for approaches that act via cell-intrinsic mechanisms.

Despite the challenges of delivery in vivo, the catalogue of ASOs targeting cancer pathways has grown. In many cases, ASOs correcting cancer-associated alternative splicing events have led to promising anticancer phenotypes in cell line and animal models (Table 1). For example, the gene encoding BCL-x (*BCL2L1*) can be alternatively spliced to produce a pro-apoptotic isoform, *BCLxS*, or an antiapoptotic isoform, *BCLxL*, and an ASO that promotes the formation of *BCLxS* induces apoptosis in glioma cell lines[214]. The *BRD9* gene encodes a poison exon that leads to degradation of its mRNA when included in *SF3B1*-mutant tumours. An ASO that forces skipping of this exon results in increased BRD9 protein levels and decreased tumour volume in UVM mouse models[30]. A similar approach has been taken to target poison exons in transcripts encoding oncogenic splicing factors. ASOs that promote inclusion of poison exons in *SRSF3* (ref. [215]) and *TRA2B* (ref. [216]) lead to alternative splicing changes in their target transcripts and decreased proliferation of cancer cells. Additional targets include regulators of p53 (for example, *MDM2*, *MDM4* and *USP5*), cell signalling (for example, *ERRB4*, *IL5R*, *STAT3*, *FGFR1* and *MSTR1*), cell death (for example, *BCL2L1*, *BIM* and *MCL1*), DNA damage (for example, *BRCA2* and *ATM*) and chromatin remodelling and transcription (for example, *BRD9* and *ERG*) (Table 1).

Novel strategies targeting alternative RNA splicing. New approaches aimed at targeting either splicing factors or specific alternative splicing events have emerged to widen the repertoire of RNA-targeting tools. One example is decoy oligonucleotides, which attenuate splicing factor activity by competing for their natural binding targets[217] (Fig. 5). Decoy oligonucleotides induce transcriptomic changes similar to knockdown of the target splicing factor, and SRSF1 decoys can limit the growth of glioma cells in vivo[217]. Another approach is the use of engineered U7 snRNAs to correct a specific alternative splicing event. This approach alters U7's specificity for histone mRNA processing and re-engineers it to block specific pre-mRNA sequences, effectively acting as an antisense molecule[218]. Stable expression of these constructs may overcome the limitation of conventional antisense therapeutics, in that they would not require multiple rounds of administration[218]. So far, this approach has been utilized in models of myotonic dystrophy, Duchenne muscular dystrophy, amyotrophic lateral sclerosis, β-thalassemia, HIV infection and spinal muscular atrophy[218]. Additionally, alterations in the sequence recognition of the U1 snRNA can enable specific targeting of exons to promote their inclusion, and has been applied to several RNA targets, including *SMN2* (spinal muscular atrophy) and *SPINK5* (Netherton syndrome)[219].

The idea of engineering programmable splicing factors started with the use of RNA-binding domains from Pumilio 1 targeted to specific pre-mRNA sequences[220]. When designed to target *BCL-X*, Pumilio 1 engineered splicing factors promoted the formation of pro-apoptotic *BCLxS* and sensitized cancer cells to chemotherapy[220]. In the CRISPR era, RNA-targeting Cas13 (CasRx) has been adapted to base edit target RNA[221] or alter splicing of pre-mRNA[222]. Building on the Cas13 RNA-targeting capability, CRISPR artificial splicing factors were developed to direct the splicing activity of an individual splicing factor to a target pre-mRNA (Fig. 5) using guide RNAs (gRNAs) targeting, for example, *SMN2* in models of spinal muscular atrophy[223] or regulatory exons of oncogenic splicing factors in breast cancer models[216]. One challenge facing CRISPR-based approaches for therapeutic splicing modulation is that the Cas machinery must be delivered and expressed in addition to the gRNA itself.

Finally, gene editing by CRISPR-based approaches enables targeting specific alternative splicing events. By engineering specific mutations, one can strengthen or abolish a specific splice site sequence in a target of interest, thereby promoting exon inclusion or skipping. For example, cytidine deaminase single-base editors have been used to programme exon skipping by mutating target DNA bases within the splice

Table 1 | Splicing-modulating antisense oligonucleotides tested in cancer models

Target gene	Induced splicing event	Tumour type	Type of cancer model	Refs.
ATM	Blocks exon inclusion	Leukaemia	Cell line	248
BCLx	Switches BCL-xL to BCL-xS	BRCA, GBM, LUAD, PRAD	Cell line	249, 250
BIM	Exon 4 inclusion	Leukaemia	Cell line	251
BRCA2	Cryptic exon skipping	BRCA	Cell line	252
BRD9	Exon 14a skipping	UVM	Cell line Xenograft mouse model	30
ERBB4	Exon 26 skipping	BRCA	Cell line Xenograft mouse model	253
ERG	Exon 4 skipping	PRAD	Cell line Xenograft mouse model	254
EZH2	Poison exon skipping	Leukaemia	Cell line	195
FGFR1	Exon α inclusion	GBM	Cell line	255
GLDC	Exon 7 skipping	LUAD	Cell line Xenograft mouse model	256
IL5R	Exon 5 skipping	Leukaemia	Cell line	257
MCL1	Exon 2 skipping	SKCM	Cell line	258
MDM2	Exon 4 skipping	UCEC	Cell line	259
MDM4	Exon 6 skipping	DLBCL, SKCM	Cell line Patient-derived xenograft mouse model	260
MKNK2	3′ untranslated region intron retention	GBM	Cell line Xenograft mouse model	92
MSTR1	Exon 11 skipping	BRCA, STAD	Cell line	261
PKM2	Exon 9 inclusion	GBM	Cell line	69
SRSF3	Poison exon inclusion	BRCA, OSCC	Cell line	215
STAT3	Exon 23 skipping	BRCA	Cell line Xenograft mouse model	262
TRA2B	Poison exon inclusion	BRCA	Cell line	216
USP5	Alternative 5′SS	GBM	Cell line	263

BRCA, breast carcinoma; DLBCL, diffuse large B cell lymphoma; GBM, glioblastoma; LUAD, lung adenocarcinoma; PRAD, prostate adenocarcinoma; OSCC, oral squamous cell carcinoma; SKCM, skin cutaneous melanoma; STAD, stomach adenocarcinoma; UCEC, uterine corpus endometrial carcinoma; UVM, uveal melanoma.

site[224,225]. Alternatively, targeted exon deletions with CRISPR–Cas9 using paired gRNAs can promote exon skipping for desired targets[226].

Immunomodulatory approaches

Peptides translated from aberrant, cancer-associated RNA isoforms are promising targets for immunotherapies. These cancer-specific neoantigens — antigenic epitopes that are not produced or presented by major histocompatibility complex (MHC) class I in healthy cells — can arise from mutations affecting splicing as well as non-mutational use of aberrant splice junctions, intron retention and other cancer-specific alternative splicing[227]. For example, alternative splicing of *CD20* in B cell lymphomas produces a T helper cell response that can selectively kill malignant B cell clones, and vaccination of humanized mice with the corresponding peptide from *CD20* spliced isoforms can produce a robust T cell response[228]. Large-scale analysis of sequencing and proteomic data has uncovered cancer-associated alternative splicing-derived epitopes that are predicted to bind MHC class I in more than half of tumour samples analysed[5]. Additionally, studies using long-read RNA sequencing (LR-seq) identified aberrant, tumour-specific isoforms, a subset of which encoded putative alternative splicing-derived neoantigens that were immunogenic in mice expressing a human MHC allele[229].

In this context, it is interesting to note that tumour mutational burden, a common measure of neoantigenic potential, does not always correlate with an individual patient's response to immune checkpoint inhibitors[230]. Discovery of alternative splicing-derived neoantigens may complement genomic analysis to determine which patients will respond to immune checkpoint therapy[231] and, additionally, represent a rich source of potential targets for immunotherapy, particularly if tumour-specific targets that are shared across many patients can be identified[227,231–233]. Splicing modulation via multiple compounds that inhibit the SF3b complex triggers an antiviral immune response and apoptosis in transplantable syngeneic mouse models of breast cancer[234], consistent with an important role for aberrant splicing in influencing tumour-immune interactions.

Another promising approach is synergistic treatment with splicing-modulating drugs and immune checkpoint inhibitors[235]. Therapeutic modulation of alternative splicing in syngeneic mouse tumour models by RBM39 degradation or PRMT inhibition induced mis-splicing-derived neoantigen presentation on tumour cells that stimulated robust antitumour immune responses and enhanced responses to checkpoint inhibition[235]. No evidence of toxicity or increased immune infiltration of healthy tissues was observed in this preclinical setting, but further work to establish safety is necessary before clinical translation.

Glossary

Branch point
A nucleotide that performs a nucleophilic attack on the 5′ splice site (5′SS) in the first step of splicing.

K homology (KH) domain
A protein domain that can bind RNA and is found in various RNA-binding proteins (RBPs), including splicing factors.

Polypyrimidine tract
A pyrimidine (C or T)-rich sequence motif upstream of many 3′ splice sites (3′SSs) that is bound by the U2AF2 subunit of the U2AF heterodimer to facilitate 3′SS recognition.

RNA splicing
A post-transcriptional mechanism that mediates the removal of introns from a pre-mRNA transcript and the ligation of exons to form a mature mRNA.

Outstanding questions and challenges

Several technical challenges and outstanding questions remain to be addressed to translate the above mechanistic findings into the clinic.

Mapping splicing alterations in tumours

Most of the studies to date have relied on short-read RNA-seq to characterize the alternative splicing repertoire in human tumours (Box 2). These approaches have revealed the complexity of the cancer transcriptome and the extraordinary magnitude of alternative splicing switches during cell transformation. However, short-read RNA-seq cannot reliably detect complex and/or full-length novel isoforms[236]. A recent LR-seq study reported that novel spliced isoforms can account for >30% of the transcriptome of breast tumours[237]. As LR-seq approaches become more robust and cost-effective, we anticipate that they will become part of the routine characterization of tumours and provide a more comprehensive view of the alternative splicing make-up of tumours and normal tissues. Obtaining precise sequences of full-length spliced isoforms will be critical for the identification of private or shared neoantigens and the development of immunotherapies that target splicing-derived peptides.

Moreover, tumours are heterogeneous at both the genomic and transcriptomic levels, and one can expect a similar complexity for alternative splicing. Yet whether distinct regions of a tumour or cell types within a tumour exhibit differences in alternative splicing remains unknown, in part because the majority of current single-cell studies are based on 3′-biased, short-read RNA-seq that cannot reliably detect alternative splicing. Recently, single-cell transcriptomic approaches coupled with LR-seq have demonstrated that full-length isoforms can be measured in single cells in the context of brain development[238–240]. Thus, single-cell LR-seq would be a very powerful strategy to define how alternative splicing contributes to tumour evolution and drug response and to identify tumour populations associated with drug resistance. Finally, single-cell LR-seq has been coupled with spatial transcriptomics to reveal how alternative splicing contributes to tissue development and disease[241]. This approach has potential utility for studying tumour initiation and progression, which have already been associated with alterations in alternative splicing.

Finally, although technologies to measure alternative splicing isoforms at the RNA level have flourished over the past 10 years, detecting and measuring the encoded protein isoforms remains very difficult. The ability to measure alternative splicing isoforms using quantitative proteomics should further enable linking alternative splicing alterations to their functional roles in human malignancies and accelerate the discovery of novel druggable targets.

Defining the function of alternative splicing switches

Work from many laboratories has identified thousands of cancer-associated alternative splicing isoforms. Yet the lack of high-throughput approaches to interrogate the function of spliced isoforms at scale impedes the discovery of clinically relevant and actionable alternative splicing alterations. Testing the function of individual isoforms is laborious, often requiring overexpression or knockdown of each target. This limits our ability to define the functional consequences of alternative splicing and identify key targets for therapeutic correction. Therefore, functional screens that allow for the simultaneous study of thousands of alternative splicing-derived isoforms are needed.

Box 2

How to detect and quantify differential splicing

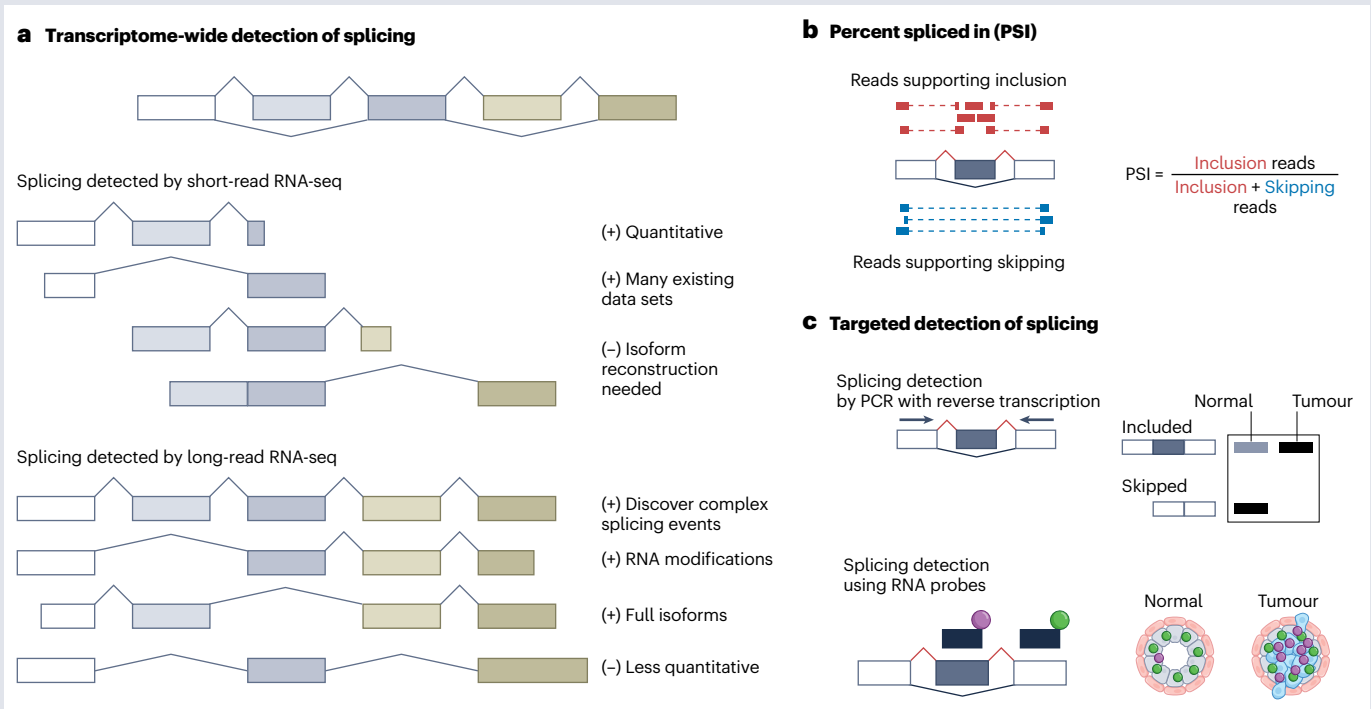
Strengths and limitations of different techniques for detecting isoforms that are differentially spliced between biological or experimental conditions (for example, cancer versus normal tissues) are discussed below.

Transcriptome-wide detection of alternative splicing isoforms can be carried out using high-throughput RNA sequencing (RNA-seq)[266]. Most cancer studies have used short-read RNA-seq (see the figure, panel **a**). Short reads are mapped to the reference transcriptome to quantify changes in splicing between conditions. Detecting novel (non-annotated) splicing involves additional steps of split read mapping, splice site inference and de novo transcript reconstruction. Differential splicing can be quantified at the level of individual splicing events (that is, inclusion or exclusion of a particular exon)[267,268], with respect to a particular isoform (that is, inclusion or exclusion of a particular exon within a full-length transcript)[269,270] or at the level of individual isoforms (that is, quantifying the abundance of one isoform with respect to all other isoforms transcribed from the parent gene)[271]. When individual splicing events are studied, alternative splicing is typically quantified using a 'percent spliced in' (PSI) or 'isoform fraction' value ranging from 0 to 100%, defined as expression of the isoforms that follow a splicing pattern of interest relative to the total expression of all transcripts of the gene (see the figure, panel **b**).

Short-read RNA-seq enables researchers to generate millions of reads for alternative splicing quantification. Because of its ubiquity, short-read data can be easily compared with public data sets such as those generated by The Cancer Genome Atlas (TCGA) or Genotype-Tissue Expression (GTEx) projects. However, isoform reconstruction and accurate quantification of full-length isoform expression are both challenging. Short-read RNA-seq permits identification of some RNA modifications directly, such as A to I editing, and others indirectly by immunoprecipitation and sequencing. Standard single-cell RNA-seq technologies, which preferentially sequence 3′ ends of RNAs, do not permit accurate splicing quantification.

Long-read RNA sequencing (LR-seq) technologies can sequence full-length RNA isoforms (see the figure, panel **a**). LR-seq can reveal complex alternative splicing, novel 5′ or 3′ untranslated regions and gene fusions. Recent LR-seq approaches enable direct RNA-seq and RNA modification detection[236,272]. However, LR-seq yields relatively few reads per sample, limiting its utility for isoform quantification. This limitation can be addressed with targeted LR-seq, such as enriching for isoforms of interest with probe capture or depleting high-abundance RNAs. Combining LR-seq for isoform identification with short-read RNA-seq for isoform quantification is effective but complex[237].

Accurately quantifying splicing is challenging due to statistical considerations. Quantifying expression of alternative splicing isoforms primarily relies upon 'informative' reads that uniquely arise from one or more, but not all, isoforms (for example, reads which cross exon–exon junctions that are only present in one isoform). Technical effects such as 3′ end biases can manifest as apparent differential alternative splicing. These challenges can be addressed by sequencing to high coverage, applying read coverage thresholds and utilizing appropriate statistical tests.



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Targeted experimental approaches can detect and quantify selected isoforms (see the figure, panel **c**). These approaches include (quantitative) PCR with reverse transcription utilizing isoform-specific primers, for which at least one primer should cross a splice junction to ensure that the assay queries mature mRNA. Digital droplet PCR can allow for absolute isoform quantification. Protein-encoding isoforms can be detected with isoform-specific antibodies. Finally, isoform-specific RNA probes enable isoform mapping and quantification with spatial resolution. Although most of these approaches are of low throughput, imaging advances may enable simultaneous detection of hundreds of isoforms[273].

Recently, CRISPR-based approaches have demonstrated that hundreds of exons can be individually deleted using paired gRNAs and screened for their effects on tumour cell growth[226]. Similarly, CRISPR-based editing can be used to mutate splice sites at scale and prevent exon inclusion[242]. However, these approaches target the DNA sequence and, therefore, could potentially also impact genome and chromatin architecture, gene transcription and other regulatory elements. Additional strategies that model the functional consequences of other alternative splicing events besides exon skipping (that is, intron retention, alternative splice sites, mutually exclusive exons) need to be developed in the future to enable testing the function of virtually any alternative splicing event (or combinations of alternative splicing events) of interest. Although further development is needed, RNA-targeting CRISPR approaches may be particularly useful in this context. Of note, many studies are biased towards studying NMD-inducing events, which are easier to model and because their putative loss-of-function consequences are easier to interpret functionally compared with other alternative splicing events.

Finally, better model systems are needed to test the functional consequences of alternative splicing alterations in malignancies and to preclinically evaluate splicing-targeting therapies. These include in vitro models that recapitulate the complexity of tumours (for example, organoids and co-culture models). Syngeneic mouse models of cancers with mutant splicing factors can also provide novel mechanistic insights and be used to test the efficacy of splicing-modulating drugs. Humanized mouse models would further enable testing the efficacy of therapies targeting human immune cells. Many functional studies of alternative splicing using in vitro and in vivo models have primarily focused on cell growth or survival as a readout, but alternative splicing switches can impact a multitude of other important cellular phenotypes. Finally, current approaches are best suited to modelling the functional consequences of a single alternative splicing switch per cell. As cancer cells typically exhibit alternative splicing alterations in many transcripts, accurately mimicking this will require modelling of combinatorial alternative splicing switches.

Origins and implications of alternative splicing switches

The past decade has revealed the extent of alterations in alternative splicing isoforms and splicing factors in cancer, but we still lack a comprehensive understanding of the functional consequences of these changes. The relative contributions of tumour-specific isoforms are still largely unknown. Is there a key set of alternative splicing isoforms that provide a growth advantage to cancer cells, or do tumours benefit from a global dysregulation of splicing, resulting in many mis-splicing events that complement each other?

Moreover, the mechanistic origins of most splicing aberrations in tumours are not yet understood. Although several splicing factors are recurrently mutated or amplified, a large proportion of solid tumours display striking changes in alternative splicing and/or splicing factor levels, yet do not bear genomic alterations directly affecting any splicing factors. Therefore, understanding the regulation of splicing factor expression in healthy tissues and tumours should facilitate the continued development of therapies targeting splicing. Regulation of splicing factors at the transcriptional level (for example, through oncogenic transcription factors such as MYC[63,93,97,243,244]) or post-transcriptional level (for example, via splicing coupled to NMD[216,226,245,246]) at least partly controls splicing factor levels in tumours. Much less is known about splicing factor regulatory mechanisms at the epigenetic, translational or post-translational levels. Although rewiring of the epigenetic landscape is a hallmark of tumours, few studies have examined how it impacts tumour-associated alternative splicing. Similarly, (post)-translational control is a crucial component of cancer development and progression, yet its impact on the splicing machinery is poorly understand. MYC activation modulates translation of the core splicing factor SF3A3, leading to downstream changes in alternative splicing and metabolic reprogramming in breast cancers[247], suggesting a key link between alternative splicing and translational control in tumours.

Alternative splicing is deeply interconnected with other molecular processes, including regulatory mechanisms at the epigenetic, transcriptional and translational levels. Therefore, cancer-driven changes in any of these mechanisms can, in turn, impact splicing outcomes, and vice versa, alterations in alternative splicing can feedback on these regulatory networks. This intricate interconnectivity can be difficult to disentangle, and studies need to be carefully designed to capture and differentiate between direct and indirect effects.

Finally, many non-genetic factors influence cancer susceptibility. These include age as well as environmental and lifestyle differences, such as diet or smoking. How these factors impact alternative splicing in pre-cancerous tissues, and whether they are associated with rewiring of the alternative splicing landscape that increases cancer risk, remains to be determined.

In sum, research over the past decade has revealed that alternative splicing dysregulation is not merely an occasional correlate of cancer but, rather, a near-ubiquitous and fundamental molecular characteristic that frequently plays a causative and even initiating role in tumorigenesis. Continued research should reveal new insights into the mechanistic origins and functional consequences of pervasive, cancer-specific splicing dysregulation and enable the creation of new cancer therapeutics that act by modulating RNA splicing.

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References

1. Black, D. L. Mechanisms of alternative pre-messenger RNA splicing. *Annu. Rev. Biochem.* **72**, 291–336 (2003).
2. Reixachs-Sole, M. & Eyras, E. Uncovering the impacts of alternative splicing on the proteome with current omics techniques. *Wiley Interdiscip. Rev. RNA* **13**, e1707 (2022).
3. Blencowe, B. J. Alternative splicing: new insights from global analyses. *Cell* **126**, 37–47 (2006).

4. Wang, E. T. et al. Alternative isoform regulation in human tissue transcriptomes. *Nature* **456**, 470–476 (2008).
This landmark study uses RNA-seq to quantify isoform expression across tissues.
5. Kahles, A. et al. Comprehensive analysis of alternative splicing across tumors from 8705 patients. *Cancer Cell* **34**, 211–224.e6 (2018).
This landmark study identifies splicing alterations across tumour types.
6. Urbanski, L. M., Leclair, N. & Anczukow, O. Alternative-splicing defects in cancer: splicing regulators and their downstream targets, guiding the way to novel cancer therapeutics. *Wiley Interdiscip. Rev. RNA* **9**, 1476 (2018).
7. Dvinge, H., Kim, E., Abdel-Wahab, O. & Bradley, R. K. RNA splicing factors as oncoproteins and tumour suppressors. *Nat. Rev. Cancer* **16**, 413–430 (2016).
8. Stanley, R. F. & Abdel-Wahab, O. Dysregulation and therapeutic targeting of RNA splicing in cancer. *Nat. Cancer* **3**, 536–546 (2022).
9. Bonnal, S. C., López-Oreja, I. & Valcárcel, J. Roles and mechanisms of alternative splicing in cancer—implications for care. *Nat. Rev. Clin. Oncol.* **17**, 457–474 (2020).
10. Hanahan, D. Hallmarks of cancer: new dimensions. *Cancer Discov.* **12**, 31–46 (2022).
11. Hanahan, D. & Weinberg, R. A. The hallmarks of cancer. *Cell* **100**, 57–70 (2000).
12. Wilkinson, M. E., Charenton, C. & Nagai, K. RNA splicing by the spliceosome. *Annu. Rev. Biochem.* **89**, 359–388 (2020).
13. Akinyi, M. V. & Frilander, M. J. At the intersection of major and minor spliceosomes: crosstalk mechanisms and their impact on gene expression. *Front. Genet.* **12**, 700744 (2021).
14. Lee, Y. & Rio, D. C. Mechanisms and regulation of alternative pre-mRNA splicing. *Annu. Rev. Biochem.* **84**, 291–323 (2015).
15. Ellis, J. D. et al. Tissue-specific alternative splicing remodels protein–protein interaction networks. *Mol. Cell* **46**, 884–892 (2012).
16. Tung, K. F., Pan, C. Y., Chen, C. H. & Lin, W. C. Top-ranked expressed gene transcripts of human protein-coding genes investigated with GTEx dataset. *Sci. Rep.* **10**, 16245 (2020).
17. Howard, J. M. & Sanford, J. R. The RNAissance family: SR proteins as multifaceted regulators of gene expression. *Wiley Interdiscip. Rev. RNA* **6**, 93–110 (2015).
18. Geuens, T., Bouhy, D. & Timmerman, V. The hnRNP family: insights into their role in health and disease. *Hum. Genet.* **135**, 851–867 (2016).
19. Papaemmanuil, E. et al. Somatic SF3B1 mutation in myelodysplasia with ring sideroblasts. *N. Engl. J. Med.* **365**, 1384–1395 (2011).
20. Yoshida, K. et al. Frequent pathway mutations of splicing machinery in myelodysplasia. *Nature* **478**, 64–69 (2011).
21. Harbour, J. W. et al. Recurrent mutations at codon 625 of the splicing factor SF3B1 in uveal melanoma. *Nat. Genet.* **45**, 133–135 (2013).
22. Martin, M. et al. Exome sequencing identifies recurrent somatic mutations in EIF1AX and SF3B1 in uveal melanoma with disomy 3. *Nat. Genet.* **45**, 933–936 (2013).
Together with Harbour et al. (2013), this paper identifies recurrent mutations in *SF3B1* in UVM.
23. Imielinski, M. et al. Mapping the hallmarks of lung adenocarcinoma with massively parallel sequencing. *Cell* **150**, 1107–1120 (2012).
24. Lee, S. C. et al. Synthetic lethal and convergent biological effects of cancer-associated spliceosomal gene mutations. *Cancer Cell* **34**, 225–241.e8 (2018).
25. Taylor, J. et al. Single-cell genomics reveals the genetic and molecular bases for escape from mutational epistasis in myeloid neoplasms. *Blood* **136**, 1477–1486 (2020).
26. Wang, L. et al. SF3B1 and other novel cancer genes in chronic lymphocytic leukemia. *N. Engl. J. Med.* **365**, 2497–2506 (2011).
This work identifies recurrent mutations in *SF3B1* in CLL.
27. Alsafadi, S. et al. Cancer-associated SF3B1 mutations affect alternative splicing by promoting alternative branchpoint usage. *Nat. Commun.* **7**, 10615 (2016).
28. Darman, R. B. et al. Cancer-associated SF3B1 hotspot mutations induce cryptic 3′ splice site selection through use of a different branch point. *Cell Rep.* **13**, 1033–1045 (2015).
This work demonstrates that recurrent *SF3B1* mutations alter branch point selection.
29. Dalton, W. B. et al. The K666N mutation in SF3B1 is associated with increased progression of MDS and distinct RNA splicing. *Blood Adv.* **4**, 1192–1196 (2020).
30. Inoue, D. et al. Spliceosomal disruption of the non-canonical BAF complex in cancer. *Nature* **574**, 432–436 (2019).
This work shows that *SF3B1* mutations disrupt chromatin remodelling to promote tumorigenesis.
31. Lieu, Y. K. et al. SF3B1 mutant-induced missplicing of MAP3K7 causes anemia in myelodysplastic syndromes. *Proc. Natl Acad. Sci. USA* **119**, 2111703119 (2022).
32. Clough, C. A. et al. Coordinated missplicing of TMEM14C and ABCB7 causes ring sideroblast formation in SF3B1-mutant myelodysplastic syndrome. *Blood* **139**, 2038–2049 (2022).
33. Yoshimi, A. et al. Coordinated alterations in RNA splicing and epigenetic regulation drive leukaemogenesis. *Nature* **574**, 273–277 (2019).
This work demonstrates genetic and functional interactions between *SRSF2* and *IDH2* in leukaemia.
34. Robertson, A. G. et al. Integrative analysis identifies four molecular and clinical subsets in uveal melanoma. *Cancer Cell* **32**, 204–220.e15 (2017).
35. Kim, E. et al. SRSF2 mutations contribute to myelodysplasia by mutant-specific effects on exon recognition. *Cancer Cell* **27**, 617–630 (2015).
36. Gallardo, M. et al. hnRNP K is a haploinsufficient tumor suppressor that regulates proliferation and differentiation programs in hematologic malignancies. *Cancer Cell* **28**, 486–499 (2015).
37. Graubert, T. A. et al. Recurrent mutations in the U2AF1 splicing factor in myelodysplastic syndromes. *Nat. Genet.* **44**, 53–57 (2012).
38. Brooks, A. N. et al. A pan-cancer analysis of transcriptome changes associated with somatic mutations in U2AF1 reveals commonly altered splicing events. *PLoS ONE* **9**, 87361 (2014).
39. Ilagan, J. O. et al. U2AF1 mutations alter splice site recognition in hematological malignancies. *Genome Res.* **25**, 14–26 (2015).
40. Smith, M. A. et al. U2AF1 mutations induce oncogenic IRAK4 isoforms and activate innate immune pathways in myeloid malignancies. *Nat. Cell Biol.* **21**, 640–650 (2019).
41. Biancon, G. et al. Precision analysis of mutant U2AF1 activity reveals deployment of stress granules in myeloid malignancies. *Mol. Cell* **82**, 1107–1122.e7 (2022).
42. Damm, F. et al. Mutations affecting mRNA splicing define distinct clinical phenotypes and correlate with patient outcome in myelodysplastic syndromes. *Blood* **119**, 3211–3218 (2012).
43. Haferlach, T. et al. Landscape of genetic lesions in 944 patients with myelodysplastic syndromes. *Leukemia* **28**, 241–247 (2014).
44. Madan, V. et al. Aberrant splicing of U12-type introns is the hallmark of ZRSR2 mutant myelodysplastic syndrome. *Nat. Commun.* **6**, 6042 (2015).
45. Inoue, D. et al. Minor intron retention drives clonal hematopoietic disorders and diverse cancer predisposition. *Nat. Genet.* **53**, 707–718 (2021).
This work shows that disruption of minor intron splicing by *ZRSR2* mutations promotes clonal advantage.
46. Wang, X., Song, X. & Yan, X. Effect of RNA splicing machinery gene mutations on prognosis of patients with MDS: a meta-analysis. *Medicine* **98**, e15743 (2019).
47. Obeng, E. A. et al. Physiologic expression of $Sf3b1^{K700E}$ causes impaired erythropoiesis, aberrant splicing, and sensitivity to therapeutic spliceosome modulation. *Cancer Cell* **30**, 404–417 (2016).
48. Shirai, C. L. et al. Mutant U2AF1 expression alters hematopoiesis and pre-mRNA splicing in vivo. *Cancer Cell* **27**, 631–643 (2015).
49. Fei, D. L. et al. Impaired hematopoiesis and leukemia development in mice with a conditional knock-in allele of a mutant splicing factor gene *U2af1*. *Proc. Natl Acad. Sci. USA* **115**, 10437–E10446 (2018).
50. Mian, S. A. et al. SF3B1 mutant MDS-initiating cells may arise from the haematopoietic stem cell compartment. *Nat. Commun.* **6**, 10004 (2015).
This work shows that *SF3B1* mutations are initiating events in MDS.
51. Fabre, M. A. et al. The longitudinal dynamics and natural history of clonal haematopoiesis. *Nature* **606**, 335–342 (2022).
52. Cancer Genome Atlas Research Network. Comprehensive molecular profiling of lung adenocarcinoma. *Nature* **511**, 543–550 (2014).
53. Ibrahimpasic, T. et al. Genomic alterations in fatal forms of non-anaplastic thyroid cancer: identification of MED12 and RBM10 as novel thyroid cancer genes associated with tumor virulence. *Clin. Cancer Res.* **23**, 5970–5980 (2017).
54. Anczuków, O. & Krainer, A. R. Splicing-factor alterations in cancers. *RNA* **22**, 1285–1301 (2016).
55. Seiler, M. et al. Somatic mutational landscape of splicing factor genes and their functional consequences across 33 cancer types. *Cell Rep.* **23**, 282–296.e4 (2018).
56. Escobar-Hoyos, L. F. et al. Altered RNA splicing by mutant p53 activates oncogenic RAS signaling in pancreatic cancer. *Cancer Cell* **38**, 198–211.e8 (2020).
57. Anczukow, O. et al. The splicing factor SRSF1 regulates apoptosis and proliferation to promote mammary epithelial cell transformation. *Nat. Struct. Mol. Biol.* **19**, 220–228 (2012).
58. Anczukow, O. et al. SRSF1-regulated alternative splicing in breast cancer. *Mol. Cell* **60**, 105–117 (2015).
59. Park, S. et al. Differential functions of splicing factors in mammary transformation and breast cancer metastasis. *Cell Rep.* **29**, 2672–2688.e7 (2019).
This work identifies the functional roles of individual SR proteins in breast cancer.
60. Karni, R. et al. The gene encoding the splicing factor SF2/ASF is a proto-oncogene. *Nat. Struct. Mol. Biol.* **14**, 185–193 (2007).
This landmark study shows that SRSF1 is a proto-oncoprotein.
61. Sebestyén, E. et al. Large-scale analysis of genome and transcriptome alterations in multiple tumors unveils novel cancer-relevant splicing networks. *Genome Res.* **26**, 732–744 (2016).
62. Ghigna, C. et al. Cell motility is controlled by SF2/ASF through alternative splicing of the Ron protooncogene. *Mol. Cell* **20**, 881–890 (2005).
63. Das, S., Anczukow, O., Akerman, M. & Krainer, A. R. Oncogenic splicing factor SRSF1 is a critical transcriptional target of MYC. *Cell Rep.* **1**, 110–117 (2012).
64. De Miguel, F. J. et al. Identification of alternative splicing events regulated by the oncogenic factor SRSF1 in lung cancer. *Cancer Res.* **74**, 1105–1115 (2014).
65. Michlewski, G., Sanford, J. R. & Caceres, J. F. The splicing factor SF2/ASF regulates translation initiation by enhancing phosphorylation of 4E-BP1. *Mol. Cell* **30**, 179–189 (2008).
66. Karni, R., Hippo, Y., Lowe, S. W. & Krainer, A. R. The splicing-factor oncoprotein SF2/ASF activates mTORC1. *Proc. Natl Acad. Sci. USA* **105**, 15323–15327 (2008).
67. Sen, S., Langiewicz, M., Jumaa, H. & Webster, N. J. Deletion of serine/arginine-rich splicing factor 3 in hepatocytes predisposes to hepatocellular carcinoma in mice. *Hepatology* **61**, 171–183 (2015).
68. Ajiro, M., Jia, R., Yang, Y., Zhu, J. & Zheng, Z. M. A genome landscape of SRSF3-regulated splicing events and gene expression in human osteosarcoma U2OS cells. *Nucleic Acids Res.* **44**, 1854–1870 (2016).

69. Wang, Z. et al. Exon-centric regulation of pyruvate kinase M alternative splicing via mutually exclusive exons. *J. Mol. Cell Biol.* **4**, 79–87 (2012).
70. Kurokawa, K. et al. Downregulation of serine/arginine-rich splicing factor 3 induces G1 cell cycle arrest and apoptosis in colon cancer cells. *Oncogene* **33**, 1407–1417 (2014).
71. Jia, R., Ajiro, M., Yu, L., McCoy, P. Jr & Zheng, Z. M. Oncogenic splicing factor SRSF3 regulates ILF3 alternative splicing to promote cancer cell proliferation and transformation. *RNA* **25**, 630–644 (2019).
72. Freytag, M. et al. Epithelial splicing regulatory protein 1 and 2 (ESRP1 and ESRP2) upregulation predicts poor prognosis in prostate cancer. *BMC Cancer* **20**, 1220 (2020).
73. Gokmen-Polar, Y. et al. Splicing factor ESRP1 controls ER-positive breast cancer by altering metabolic pathways. *EMBO Rep.* **20**, 46078 (2019).
74. Shapiro, I. M. et al. An EMT-driven alternative splicing program occurs in human breast cancer and modulates cellular phenotype. *PLoS Genet.* **7**, 1002218 (2011).
75. Munkley, J. et al. Androgen-regulated transcription of ESRP2 drives alternative splicing patterns in prostate cancer. *eLife* **8**, 47678 (2019).
76. Ishii, H. et al. Epithelial splicing regulatory proteins 1 (ESRP1) and 2 (ESRP2) suppress cancer cell motility via different mechanisms. *J. Biol. Chem.* **289**, 27386–27399 (2014).
77. Bechara, E. G., Sebestyen, E., Bernardis, I., Eyras, E. & Valcarcel, J. RBM5, 6, and 10 differentially regulate NUMB alternative splicing to control cancer cell proliferation. *Mol. Cell* **52**, 720–733 (2013).
This work demonstrates the role of RBMs in tumorigenesis.
78. Rintala-Maki, N. D. et al. Expression of RBM5-related factors in primary breast tissue. *J. Cell Biochem.* **100**, 1440–1458 (2007).
79. Inoue, A. RBM10: structure, functions, and associated diseases. *Gene* **783**, 145463 (2021).
80. Lu, W. et al. QKI impairs self-renewal and tumorigenicity of oral cancer cells via repression of SOX2. *Cancer Biol. Ther.* **15**, 1174–1184 (2014).
81. Zong, F. Y. et al. The RNA-binding protein QKI suppresses cancer-associated aberrant splicing. *PLoS Genet.* **10**, 1004289 (2014).
82. Bandopadhayay, P. et al. MYB-QKI rearrangements in angiocentric glioma drive tumorigenicity through a tripartite mechanism. *Nat. Genet.* **48**, 273–282 (2016).
83. Shirakihara, T. et al. TGF-β regulates isoform switching of FGF receptors and epithelial–mesenchymal transition. *EMBO J.* **30**, 783–795 (2011).
84. Warzecha, C. C. et al. An ESRP-regulated splicing programme is abrogated during the epithelial–mesenchymal transition. *EMBO J.* **29**, 3286–3300 (2010).
This work demonstrates the important role played by regulated splicing during EMT.
85. Warzecha, C. C., Shen, S., Xing, Y. & Carstens, R. P. The epithelial splicing factors ESRP1 and ESRP2 positively and negatively regulate diverse types of alternative splicing events. *RNA Biol.* **6**, 546–562 (2009).
86. Yae, T. et al. Alternative splicing of CD44 mRNA by ESRP1 enhances lung colonization of metastatic cancer cell. *Nat. Commun.* **3**, 883 (2012).
This work demonstrates the role of a CD44 isoform in lung metastasis in vivo.
87. Sutherland, L. C., Wang, K. & Robinson, A. G. RBM5 as a putative tumor suppressor gene for lung cancer. *J. Thorac. Oncol.* **5**, 294–298 (2010).
88. Jamsai, D. et al. In vivo evidence that RBM5 is a tumour suppressor in the lung. *Sci. Rep.* **7**, 16323 (2017).
89. Zhao, Y. et al. The tumor suppressing effects of QKI-5 in prostate cancer: a novel diagnostic and prognostic protein. *Cancer Biol. Ther.* **15**, 108–118 (2014).
90. Frampton, G. M. et al. Activation of MET via diverse exon 14 splicing alterations occurs in multiple tumor types and confers clinical sensitivity to MET inhibitors. *Cancer Discov.* **5**, 850–859 (2015).
91. Jung, H. et al. Intron retention is a widespread mechanism of tumor-suppressor inactivation. *Nat. Genet.* **47**, 1242–1248 (2015).
92. Mogilevsky, M. et al. Modulation of MKNK2 alternative splicing by splice-switching oligonucleotides as a novel approach for glioblastoma treatment. *Nucleic Acids Res.* **46**, 11396–11404 (2018).
93. Koh, C. M. et al. MYC regulates the core pre-mRNA splicing machinery as an essential step in lymphomagenesis. *Nature* **523**, 96–100 (2015).
This work identifies a MYC-driven splicing vulnerability.
94. Ben-Hur, V. et al. S6K1 alternative splicing modulates its oncogenic activity and regulates mTORC1. *Cell Rep.* **3**, 103–115 (2013).
95. Amaral, C. L. et al. S6Ks isoforms contribute to viability, migration, docetaxel resistance and tumor formation of prostate cancer cells. *BMC Cancer* **16**, 602 (2016).
96. Mei, H., Wang, Y., Fan, J. & Lin, Z. Alternative splicing of S6K1 promotes non-small cell lung cancer survival. *Tumour Biol.* **37**, 13369–13376 (2016).
97. David, C. J., Chen, M., Assanah, M., Canoll, P. & Manley, J. L. HnRNP proteins controlled by c-Myc deregulate pyruvate kinase mRNA splicing in cancer. *Nature* **463**, 364–368 (2010).
98. Clower, C. V. et al. The alternative splicing repressors hnRNP A1/A2 and PTB influence pyruvate kinase isoform expression and cell metabolism. *Proc. Natl Acad. Sci. USA* **107**, 1894–1899 (2010).
99. Dayton, T. L., Jacks, T. & Vander Heiden, M. G. PKM2, cancer metabolism, and the road ahead. *EMBO Rep.* **17**, 1721–1730 (2016).
100. Wang, Z., Jeon, H. Y., Rigo, F., Bennett, C. F. & Krainer, A. R. Manipulation of PK-M mutually exclusive alternative splicing by antisense oligonucleotides. *Open. Biol.* **2**, 120133 (2012).
101. Boise, L. H. et al. bcl-x, a bcl-2-related gene that functions as a dominant regulator of apoptotic cell death. *Cell* **74**, 597–608 (1993).
102. Wu, L., Mao, C. & Ming, X. Modulation of Bcl-x alternative splicing induces apoptosis of human hepatic stellate cells. *Biomed. Res. Int.* **2016**, 7478650 (2016).
103. Dole, M. G. et al. Bcl-xS enhances adenoviral vector-induced apoptosis in neuroblastoma cells. *Cancer Res.* **56**, 5734–5740 (1996).
104. Minn, A. J., Boise, L. H. & Thompson, C. B. Bcl-x(S) anatagonizes the protective effects of Bcl-x(L). *J. Biol. Chem.* **271**, 6306–6312 (1996).
105. Paronetto, M. P., Achsel, T., Massiello, A., Chalfant, C. E. & Sette, C. The RNA-binding protein Sam68 modulates the alternative splicing of Bcl-x. *J. Cell Biol.* **176**, 929–939 (2007).
106. Zhou, A., Ou, A. C., Cho, A., Benz, E. J. Jr & Huang, S. C. Novel splicing factor RBM25 modulates Bcl-x pre-mRNA 5′ splice site selection. *Mol. Cell Biol.* **28**, 5924–5936 (2008).
107. Bielli, P., Bordi, M., Di Biasio, V. & Sette, C. Regulation of BCL-X splicing reveals a role for the polypyrimidine tract binding protein (PTBP1/hnRNP I) in alternative 5′ splice site selection. *Nucleic Acids Res.* **42**, 12070–12081 (2014).
108. Wang, Y. et al. The splicing factor RBM4 controls apoptosis, proliferation, and migration to suppress tumor progression. *Cancer Cell* **26**, 374–389 (2014).
This work demonstrates the role of RBMs in tumorigenesis in vivo.
109. Moore, M. J., Wang, Q., Kennedy, C. J. & Silver, P. A. An alternative splicing network links cell-cycle control to apoptosis. *Cell* **142**, 625–636 (2010).
110. Garneau, D., Revil, T., Fisette, J. F. & Chabot, B. Heterogeneous nuclear ribonucleoprotein F/H proteins modulate the alternative splicing of the apoptotic mediator Bcl-x. *J. Biol. Chem.* **280**, 22641–22650 (2005).
111. Revil, T., Pelletier, J., Toutant, J., Cloutier, A. & Chabot, B. Heterogeneous nuclear ribonucleoprotein K represses the production of pro-apoptotic Bcl-xS splice isoform. *J. Biol. Chem.* **284**, 21458–21467 (2009).
112. Cloutier, P. et al. Antagonistic effects of the SRp30c protein and cryptic 5′ splice sites on the alternative splicing of the apoptotic regulator Bcl-x. *J. Biol. Chem.* **283**, 21315–21324 (2008).
113. Pabla, N., Bhatt, K. & Dong, Z. Checkpoint kinase 1 (Chk1)-short is a splice variant and endogenous inhibitor of Chk1 that regulates cell cycle and DNA damage checkpoints. *Proc. Natl Acad. Sci. USA* **109**, 197–202 (2012).
114. Hu, G. et al. Clinical and functional significance of CHK1-S, an alternatively spliced isoform of the CHK1 gene, in hepatocellular carcinoma. *J. Cancer* **11**, 1792–1799 (2020).
115. Wong, M. S., Shay, J. W. & Wright, W. E. Regulation of human telomerase splicing by RNA:RNA pairing. *Nat. Commun.* **5**, 3306 (2014).
116. Sayed, M. E. et al. NOVA1 directs PTBP1 to hTERT pre-mRNA and promotes telomerase activity in cancer cells. *Oncogene* **38**, 2937–2952 (2019).
117. Ludlow, A. T. et al. NOVA1 regulates hTERT splicing and cell growth in non-small cell lung cancer. *Nat. Commun.* **9**, 3112 (2018).
118. Listerman, I., Sun, J., Gazzaniga, F. S., Lukas, J. L. & Blackburn, E. H. The major reverse transcriptase-incompetent splice variant of the human telomerase protein inhibits telomerase activity but protects from apoptosis. *Cancer Res.* **73**, 2817–2828 (2013).
119. Pont, A. R., Sadri, N., Hsiao, S. J., Smith, S. & Schneider, R. J. mRNA decay factor AUF1 maintains normal aging, telomere maintenance, and suppression of senescence by activation of telomerase transcription. *Mol. Cell* **47**, 5–15 (2012).
120. Kang, X., Chen, W., Kim, R. H., Kang, M. K. & Park, N. H. Regulation of the hTERT promoter activity by MSH2, the hnRNPs K and D, and GRHL2 in human oral squamous cell carcinoma cells. *Oncogene* **28**, 565–574 (2009).
121. DiFeo, A., Martignetti, J. A. & Narla, G. The role of KLF6 and its splice variants in cancer therapy. *Drug. Resist. Updat.* **12**, 1–7 (2009).
122. Hatami, R. et al. KLF6-SV1 drives breast cancer metastasis and is associated with poor survival. *Sci. Transl. Med.* **5**, 12 (2013).
123. Yea, S. et al. Ras promotes growth by alternative splicing-mediated inactivation of the KLF6 tumor suppressor in hepatocellular carcinoma. *Gastroenterology* **134**, 1521–1531 (2008).
124. Botella, L. M. et al. TGF-β regulates the expression of transcription factor KLF6 and its splice variants and promotes co-operative transactivation of common target genes through a Smad3–Sp1–KLF6 interaction. *Biochem. J.* **419**, 485–495 (2009).
125. DiFeo, A. et al. A functional role for KLF6–SV1 in lung adenocarcinoma prognosis and chemotherapy response. *Cancer Res.* **68**, 965–970 (2008).
126. Tanaka, N., Yoshida, H., Suzuki, Y. & Harigaya, K. Relative expression of hMena11a and hMenaINV splice isoforms is a useful biomarker in development and progression of human breast carcinoma. *Int. J. Oncol.* **45**, 1921–1928 (2014).
127. Oudin, M. J. et al. Characterization of the expression of the pro-metastatic Mena^{INV} isoform during breast tumor progression. *Clin. Exp. Metastasis* **33**, 249–261 (2016).
This work characterizes the expression of MENA isoforms in primary tumours.
128. Goswami, S. et al. Identification of invasion specific splice variants of the cytoskeletal protein Mena present in mammary tumor cells during invasion in vivo. *Clin. Exp. Metastasis* **26**, 153–159 (2009).
129. Di Modugno, F. et al. Splicing program of human MENA produces a previously undescribed isoform associated with invasive, mesenchymal-like breast tumors. *Proc. Natl Acad. Sci. USA* **109**, 19280–19285 (2012).
130. Bria, E. et al. Prognostic impact of alternative splicing-derived hMENA isoforms in resected, node-negative, non-small-cell lung cancer. *Oncotarget* **5**, 11054–11063 (2014).
131. Balsamo, M. et al. The alternatively-included 11a sequence modifies the effects of Mena on actin cytoskeletal organization and cell behavior. *Sci. Rep.* **6**, 35298 (2016).
132. Di Modugno, F. et al. Molecular cloning of hMena (ENAH) and its splice variant hMena+11a: epidermal growth factor increases their expression and stimulates hMena+11a phosphorylation in breast cancer cell lines. *Cancer Res.* **67**, 2657–2665 (2007).
133. Philippar, U. et al. A Mena invasion isoform potentiates EGF-induced carcinoma cell invasion and metastasis. *Dev. Cell* **15**, 813–828 (2008).
This work identifies MENA isoforms.

134. Houck, K. A. et al. The vascular endothelial growth factor family: identification of a fourth molecular species and characterization of alternative splicing of RNA. *Mol. Endocrinol.* **5**, 1806–1814 (1991).
135. Bates, D. O. et al. VEGF165b, an inhibitory splice variant of vascular endothelial growth factor, is down-regulated in renal cell carcinoma. *Cancer Res.* **62**, 4123–4131 (2002).
This work identifies VEGF isoforms.
136. Woolard, J. et al. VEGF165b, an inhibitory vascular endothelial growth factor splice variant: mechanism of action, in vivo effect on angiogenesis and endogenous protein expression. *Cancer Res.* **64**, 7822–7835 (2004).
137. Nowak, D. G. et al. Regulation of vascular endothelial growth factor (VEGF) splicing from pro-angiogenic to anti-angiogenic isoforms: a novel therapeutic strategy for angiogenesis. *J. Biol. Chem.* **285**, 5532–5540 (2010).
138. Harper, S. J. & Bates, D. O. VEGF-A splicing: the key to anti-angiogenic therapeutics? *Nat. Rev. Cancer* **8**, 880–887 (2008).
139. Pritchard-Jones, R. O. et al. Expression of $VEGF_{xxx}b$, the inhibitory isoforms of VEGF, in malignant melanoma. *Br. J. Cancer* **97**, 223–230 (2007).
140. Varey, A. H. et al. VEGF 165 b, an antiangiogenic VEGF-A isoform, binds and inhibits bevacizumab treatment in experimental colorectal carcinoma: balance of pro- and antiangiogenic VEGF-A isoforms has implications for therapy. *Br. J. Cancer* **98**, 1366–1379 (2008).
141. Biselli-Chicote, P. M. et al. Overexpression of antiangiogenic vascular endothelial growth factor isoform and splicing pegulatory factors in oral, laryngeal and pharyngeal squamous cell carcinomas. *Asian Pac. J. Cancer Prev.* **18**, 2171–2177 (2017).
142. Rennel, E. et al. The endogenous anti-angiogenic VEGF isoform, VEGF165b inhibits human tumour growth in mice. *Br. J. Cancer* **98**, 1250–1257 (2008).
143. Robertson, C. The extracellular matrix in breast cancer predicts prognosis through composition, splicing, and crosslinking. *Exp. Cell Res.* **343**, 73–81 (2016).
144. Nam, J. M., Onodera, Y., Bissell, M. J. & Park, C. C. Breast cancer cells in three-dimensional culture display an enhanced radioresponse after coordinate targeting of integrin α5β1 and fibronectin. *Cancer Res.* **70**, 5238–5248 (2010).
145. Schiefner, A., Gebauer, M. & Skerra, A. Extra-domain B in oncofetal fibronectin structurally promotes fibrillar head-to-tail dimerization of extracellular matrix protein. *J. Biol. Chem.* **287**, 17578–17588 (2012).
146. Fukuda, T. et al. Mice lacking the EDB segment of fibronectin develop normally but exhibit reduced cell growth and fibronectin matrix assembly in vitro. *Cancer Res.* **62**, 5603–5610 (2002).
147. Borsi, L. et al. Expression of different tenascin isoforms in normal, hyperplastic and neoplastic human breast tissues. *Int. J. Cancer* **52**, 688–692 (1992).
148. Briones-Orta, M. A. et al. Osteopontin splice variants and polymorphisms in cancer progression and prognosis. *Biochim. Biophys. Acta* **1868**, 93–108A (2017).
149. Srebrow, A., Blaustein, M. & Kornblihtt, A. R. Regulation of fibronectin alternative splicing by a basement membrane-like extracellular matrix. *FEBS Lett.* **514**, 285–289 (2002).
150. Bordeleau, F. et al. Tissue stiffness regulates serine/arginine-rich protein-mediated splicing of the extra domain B-fibronectin isoform in tumors. *Proc. Natl Acad. Sci. USA* **112**, 8314–8319 (2015).
151. Martinez, N. M. & Lynch, K. W. Control of alternative splicing in immune responses: many regulators, many predictions, much still to learn. *Immunol. Rev.* **253**, 216–236 (2013).
152. O'Connor, B. P. et al. Regulation of Toll-like receptor signaling by the SF3a mRNA splicing complex. *PLoS Genet.* **11**, 1004932 (2015).
153. Adib-Conquy, M. et al. Up-regulation of MyD88s and SIGIRR, molecules inhibiting Toll-like receptor signaling, in monocytes from septic patients. *Crit. Care Med.* **34**, 2377–2385 (2006).
154. De Arras, L. et al. Comparative genomics RNAi screen identifies Eftud2 as a novel regulator of innate immunity. *Genetics* **197**, 485–496 (2014).
155. Poulikakos, P. I. et al. RAF inhibitor resistance is mediated by dimerization of aberrantly spliced BRAF(V600E). *Nature* **480**, 387–390 (2011).
156. Wang, Y. et al. The BRCA1- Δ11q alternative splice isoform bypasses germline mutations and promotes therapeutic resistance to PARP inhibition and cisplatin. *Cancer Res.* **76**, 2778–2790 (2016).
157. Ozden, O. et al. Expression of an oncogenic BARD1 splice variant impairs homologous recombination and predicts response to PARP-1 inhibitor therapy in colon cancer. *Sci. Rep.* **6**, 26273 (2016).
158. Ng, K. P. et al. A common BIM deletion polymorphism mediates intrinsic resistance and inferior responses to tyrosine kinase inhibitors in cancer. *Nat. Med.* **18**, 521–528 (2012).
This work links resistance to tyrosine kinase inhibitors with *BIM* splicing.
159. Castiglioni, F. et al. Role of exon-16-deleted HER2 in breast carcinomas. *Endocr. Relat. Cancer* **13**, 221–232 (2006).
160. Alajati, A. et al. Mammary tumor formation and metastasis evoked by a HER2 splice variant. *Cancer Res.* **73**, 5320–5327 (2013).
161. Dehm, S. M., Schmidt, L. J., Heemers, H. V., Vessella, R. L. & Tindall, D. J. Splicing of a novel androgen receptor exon generates a constitutively active androgen receptor that mediates prostate cancer therapy resistance. *Cancer Res.* **68**, 5469–5477 (2008).
This work identifies a constitutively active AR isoform that contributes to therapy resistance.
162. Antonarakis, E. S. et al. AR-V7 and resistance to enzalutamide and abiraterone in prostate cancer. *N. Engl. J. Med.* **371**, 1028–1038 (2014).
163. Sun, S. et al. Castration resistance in human prostate cancer is conferred by a frequently occurring androgen receptor splice variant. *J. Clin. Invest.* **120**, 2715–2730 (2010).
This work identifies a constitutively active AR isoform that contributes to castration resistance.
164. Thiebaut, C. et al. The role of ERα36 in development and tumor malignancy. *Int. J. Mol. Sci.* **21**, 4116 (2020).
165. Zheng, S., Asnani, M. & Thomas-Tikhonenko, A. Escape from ALL-CARTaz: leukemia immunoediting in the age of chimeric antigen receptors. *Cancer J.* **25**, 217–222 (2019).
166. Sotillo, E. et al. Convergence of acquired mutations and alternative splicing of CD19 enables resistance to CART-19 immunotherapy. *Cancer Discov.* **5**, 1282–1295 (2015).
This work demonstrates that alternative splicing can enable escape from CAR T cell therapy.
167. Zheng, S. et al. Modulation of CD22 protein expression in childhood leukemia by pervasive splicing aberrations: implications for CD22-directed immunotherapies. *Blood Cancer Discov.* **3**, 103–115 (2022).
168. Nakajima, H. et al. New antitumor substances, FR901463, FR901464 and FR901465. II. Activities against experimental tumors in mice and mechanism of action. *J. Antibiot.* **49**, 1204–1211 (1996).
169. Thompson, C. F., Jamison, T. F. & Jacobsen, E. N. FR901464: total synthesis, proof of structure, and evaluation of synthetic analogues. *J. Am. Chem. Soc.* **123**, 9974–9983 (2001).
170. Kaida, D. et al. Spliceostatin A targets SF3b and inhibits both splicing and nuclear retention of pre-mRNA. *Nat. Chem. Biol.* **3**, 576–583 (2007).
171. Osman, S. et al. Structural requirements for the antiproliferative activity of pre-mRNA splicing inhibitor FR901464. *Chemistry* **17**, 895–904 (2011).
172. Albert, B. J., Sivaramakrishnan, A., Naka, T., Czaicki, N. L. & Koide, K. Total syntheses, fragmentation studies, and antitumor/antiproliferative activities of FR901464 and its low picomolar analogue. *J. Am. Chem. Soc.* **129**, 2648–2659 (2007).
173. Liu, X. et al. Genomics-guided discovery of thailanstatins A, B, and C As pre-mRNA splicing inhibitors and antiproliferative agents from *Burkholderia thailandensis* MSMB43. *J. Nat. Prod.* **76**, 685–693 (2013).
174. Corrionero, A., Minana, B. & Valcarcel, J. Reduced fidelity of branch point recognition and alternative splicing induced by the anti-tumor drug spliceostatin A. *Genes. Dev.* **25**, 445–459 (2011).
This work characterizes the mechanism of action of spliceostatin A.
175. Seiler, M. et al. H3B-8800, an orally available small-molecule splicing modulator, induces lethality in spliceosome-mutant cancers. *Nat. Med.* **24**, 497–504 (2018).
176. Hong, D. S. et al. A phase I, open-label, single-arm, dose-escalation study of E7107, a precursor messenger ribonucleic acid (pre-mRNA) splicesome inhibitor administered intravenously on days 1 and 8 every 21 days to patients with solid tumors. *Invest. N. Drugs* **32**, 436–444 (2014).
177. Eskens, F. A. et al. Phase I pharmacokinetic and pharmacodynamic study of the first-in-class spliceosome inhibitor E7107 in patients with advanced solid tumors. *Clin. Cancer Res.* **19**, 6296–6304 (2013).
178. Steensma, D. P. et al. Phase I first-in-human dose escalation study of the oral SF3B1 modulator H3B-8800 in myeloid neoplasms. *Leukemia* **35**, 3542–3550 (2021).
179. O'Brien, K., Matlin, A. J., Lowell, A. M. & Moore, M. J. The biflavonoid isoginkgetin is a general inhibitor of pre-mRNA splicing. *J. Biol. Chem.* **283**, 33147–33154 (2008).
180. Vanzyl, E. J. et al. The spliceosome inhibitors isoginkgetin and pladienolide B induce ATF3-dependent cell death. *PLoS ONE* **15**, e0224953 (2020).
181. Yoon, S. O., Shin, S., Lee, H. J., Chun, H. K. & Chung, A. S. Isoginkgetin inhibits tumor cell invasion by regulating phosphatidylinositol 3-kinase/Akt-dependent matrix metalloproteinase-9 expression. *Mol. Cancer Ther.* **5**, 2666–2675 (2006).
182. Darrigrand, R. et al. Isoginkgetin derivative IP2 enhances the adaptive immune response against tumor antigens. *Commun. Biol.* **4**, 269 (2021).
183. Han, T. et al. Anticancer sulfonamides target splicing by inducing RBM39 degradation via recruitment to DCAF15. *Science* **356**, 3755 (2017).
This work identifies RBM39 as the molecular target of indisulam.
184. Ting, T. C. et al. Aryl sulfonamides degrade RBM39 and RBM23 by recruitment to CRL4–DCAF15. *Cell Rep.* **29**, 1499–1510.e6 (2019).
185. Bussiere, D. E. et al. Structural basis of indisulam-mediated RBM39 recruitment to DCAF15 E3 ligase complex. *Nat. Chem. Biol.* **16**, 15–23 (2020).
186. Tari, M. et al. $U2AF^{65}$ assemblies drive sequence-specific splice site recognition. *EMBO Rep.* **20**, 47604 (2019).
187. Stepanyuk, G. A. et al. UHM–ULM interactions in the RBM39–U2AF65 splicing-factor complex. *Acta Crystallogr. D. Struct. Biol.* **72**, 497–511 (2016).
188. Loerch, S., Maucuer, A., Manceau, V., Green, M. R. & Kielkopf, C. L. Cancer-relevant splicing factor CAPERα engages the essential splicing factor SF3b155 in a specific ternary complex. *J. Biol. Chem.* **289**, 17325–17337 (2014).
189. Kralovicova, J. et al. PUF60-activated exons uncover altered 3′ splice-site selection by germline missense mutations in a single RRM. *Nucleic Acids Res.* **46**, 6166–6187 (2018).
190. Mai, S. et al. Global regulation of alternative RNA splicing by the SR-rich protein RBM39. *Biochim. Biophys. Acta* **1859**, 1014–1024 (2016).
191. Xu, Y., Nijhuis, A. & Keun, H. C. RNA-binding motif protein 39 (RBM39): an emerging cancer target. *Br. J. Pharmacol.* **179**, 2795–2812 (2020).
192. Hamid, O. et al. A randomized, open-label clinical trial of tasisulam sodium versus paclitaxel as second-line treatment in patients with metastatic melanoma. *Cancer* **120**, 2016–2024 (2014).

193. Bezzi, M. et al. Regulation of constitutive and alternative splicing by PRMT5 reveals a role for Mdm4 pre-mRNA in sensing defects in the spliceosomal machinery. *Genes Dev.* **27**, 1903–1916 (2013).
194. Radzisheuskaya, A. et al. PRMT5 methylome profiling uncovers a direct link to splicing regulation in acute myeloid leukemia. *Nat. Struct. Mol. Biol.* **26**, 999–1012 (2019).
195. Fong, J. Y. et al. Therapeutic targeting of RNA splicing catalysis through inhibition of protein arginine methylation. *Cancer Cell* **36**, 194–209.e9 (2019).
196. Gammons, M. V. et al. Topical antiangiogenic SRPK1 inhibitors reduce choroidal neovascularization in rodent models of exudative AMD. *Invest. Ophthalmol. Vis. Sci.* **54**, 6052–6062 (2013).
197. Hatcher, J. M. et al. SRPKIN-1: a covalent SRPK1/2 inhibitor that potently converts VEGF from pro-angiogenic to anti-angiogenic isoform. *Cell Chem. Biol.* **25**, 460–470.e6 (2018).
198. Batson, J. et al. Development of potent, selective SRPK1 inhibitors as potential topical therapeutics for neovascular eye disease. *ACS Chem. Biol.* **12**, 825–832 (2017).
199. Sakuma, M., Iida, K. & Hagiwara, M. Deciphering targeting rules of splicing modulator compounds: case of TG003. *BMC Mol. Biol.* **16**, 16 (2015).
200. Babu, N. et al. Phosphoproteomic analysis identifies CLK1 as a novel therapeutic target in gastric cancer. *Gastric Cancer* **23**, 796–810 (2020).
201. Uzor, S. et al. CDC2-like (CLK) protein kinase inhibition as a novel targeted therapeutic strategy in prostate cancer. *Sci. Rep.* **11**, 7963 (2021).
202. Sohail, M. et al. A novel class of inhibitors that target SRSF10 and promote p53-mediated cytotoxicity on human colorectal cancer cells. *Nar. Cancer* **3**, 19 (2021).
203. Hluchy, M. et al. CDK11 regulates pre-mRNA splicing by phosphorylation of SF3B1. *Nature* **609**, 829–834 (2022).
204. Sheridan, C. First small-molecule drug targeting RNA gains momentum. *Nat. Biotechnol.* **39**, 6–8 (2021).
205. Baranello, G. et al. Risdiplam in type 1 spinal muscular atrophy. *N. Engl. J. Med.* **384**, 915–923 (2021).
This work demonstrates that risdiplam increases SMN protein levels in patients with spinal muscular atrophy.
206. Sivaramakrishnan, M. et al. Binding to SMN2 pre-mRNA–protein complex elicits specificity for small molecule splicing modifiers. *Nat. Commun.* **8**, 1476 (2017).
207. Costales, M. G., Childs-Disney, J. L., Haniff, H. S. & Disney, M. D. How we think about targeting RNA with small molecules. *J. Med. Chem.* **63**, 8880–8900 (2020).
208. Umuhire Juru, A. & Hargrove, A. E. Frameworks for targeting RNA with small molecules. *J. Biol. Chem.* **296**, 100191 (2021).
209. Havens, M. A. & Hastings, M. L. Splice-switching antisense oligonucleotides as therapeutic drugs. *Nucleic Acids Res.* **44**, 6549–6563 (2016).
210. Bennett, C. F. & Swayze, E. E. RNA targeting therapeutics: molecular mechanisms of antisense oligonucleotides as a therapeutic platform. *Annu. Rev. Pharmacol. Toxicol.* **50**, 259–293 (2010).
211. Kim, Y. et al. Enhanced potency of GalNAc-conjugated antisense oligonucleotides in hepatocellular cancer models. *Mol. Ther.* **27**, 1547–1557 (2019).
212. Scharner, J., Qi, S., Rigo, F., Bennett, C. F. & Krainer, A. R. Delivery of GalNAc-conjugated splice-switching ASOs to non-hepatic cells through ectopic expression of asialoglycoprotein receptor. *Mol. Ther. Nucleic Acids* **16**, 313–325 (2019).
213. Juliano, R. L. The delivery of therapeutic oligonucleotides. *Nucleic Acids Res.* **44**, 6518–6548 (2016).
214. Li, Z. et al. Pro-apoptotic effects of splice-switching oligonucleotides targeting Bcl-x pre-mRNA in human glioma cell lines. *Oncol. Rep.* **35**, 1013–1019 (2016).
215. Sun, Y., Yan, L., Guo, J., Shao, J. & Jia, R. Downregulation of SRSF3 by antisense oligonucleotides sensitizes oral squamous cell carcinoma and breast cancer cells to paclitaxel treatment. *Cancer Chemother. Pharmacol.* **84**, 1133–1143 (2019).
216. Leclair, N. K. et al. Poison exon splicing regulates a coordinated network of SR protein expression during differentiation and tumorigenesis. *Mol. Cell* **80**, 648–665.e9 (2020).
This work identifies functional roles for poison exons in tumorigenesis.
217. Denichenko, P. et al. Specific inhibition of splicing factor activity by decoy RNA oligonucleotides. *Nat. Commun.* **10**, 1590 (2019).
218. Gadgil, A. & Raczynska, K. D. U7 snRNA: a tool for gene therapy. *J. Gene Med.* **23**, 3321 (2021).
219. Rogalska, M. E. et al. Therapeutic activity of modified U1 core spliceosomal particles. *Nat. Commun.* **7**, 11168 (2016).
220. Wang, Y., Cheong, C. G., Hall, T. M. & Wang, Z. Engineering splicing factors with designed specificities. *Nat. Methods* **6**, 825–830 (2009).
221. Cox, D. B. T. et al. RNA editing with CRISPR–Cas13. *Science* **358**, 1019–1027 (2017).
222. Konermann, S. et al. Transcriptome engineering with RNA-targeting type VI-D CRISPR effectors. *Cell* **173**, 665–676.e14 (2018).
This work uses RNA-targeting Cas to manipulate alternative splicing.
223. Du, M., Jillette, N., Zhu, J. J., Li, S. & Cheng, A. W. CRISPR artificial splicing factors. *Nat. Commun.* **11**, 2973 (2020).
224. Gapinske, M. et al. CRISPR–SKIP: programmable gene splicing with single base editors. *Genome Biol.* **19**, 107 (2018).
225. Banas, K. et al. Exon skipping induced by CRISPR-directed gene editing regulates the response to chemotherapy in non-small cell lung carcinoma cells. *Gene Ther.* **29**, 357–367 (2022).
226. Thomas, J. D. et al. RNA isoform screens uncover the essentiality and tumor-suppressor activity of ultraconserved poison exons. *Nat. Genet.* **52**, 84–94 (2020).
This work reveals tumour-suppressive roles for poison exons.
227. Smith, C. C. et al. Alternative tumour-specific antigens. *Nat. Rev. Cancer* **19**, 465–478 (2019).
228. Vauchy, C. et al. CD20 alternative splicing isoform generates immunogenic CD4 helper T epitopes. *Int. J. Cancer* **137**, 116–126 (2015).
229. Oka, M. et al. Aberrant splicing isoforms detected by full-length transcriptome sequencing as transcripts of potential neoantigens in non-small cell lung cancer. *Genome Biol.* **22**, 9 (2021).
230. Snyder, A. et al. Genetic basis for clinical response to CTLA-4 blockade in melanoma. *N. Engl. J. Med.* **371**, 2189–2199 (2014).
231. Frankiw, L., Baltimore, D. & Li, G. Alternative mRNA splicing in cancer immunotherapy. *Nat. Rev. Immunol.* **19**, 675–687 (2019).
232. Slansky, J. E. & Spellman, P. T. Alternative splicing in tumors—a path to immunogenicity? *N. Engl. J. Med.* **380**, 877–880 (2019).
233. Hoyos, L. E. & Abdel-Wahab, O. Cancer-specific splicing changes and the potential for splicing-derived neoantigens. *Cancer Cell* **34**, 181–183 (2018).
234. Bowling, E. A. et al. Spliceosome-targeted therapies trigger an antiviral immune response in triple-negative breast cancer. *Cell* **184**, 384–403.e21 (2021).
This work shows that inhibition of the SF3b complex triggers antiviral signalling.
235. Lu, S. X. et al. Pharmacologic modulation of RNA splicing enhances anti-tumor immunity. *Cell* **184**, 4032–4047.e31 (2021).
This work demonstrates that splicing modulation triggers neoantigen production and synergizes with immune checkpoint blockade.
236. De Paoli-Iseppi, R., Gleeson, J. & Clark, M. B. Isoform age—splice isoform profiling using long-read technologies. *Front. Mol. Biosci.* **8**, 711733 (2021).
237. Veiga, D. F. T. et al. A comprehensive long-read isoform analysis platform and sequencing resource for breast cancer. *Sci. Adv.* **8**, eabg6711 (2022).
This work identifies full-length isoforms in primary tumours using LR-seq.
238. Tian, L. et al. Comprehensive characterization of single-cell full-length isoforms in human and mouse with long-read sequencing. *Genome Biol.* **22**, 310 (2021).
239. Gupta, I. et al. Single-cell isoform RNA sequencing characterizes isoforms in thousands of cerebellar cells. *Nat. Biotechnol.* **36**, 1197–1202 (2018).
240. Hardwick, S. A. et al. Single-nuclei isoform RNA sequencing unlocks barcoded exon connectivity in frozen brain tissue. *Nat. Biotechnol.* **40**, 1082–1092 (2022).
241. Joglekar, A. et al. A spatially resolved brain region- and cell type-specific isoform atlas of the postnatal mouse brain. *Nat. Commun.* **12**, 463 (2021).
242. Mou, H. et al. CRISPR/Cas9-mediated genome editing induces exon skipping by alternative splicing or exon deletion. *Genome Biol.* **18**, 108 (2017).
243. Urbanski, L. et al. MYC regulates a pan-cancer network of co-expressed oncogenic splicing factors. *Cell Rep.* **41**, 11704 (2022).
244. Hsu, T. Y. et al. The spliceosome is a therapeutic vulnerability in MYC-driven cancer. *Nature* **525**, 384–388 (2015).
This work identifies a MYC-driven splicing vulnerability.
245. Rossbach, O. et al. Auto- and cross-regulation of the hnRNP L proteins by alternative splicing. *Mol. Cell Biol.* **29**, 1442–1451 (2009).
246. Lareau, L. F., Inada, M., Green, R. E., Wengrod, J. C. & Brenner, S. E. Unproductive splicing of SR genes associated with highly conserved and ultraconserved DNA elements. *Nature* **446**, 926–929 (2007).
This landmark study identifies conserved poison exons in genes encoding SR proteins.
247. Ciesla, M. et al. Oncogenic translation directs spliceosome dynamics revealing an integral role for SF3A3 in breast cancer. *Mol. Cell* **81**, 1453–1468.e12 (2021).
248. Kralovicova, J., Moreno, P. M., Cross, N. C., Pego, A. P. & Vorechovsky, I. Antisense oligonucleotides modulating activation of a nonsense-mediated RNA decay switch exon in the ATM gene. *Nucleic Acid. Ther.* **26**, 392–400 (2016).
249. Mercatante, D. R., Bortner, C. D., Cidlowski, J. A. & Kole, R. Modification of alternative splicing of Bcl-x pre-mRNA in prostate and breast cancer cells. analysis of apoptosis and cell death. *J. Biol. Chem.* **276**, 16411–16417 (2001).
250. Taylor, J. K., Zhang, Q. Q., Wyatt, J. R. & Dean, N. M. Induction of endogenous Bcl-xS through the control of Bcl-x pre-mRNA splicing by antisense oligonucleotides. *Nat. Biotechnol.* **17**, 1097–1100 (1999).
251. Liu, J. et al. Overcoming imatinib resistance conferred by the BIM deletion polymorphism in chronic myeloid leukemia with splice-switching antisense oligonucleotides. *Oncotarget* **8**, 77567–77585 (2017).
252. Anczuków, O. et al. BRCA2 deep intronic mutation causing activation of a cryptic exon: opening toward a new preventive therapeutic strategy. *Clin. Cancer Res.* **18**, 4903–4909 (2012).
253. Nielsen, T. O., Sorensen, S., Dagnaes-Hansen, F., Kjems, J. & Sorensen, B. S. Directing HER4 mRNA expression towards the CYT2 isoform by antisense oligonucleotide decreases growth of breast cancer cells in vitro and in vivo. *Br. J. Cancer* **108**, 2291–2298 (2013).
254. Li, L. et al. Targeting the ERG oncogene with splice-switching oligonucleotides as a novel therapeutic strategy in prostate cancer. *Br. J. Cancer* **123**, 1024–1032 (2020).
255. Bruno, I. G., Jin, W. & Cote, G. J. Correction of aberrant FGFR1 alternative RNA splicing through targeting of intronic regulatory elements. *Hum. Mol. Genet.* **13**, 2409–2420 (2004).
256. Lin, J. et al. Induced-decay of glycine decarboxylase transcripts as an anticancer therapeutic strategy for non-small-cell lung carcinoma. *Mol. Ther. Nucleic Acids* **9**, 263–273 (2017).
257. Karras, J. G., McKay, R. A., Lu, T., Dean, N. M. & Monia, B. P. Antisense inhibition of membrane-bound human interleukin-5 receptor-α chain does not affect soluble receptor expression and induces apoptosis in TF-1 cells. *Antisense Nucleic Acid. Drug Dev.* **10**, 347–357 (2000).

258. Shieh, J. J., Liu, K. T., Huang, S. W., Chen, Y. J. & Hsieh, T. Y. Modification of alternative splicing of Mcl-1 pre-mRNA using antisense morpholino oligonucleotides induces apoptosis in basal cell carcinoma cells. *J. Invest. Dermatol.* **129**, 2497–2506 (2009).
259. Shiraishi, T., Eysturskarth, J. & Nielsen, P. E. Modulation of mdm2 pre-mRNA splicing by 9-aminoacridine-PNA (peptide nucleic acid) conjugates targeting intron–exon junctions. *BMC Cancer* **10**, 342 (2010).
260. Dewaele, M. et al. Antisense oligonucleotide-mediated MDM4 exon 6 skipping impairs tumor growth. *J. Clin. Invest.* **126**, 68–84 (2016).
261. Ghigna, C. et al. Pro-metastatic splicing of Ron proto-oncogene mRNA can be reversed: therapeutic potential of bifunctional oligonucleotides and indole derivatives. *RNA Biol.* **7**, 495–503 (2010).
262. Zammarchi, F. et al. Antitumorigenic potential of STAT3 alternative splicing modulation. *Proc. Natl Acad. Sci. USA* **108**, 17779–17784 (2011).
This work demonstrates that ASOs can be used to manipulate splicing in in vivo cancer models.
263. Izaguirre, D. I. et al. PTBP1-dependent regulation of USP5 alternative RNA splicing plays a role in glioblastoma tumorigenesis. *Mol. Carcinog.* **51**, 895–906 (2012).
264. Shen, H., Zheng, X., Luecke, S. & Green, M. R. The U2AF35-related protein Urp contacts the 3′ splice site to promote U12-type intron splicing and the second step of U2-type intron splicing. *Genes Dev.* **24**, 2389–2394 (2010).
265. Dvinge, H. & Bradley, R. K. Widespread intron retention diversifies most cancer transcriptomes. *Genome Med.* **7**, 45 (2015).
266. Mehmood, A. et al. Systematic evaluation of differential splicing tools for RNA-seq studies. *Brief. Bioinform* **21**, 2052–2065 (2020).
267. Shen, S. et al. rMATS: robust and flexible detection of differential alternative splicing from replicate RNA-seq data. *Proc. Natl Acad. Sci. USA* **111**, 5593–5601 (2014).
268. Katz, Y., Wang, E. T., Airoldi, E. M. & Burge, C. B. Analysis and design of RNA sequencing experiments for identifying isoform regulation. *Nat. Methods* **7**, 1009–1015 (2010).
269. Vaquero-Garcia, J. et al. A new view of transcriptome complexity and regulation through the lens of local splicing variations. *eLife* **5**, 11752 (2016).
270. Trincado, J. L. et al. SUPPA2: fast, accurate, and uncertainty-aware differential splicing analysis across multiple conditions. *Genome Biol.* **19**, 40 (2018).
271. Li, Y. I. et al. Annotation-free quantification of RNA splicing using LeafCutter. *Nat. Genet.* **50**, 151–158 (2018).
272. Leger, A. et al. RNA modifications detection by comparative Nanopore direct RNA sequencing. *Nat. Commun.* **12**, 7198 (2021).
273. He, S. et al. High-plex imaging of RNA and proteins at subcellular resolution in fixed tissue by spatial molecular imaging. *Nat. Biotechnol.* https://doi.org/10.1038/s41587-022-01483-z (2022).

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Author contributions
The authors contributed equally to all aspects of the article.

Competing interests
R.K.B. is an inventor on patent applications filed by Fred Hutchinson Cancer Center related to modulating splicing for cancer therapy. O.A. is an inventor on a patent application filed by The Jackson Laboratory related to modulating splicing factors.

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