Molecular Therapy
Review

# Impacts and mechanisms of alternative mRNA splicing in cancer metabolism, immune response, and therapeutics

Qiu Peng,[1,2,4] Yujuan Zhou,[1,3,4] Linda Oyang,[1] Nayiyuan Wu,[1] Yanyan Tang,[1] Min Su,[1] Xia Luo,[1] Ying Wang,[1] Xiaowu Sheng,[1] Jian Ma,[1,2] and Qianjin Liao[1,3]

[1]Hunan Key Laboratory of Cancer Metabolism, Hunan Cancer Hospital and the Affiliated Cancer Hospital of Xiangya School of Medicine, Central South University, Changsha, 410013 Hunan, China; [2]Cancer Research Institute and School of Basic Medical Science, Central South University, Changsha, China; [3]Hunan Key Laboratory of Translational Radiation Oncology, 283 Tongzipo Road, Changsha 410013, Hunan, China

**Alternative pre-mRNA splicing (AS) provides the potential to produce diversity at RNA and protein levels. Disruptions in the regulation of pre-mRNA splicing can lead to diseases. With the development of transcriptome and genome sequencing technology, increasing diseases have been identified to be associated with abnormal splicing of mRNAs. In tumors, abnormal alternative splicing frequently plays critical roles in cancer pathogenesis and may be considered as new biomarkers and therapeutic targets for cancer intervention. Metabolic abnormalities and immune disorders are important hallmarks of cancer. AS produces multiple different isoforms and diversifies protein expression, which is utilized by the immune and metabolic reprogramming systems to expand gene functions. The abnormal splicing events contributed to tumor progression, partially due to effects on immune response and metabolic reprogramming. Herein, we reviewed the vital role of alternative splicing in regulating cancer metabolism and immune response. We discussed how alternative splicing regulates metabolic reprogramming of cancer cells and antitumor immune response, and the possible strategies to targeting alternative splicing pathways or splicing-regulated metabolic pathway in the context of anticancer immunotherapy. Further, we highlighted the challenges and discuss the perspectives for RNA-based strategies for the treatment of cancer with abnormally alternative splicing isoforms.**

## INTRODUCTION

Alternative RNA splicing (AS) is a primary mechanism of post-transcriptional gene modulations that removes introns and links exons specifically to form distinct mature messenger RNA (mRNA) and extremely diversifies the repertoire of the transcriptome and proteome.[1] Increasing evidence has shown that ~95% of human genes express more than one alternative splice isoform, which has become one of the significant molecular markers of cancer and potential targets for the development of new therapeutics.[2–4]

Tumor cells change their metabolism profile through a variety of mechanisms to promote tumor progression. The metabolic pathways used by rapidly proliferating tumor cells to generate macromolecules necessary for growth and survival, as well as the underlying mechanisms controlled by tumor cells to meet their energy requirements, have become the core hallmark of cancer for many years.[5,6] Even under normoxic conditions, these tumor cells are more prone to aerobic glycolysis than oxidative phosphate.[7–9] This metabolic reprogramming has been shown to supply the building blocks that support the rapid proliferation of tumor cells. The increased lactic acid and other metabolites produced by aerobic glycolysis are related to the promotion of cell invasion.[10] Furthermore, metabolic changes in tumor cells also are involved in the regulation of the stemness and chemical resistance of cancer. Due to the importance of metabolism in tumor progression, increasing studies have begun to develop new therapeutic drugs targeting cancer metabolism.[11–14] In tumor cells, the Warburg effect has been indicated to be regulated by alternative splicing.[15] The pyruvate kinase (PKM) deserves special mention. It catalyzes the last step of glycolysis, which converts phosphoenolpyruvate into pyruvate. The alternative use of two mutually exclusive exons 9 or 10 led to the formation of PKM1 or PKM2 subtypes, respectively. It has been found that PKM1 promotes oxidative phosphorylation, while PKM2 is involved in promoting aerobic glycolysis and is up-regulated in a variety of cancers.[16,17] In addition, hypoxia-induced alternative splicing has also been reported to be a powerful driving force for tumor pathogenesis and progression.[18]

The immune system is responsible for monitoring and defending against the invasion of different foreign pathogens and maintaining

https://doi.org/10.1016/j.ymthe.2021.11.010.

[4]These authors contributed equally

**Correspondence:** Jian Ma, Hunan Key Laboratory of Cancer Metabolism, Hunan Cancer Hospital and The Affiliated Cancer Hospital of Xiangya School of Medicine, Central South University, 283 Tongzipo Road, Changsha 410013, Hunan, China.
**E-mail:** majian@csu.edu.cn
**Correspondence:** Qianjin Liao, Hunan Key Laboratory of Cancer Metabolism, Hunan Cancer Hospital and The Affiliated Cancer Hospital of Xiangya School of Medicine, Central South University, 283 Tongzipo Road, Changsha 410013, Hunan, China.
**E-mail:** liaoqianjin@hnca.org.cn

 

internal homeostasis by maintaining appropriate immune tolerance and regulation.[19] Immune surveillance and immune defense are the main immune responses. According to the type of exogenous antigen, the immune response can comprehensively construct an immune defense through a variety of immune cells, and exert anti-virus, antibacterial, and antitumor functions.[19] Although tumor cells have developed an incredible strategy to evade the elimination of the immune system,[20] immunotherapy is producing effective treatments for some previously incurable cancers in recent years.[21–23] Alternative processing of mRNAs, with the potential to generate multiple functionally distinct mRNAs and protein isoforms in many cancers, may provide the potential of a widespread target space.[24] Moreover, immune cells divide and differentiate continuously throughout their lives. The cells of the adaptive immune system are highly evolved cells in vertebrates. Therefore, it is not surprising that immune cells use alternative splicing to ensure high transcriptional diversity and regulate gene expression.[25] Immune activation is the basis for the host response to pathogenic infections and is known to be affected by alternative splicing.[26] Recent studies suggest that pathogen co-opting of host RNA splicing machinery may be part of a larger plan to perturb the infection-mediated host response mechanism.[27,28] In addition, several studies have reported that abnormal alternative RNA splicing can affect diseases by regulating the activation of immune cells.[29,30]

In this review, we focused on how alternative RNA splicing is altered in cancer and functionally drives cancer initiation and maintenance by regulating immune response and metabolic reprogramming. In addition, we discuss emerging new strategies of how to regulate pathologic splicing products in tumors. For any pharmacological regulation, including mutations in the splicing site mutations or the aberrant expression of splicing factors (trans-effect changes) or mutations, this will create opportunities for the production of a drug that may affect tumors in a truly targeted way. Furthermore, we explore emerging evidence suggesting that pre-mRNA splicing-derived aberrant isoforms can be suitable tumor-specific antigens for cancer immunotherapy. Finally, we highlight various strategies for targeting splicing defects in cancer and discuss the possible role of splicing dysregulation in cancer immunotherapy.

## MOLECULAR MECHANISMS OF PRE-mRNA SPLICING

Splicing occurs through the synergistic action of multiple subunit complexes. The core of the spliceosome is composed of small ribosomal proteins, U1, U2, U4, U5, and U6, and hundreds of proteins.[31,32] *Cis*-acting regulatory sequences, splicing factors, and other RNA-binding proteins that recognize and bind splice sites constitute a common mechanism for establishing and maintaining alternative splicing patterns.[33] These sites can be introns or exons, splicing enhancers, or splicing silencers (exon-splicing enhancers or silencers [ESE or ESS], and intron-splicing enhancers or silencers [ISE or ISS]). Generally, the *cis*-elements on the precursor RNA can recruit *trans*-splicing factors and influence splicing through various mechanisms.[34] There are two common types of splicing factors: one is serine and arginine-rich splicing factor (SR protein), and the other is nuclear heterogeneous ribonucleoprotein (hnRNPs). In exons, SR proteins usually bind ESE to activate splicing, while hnRNPs usually bind ESS to inhibit splicing.[35] The activities of splicing factors are often interdependent, which means that their activities can change when they bind to different positions of pre-mRNA. For example, SR proteins promote splicing when they bind to exons, but inhibit splicing when they bind to introns.

The activity of hnRNPs is more complicated because different members can act as activators or inhibitors when binding the same intron or exon. The interaction between splicing factors and *cis*-regulatory elements usually constitutes a complex network responsible for the plasticity of splicing regulation.[36] In addition to the aforementioned core components (small nuclear ribonucleoproteins [snRNPs], SR proteins, and hnRNPs), hundreds of RNA-binding proteins are widely believed to be involved in the regulation of alternative splicing.[37,38] The regulatory complexity of these RNA-binding proteins can diversify the RNA products for alternative splicing. This review summarizes a simplified spliceosome assembly pathway and core splicing factors required for exons or introns definition (Figure 1).

The regulation of alternative RNA splicing largely occurs co-transcriptionally, and the selection of alternative splicing sites is influenced by RNA polymerase II elongation rate, chromatin remodelers, and histone deacetylase inhibitors.[39] RNA polymerase II is thought to accumulate at promoters and co-transcriptional alternative splicing sites where RNA synthesis is slowed. Brodsk et al. reported that RNA polymerase II accumulates at many constitutively spliced exons, but is biased for alternatively spliced exons.[40] Consistent with this idea, others have also found that polymerase density is higher on alternative exons than on constitutive exons.[41,42] Histone posttranslational modifications (PTMs) can occur at multiple locations within a given polypeptide chain and plays an important role in regulating chromatin structure.[43] Some studies found an obvious enrichment of the H3K36me3 mark on exons by analyzing genome-wide chromatin immunoprecipitation-sequencing (ChIP-seq) data. Notably, this histone mark seems to correlate more significantly with constitutive exons than with alternative exons.[44,45]

The important contribution of oncogenic signaling pathways in the regulation of alternative RNA splicing occurs through modifications of RNA-binding protein (RBP) function or occupancy of histone modifications on the body of transcribed genes. Blaustein et al. showed that epithelial-mesenchymal interaction regulates the inclusion of the fibronectin alternative exon EDA via PI3-kinase in mouse mammary epithelial cells, and the PI3-kinase pathway is mediated by the action of two SR proteins, SF2/ASF and 9G8.[46,47] Patel et al. provided *in vitro* and *in vivo* evidence showing that Akt2 kinase directly phosphorylated splicing factor SRp40, thereby connecting the insulin and PI3-kinase/Akt signaling pathway with phosphorylation of a site on a nuclear splicing protein and promoting exon inclusion.[48] In addition, Sanidas et al. showed that the RNA processing regulator IWS1 and AKT play a central role in the regulation of the alternative

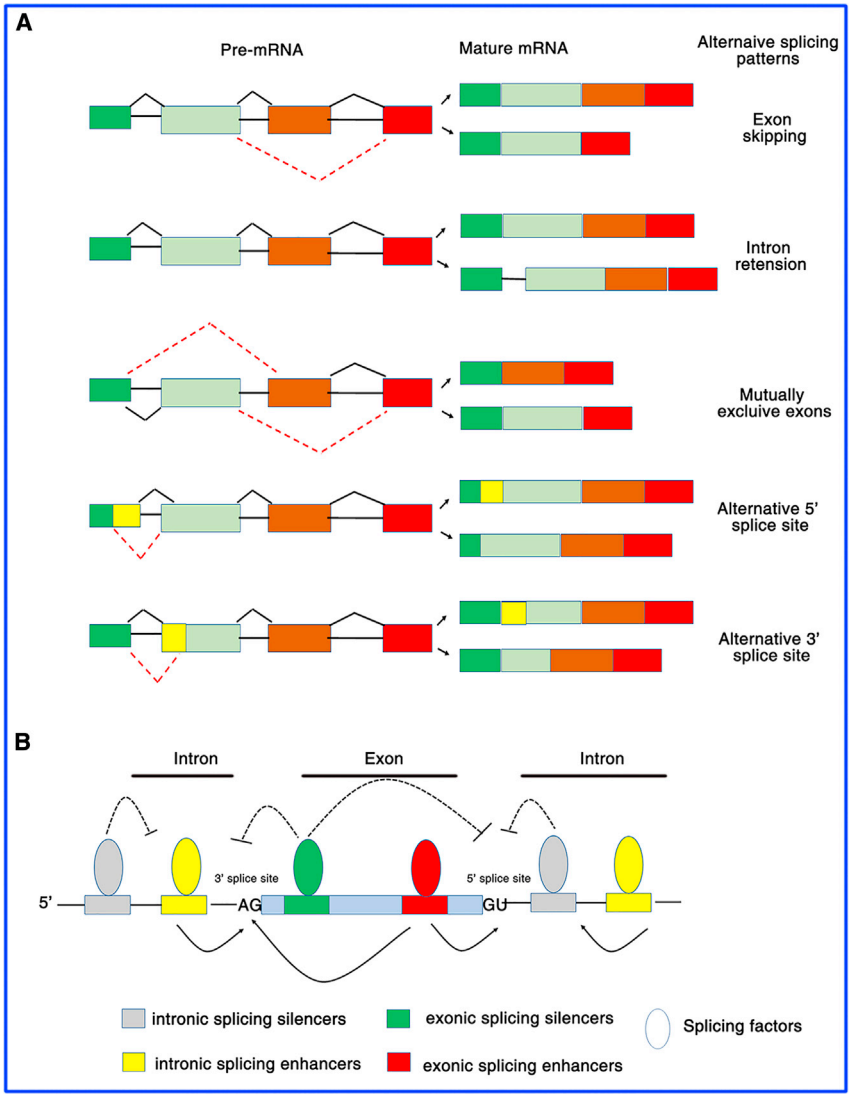


**Figure 1. Regulation of alternative pre-mRNA splicing in tumor**

(A) Schematic diagram of five examples of alternative splicing modes is exon skipping, intron retention, mutually exclusive exons, alternative 5'splice site, and alternative 3'splice site. Shown on the right is the mature mRNA transcript from each alternative splicing event. (B) The complex interaction of *cis*-acting elements and *trans*-factors in alternative splicing regulation. RNA-binding proteins, serine/arginine-rich proteins, and heterogeneous ribonucleoproteins bind to exon or intron *cis*-regulatory elements to promote or prevent splicing factor recognition of the splice site.

splicing of FGFR2, promoting the skipping of exon 8 from the mature FGFR2 mRNA transcript. The exclusion of the FGFR2 exon 8 depends on the phosphorylation of IWS1 by Akt3 and Akt1 at Ser720/Thr721, which is required for the recruitment of SETD2 to the RNA Pol II complex. This results in the trimethylation of histone H3 at K36 in the body of the transcribed FGFR2 gene, triggering the skipping of exon 8 from the mature transcript.[49] Recently, also it is shown that AKT3-mediated IWS1 phosphorylation promotes the proliferation of EGFR-mutant lung adenocarcinomas through cell cycle-regulated U2AF2 RNA splicing.[50] In addition to the PIK3/AKT pathway, roles of the Wnt signaling, nuclear factor (NF)-κB pathway, and Hippo signaling pathway in the regulation of alternative splicing of several cancer-related genes have also been established.[51–53] Interestingly, Xu et al. recently found that estrogen receptor α (ERα) is a potent non-canonical RBP. They found that ERα RNA binding function is uncoupled from its activity to bind DNA and critical for breast cancer progression. Mechanistically, ERα controls different steps of RNA metabolism. In particular, they demonstrated that ERα RNA binding mediates alternative splicing of XBP1 and translation of the eIF4G2 and MCL1 mRNAs, which facilitates survival in stress conditions and sustains tamoxifen resistance of cancer cells.[54]

RNA secondary structure is emerging as an important layer in splicing regulation. Huang et al. found that the I-8 element contains an RNA G-quadruplex structure. The RNA sequence with the capacity of forming the secondary structure of G-quadruplex itself, rather than the linear G tract, promotes alternative splicing and the production of the epithelial-specific CD44v isoform. They further demonstrated that hnRNP-F regulates G-quadruplex-associated alternative splicing. Depletion of hnRNP-F induces EMT-associated CD44 splice isoform switch and promotes the EMT phenotype, a critical process that drives tumor metastasis.[55] Recently, Fish et al. reported a systematic effort to decipher the RNA structural code that shapes pathological splicing during breast cancer metastasis. They found a previously unknown structural splicing enhancer that is enriched near cassette exons with increased inclusion in highly metastatic cells and showed that the spliceosomal protein SNRPA1interacts with these enhancers to promote cassette exon inclusion. This interaction enhances metastatic lung colonization and cancer cell invasion, in part through SNRPA1-mediated regulation of PLEC alternative splicing, which can be counteracted by splicing modulating morpholinos.[56]

## DYSREGULATION OF ALTERNATIVE SPLICING IN CANCER

Splicing abnormalities caused by splicing *cis*-element mutations or splicing factor mutations and expression dysregulation are widespread in cancer, and significantly promote the progression of

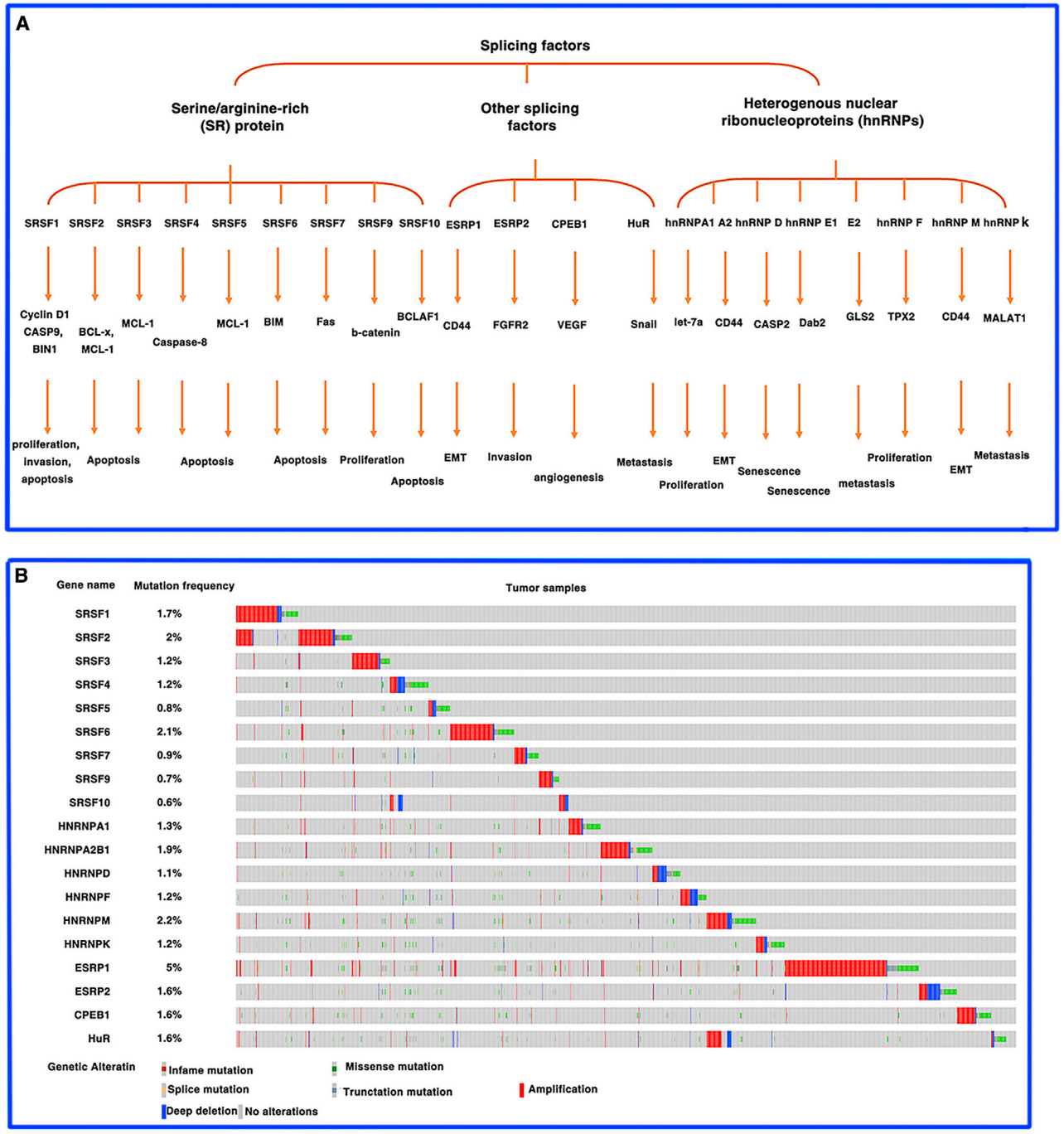


**Figure 2. Dysregulation of alternative splicing in cancer**

(A) Roles of splicing factors in cancer. Simply, some well-known splicing regulatory proteins are involved in abnormally regulating the expression of tumor-related isoforms of the cancer hallmarks due to their expression dysregulations or mutations. (B) The TCGA database shows that the well-known splicing factor changes have been detected in most human tumors. In several patient cohorts, genetic alterations (including expression changes and repeated somatic mutations of splicing factors) were detected in more than 2% of tumors. Splicing factor amplification is depicted in red, deep deletion in blue, inframe mutation in purple, splice mutation in yellow, and somatic mutations in green.

cancer[57,58] (Figure 2). Increasing evidence shows that cancer-related splicing abnormalities affect almost all aspects of cancer biology.[59] Cancer cells selectively express potentially oncogenic splicing isoforms of affected genes to gain a growth advantage. Therefore, splicing dysregulation is a molecular marker of cancer and has an oncogenic effect in some cancers.[60,61]

### Abnormal expression of splicing factors in cancer

In addition to genome mutations, changes in the copy number or expression levels (up-regulation or down-regulation) of splicing factors can also interfere with the cancer pathogenesis. In fact, the abnormal expression of some splicing factors has been frequently reported to be closely related to the development and progression of cancer.[62,63] A variety of splicing factors deregulated in cancers have been shown to exhibit oncogenic or tumor suppressive functions, which can be categorized into SR proteins, hnRNP proteins, and other splicing factors.[64,65] A recent analysis of the expression levels of 1,348 RBPs found that extensive changes in RNA-binding protein expression are related to various alternative splicing changes in cancer driving and oncogenic pathways.[66] One of the best characterized is SRSF1, an oncogenic SR protein involved in both constitutive and alternative splicing. It is up-regulated in a variety of types of tumors, including colon, breast, glioblastoma, and lung.[61] Moreover, overexpression of SRSF1 induced an increase in the expression levels of oncogenic isoforms of RON, MNK2, and S6K1, and anti-apoptotic protein isoforms Bcl-xL and MCL-1L, and a decrease of tumor suppressor protein isoform of BIN1.[67–69] Anczukow et al. identified the targets of SRSF1 in human breast tumors by using RNA sequencing. De novo discovery of the binding motif and construction of regulatory maps provided insights into SRSF1 regulatory mechanisms and uncovered oncogenic splicing events representing potential therapeutic targets.[70] In addition, they also found that SRSF1 overexpression promoted alternative splicing of BIM and BIN1 to produce isoforms that lack pro-apoptotic functions and contribute to the phenotype. Finally, SRSF1 cooperated specifically with MYC to transform mammary epithelial cells, in part by potentiating eIF4E activation, and these cooperating oncogenes are significantly co-expressed in human breast tumors.[65] SRSF3 and SRSF6 are also overexpressed in certain cancer types and are considered to be oncoproteins.[71,72] The importance of hnRNPs in cancer is reflected in the fact that these proteins are often associated with the prognosis of cancer patients.[73] For example, hnRNP K is considered to be a tumor suppressor, and its expression is reduced in hematological malignancies and is related to prognosis.[74] Recently, hnRNP A2 has been found to be related to enhancing the expression of anti-apoptotic proteins BIN1 and CASP9 and reducing the expression of pro-apoptotic proteins Bcl-xS.[67] In addition, other splicing factors, such as PTBP1, whose increased expression can induce the expression of tumor-associated splicing isoforms, including RAC1, NUMB, and PKM.[75] RBM4 has also been shown to function as a tumor suppressor in cancers by suppressing the expression of anti-apoptotic splice isoform of BCL-X.[76]

### Mutations of splicing factors in cancer

Somatic mutations that affect the components of the early spliceosome complex are often described in cancer, especially in hematological malignancies.[33] Among the splicing factors most affected by these mutations are SF3B1, SRSF2, U2AF1, and ZRSR2.[77] SF3B1 is a subunit of U2 snRNP, which can recognize the branchpoint site and is the most common mutant of splicing regulator in many cancers. The mutation frequency in breast cancer can reach about 81%.[78] Cancer-related SF3B1 mutations are located in HEAT domains, which are involved in the protein-protein interactions and clustered in hotspot mutations, namely E622, R625, K700, H662, and K666. Specifically, it was recently demonstrated that hotspot mutations affect residues of SF3B1 that are in close proximity to the polypyrimidine tract within the intron, and consequently connected to abnormal branchpoint site usage.[79–81] SRSF2 is a member of the SR protein family that promotes splicing by binding to the ESE sequences through its RNA recognition motif.[82] SRSF2 mutations have been found in hematological malignancies, particularly in patients with chronic myelomonocytic leukemia (CMML, 28%–47%).[58] SRSF2 mutations are concentrated on P95 residues, leading to changes in RNA-binding preference, which is conducive to the recognition of C-rich CCNGs rather than G-rich ESEs, and leads to changes in the splicing of hundreds of mRNAs.[83,84] Moreover, through analyses of transcriptomes from 982 patients with acute myeloid leukemia, Yoshimi et al. identified frequent overlap of mutations in IDH2 and SRSF2 that together promote leukemogenesis through coordinated effects on the epigenome and RNA splicing. While mutations in either IDH2 or SRSF2 imparted distinct splicing events, co-expression of mutant IDH2 altered the splicing effects of mutant SRSF2 and resulted in more profound splicing changes than either mutation alone.[85] U2AF1, required for recognition of the AG dinucleotide-dependent 3′ splicing site recognized by the main spliceosome, hotspot mutations were reported to occur at residue S34 or Q157 located in the zinc finger domains in myeloid malignancies, thus affecting the recognition of the splice sites.[86,87] ZRSR2 is also a member of SR protein family and affects the 3′ splicing sites through U12. Compared with SF3B1, U2AF2, and SRSF2, ZRSR2 mutations spread throughout the entire gene, often leading to protein truncation, indicating that this is a pathogenesis caused by loss of function.[88]

### Mutations of splicing regulatory *cis*-elements in cancer

Mutations in splicing regulatory *cis*-elements of tumor-associated genes can result in splicing abnormalities that promote tumor progression. Recently, a global exome analysis of genes with splice site mutations in more than 8,000 tumor samples identified that more than 1,000 genes were affected by mutations that may produce new alternative splice sites.[89] High-throughput sequencing has been used to analyze the frequency of genomic mutations in different types of tumors. The search for single nucleotide variants revealed 1.5% mutations in splice sites.[90] For example, the mutation from G to A in exon 10 of NEIL1 can cause the shortening of mature mRNAs, and then through the translation produces abnormal protein molecules that do not have DNA repair functions, thereby promoting the occurrence of gastric cancer.[91] Tao et al. discovered a new alternative splicing site on the MYH-IVS10-2 A'G (c.892-2 A→G) heterozygous gene in two Japanese family gastric cancer patients. This form of alternative splicing can produce abnormal mRNA transcripts and translate a shortened MYH protein, which has impaired DNA repair functions.[92] In addition, this mutation site in the CDH1 gene was also identified in a 52-year-old patient with signet ring cell carcinoma. This site can cause changes in traditional splicing sites, resulting in three different CDH1 transcripts.[93]

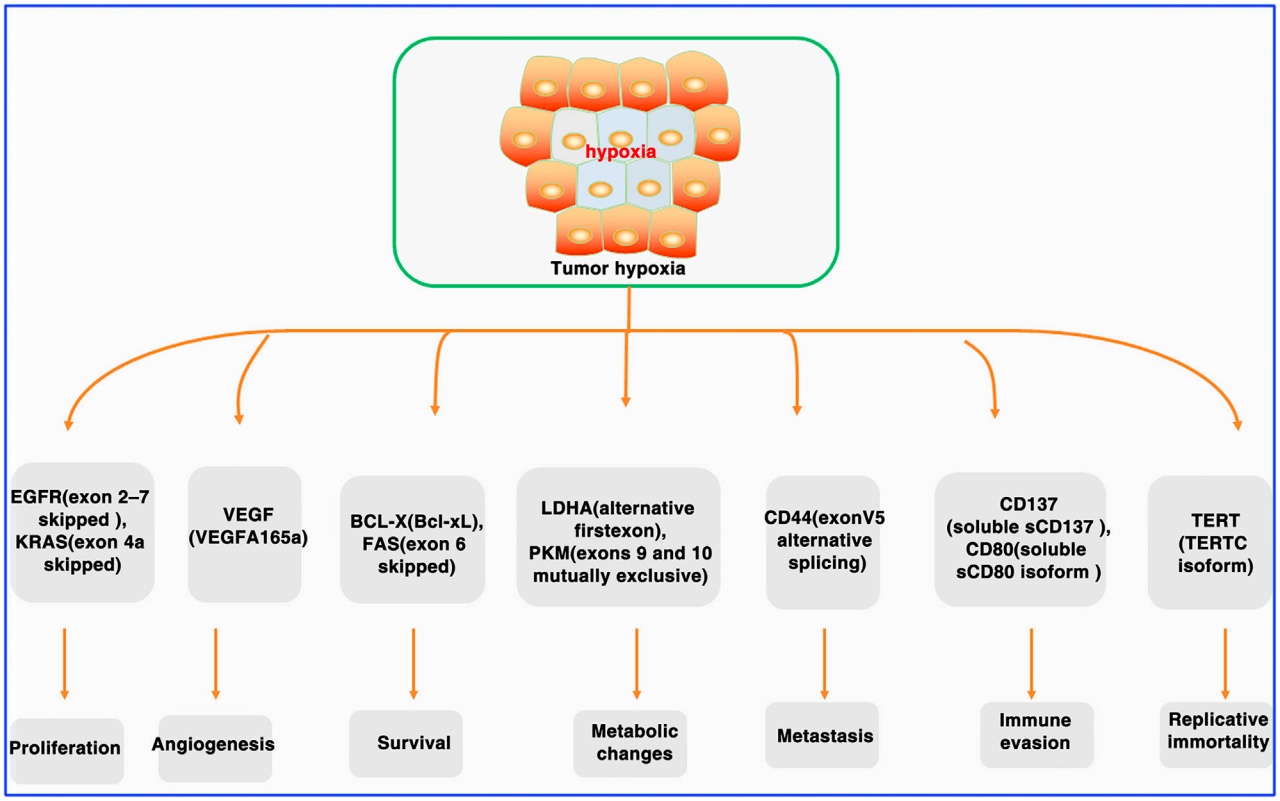


**Figure 3. Schematic of tumor hypoxia-induced splicing events in several hallmarks of cancer**

Schematic representations of the many ways that tumor hypoxia-induced alternative splicing promotes proliferation, angiogenesis, apoptosis avoidance, metabolic changes, metastasis, immune evasion, and chromosomal instability.

## ALTERNATIVE SPLICING AND TUMOR METABOLISM

Tumor cells have a unique metabolic system, which is an important hallmark that distinguishes tumor cells from normal cells. Most normal tissues can generate sufficient energy supplies through the tricarboxylic acid cycle and oxidative phosphorylation under aerobic conditions. Fast-proliferating cells, such as tumor cells, use a different metabolic method from the former, that is, when oxygen is abundant, a large amount of glucose is converted into lactic acid for energy supply. This phenomenon is called the Warburg effect or aerobic glycolysis.[94] Although this phenomenon has a very low efficiency in generating energy, glycolytic metabolites will participate in the synthesis of molecules that are important for cell proliferation, thereby promoting tumor growth.[95]

During tumor progression, tumor cells frequently experience hypoxia, due to overgrowth. Hypoxia is a common feature of most solid tumors and plays an important role in tumor metabolism regulation, invasion, metastasis, and other aspects.[96] The response to hypoxia includes a series of adaptive mechanisms that promote cell survival. Hypoxia alters metabolism and is a key component of maintaining the type of glycolytic metabolism.[9] There is abundant evidence showing that hypoxia-induced alternative splicing events are a potent driving force in tumors[18] (Figure 3). Hypoxia-induced alternative splicing is essential for the adaptation of the tumor cell microenvironment. It is one of the most important functions of tumor hypoxia responses.[97] The vascular endothelial growth factors (VEGFs), the key regulators of angiogenesis, are primarily regulated by hypoxia and are direct targets of HIF-1α and HIF-2α. Tumor hypoxia is also known to increase the expression of pro-angiogenic VEGF isoforms. However, the anti-angiogenic VEGF isoform formation is promoted preferentially under normoxia.[98–100] In addition, the hypoxia-induced alternative pre-mRNA splicing affects tumor metabolic reprogramming under anaerobic conditions, which initiates with HIF1α stabilization and promotion of PKM2 splice isoform expression, at the expense of the oxidative phosphorylation-promoting PKM1 isoform, leading to a metabolic shift to glycolysis.[18] Lactate dehydrogenase (LDH/LDHA) is an HIF-target gene, and its overexpression is associated with poor prognosis in several cancers. In cancer cells, hypoxia promotes alternative LDHA-001splicing and reduces LDHA-201 isoform expression, resulting in the loss of LDHA-201 expression through nonsense-mediated mRNA decay.[101] The effects of hypoxia on general splicing mechanisms include the imbalance of splicing factor expression of hnRNPA1, hnRNP M, SRSF1, SRSF3, SAM68, Hur, PRPF40b, and RBM4, and the activation and increased expression of SR splicing factor kinases CLK1 and SRPK1, which promote

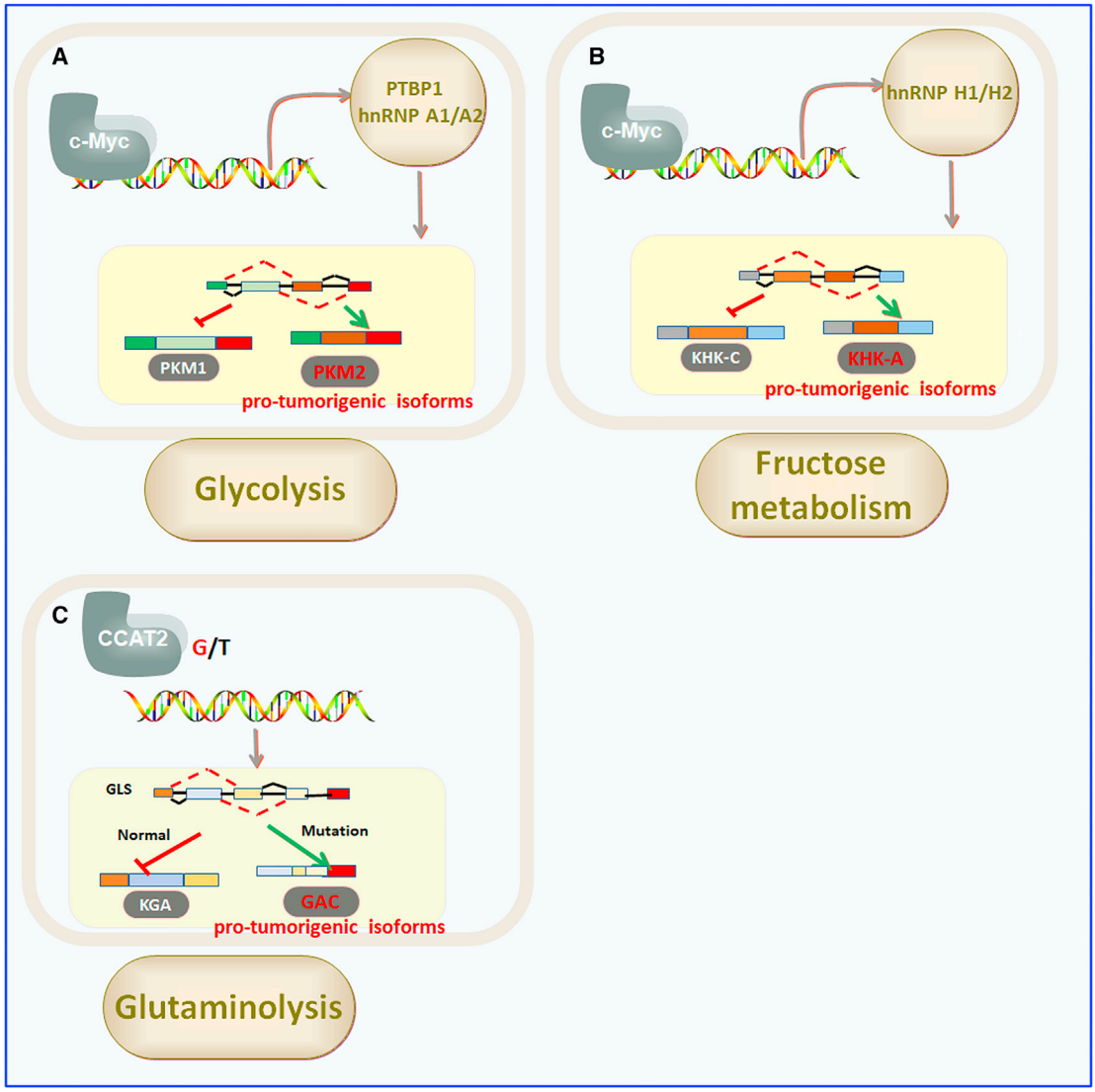


**Figure 4. Dysregulation of alternative splicing events in cancer metabolism**

(A) c-Myc-mediated transcription of PTBP1 and hnRNP A1/A2 promotes the formation of the pro-tumorigenic isoform PKM2, which contains exon 10 and excludes exon 9. (B) Mutually exclusive exons define the formation of KHK-A or KHK-C. KHK-A has tumorigenic activity, and its formation is promoted by the expression of hnRNP H1 and hnRNP H2 mediated by c-Myc. (C) The regulation of GLS alternative splicing is complex, involving the activity of LncRNA CCAT2, which binds to other proteins in an allele-specific manner. Alleles containing SNP G promote the formation of a shorter isoform (lacking exons 16–18), while alleles containing T promote the formation of a longer isoform.

hyperphosphorylation and activity of SR splicing factors, change intracellular localization of splicing factors, and the ability to interact with other proteins and pre-mRNAs, leading to transcription of hypoxic adaptive genes and promoting tumor progression.[76,102–105] Recently, Han et al. found a large number of alternative splicing events, including intron retention, exon skipping, and alternative first exon usage that was regulated by acute and chronic hypoxia where intron retention was the most dominant type of hypoxia-induced alternative splicing. Many of these genes are involved in metabolism, cancer cell proliferation, migration, and invasion, suggesting they may modulate or be involved in additional features of tumorigenic development that extend beyond the known functions of canonical full-length transcripts.[101] There is increasing evidence that cancer-related alternative splicing helps promote tumor growth and proliferation, partly because of the effects of metabolic reprogramming. Herein, we discuss the role of splicing in regulating cancer metabolism and put forward evidence to support the view that in many types of cancers, alternative splicing acts as a molecular switch to change metabolism and drive tumorigenesis (Figure 4).

### Alternative splicing regulates glucose metabolism

Compared with normal cells, tumor cells preferentially metabolize glucose through aerobic glycolysis, and some of the genes encoding enzymes are prone to alternative splicing. Glycolysis consists of a series of 10 enzyme-catalyzed reactions, three of which are irreversible and are called commitment steps.[13] Pyruvate kinase catalyzes the last step of glycolysis and converts phosphoenolpyruvate into pyruvate, which is a key determinant of how glucose is used in cancerous and differentiated cells. Pyruvate kinase muscle isozyme PKM produces PKM1 and PKM2 splice isoforms using mutually exclusive exon 9 and exon 10, respectively. PKM1 is expressed in most normal cells and promotes oxidative phosphorylation, while PKM2 is up-regulated in tumor cells and promotes aerobic glycolysis.[106] Christofk et al. proved that the replacement of PKM2 in tumor cells with PKM1 can significantly reduce the production of lactic acid and the size of the tumor, indicating that the presence of PKM1 or PKM2 in tumor cells can directly affect the metabolic phenotype of the tumors.[16] By regulating the expression of PKM genes, inhibiting the expression of PKM2 and promoting the production of PKM1 is of great significance for inhibiting tumor progression. Studies have shown that the alternative splicing of PKM is determined by splicing regulators hnRNPA1, hnRNPA2, PTB, and SRSF3, etc. It suggests that splicing factors are involved in the role of alternative splicing in tumor metabolism.[107]

### Alternative splicing regulates glutamine metabolism

In addition to aerobic glycolysis, enhanced glutamine metabolism is another important feature of cancer cells.[14] Glutamine is an important precursor of biosynthetic reactions, supplementing the carbon sources of the tricarboxylic acid cycle, and can produce glutathione to regulate redox homeostasis.[6,108–110] Glutaminase metabolizes glutamine into glutamate in the mitochondria, which is hydrolyzed to α-ketoglutarate (α-KG), an important anaplerotic source for the tricarboxylic acid cycle in fast-growing cells.[111] Glutaminase exists in two alternatively spliced variants: glutaminase C, a 598-amino acid long protein that lacks the last 16 to 18 exons, and kidney-type glutaminase.[112,113] Compared with normal tissues, glutaminase C isoform is found to be dominant in adenoma, glioma, colorectal cancer, and breast tumors. Specific inhibition of glutaminase C isoform with small molecule inhibitors can inhibit tumor progression.[114–116] A mechanism of the priority expression of glutaminase C isoform and its regulation was elusive until recently. Previous studies reported that the kidney-type glutaminase isoform contains miR-23a/b target sites in rat cells. In addition, although c-Myc transcriptional inhibition of miR-23a/b leads to the de-suppression of kidney-type glutaminase isoform, a large-scale analysis of clinical samples from the TCGA dataset found that tumors often switch the use of 3′UTR from kidney-type glutaminase isoform to glutaminase C isoform, thus avoiding the need to inhibit miR-23a/b.[117,118] Recently, Masamha et al. reported that one of the main targets of CFIm25 is glutaminase. After CFIm25 was down-regulated, glutaminase underwent splicing alternative polyadenylation changes, and kidney-type glutaminase isoform expression was significantly increased. Interestingly, CFIm25 knockdown also affects alternative splicing, and glutaminase C isoform exon inclusion is inhibited in cells that normally use glutaminase C isoform.[119]

### Alternative splicing regulates fructose metabolism

In order to meet the needs of their own growth, tumor cells will use all available nutrients. Fructose, as the isomer of glucose, is a better carbon source. Glycerol-3-phosphate and glyceraldehyde-3-phosphate, which are metabolized by fructose in tumor cells, can provide a material basis for tumor cells to synthesize phospholipids and triglycerides. In addition, the intermediate products of fructose metabolism can enter the non-oxidative pentose phosphate pathway, which provides ribose 5-phosphate for the synthesis of ribose in tumor cells and produces a large amount of NAPDH.[120] Fructose metabolism begins with its phosphorylation by the enzyme ketohexokinase (KHK), which exists in two alternatively spliced variants, KHK-A and KHK-C.[121] KHK-C is tissue-specific and is predominantly expressed in liver, intestine, and kidney,[122] while the KHK isoform has been linked to the development of disorders, such as cancer.[123] KHK-C and KHK-A were produced by specific excision of adjacent exons 3A and 3C, respectively. The expression of these two isoforms is tissue-specific, but usually mutually exclusive. Some studies have emphasized the importance of KHK-C expression in the progression of diabetes, liver diseases, or hypertension.[124–126] Recently, it was found that KHK-A, as a protein kinase, directly phosphorylates phosphoribosyl pyrophosphate synthetase 1 from in the de novo nucleic acid synthesis pathway in hepatocellular carcinoma cells.[127] Li et al. identified hnRNP H1 and hnRNP H2 as the major splicing proteins binding to the splicing exons and adjacent introns of KHK. Mutagenesis of the binding motif and minigene reported assay further identified their role in regulating this splicing event.[127]

### Alternative splicing regulates fatty acid metabolism

Compared with aerobic glycolysis, the increase of lipid fatty acid metabolism has received less attention, but recently it has been considered as another important metabolic abnormality that is necessary for tumor progression.[128] Fatty acids are a kind of carboxylic acid with a long aliphatic chain. It usually appears as an even number of carbons, which can be saturated or unsaturated. Fatty acids are essential for energy storage, membrane synthesis, and signal molecule production.[129] Fatty acids need to be converted to acyl coenzyme A for subsequent metabolism, including anabolism or catabolism. Acyl coenzyme A synthetase located on the endoplasmic reticulum and mitochondrial outer membrane, catalyzes the conversion of fatty acids in the presence of ATP, coenzyme A, and Mg2.[130] Long-chain acyl-CoA synthetase (ACSL) family members include five different ACSL isoforms, each encoded by a separate gene and have multiple spliced variants.[131] ACSL4 is a member of the ACSL family. Its expression in liver cancer tissues is significantly higher than that in normal tissues, and is involved in tumorigenesis by regulating cAMP and p38 MAPK pathways.[132] In addition, ACSl4 is also highly expressed in breast cancer cells, which promotes tumor growth *in vivo* and *in vitro*.[133]

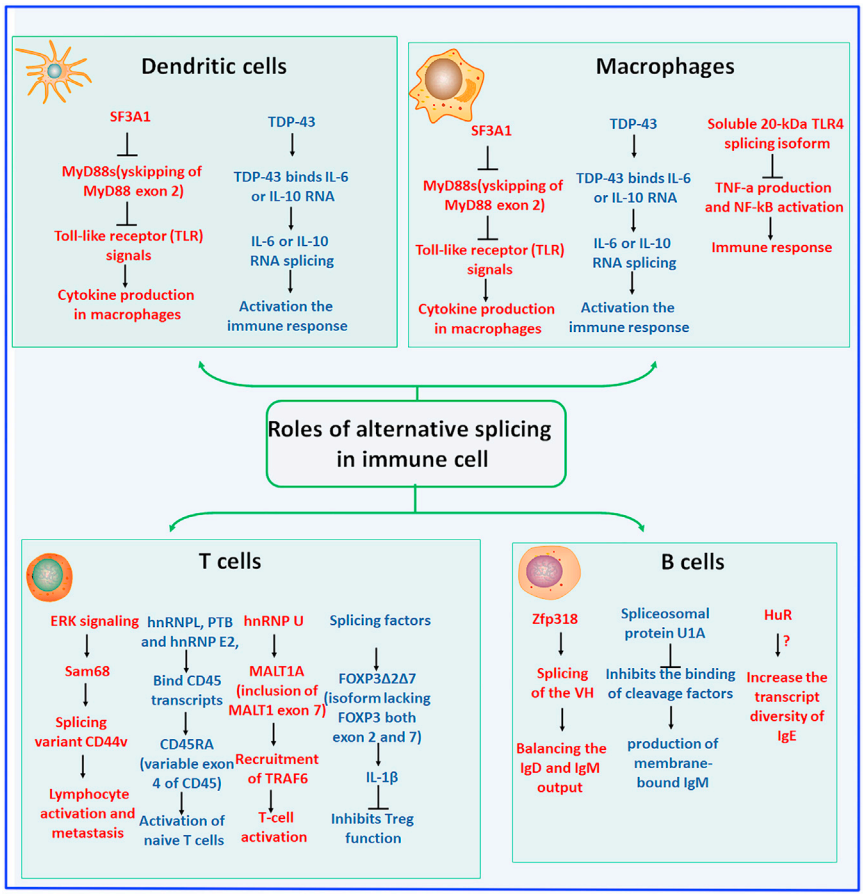


**Figure 5. Roles of abnormal alternative splicing events in immune cells**

Schematic shows abnormal alternative splicing isoforms in dendritic cells, macrophages, B cells and T cells. In addition, we describe the mechanism of alternative splicing isoforms in important genes for immune cell differentiation and function, such as MyD88/interleukin (IL)-6/IL-10 in dendritic cells, MyD88/IL-6/IL-10/TLR4 in macrophages, CD45/CD44/FOXP3/MALT1 in T cells or IgM/IgD/IgE in B cells.

## ALTERNATIVE SPLICING AND TUMOR IMMUNE RESPONSE

Tumor cells can modify their surface antigens to change the microenvironment of tumor tissues; avoid the surveillance, recognition, and attack of the immune system, and continue to grow and proliferate. A positive correlation has been found between tumor mutation loads and response to immune checkpoint blockade. Cancer neoantigens derived from somatic mutations activate an adaptive immune response to kill cancer cells. Similarly, tumor-associated splicing events with neoantigen-generating capacities have been proposed as predictors for the response of immunotherapy.[64] Increasing clinical studies have revealed remarkable outcomes both for TCR-T cell therapy targeting cancer germline antigens and for neoantigen-based vaccines.[134] Coding mutation-derived neoantigens are best studied, but neoantigens can also arise from other processes, such as altered splicing events. Some studies analyzing the high-throughput sequencing data concluded that tumor-specific alternative splicing is abundant and may generate neoepitopes that contribute to the epitope repertoire.[89,135] Increased studies have shown that splicing disorders directly affect genes that play a key role in the immune pathway, thereby affecting the effectiveness of cancer immunotherapy[24,136,137] (Figure 5). Identifying the role of different splice isoforms in specific cancers and the regulatory role of splicing factors is of great significance for revealing the mechanisms of tumor evasion of immune response and the immune mechanisms of tumors.

### Alternative splicing in macrophages

The ImmGen Consortium found through high-throughput sequencing that alternative splicing is ubiquitous. About 60% of genes show different alternative splicing isoforms in T or B cells, of which about 70% of alternative splicing events are related to lineage differentiation, and more than 7,000 previously unreported subtypes can be identified.[138] A prominent example of alternative splicing in innate immune signal transduction is MyD88. One of its subtypes, MyD88s, has been shown to regulate the production of inflammatory factors in macrophages, thus limiting the innate immune activation downstream of TLR signaling.[139] The regulation of the splicing factors Eftud2 on MyD88s may indicate the link between alternative splicing and innate immune response.[140] In addition, in the TLR4 signaling pathway, the splicing factors SF3A1, SF3A2, SF3A3, or SF3B1 are essential for the generation of the long subtype of MyD88, because their knockout will lead to an increase in the short subtype, thereby inhibiting the MyD88 signaling pathway.[139] In addition, for TLR4, there is a soluble isoform, that is, a 144-base pair insertion between exons 2 and 3, which results in a premature stop codon, and this subtype can be induced in an inflammatory response. This isoform is functionally different from the membrane-bound isoform because it inhibits the production of tumor necrosis factor-α and NF-κB signals in the macrophage cell line, and therefore serves as a negative feedback mechanism to limit excessive inflammation response.[141]

### Alternative splicing in dendritic cells

The maturation of dendritic cells is a key step in inducing adaptive immune responses. Jia et al. previously found that the expression of the splicing factor hnRNP C was significantly increased after LPS stimulation. hnRNP C knockout or overexpression down-regulates or up-regulates the expression of NF-κB p65 and its downstream targets CD80 and CD40. These results indicate that the splicing factor hnRNP C regulates the maturation of dendritic cells by affecting the expression of p65, CD80, and CD40.[142] Geng et al. found that

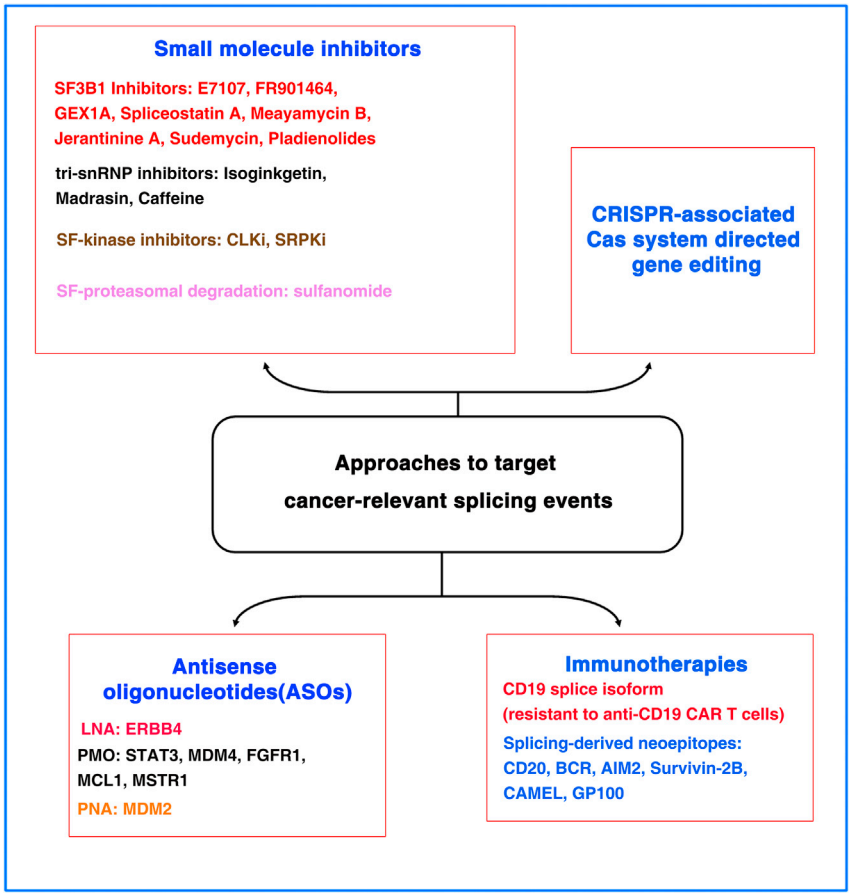


**Figure 6. Methods for therapeutic modulation of abnormal alternative splicing events in tumor**

Several representative approaches are depicted in the simplified splicing regulation diagram, including small molecules targeting the spliceosome compound, ASOs specifically designed to block the binding of splicing factors to the pre-mRNA molecule, CRISPR-associated (Cas) system bound to the vicinity of abnormal genomic sequences, as well as antibodies or CAR-T cells against tumor-specific neo-antigen due to alternative splicing. CAR, chimeric antigen receptor; LNA, locked nucleic acid; PMO, phosphorodiamidate morpholino oligomer; PNA, peptide nucleic acid.

the specific deletion of splicing factor Ptbp1 in DCs can increase the expression of major histocompatibility complex (MHC) II. Functionally, deletion of Ptbp1 in dendritic cells can enhance antitumor immunity by regulating Pkm alternative splicing. These findings reveal the role of splicing factor PTBP1 in dendritic cells, suggesting that PTBP1 may become a new target for antitumor therapy.[143]

### Alternative splicing in B cells

Alternative splicing can also increase the diversity of immunoglobulin (Ig)E transcripts in B cells. IgE exists in various subtypes, including secreted and membrane-bound.[144,145] Studies have shown that the generation of different IgE subtypes depends on different stimuli and follows developmental characteristics,[146] highlighting the differentiation-related regulation of splicing variant expression.[147] An example of an effective regulator for these alternative splicing events in B cells is the splicing protein HuR.[148,149] The loss of HuR leads to defective class-switching and B cell death. It also emphasizes the importance of tightly regulated splicing mechanisms in the differentiation and activation of B cells.

### Alternative splicing in T cells

There are also some examples to illustrate the role of alternative splicing in T cell regulation and differentiation. The expression pattern of CD45 isoforms is different at different stages of lymphocyte development and is used as a marker between naive (CD45RA+ or CD45RB+) and memory (CD45RO+) T cells.[150] The CD45 gene contains 34 exons, of which exons 4, 5, and 6 can be alternatively spliced.[151] Minigene and RNA immunoprecipitation experiments showed that splicing factor hnRNP LL is related to the exclusion of exons 4 and 6 in CD45.[152] hnRNP LL is tissue-specific and is preferentially expressed in activated T lymphocytes.[152] Its high expression promotes CD45 splicing, resulting in a decrease in high molecular weight subtypes and an increase in low molecular weight subtypes. These indicate that the transformation of the CD45 splicing isoform is critical to the development and function of T lymphocytes. In addition, MALT1 exhibits lymphocyte activation function by connecting TCR/CD28 with downstream signaling pathways.[153] There are two splice isoforms of MALT1, MALT1A and MALT1B. MALT1A containing exon 7 can promote the recruitment of TRAF6 and enhance the function of the MALT1 scaffold, but it cannot enhance the activity of protease.[30] Naive CD4+ T cells mainly express MALT1B, and activated CD4+ T cells mainly express MALT1A. Down-regulation of splicing protein hnRNP U promotes the expression of MALT1A and T cell activation.[30]

## ALTERNATIVE SPLICING AND CANCER THERAPY

The existence of tumor-specific splicing isoforms drives or promotes tumor growth, making them potential therapeutic targets. The diversity of alternative splicing regulation changes suggests that multiple strategies can be used to target abnormal splicing programs in cancer (Figure 6). Thus far, there are many ways to interfere with RNA splicing, including directly targeting RNA splicing factors, blocking kinases that regulate splicing factors, and using oligonucleotides that can regulate RNA splicing factors, etc.[154] Herein, we highlight the key advances in the development of treatments for oncogenic alternative splicing (Table 1).

**Table 1. The application of alternative splicing in cancer therapy**

| Tumor type | Splicing-based treatment strategy | Drug names | Inhibition target | References |
|---|---|---|---|---|
| Colorectal, esophageal | Targeting the core spliceosome | E7107 | SF3b | [155] |
| Colorectal | Targeting the core spliceosome | FR901464 | SF3b | [156] |
| Melanoma | Targeting the core spliceosome | GEX1 | SF3b | [157] |
| Chronic lymphocytic leukemia | Targeting the core spliceosome | Spliceostatin A | SF3b | [158] |
| Head and neck | Targeting the core spliceosome | Meayamycin B | SF3b | [159] |
| Breast | Targeting the core spliceosome | Isoginkgetin | U4/U6-U5 tri-snRNP | [160] |
| Not determined | Targeting the core spliceosome | Ubistatin A | U4/U6-U5 tri-snRNP | [161] |
| Lung | Targeting regulatory splicing factors | NB-506 | SF2/ASF | [162] |
| Hepatocellular | Targeting regulatory splicing factors | GSK3326595 | PRMT5 | [163] |
| Lung | Targeting regulatory splicing factors | JNJ-64619178 | PRMT5 | [164] |
| 12 tumor types | Targeting regulatory splicing factors | GSK3368715 | PRMT1 | [165] |
| Melanoma | Targeting regulatory splicing factors | SRPIN340 | SRPK1 and SRPK2 | [166] |
| Prostate | Targeting regulatory splicing factors | Indisulam | RBM39 | [167] |
| Breast | Splicing isoform-specific targeting | Oligonucleotides | BRCA1 | [168] |
| Pancreatic | Splicing isoform-specific targeting | Oligonucleotides | PKM | [169] |
| Glioma | Splicing isoform-specific targeting | Oligonucleotides | Bcl | [170] |
| Melanoma | Splicing isoform-specific targeting | Oligonucleotides | MDM4 | [171] |

### Small molecules against splicing modulators

The use of small molecule inhibitors to target mutations or abnormally expressed splicing factors is the first and one of the best researched methods of splicing-based therapy. The earliest splicing regulators is FR901464 and its acetylated derivative spliceostatin A (SSA). SSA is a natural product extracted from *Pseudomonas* spp. It was first described in human and mouse tumors in several xenograft models with antitumor effects.[172–174] Mechanistically, these compounds non-covalently bind to SF3B1, disrupting the interaction between the SF3B/U2AF1/U2-snRNP complex and the pre-mRNA, leading to disordered pre-mRNA splicing.[175,176] The first splicing modulator to enter phase 1 clinical trials, pladienolide B synthetic E7107, was tested in solid tumors where spliceosome mutations are fairly uncommon and minimal clinical activity was observed.[155,177] In addition, H3B-8800 is a silosadienolide derivative, which has been shown to have a stronger inhibitory effect on cancer cells with mutations in SF3B1, SRSF2, U2AF1, and U2AF1 genes.[178] This drug candidate is currently in phase I clinical trials [NCT02841540]. Other new small molecules targeting different stages of the splice pathway have been discovered. For example, N-palmitoyl-l-leucine can block the later stages of spliceosome assembly, Cp028 can inhibit the middle stage of the spliceosome activation process, and jerantine An up-regulates the SF3b protein in breast cancer cells, resulting in an increase in unspliced pre-mRNA.[179–181] A recent study showed that by reducing the expression of pro-angiogenic VEGF isoforms, the compound SRPIN340 inhibits SRPK1 (which phosphorylates splicing factors SRSF1) and significantly reduces tumor growth in metastatic melanoma.[166] Another promising splicing-related therapeutic target is PRMT5, an arginine methyltransferase that methylates U2 snRNP's Sm protein.[64] Pharmacological inhibition of the PRMT5 has exhibited antitumor potency associated with splicing defects in many cancer types.[182] Tumor cells are shown to be sensitive to PRMT5 inhibition, in part due to the general inhibition of splicing.[64] Several PRMT5 inhibitors are currently in clinical trials for patients with relapsed/refractory solid tumors. These include compounds GSK3326595, PF06939999, and JNJ-64619178.[183] These findings indicate that small molecules, which are general inhibitors of splicing, may provide a new way to develop new anticancer drugs.

### Antisense oligonucleotides for alternative splicing in cancer therapy

RNA-targeted therapy appeared in 1978, when Zamecnik and Stephenson first described this therapy, a chemically modified oligonucleotide that can inhibit Rous sarcoma virus gene expression and virus replication.[184,185] As a result, antisense oligonucleotides (ASOs) have been extensively explored in the process of drug development, and have proven to be a useful alternative to target specificity in the treatment of splicing-related cancers. ASOs are synthetic molecules composed of short single-stranded nucleic acid sequences, generally about 20 nucleotides, which are combined with complementary pre-mRNA sequences through base pairs.[186] Targeted RNA therapy has been applied to the clinic, and many clinical trials of ASOs are under way.[187–189] Previous studies reported that nusinersen, an antisense oligonucleotide for intron silencing in SMN2, enhances the inclusion of exon 7 in SMN2 mRNA, and restores functional SMN protein levels in patients with spinal muscle atrophy with SMN1 inactivating mutations.[190,191] Preclinical studies have shown that ASOs may also have therapeutic values in oncology.[192] By using ASOs to target oncogenic splice variants or key oncogenes, promising

preclinical results have also been achieved. For example, it is reported that ASO targeting MDM4 with exon 6 skipping can reduce the expression of MDM4, thereby inhibiting the growth of melanoma and diffuse large B cell lymphoma, and improving the sensitivity to MAPK targeted therapy.[171] Bcl-x ASO was designed to induce splicing switches, which is conducive to the production of pro-apoptotic Bcl-xS isoforms. *In vitro* experiments have shown that in various cancer cell lines, these ASO treatments are used to transfer splicing from Bcl-xL to Bcl-xS.[193] In addition, the Bcl-xS protein induced by ASO makes cancer cells sensitive to chemotherapy drugs or UV radiation.[194] Recent preclinical data have evaluated the application of ASOs in targeting key cancer driver genes, showing promise. AZD9150 is an anti-transcription factor STAT3 ASO drug, which can reduce the expression of STAT3 in a variety of preclinical tumor models, and shows antitumor activity in lymphoma and lung cancer models.[195] AZD4785 is another ASO targeting KRAS-driven cancers, depleting KRAS and inhibiting downstream effector pathways and anti-proliferative effects.[196] In addition, a related ASO method is to design a bait oligonucleotide composed of RNA motifs recognized by a given splicing factor, which can down-regulate its splicing activity. When splicing factors are overexpressed or overactive in cancer cells, this may be a promising treatment.[197]

#### CRISPR-Cas9-guided alternative splicing in cancer therapy

The clustered regularly interspaced short palindromic repeats (CRISPR) and the CRISPR-related (Cas) 9 protein system have become the forefront of gene editing technology, and their clinical applications have been studied in many malignant and non-malignant tumors.[198] The CRISPR-Cas system can be designed to use a single guide RNA (sgRNA) to destroy or edit specific splice sites, use a pair of sgRNAs to remove specific exons or regulate *cis*-elements, or use template-mediated homologous recombination to correct splicing abnormalities caused by gene mutations.[199–201] An innovative knockin system based on CRISPR-Cas9 was used to explore the functional significance of SF3B1 mutations leading to alternative splicing events in cancer cell lines.[202] Although research on CRISPR-Cas9 for targeting oncogenic alternative splicing events is still insufficient, the specificity of these events for various cancer types and the easy customization of the CRISPR-Cas9 system indicate that CRISPR-Cas9 can effectively target splicing mutations.

#### Alternative splicing for cancer immunotherapy

Immunotherapy is producing effective treatments for some previously incurable cancers.[136,137] According to certain different patterns of mRNA alternative splicing between tumor and normal tissues, neoantigens are produced by abnormally spliced mRNA but not recognized by the immune system, including splicing mutations, gene fusion, and other processes.[203] Some studies have shown that splicing disorders directly affect genes that play a key role in the immune pathway, thereby affecting the effectiveness of cancer immunotherapy.[24] A study on the B cell marker CD20 showed that the alternative splicing subtype of 168 nucleotides spliced in exons 3 to 7 is only present in some patient-derived B lymphoma cell lines rather than normal cells, and can produce CD20-derived peptides with HLA-DR1 binding epitope and vaccination, thereby inducing specific CD4+ and CD8+ responses in transgenic mice.[204] The other recent research on CD19 exon 2 skipping results in a stable subtype that is not recognized by T cells expressing CD19-specific chimeric antigen receptor (CAR-T). Therefore, it is resistant to CD19 CAR-T in the treatment of B cell acute lymphoblastic leukemia.[205] In addition, a study found that two secreted splice variants of programmed death ligand 1 (PD-L1) trigger resistance to PD-L1 blockade in non-small cell lung cancer.[206] Lu et al. showed that pharmacologic regulation of splicing through specific drug classes generates bona fide neoantigens and elicits antitumor immunity, increasing checkpoint immunotherapy. Splicing regulation inhibits tumor growth and enhances checkpoint blockade in a manner dependent on host T cells and peptides presented on tumor MHC class I. Splicing regulation induces stereotyped splicing changes across tumor types, altering the MHC I-bound immunopeptidome to yield splicing-derived neoepitopes that trigger an antitumor T cell response *in vivo*. These data address the central question of whether altered RNA splicing generates immunologically meaningful neoantigens to provoke an effective antitumor immune response.[207] Although the use of tumor-specific mRNA splicing events to expand the immunotherapy target space is progressing significantly, a lot of work is still needed.

## CONCLUSIONS

Alternative splicing is a vital step in gene expression and alternative splicing generates transcriptome diversity in eukaryotic cells. However, although alternative splicing is the main driver of bio-diversity and plays a role in many characteristics of cancer, it has been neglected for a long time in the analysis of tumor characteristics, and it has also been neglected as a source of new biomarkers and therapeutic targets for drug development. In recent years, great progress has been made in the research of alternative splicing, cancer metabolism, and tumor immunity, and the relationship between them has become increasingly clear. In cancer, abnormal alternative splicing may deregulate every "cancer feature" proposed by Hanahan and Weinberg.[208] Tumor metabolic disorders and tumor immune evasion are important characteristics that distinguish tumor cells from normal cells. Understanding the role of abnormal splicing events in tumor metabolism disorders and tumor immune evasion will facilitate the development of specifically targeted tumor drugs. This review article presents the aberrant-splicing events could be involved in tumorigenesis as oncogenic drivers by regulating tumor metabolism and tumor immune evasion. Although important advances have been made in the role of abnormal splicing in tumors, there is still a great deal of work left over. First of all, the data obtained of splicing events are mainly from the second generation of RNA sequencing, which has a major drawback that the read length is too short, resulting in inaccuracy. Therefore, with the development of third-generation sequencing, the combination of second-generation and third-generation sequencing is expected to produce more accurate and high-throughput splicing events. Targeting splicing has also been explored as a new treatment option for cancer, including small molecules, ASOs, novel RNA-based CRISPR-Cas9 editing technology, and immunotherapy that modulates splicing. Despite exciting progress

in this area, these targeted anticancer therapies based on alternative splicing are still far from reaching the clinic. For example, a number of drugs or inhibitors that target splicing are undergoing clinical trials in cancer, but they are still far from being used. More research is needed to find more precise targeting mechanisms. Therefore, it is imperative to reveal in more detail how alternative splicing changes actually drive tumorigenesis and how it is linked to altered tumor metabolism, and tumor immune and other tumor-associated signaling pathways.

## ACKNOWLEDGMENTS

This work was supported in part by grants from the following sources: the National Natural Science Foundation of China (82173142, 81972636, 81872281, 81772842), the Natural Science Foundation of Hunan Province (2020JJ5336, 2019JJ40175, 2019JJ40183), the Research Project of Health Commission of Hunan Province (202109031837, 20201020, C2019066), Hunan Provincial Science and Technology Department (2020TP1018), Ascend Foundation of National Cancer Center (NCC2018b68), and supported by Hunan Cancer Hospital Climb Plan (ZX2020001-3, YF2020002).

## AUTHOR CONTRIBUTIONS

Q.P., Y.Z., L.O., N.W., Y.T., M.S., X.L., Y.W., and X.S. collected the related paper and drafted the manuscript. J.M. and Q.L. revised and finalized the manuscript. All authors read and approved the final manuscript.

## DECLARATION OF INTERESTS

The authors declare that they have no competing interests.

## REFERENCES

1. Du, J.X., Zhu, G.Q., Cai, J.L., Wang, B., Luo, Y.H., Chen, C., Cai, C.Z., Zhang, S.J., Zhou, J., Fan, J., et al. (2021). Splicing factors: insights into their regulatory network in alternative splicing in cancer. Cancer Lett. *501*, 83–104.
2. Zhang, Y., Qian, J., Gu, C., and Yang, Y. (2021). Alternative splicing and cancer: a systematic review. Signal Transduct. Target. Ther. *6*, 78.
3. Kim, M.S., Pinto, S.M., Getnet, D., Nirujogi, R.S., Manda, S.S., Chaerkady, R., Madugundu, A.K., Kelkar, D.S., Isserlin, R., Jain, S., et al. (2014). A draft map of the human proteome. Nature *509*, 575–581.
4. Baralle, F.E., and Giudice, J. (2017). Alternative splicing as a regulator of development and tissue identity. Nat. Rev. Mol. Cell Biol. *18*, 437–451.
5. Schulze, A., and Harris, A.L. (2012). How cancer metabolism is tuned for proliferation and vulnerable to disruption. Nature *491*, 364–373.
6. Pavlova, N.N., and Thompson, C.B. (2016). The emerging hallmarks of cancer metabolism. Cell Metab. *23*, 27–47.
7. Warburg, O. (1956). On respiratory impairment in cancer cells. Science *124*, 269–270.
8. Vander Heiden, M.G., Cantley, L.C., and Thompson, C.B. (2009). Understanding the Warburg effect: the metabolic requirements of cell proliferation. Science *324*, 1029–1033.
9. Al Tameemi, W., Dale, T.P., Al-Jumaily, R.M.K., and Forsyth, N.R. (2019). Hypoxia-modified cancer cell metabolism. Front. Cell Dev. Biol. *7*, 4.
10. Bhattacharya, R., Ray Chaudhuri, S., and Roy, S.S. (2018). FGF9-induced ovarian cancer cell invasion involves VEGF-A/VEGFR2 augmentation by virtue of ETS1 up-regulation and metabolic reprogramming. J. Cell Biochem. *119*, 8174–8189.
11. Yang, M., and Vousden, K.H. (2016). Serine and one-carbon metabolism in cancer. Nat. Rev. Cancer *16*, 650–662.
12. Mitra, T., and Bhattacharya, R. (2020). Phytochemicals modulate cancer aggressiveness: a review depicting the anticancer efficacy of dietary polyphenols and their combinations. J. Cell Physiol. *235*, 7696–7708.
13. Hay, N. (2016). Reprogramming glucose metabolism in cancer: can it be exploited for cancer therapy? Nat. Rev. Cancer *16*, 635–649.
14. Altman, B.J., Stine, Z.E., and Dang, C.V. (2016). From Krebs to clinic: glutamine metabolism to cancer therapy. Nat. Rev. Cancer *16*, 619–634.
15. Roy Burman, D., Das, S., Das, C., and Bhattacharya, R. (2021). Alternative splicing modulates cancer aggressiveness: role in EMT/metastasis and chemoresistance. Mol. Biol. Rep. *48*, 897–914.
16. Christofk, H.R., Vander Heiden, M.G., Harris, M.H., Ramanathan, A., Gerszten, R.E., Wei, R., Fleming, M.D., Schreiber, S.L., and Cantley, L.C. (2008). The M2 splice isoform of pyruvate kinase is important for cancer metabolism and tumour growth. Nature *452*, 230–233.
17. Bielli, P., Pagliarini, V., Pieraccioli, M., Caggiano, C., and Sette, C. (2019). Splicing dysregulation as oncogenic driver and passenger factor in brain tumors. Cells *9*, 10.
18. Farina, A.R., Cappabianca, L., Sebastiano, M., Zelli, V., Guadagni, S., and Mackay, A.R. (2020). Hypoxia-induced alternative splicing: the 11th hallmark of cancer. J. Exp. Clin. Cancer Res. *39*, 110.
19. Chen, X., Yang, T., Wang, W., Xi, W., Zhang, T., Li, Q., Yang, A., and Wang, T. (2019). Circular RNAs in immune responses and immune diseases. Theranostics *9*, 588–607.
20. Natua, S., Ashok, C., and Shukla, S. (2021). Hypoxia-induced alternative splicing in human diseases: the pledge, the turn, and the prestige. Cell Mol. Life Sci. *78*, 2729–2747.
21. Sahin, U., Derhovanessian, E., Miller, M., Kloke, B.P., Simon, P., Lower, M., Bukur, V., Tadmor, A.D., Luxemburger, U., Schrors, B., et al. (2017). Personalized RNA mutanome vaccines mobilize poly-specific therapeutic immunity against cancer. Nature *547*, 222–226.
22. Keskin, D.B., Anandappa, A.J., Sun, J., Tirosh, I., Mathewson, N.D., Li, S., Oliveira, G., Giobbie-Hurder, A., Felt, K., Gjini, E., et al. (2019). Neoantigen vaccine generates intratumoral T cell responses in phase Ib glioblastoma trial. Nature *565*, 234–239.
23. Hilf, N., Kuttruff-Coqui, S., Frenzel, K., Bukur, V., Stevanovic, S., Gouttefangeas, C., Platten, M., Tabatabai, G., Dutoit, V., van der Burg, S.H., et al. (2019). Actively personalized vaccination trial for newly diagnosed glioblastoma. Nature *565*, 240–245.
24. Frankiw, L., Baltimore, D., and Li, G. (2019). Alternative mRNA splicing in cancer immunotherapy. Nat. Rev. Immunol. *19*, 675–687.
25. Schaub, A., and Glasmacher, E. (2017). Splicing in immune cells-mechanistic insights and emerging topics. Int. Immunol. *29*, 173–181.
26. Chauhan, K., Kalam, H., Dutt, R., and Kumar, D. (2019). RNA splicing: a new paradigm in host-pathogen interactions. J. Mol. Biol. *431*, 1565–1575.
27. Kalam, H., Fontana, M.F., and Kumar, D. (2017). Alternate splicing of transcripts shape macrophage response to *Mycobacterium tuberculosis* infection. PLoS Pathog. *13*, e1006236.
28. De Maio, F.A., Risso, G., Iglesias, N.G., Shah, P., Pozzi, B., Gebhard, L.G., Mammi, P., Mancini, E., Yanovsky, M.J., Andino, R., et al. (2016). The dengue virus NS5 protein intrudes in the cellular spliceosome and modulates splicing. PLoS Pathog. *12*, e1005841.
29. Webb, L.M., Sengupta, S., Edell, C., Piedra-Quintero, Z.L., Amici, S.A., Miranda, J.N., Bevins, M., Kennemer, A., Laliotis, G., Tsichlis, P.N., et al. (2020). Protein arginine methyltransferase 5 promotes cholesterol biosynthesis-mediated Th17 responses and autoimmunity. J. Clin. Invest. *130*, 1683–1698.
30. Meininger, I., Griesbach, R.A., Hu, D., Gehring, T., Seeholzer, T., Bertossi, A., Kranich, J., Oeckinghaus, A., Eitelhuber, A.C., Greczmiel, U., et al. (2016). Alternative splicing of MALT1 controls signalling and activation of CD4(+) T cells. Nat. Commun. *7*, 11292.
31. Maniatis, T., and Reed, R. (1987). The role of small nuclear ribonucleoprotein particles in pre-mRNA splicing. Nature *325*, 673–678.

32. Fischer, U., Englbrecht, C., and Chari, A. (2011). Biogenesis of spliceosomal small nuclear ribonucleoproteins. Wiley Interdiscip. Rev. RNA *2*, 718–731.

33. Bessa, C., Matos, P., Jordan, P., and Goncalves, V. (2020). Alternative splicing: expanding the landscape of cancer biomarkers and therapeutics. Int. J. Mol. Sci. *21*, 9032.

34. Braunschweig, U., Gueroussov, S., Plocik, A.M., Graveley, B.R., and Blencowe, B.J. (2013). Dynamic integration of splicing within gene regulatory pathways. Cell *152*, 1252–1269.

35. Sahebi, M., Hanafi, M.M., van Wijnen, A.J., Azizi, P., Abiri, R., Ashkani, S., and Taheri, S. (2016). Towards understanding pre-mRNA splicing mechanisms and the role of SR proteins. Gene *587*, 107–119.

36. Chen, M., and Manley, J.L. (2009). Mechanisms of alternative splicing regulation: insights from molecular and genomics approaches. Nat. Rev. Mol. Cell Biol. *10*, 741–754.

37. Castello, A., Fischer, B., Eichelbaum, K., Horos, R., Beckmann, B.M., Strein, C., Davey, N.E., Humphreys, D.T., Preiss, T., Steinmetz, L.M., et al. (2012). Insights into RNA biology from an atlas of mammalian mRNA-binding proteins. Cell *149*, 1393–1406.

38. Barbosa-Morais, N.L., Carmo-Fonseca, M., and Aparicio, S. (2006). Systematic genome-wide annotation of spliceosomal proteins reveals differential gene family expansion. Genome Res. *16*, 66–77.

39. Luco, R.F., Pan, Q., Tominaga, K., Blencowe, B.J., Pereira-Smith, O.M., and Misteli, T. (2010). Regulation of alternative splicing by histone modifications. Science *327*, 996–1000.

40. Brodsky, A.S., Meyer, C.A., Swinburne, I.A., Hall, G., Keenan, B.J., Liu, X.S., Fox, E.A., and Silver, P.A. (2005). Genomic mapping of RNA polymerase II reveals sites of co-transcriptional regulation in human cells. Genome Biol. *6*, R64.

41. Zhou, H.L., Hinman, M.N., Barron, V.A., Geng, C., Zhou, G., Luo, G., Siegel, R.E., and Lou, H. (2011). Hu proteins regulate alternative splicing by inducing localized histone hyperacetylation in an RNA-dependent manner. Proc. Natl. Acad. Sci. U S A *108*, E627–E635.

42. Ameyar-Zazoua, M., Rachez, C., Souidi, M., Robin, P., Fritsch, L., Young, R., Morozova, N., Fenouil, R., Descostes, N., Andrau, J.C., et al. (2012). Argonaute proteins couple chromatin silencing to alternative splicing. Nat. Struct. Mol. Biol. *19*, 998–1004.

43. Zhou, H.L., Luo, G., Wise, J.A., and Lou, H. (2014). Regulation of alternative splicing by local histone modifications: potential roles for RNA-guided mechanisms. Nucleic Acids Res. *42*, 701–713.

44. Spies, N., Nielsen, C.B., Padgett, R.A., and Burge, C.B. (2009). Biased chromatin signatures around polyadenylation sites and exons. Mol. Cell *36*, 245–254.

45. Kolasinska-Zwierz, P., Down, T., Latorre, I., Liu, T., Liu, X.S., and Ahringer, J. (2009). Differential chromatin marking of introns and expressed exons by H3K36me3. Nat. Genet. *41*, 376–381.

46. Blaustein, M., Pelisch, F., Tanos, T., Munoz, M.J., Wengier, D., Quadrana, L., Sanford, J.R., Muschietti, J.P., Kornblihtt, A.R., Caceres, J.F., et al. (2005). Concerted regulation of nuclear and cytoplasmic activities of SR proteins by AKT. Nat. Struct. Mol. Biol. *12*, 1037–1044.

47. Blaustein, M., Pelisch, F., Coso, O.A., Bissell, M.J., Kornblihtt, A.R., and Srebrow, A. (2004). Mammary epithelial-mesenchymal interaction regulates fibronectin alternative splicing via phosphatidylinositol 3-kinase. J. Biol. Chem. *279*, 21029–21037.

48. Patel, N.A., Kaneko, S., Apostolatos, H.S., Bae, S.S., Watson, J.E., Davidowitz, K., Chappell, D.S., Birnbaum, M.J., Cheng, J.Q., and Cooper, D.R. (2005). Molecular and genetic studies imply Akt-mediated signaling promotes protein kinase CbetaII alternative splicing via phosphorylation of serine/arginine-rich splicing factor SRp40. J. Biol. Chem. *280*, 14302–14309.

49. Sanidas, I., Polytarchou, C., Hatziapostolou, M., Ezell, S.A., Kottakis, F., Hu, L., Guo, A., Xie, J., Comb, M.J., Iliopoulos, D., et al. (2014). Phosphoproteomics screen reveals akt isoform-specific signals linking RNA processing to lung cancer. Mol. Cell *53*, 577–590.

50. Laliotis, G.I., Chavdoula, E., Paraskevopoulou, M.D., Kaba, A., La Ferlita, A., Singh, S., Anastas, V., Nair, K.A., 2nd, Orlacchio, A., Taraslia, V., et al. (2021). AKT3-mediated IWS1 phosphorylation promotes the proliferation of EGFR-mutant lung adenocarcinomas through cell cycle-regulated U2AF2 RNA splicing. Nat. Commun. *12*, 4624.

51. Sumithra, B., Saxena, U., and Das, A.B. (2016). Alternative splicing within the Wnt signaling pathway: role in cancer development. Cell Oncol. (Dordr) *39*, 1–13.

52. Leeman, J.R., and Gilmore, T.D. (2008). Alternative splicing in the NF-kappaB signaling pathway. Gene *423*, 97–107.

53. Bangru, S., Arif, W., Seimetz, J., Bhate, A., Chen, J., Rashan, E.H., Carstens, R.P., Anakk, S., and Kalsotra, A. (2018). Alternative splicing rewires Hippo signaling pathway in hepatocytes to promote liver regeneration. Nat. Struct. Mol. Biol. *25*, 928–939.

54. Xu, Y., Huangyang, P., Wang, Y., Xue, L., Devericks, E., Nguyen, H.G., Yu, X., Oses-Prieto, J.A., Burlingame, A.L., Miglani, S., et al. (2021). ERalpha is an RNA-binding protein sustaining tumor cell survival and drug resistance. Cell *184*, 5215–5229.e7.

55. Huang, H., Zhang, J., Harvey, S.E., Hu, X., and Cheng, C. (2017). RNA G-quadruplex secondary structure promotes alternative splicing via the RNA-binding protein hnRNPF. Genes Dev. *31*, 2296–2309.

56. Fish, L., Khoroshkin, M., Navickas, A., Garcia, K., Culbertson, B., Hanisch, B., Zhang, S., Nguyen, H.C.B., Soto, L.M., Dermit, M., et al. (2021). A prometastatic splicing program regulated by SNRPA1 interactions with structured RNA elements. Science *372*, eabc7531.

57. Song, X., Zeng, Z., Wei, H., and Wang, Z. (2018). Alternative splicing in cancers: from aberrant regulation to new therapeutics. Semin. Cell Dev. Biol. *75*, 13–22.

58. Anczukow, O., and Krainer, A.R. (2016). Splicing-factor alterations in cancers. RNA *22*, 1285–1301.

59. Siegfried, Z., and Karni, R. (2018). The role of alternative splicing in cancer drug resistance. Curr. Opin. Genet. Dev. *48*, 16–21.

60. Zhao, J., Sun, Y., Huang, Y., Song, F., Huang, Z., Bao, Y., Zuo, J., Saffen, D., Shao, Z., Liu, W., et al. (2017). Functional analysis reveals that RBM10 mutations contribute to lung adenocarcinoma pathogenesis by deregulating splicing. Sci. Rep. *7*, 40488.

61. Urbanski, L.M., Leclair, N., and Anczukow, O. (2018). Alternative-splicing defects in cancer: splicing regulators and their downstream targets, guiding the way to novel cancer therapeutics. Wiley Interdiscip. Rev. RNA *9*, e1476.

62. Zhang, J., and Manley, J.L. (2013). Misregulation of pre-mRNA alternative splicing in cancer. Cancer Discov. *3*, 1228–1237.

63. Sveen, A., Kilpinen, S., Ruusulehto, A., Lothe, R.A., and Skotheim, R.I. (2016). Aberrant RNA splicing in cancer; expression changes and driver mutations of splicing factor genes. Oncogene *35*, 2413–2427.

64. Wang, Y., Bao, Y., Zhang, S., and Wang, Z. (2020). Splicing dysregulation in cancer: from mechanistic understanding to a new class of therapeutic targets. Sci. China Life Sci. *63*, 469–484.

65. Anczukow, O., Rosenberg, A.Z., Akerman, M., Das, S., Zhan, L., Karni, R., Muthuswamy, S.K., and Krainer, A.R. (2012). The splicing factor SRSF1 regulates apoptosis and proliferation to promote mammary epithelial cell transformation. Nat. Struct. Mol. Biol. *19*, 220–228.

66. Sebestyen, E., Singh, B., Minana, B., Pages, A., Mateo, F., Pujana, M.A., Valcarcel, J., and Eyras, E. (2016). Large-scale analysis of genome and transcriptome alterations in multiple tumors unveils novel cancer-relevant splicing networks. Genome Res. *26*, 732–744.

67. Kedzierska, H., and Piekielko-Witkowska, A. (2017). Splicing factors of SR and hnRNP families as regulators of apoptosis in cancer. Cancer Lett. *396*, 53–65.

68. Karni, R., de Stanchina, E., Lowe, S.W., Sinha, R., Mu, D., and Krainer, A.R. (2007). The gene encoding the splicing factor SF2/ASF is a proto-oncogene. Nat. Struct. Mol. Biol. *14*, 185–193.

69. Ghigna, C., Giordano, S., Shen, H., Benvenuto, F., Castiglioni, F., Comoglio, P.M., Green, M.R., Riva, S., and Biamonti, G. (2005). Cell motility is controlled by SF2/ASF through alternative splicing of the Ron protooncogene. Mol. Cell *20*, 881–890.

70. Anczukow, O., Akerman, M., Clery, A., Wu, J., Shen, C., Shirole, N.H., Raimer, A., Sun, S., Jensen, M.A., Hua, Y., et al. (2015). SRSF1-Regulated alternative splicing in breast cancer. Mol. Cell *60*, 105–117.

71. Jia, R., Li, C., McCoy, J.P., Deng, C.X., and Zheng, Z.M. (2010). SRp20 is a proto-oncogene critical for cell proliferation and tumor induction and maintenance. Int. J. Biol. Sci. *6*, 806–826.

72. Jensen, M.A., Wilkinson, J.E., and Krainer, A.R. (2014). Splicing factor SRSF6 promotes hyperplasia of sensitized skin. Nat. Struct. Mol. Biol. *21*, 189–197.

73. Huelga, S.C., Vu, A.Q., Arnold, J.D., Liang, T.Y., Liu, P.P., Yan, B.Y., Donohue, J.P., Shiue, L., Hoon, S., Brenner, S., et al. (2012). Integrative genome-wide analysis reveals cooperative regulation of alternative splicing by hnRNP proteins. Cell Rep. *1*, 167–178.

74. Gallardo, M., Lee, H.J., Zhang, X., Bueso-Ramos, C., Pageon, L.R., McArthur, M., Multani, A., Nazha, A., Manshouri, T., Parker-Thornburg, J., et al. (2015). hnRNP K is a haploinsufficient tumor suppressor that regulates proliferation and differentiation programs in hematologic malignancies. Cancer Cell *28*, 486–499.

75. Hollander, D., Donyo, M., Atias, N., Mekahel, K., Melamed, Z., Yannai, S., Lev-Maor, G., Shilo, A., Schwartz, S., Barshack, I., et al. (2016). A network-based analysis of colon cancer splicing changes reveals a tumorigenesis-favoring regulatory pathway emanating from ELK1. Genome Res. *26*, 541–553.

76. Wang, Y., Chen, D., Qian, H., Tsai, Y.S., Shao, S., Liu, Q., Dominguez, D., and Wang, Z. (2014). The splicing factor RBM4 controls apoptosis, proliferation, and migration to suppress tumor progression. Cancer Cell *26*, 374–389.

77. Yoshida, K., Sanada, M., Shiraishi, Y., Nowak, D., Nagata, Y., Yamamoto, R., Sato, Y., Sato-Otsubo, A., Kon, A., Nagasaki, M., et al. (2011). Frequent pathway mutations of splicing machinery in myelodysplasia. Nature *478*, 64–69.

78. Bonnal, S.C., Lopez-Oreja, I., and Valcarcel, J. (2020). Roles and mechanisms of alternative splicing in cancer - implications for care. Nat. Rev. Clin. Oncol. *17*, 457–474.

79. Tang, Q., Rodriguez-Santiago, S., Wang, J., Pu, J., Yuste, A., Gupta, V., Moldon, A., Xu, Y.Z., and Query, C.C. (2016). SF3B1/Hsh155 HEAT motif mutations affect interaction with the spliceosomal ATPase Prp5, resulting in altered branch site selectivity in pre-mRNA splicing. Genes Dev. *30*, 2710–2723.

80. Darman, R.B., Seiler, M., Agrawal, A.A., Lim, K.H., Peng, S., Aird, D., Bailey, S.L., Bhavsar, E.B., Chan, B., Colla, S., et al. (2015). Cancer-associated SF3B1 hotspot mutations induce cryptic 3' splice site selection through use of a different branch point. Cell Rep. *13*, 1033–1045.

81. Carrocci, T.J., Zoerner, D.M., Paulson, J.C., and Hoskins, A.A. (2017). SF3b1 mutations associated with myelodysplastic syndromes alter the fidelity of branchsite selection in yeast. Nucleic Acids Res. *45*, 4837–4852.

82. Daubner, G.M., Clery, A., Jayne, S., Stevenin, J., and Allain, F.H. (2012). A syn-anti conformational difference allows SRSF2 to recognize guanines and cytosines equally well. EMBO J. *31*, 162–174.

83. Zhang, J., Lieu, Y.K., Ali, A.M., Penson, A., Reggio, K.S., Rabadan, R., Raza, A., Mukherjee, S., and Manley, J.L. (2015). Disease-associated mutation in SRSF2 misregulates splicing by altering RNA-binding affinities. Proc. Natl. Acad. Sci. U S A *112*, E4726–E4734.

84. Kim, E., Ilagan, J.O., Liang, Y., Daubner, G.M., Lee, S.C., Ramakrishnan, A., Li, Y., Chung, Y.R., Micol, J.B., Murphy, M.E., et al. (2015). SRSF2 mutations contribute to myelodysplasia by mutant-specific effects on exon recognition. Cancer Cell *27*, 617–630.

85. Yoshimi, A., Lin, K.T., Wiseman, D.H., Rahman, M.A., Pastore, A., Wang, B., Lee, S.C., Micol, J.B., Zhang, X.J., de Botton, S., et al. (2019). Coordinated alterations in RNA splicing and epigenetic regulation drive leukaemogenesis. Nature *574*, 273–277.

86. Ilagan, J.O., Ramakrishnan, A., Hayes, B., Murphy, M.E., Zebari, A.S., Bradley, P., and Bradley, R.K. (2015). U2AF1 mutations alter splice site recognition in hematological malignancies. Genome Res. *25*, 14–26.

87. Fei, D.L., Motowski, H., Chatrikhi, R., Prasad, S., Yu, J., Gao, S., Kielkopf, C.L., Bradley, R.K., and Varmus, H. (2016). Wild-type U2AF1 antagonizes the splicing program characteristic of U2AF1-mutant tumors and is required for cell survival. PLoS Genet. *12*, e1006384.

88. Madan, V., Kanojia, D., Li, J., Okamoto, R., Sato-Otsubo, A., Kohlmann, A., Sanada, M., Grossmann, V., Sundaresan, J., Shiraishi, Y., et al. (2015). Aberrant splicing of U12-type introns is the hallmark of ZRSR2 mutant myelodysplastic syndrome. Nat. Commun. *6*, 6042.

89. Jayasinghe, R.G., Cao, S., Gao, Q., Wendl, M.C., Vo, N.S., Reynolds, S.M., Zhao, Y., Climente-Gonzalez, H., Chai, S., Wang, F., et al. (2018). Systematic analysis of splice-site-creating mutations in cancer. Cell Rep. *23*, 270–281.e3.

90. Jung, H., Lee, D., Lee, J., Park, D., Kim, Y.J., Park, W.Y., Hong, D., Park, P.J., and Lee, E. (2015). Intron retention is a widespread mechanism of tumor-suppressor inactivation. Nat. Genet. *47*, 1242–1248.

91. Shinmura, K., Tao, H., Goto, M., Igarashi, H., Taniguchi, T., Maekawa, M., Takezaki, T., and Sugimura, H. (2004). Inactivating mutations of the human base excision repair gene NEIL1 in gastric cancer. Carcinogenesis *25*, 2311–2317.

92. Tao, H., Shinmura, K., Hanaoka, T., Natsukawa, S., Shaura, K., Koizumi, Y., Kasuga, Y., Ozawa, T., Tsujinaka, T., Li, Z., et al. (2004). A novel splice-site variant of the base excision repair gene MYH is associated with production of an aberrant mRNA transcript encoding a truncated MYH protein not localized in the nucleus. Carcinogenesis *25*, 1859–1866.

93. Matsukuma, K.E., Mullins, F.M., Dietz, L., Zehnder, J.L., Ford, J.M., Chun, N.M., and Schrijver, I. (2010). Hereditary diffuse gastric cancer due to a previously undescribed CDH1 splice site mutation. Hum. Pathol. *41*, 1200–1203.

94. Warburg, O. (1956). On the origin of cancer cells. Science *123*, 309–314.

95. Harris, I., McCracken, S., and Mak, T.W. (2012). PKM2: a gatekeeper between growth and survival. Cell Res. *22*, 447–449.

96. Semenza, G.L. (2002). Involvement of hypoxia-inducible factor 1 in human cancer. Intern. Med. *41*, 79–83.

97. Bowler, E., and Oltean, S. (2019). Alternative splicing in angiogenesis. Int. J. Mol. Sci. *20*, 2067.

98. Qiu, Y., Hoareau-Aveilla, C., Oltean, S., Harper, S.J., and Bates, D.O. (2009). The anti-angiogenic isoforms of VEGF in health and disease. Biochem. Soc. Trans. *37*, 1207–1213.

99. Carroll, V.A., and Ashcroft, M. (2006). Role of hypoxia-inducible factor (HIF)-1alpha versus HIF-2alpha in the regulation of HIF target genes in response to hypoxia, insulin-like growth factor-I, or loss of von Hippel-Lindau function: implications for targeting the HIF pathway. Cancer Res. *66*, 6264–6270.

100. Barratt, S.L., Blythe, T., Ourradi, K., Jarrett, C., Welsh, G.I., Bates, D.O., and Millar, A.B. (2018). Effects of hypoxia and hyperoxia on the differential expression of VEGF-A isoforms and receptors in Idiopathic Pulmonary Fibrosis (IPF). Respir. Res. *19*, 9.

101. Han, J., Li, J., Ho, J.C., Chia, G.S., Kato, H., Jha, S., Yang, H., Poellinger, L., and Lee, K.L. (2017). Hypoxia is a key driver of alternative splicing in human breast cancer cells. Sci. Rep. *7*, 4108.

102. Uniacke, J., Holterman, C.E., Lachance, G., Franovic, A., Jacob, M.D., Fabian, M.R., Payette, J., Holcik, M., Pause, A., and Lee, S. (2012). An oxygen-regulated switch in the protein synthesis machinery. Nature *486*, 126–129.

103. Lorenzini, P.A., Chew, R.S.E., Tan, C.W., Yong, J.Y., Zhang, F., Zheng, J., and Roca, X. (2019). Human PRPF40B regulates hundreds of alternative splicing targets and represses a hypoxia expression signature. RNA *25*, 905–920.

104. Jakubauskiene, E., Vilys, L., Makino, Y., Poellinger, L., and Kanopka, A. (2015). Increased serine-arginine (SR) protein phosphorylation changes pre-mRNA splicing in hypoxia. J. Biol. Chem. *290*, 18079–18089.

105. Chen, T.M., Lai, M.C., Li, Y.H., Chan, Y.L., Wu, C.H., Wang, Y.M., Chien, C.W., Huang, S.Y., Sun, H.S., and Tsai, S.J. (2019). hnRNPM induces translation switch under hypoxia to promote colon cancer development. EBioMedicine *41*, 299–309.

106. Chen, M., Zhang, J., and Manley, J.L. (2010). Turning on a fuel switch of cancer: hnRNP proteins regulate alternative splicing of pyruvate kinase mRNA. Cancer Res. *70*, 8977–8980.

107. Mazurek, S. (2011). Pyruvate kinase type M2: a key regulator of the metabolic budget system in tumor cells. Int. J. Biochem. Cell Biol. *43*, 969–980.

108. Jiang, L., Shestov, A.A., Swain, P., Yang, C., Parker, S.J., Wang, Q.A., Terada, L.S., Adams, N.D., McCabe, M.T., Pietrak, B., et al. (2016). Reductive carboxylation supports redox homeostasis during anchorage-independent growth. Nature *532*, 255–258.

109. DeBerardinis, R.J., and Chandel, N.S. (2016). Fundamentals of cancer metabolism. Sci. Adv. *2*, e1600200.

110. Cluntun, A.A., Lukey, M.J., Cerione, R.A., and Locasale, J.W. (2017). Glutamine metabolism in cancer: understanding the heterogeneity. Trends Cancer *3*, 169–180.

111. Le, A., Lane, A.N., Hamaker, M., Bose, S., Gouw, A., Barbi, J., Tsukamoto, T., Rojas, C.J., Slusher, B.S., Zhang, H., et al. (2012). Glucose-independent glutamine metabolism via TCA cycling for proliferation and survival in B cells. Cell Metab. *15*, 110–121.

112. Elgadi, K.M., Meguid, R.A., Qian, M., Souba, W.W., and Abcouwer, S.F. (1999). Cloning and analysis of unique human glutaminase isoforms generated by tissue-specific alternative splicing. Physiol. Genomics *1*, 51–62.

113. Cassago, A., Ferreira, A.P., Ferreira, I.M., Fornezari, C., Gomes, E.R., Greene, K.S., Pereira, H.M., Garratt, R.C., Dias, S.M., and Ambrosio, A.L. (2012). Mitochondrial localization and structure-based phosphate activation mechanism of Glutaminase C with implications for cancer metabolism. Proc. Natl. Acad. Sci. U S A *109*, 1092–1097.

114. Wang, J.B., Erickson, J.W., Fuji, R., Ramachandran, S., Gao, P., Dinavahi, R., Wilson, K.F., Ambrosio, A.L., Dias, S.M., Dang, C.V., et al. (2010). Targeting mitochondrial glutaminase activity inhibits oncogenic transformation. Cancer Cell *18*, 207–219.

115. Szeliga, M., Sidoryk, M., Matyja, E., Kowalczyk, P., and Albrecht, J. (2005). Lack of expression of the liver-type glutaminase (LGA) mRNA in human malignant gliomas. Neurosci. Lett. *374*, 171–173.

116. Szeliga, M., Matyja, E., Obara, M., Grajkowska, W., Czernicki, T., and Albrecht, J. (2008). Relative expression of mRNAS coding for glutaminase isoforms in CNS tissues and CNS tumors. Neurochem. Res. *33*, 808–813.

117. Xia, Z., Donehower, L.A., Cooper, T.A., Neilson, J.R., Wheeler, D.A., Wagner, E.J., and Li, W. (2014). Dynamic analyses of alternative polyadenylation from RNA-seq reveal a 3'-UTR landscape across seven tumour types. Nat. Commun. *5*, 5274.

118. Laterza, O.F., and Curthoys, N.P. (2000). Specificity and functional analysis of the pH-responsive element within renal glutaminase mRNA. Am. J. Physiol. Ren. Physiol *278*, F970–F977.

119. Masamha, C.P., Xia, Z., Peart, N., Collum, S., Li, W., Wagner, E.J., and Shyu, A.B. (2016). CFIm25 regulates glutaminase alternative terminal exon definition to modulate miR-23 function. RNA *22*, 830–838.

120. Picon-Ruiz, M., Morata-Tarifa, C., Valle-Goffin, J.J., Friedman, E.R., and Slingerland, J.M. (2017). Obesity and adverse breast cancer risk and outcome: mechanistic insights and strategies for intervention. CA Cancer J. Clin. *67*, 378–397.

121. Heinz, F., Lamprecht, W., and Kirsch, J. (1968). Enzymes of fructose metabolism in human liver. J. Clin. Invest. *47*, 1826–1832.

122. Diggle, C.P., Shires, M., Leitch, D., Brooke, D., Carr, I.M., Markham, A.F., Hayward, B.E., Asipu, A., and Bonthron, D.T. (2009). Ketohexokinase: expression and localization of the principal fructose-metabolizing enzyme. J. Histochem. Cytochem. *57*, 763–774.

123. Eberhart, T., Schonenberger, M.J., Walter, K.M., Charles, K.N., Faust, P.L., and Kovacs, W.J. (2020). Peroxisome-deficiency and HIF-2alpha signaling are negative regulators of ketohexokinase expression. Front. Cell Dev. Biol. *8*, 566.

124. Lanaspa, M.A., Ishimoto, T., Cicerchi, C., Tamura, Y., Roncal-Jimenez, C.A., Chen, W., Tanabe, K., Andres-Hernando, A., Orlicky, D.J., Finol, E., et al. (2014). Endogenous fructose production and fructokinase activation mediate renal injury in diabetic nephropathy. J. Am. Soc. Nephrol. *25*, 2526–2538.

125. Hayasaki, T., Ishimoto, T., Doke, T., Hirayama, A., Soga, T., Furuhashi, K., Kato, N., Kosugi, T., Tsuboi, N., Lanaspa, M.A., et al. (2019). Fructose increases the activity of sodium hydrogen exchanger in renal proximal tubules that is dependent on ketohexokinase. J. Nutr. Biochem. *71*, 54–62.

126. Doke, T., Ishimoto, T., Hayasaki, T., Ikeda, S., Hasebe, M., Hirayama, A., Soga, T., Kato, N., Kosugi, T., Tsuboi, N., et al. (2018). Lacking ketohexokinase-A exacerbates renal injury in streptozotocin-induced diabetic mice. Metabolism *85*, 161–170.

127. Li, X., Qian, X., Peng, L.X., Jiang, Y., Hawke, D.H., Zheng, Y., Xia, Y., Lee, J.H., Cote, G., Wang, H., et al. (2016). A splicing switch from ketohexokinase-C to ketohexokinase-A drives hepatocellular carcinoma formation. Nat. Cell Biol. *18*, 561–571.

128. Swierczynski, J., Hebanowska, A., and Sledzinski, T. (2014). Role of abnormal lipid metabolism in development, progression, diagnosis and therapy of pancreatic cancer. World J. Gastroenterol. *20*, 2279–2303.

129. Currie, E., Schulze, A., Zechner, R., Walther, T.C., and Farese, R.V., Jr. (2013). Cellular fatty acid metabolism and cancer. Cell Metab. *18*, 153–161.

130. Yan, S., Yang, X.F., Liu, H.L., Fu, N., Ouyang, Y., and Qing, K. (2015). Long-chain acyl-CoA synthetase in fatty acid metabolism involved in liver and other diseases: an update. World J. Gastroenterol. *21*, 3492–3498.

131. Soupene, E., and Kuypers, F.A. (2008). Mammalian long-chain acyl-CoA synthetases. Exp. Biol. Med. (Maywood) *233*, 507–521.

132. Liang, Y.C., Wu, C.H., Chu, J.S., Wang, C.K., Hung, L.F., Wang, Y.J., Ho, Y.S., Chang, J.G., and Lin, S.Y. (2005). Involvement of fatty acid-CoA ligase 4 in hepatocellular carcinoma growth: roles of cyclic AMP and p38 mitogen-activated protein kinase. World J. Gastroenterol. *11*, 2557–2563.

133. Wu, X., Li, Y., Wang, J., Wen, X., Marcus, M.T., Daniels, G., Zhang, D.Y., Ye, F., Wang, L.H., Du, X., et al. (2013). Long chain fatty Acyl-CoA synthetase 4 is a biomarker for and mediator of hormone resistance in human breast cancer. PLoS One *8*, e77060.

134. Paucek, R.D., Baltimore, D., and Li, G. (2019). The cellular immunotherapy revolution: arming the immune system for precision therapy. Trends Immunol. *40*, 292–309.

135. Kahles, A., Lehmann, K.V., Toussaint, N.C., Huser, M., Stark, S.G., Sachsenberg, T., Stegle, O., Kohlbacher, O., Sander, C., and Gunnar, R.; Cancer Genome Atlas Research, Network (2018). Comprehensive analysis of alternative splicing across tumors from 8,705 patients. Cancer Cell *34*, 211–224.e6.

136. Zhao, Z., Xiao, X., Saw, P.E., Wu, W., Huang, H., Chen, J., and Nie, Y. (2020). Chimeric antigen receptor T cells in solid tumors: a war against the tumor microenvironment. Sci. China Life Sci. *63*, 180–205.

137. Tian, M., Shi, Y., Liu, W., and Fan, J. (2019). Immunotherapy of hepatocellular carcinoma: strategies for combinatorial intervention. Sci. China Life Sci. *62*, 1138–1143.

138. Ergun, A., Doran, G., Costello, J.C., Paik, H.H., Collins, J.J., Mathis, D., Benoist, C., and ImmGen, C. (2013). Differential splicing across immune system lineages. Proc. Natl. Acad. Sci. U S A *110*, 14324–14329.

139. De Arras, L., and Alper, S. (2013). Limiting of the innate immune response by SF3A-dependent control of MyD88 alternative mRNA splicing. PLoS Genet. *9*, e1003855.

140. De Arras, L., Laws, R., Leach, S.M., Pontis, K., Freedman, J.H., Schwartz, D.A., and Alper, S. (2014). Comparative genomics RNAi screen identifies Eftud2 as a novel regulator of innate immunity. Genetics *197*, 485–496.

141. Iwami, K.I., Matsuguchi, T., Masuda, A., Kikuchi, T., Musikacharoen, T., and Yoshikai, Y. (2000). Cutting edge: naturally occurring soluble form of mouse Toll-like receptor 4 inhibits lipopolysaccharide signaling. J. Immunol. *165*, 6682–6686.

142. Jia, R., Li, X., Yu, C., Fan, M., and Guo, J. (2013). The splicing factor hnRNP C regulates expression of co-stimulatory molecules CD80 and CD40 in dendritic cells. Immunol. Lett. *153*, 27–32.

143. Geng, G., Xu, C., Peng, N., Li, Y., Liu, J., Wu, J., Liang, J., Zhu, Y., and Shi, L. (2021). PTBP1 is necessary for dendritic cells to regulate T-cell homeostasis and antitumour immunity. Immunology *163*, 74–85.

144. Vindevoghel, L., Kon, A., Lechleider, R.J., Uitto, J., Roberts, A.B., and Mauviel, A. (1998). Smad-dependent transcriptional activation of human type VII collagen gene (COL7A1) promoter by transforming growth factor-beta. J. Biol. Chem. *273*, 13053–13057.

145. Anand, S., Batista, F.D., Tkach, T., Efremov, D.G., and Burrone, O.R. (1997). Multiple transcripts of the murine immunoglobulin epsilon membrane locus are generated by alternative splicing and differential usage of two polyadenylation sites. Mol. Immunol. *34*, 175–183.

146. Saxon, A., Diaz-Sanchez, D., and Zhang, K. (1997). Regulation of the expression of distinct human secreted IgE proteins produced by alternative RNA splicing. Biochem. Soc. Trans. *25*, 383–387.

147. Diaz-Sanchez, D., Zhang, K., Nutman, T.B., and Saxon, A. (1995). Differential regulation of alternative 3' splicing of epsilon messenger RNA variants. J. Immunol. *155*, 1930–1941.

148. Mukherjee, N., Corcoran, D.L., Nusbaum, J.D., Reid, D.W., Georgiev, S., Hafner, M., Ascano, M., Jr., Tuschl, T., Ohler, U., and Keene, J.D. (2011). Integrative regulatory

mapping indicates that the RNA-binding protein HuR couples pre-mRNA processing and mRNA stability. Mol. Cell *43*, 327–339.

149. Diaz-Munoz, M.D., Bell, S.E., Fairfax, K., Monzon-Casanova, E., Cunningham, A.F., Gonzalez-Porta, M., Andrews, S.R., Bunik, V.I., Zarnack, K., Curk, T., et al. (2015). The RNA-binding protein HuR is essential for the B cell antibody response. Nat. Immunol. *16*, 415–425.

150. Chang, X. (2016). RNA-binding protein hnRNPLL as a critical regulator of lymphocyte homeostasis and differentiation. Wiley Interdiscip. Rev. RNA *7*, 295–302.

151. Lynch, K.W. (2004). Consequences of regulated pre-mRNA splicing in the immune system. Nat. Rev. Immunol. *4*, 931–940.

152. Preussner, M., Schreiner, S., Hung, L.H., Porstner, M., Jack, H.M., Benes, V., Ratsch, G., and Bindereif, A. (2012). HnRNP L and L-like cooperate in multiple-exon regulation of CD45 alternative splicing. Nucleic Acids Res. *40*, 5666–5678.

153. Ruefli-Brasse, A.A., French, D.M., and Dixit, V.M. (2003). Regulation of NF-kappaB-dependent lymphocyte activation and development by paracaspase. Science *302*, 1581–1584.

154. Dong, X., and Chen, R. (2020). Understanding aberrant RNA splicing to facilitate cancer diagnosis and therapy. Oncogene *39*, 2231–2242.

155. Eskens, F.A., Ramos, F.J., Burger, H., O'Brien, J.P., Piera, A., de Jonge, M.J., Mizui, Y., Wiemer, E.A., Carreras, M.J., Baselga, J., et al. (2013). 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.

156. Yamano, T., Kubo, S., Yano, A., Kominato, T., Tanaka, S., Ikeda, M., and Tomita, N. (2019). Splicing modulator FR901464 is a potential agent for colorectal cancer in combination therapy. Oncotarget *10*, 352–367.

157. Lagisetti, C., Yermolina, M.V., Sharma, L.K., Palacios, G., Prigaro, B.J., and Webb, T.R. (2014). Pre-mRNA splicing-modulatory pharmacophores: the total synthesis of herboxidiene, a pladienolide-herboxidiene hybrid analog and related derivatives. ACS Chem. Biol. *9*, 643–648.

158. Larrayoz, M., Blakemore, S.J., Dobson, R.C., Blunt, M.D., Rose-Zerilli, M.J., Walewska, R., Duncombe, A., Oscier, D., Koide, K., Forconi, F., et al. (2016). The SF3B1 inhibitor spliceostatin A (SSA) elicits apoptosis in chronic lymphocytic leukaemia cells through downregulation of Mcl-1. Leukemia *30*, 351–360.

159. Gao, Y., Trivedi, S., Ferris, R.L., and Koide, K. (2014). Regulation of HPV16 E6 and MCL1 by SF3B1 inhibitor in head and neck cancer cells. Sci. Rep. *4*, 6098.

160. Yoon, S.O., Shin, S., Lee, H.J., Chun, H.K., and Chung, A.S. (2006). Isoginkgetin inhibits tumor cell invasion by regulating phosphatidylinositol 3-kinase/Akt-dependent matrix metalloproteinase-9 expression. Mol. Cancer Ther. *5*, 2666–2675.

161. Bellare, P., Small, E.C., Huang, X., Wohlschlegel, J.A., Staley, J.P., and Sontheimer, E.J. (2008). A role for ubiquitin in the spliceosome assembly pathway. Nat. Struct. Mol. Biol. *15*, 444–451.

162. Fukuda, M., Nishio, K., Kanzawa, F., Ogasawara, H., Ishida, T., Arioka, H., Bojanowski, K., Oka, M., and Saijo, N. (1996). Synergism between cisplatin and topoisomerase I inhibitors, NB-506 and SN-38, in human small cell lung cancer cells. Cancer Res. *56*, 789–793.

163. Luo, Y., Gao, Y., Liu, W., Yang, Y., Jiang, J., Wang, Y., Tang, W., Yang, S., Sun, L., Cai, J., et al. (2021). Myelocytomatosis-protein arginine N-methyltransferase 5 axis defines the tumorigenesis and immune response in hepatocellular carcinoma. Hepatology *74*, 1932–1951.

164. Brehmer, D., Beke, L., Wu, T., Millar, H.J., Moy, C., Sun, W., Mannens, G., Pande, V., Boeckx, A., van Heerde, E., et al. (2021). Discovery and pharmacological characterization of JNJ-64619178, a novel small molecule inhibitor of PRMT5 with potent anti-tumor activity. Mol. Cancer Ther. https://doi.org/10.1158/1535-7163.MCT-21-0367.

165. Fedoriw, A., Rajapurkar, S.R., O'Brien, S., Gerhart, S.V., Mitchell, L.H., Adams, N.D., Rioux, N., Lingaraj, T., Ribich, S.A., Pappalardi, M.B., et al. (2019). Anti-tumor activity of the type I PRMT inhibitor, GSK3368715, synergizes with PRMT5 inhibition through MTAP loss. Cancer Cell *36*, 100–114.e125.

166. Gammons, M.V., Lucas, R., Dean, R., Coupland, S.E., Oltean, S., and Bates, D.O. (2014). Targeting SRPK1 to control VEGF-mediated tumour angiogenesis in metastatic melanoma. Br. J. Cancer *111*, 477–485.

167. Melnyk, J.E., Steri, V., Nguyen, H.G., Hann, B., Feng, F.Y., and Shokat, K.M. (2020). The splicing modulator sulfonamide indisulam reduces AR-V7 in prostate cancer cells. Bioorg. Med. Chem. *28*, 115712.

168. Smith, L.D., Leme de Calais, F., Raponi, M., Mellone, M., Buratti, E., Blaydes, J.P., and Baralle, D. (2017). Novel splice-switching oligonucleotide promotes BRCA1 aberrant splicing and susceptibility to PARP inhibitor action. Int. J. Cancer *140*, 1564–1570.

169. Calabretta, S., Bielli, P., Passacantilli, I., Pilozzi, E., Fendrich, V., Capurso, G., Fave, G.D., and Sette, C. (2016). Modulation of PKM alternative splicing by PTBP1 promotes gemcitabine resistance in pancreatic cancer cells. Oncogene *35*, 2031–2039.

170. Li, Z., Li, Q., Han, L., Tian, N., Liang, Q., Li, Y., Zhao, X., Du, C., and Tian, Y. (2016). Pro-apoptotic effects of splice-switching oligonucleotides targeting Bcl-x pre-mRNA in human glioma cell lines. Oncol. Rep. *35*, 1013–1019.

171. Dewaele, M., Tabaglio, T., Willekens, K., Bezzi, M., Teo, S.X., Low, D.H., Koh, C.M., Rambow, F., Fiers, M., Rogiers, A., et al. (2016). Antisense oligonucleotide-mediated MDM4 exon 6 skipping impairs tumor growth. J. Clin. Invest. *126*, 68–84.

172. Nakajima, H., Takase, S., Terano, H., and Tanaka, H. (1997). New antitumor substances, FR901463, FR901464 and FR901465. III. Structures of FR901463, FR901464 and FR901465. J. Antibiot. (Tokyo) *50*, 96–99.

173. Nakajima, H., Sato, B., Fujita, T., Takase, S., Terano, H., and Okuhara, M. (1996). New antitumor substances, FR901463, FR901464 and FR901465. I. Taxonomy, fermentation, isolation, physico-chemical properties and biological activities. J. Antibiot. (Tokyo) *49*, 1196–1203.

174. Nakajima, H., Hori, Y., Terano, H., Okuhara, M., Manda, T., Matsumoto, S., and Shimomura, K. (1996). New antitumor substances, FR901463, FR901464 and FR901465. II. Activities against experimental tumors in mice and mechanism of action. J. Antibiot. (Tokyo) *49*, 1204–1211.

175. Roybal, G.A., and Jurica, M.S. (2010). Spliceostatin A inhibits spliceosome assembly subsequent to prespliceosome formation. Nucleic Acids Res. *38*, 6664–6672.

176. Corrionero, A., Minana, B., and Valcarcel, J. (2011). Reduced fidelity of branch point recognition and alternative splicing induced by the anti-tumor drug spliceostatin A. Genes Dev. *25*, 445–459.

177. Hong, D.S., Kurzrock, R., Naing, A., Wheler, J.J., Falchook, G.S., Schiffman, J.S., Faulkner, N., Pilat, M.J., O'Brien, J., and LoRusso, P. (2014). 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. New Drugs *32*, 436–444.

178. Seiler, M., Yoshimi, A., Darman, R., Chan, B., Keaney, G., Thomas, M., Agrawal, A.A., Caleb, B., Csibi, A., Sean, E., et al. (2018). H3B-8800, an orally available small-molecule splicing modulator, induces lethality in spliceosome-mutant cancers. Nat. Med. *24*, 497–504.

179. Sidarovich, A., Will, C.L., Anokhina, M.M., Ceballos, J., Sievers, S., Agafonov, D.E., Samatov, T., Bao, P., Kastner, B., Urlaub, H., et al. (2017). Identification of a small molecule inhibitor that stalls splicing at an early step of spliceosome activation. Elife *6*, e23533.

180. Effenberger, K.A., James, R.C., Urabe, V.K., Dickey, B.J., Linington, R.G., and Jurica, M.S. (2015). The natural product N-Palmitoyl-l-leucine selectively inhibits late assembly of human spliceosomes. J. Biol. Chem. *290*, 27524–27531.

181. Chung, F.F., Tan, P.F., Raja, V.J., Tan, B.S., Lim, K.H., Kam, T.S., Hii, L.W., Tan, S.H., See, S.J., Tan, Y.F., et al. (2017). Jerantinine A induces tumor-specific cell death through modulation of splicing factor 3b subunit 1 (SF3B1). Sci. Rep. *7*, 42504.

182. Wang, E., and Aifantis, I. (2020). RNA splicing and cancer. Trends Cancer *6*, 631–644.

183. Obeng, E.A., Stewart, C., and Abdel-Wahab, O. (2019). Altered RNA processing in cancer pathogenesis and therapy. Cancer Discov. *9*, 1493–1510.

184. Zamecnik, P.C., and Stephenson, M.L. (1978). Inhibition of Rous sarcoma virus replication and cell transformation by a specific oligodeoxynucleotide. Proc. Natl. Acad. Sci. U S A *75*, 280–284.

185. Stephenson, M.L., and Zamecnik, P.C. (1978). Inhibition of Rous sarcoma viral RNA translation by a specific oligodeoxyribonucleotide. Proc. Natl. Acad. Sci. U S A *75*, 285–288.

186. Dean, N.M., and Bennett, C.F. (2003). Antisense oligonucleotide-based therapeutics for cancer. Oncogene *22*, 9087–9096.

187. Liu, J., and Guo, B. (2020). RNA-based therapeutics for colorectal cancer: updates and future directions. Pharmacol. Res. *152*, 104550.

188. Levin, A.A. (2019). Treating disease at the RNA level with oligonucleotides. N. Engl. J. Med. *380*, 57–70.

189. Bennett, C.F. (2019). Therapeutic antisense oligonucleotides are coming of age. Annu. Rev. Med. *70*, 307–321.

190. Rigo, F., Chun, S.J., Norris, D.A., Hung, G., Lee, S., Matson, J., Fey, R.A., Gaus, H., Hua, Y., Grundy, J.S., et al. (2014). Pharmacology of a central nervous system delivered 2'-O-methoxyethyl-modified survival of motor neuron splicing oligonucleotide in mice and nonhuman primates. J. Pharmacol. Exp. Ther. *350*, 46–55.

191. Hua, Y., Vickers, T.A., Okunola, H.L., Bennett, C.F., and Krainer, A.R. (2008). Antisense masking of an hnRNP A1/A2 intronic splicing silencer corrects SMN2 splicing in transgenic mice. Am. J. Hum. Genet. *82*, 834–848.

192. Desterro, J., Bak-Gordon, P., and Carmo-Fonseca, M. (2020). Targeting mRNA processing as an anticancer strategy. Nat. Rev. Drug Discov. *19*, 112–129.

193. Mercatante, D.R., Bortner, C.D., Cidlowski, J.A., and Kole, R. (2001). 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.

194. Mercatante, D.R., Mohler, J.L., and Kole, R. (2002). Cellular response to an antisense-mediated shift of Bcl-x pre-mRNA splicing and antineoplastic agents. J. Biol. Chem. *277*, 49374–49382.

195. Hong, D., Kurzrock, R., Kim, Y., Woessner, R., Younes, A., Nemunaitis, J., Fowler, N., Zhou, T., Schmidt, J., Jo, M., et al. (2015). AZD9150, a next-generation antisense oligonucleotide inhibitor of STAT3 with early evidence of clinical activity in lymphoma and lung cancer. Sci. Transl. Med. *7*, 314ra185.

196. Ross, S.J., Revenko, A.S., Hanson, L.L., Ellston, R., Staniszewska, A., Whalley, N., Pandey, S.K., Revill, M., Rooney, C., Buckett, L.K., et al. (2017). Targeting KRAS-dependent tumors with AZD4785, a high-affinity therapeutic antisense oligonucleotide inhibitor of KRAS. Sci. Transl. Med. *9*, eaal5253.

197. Denichenko, P., Mogilevsky, M., Clery, A., Welte, T., Biran, J., Shimshon, O., Barnabas, G.D., Danan-Gotthold, M., Kumar, S., Yavin, E., et al. (2019). Specific inhibition of splicing factor activity by decoy RNA oligonucleotides. Nat. Commun. *10*, 1590.

198. Marzese, D.M., Manughian-Peter, A.O., Orozco, J.I.J., and Hoon, D.S.B. (2018). Alternative splicing and cancer metastasis: prognostic and therapeutic applications. Clin. Exp. Metastasis *35*, 393–402.

199. Garcia-Tunon, I., Alonso-Perez, V., Vuelta, E., Perez-Ramos, S., Herrero, M., Mendez, L., Hernandez-Sanchez, J.M., Martin-Izquierdo, M., Saldana, R., Sevilla, J., et al. (2019). Splice donor site sgRNAs enhance CRISPR/Cas9-mediated knockout efficiency. PLoS One *14*, e0216674.

200. Gapinske, M., Luu, A., Winter, J., Woods, W.S., Kostan, K.A., Shiva, N., Song, J.S., and Perez-Pinera, P. (2018). CRISPR-SKIP: programmable gene splicing with single base editors. Genome Biol. *19*, 107.

201. Anzalone, A.V., Randolph, P.B., Davis, J.R., Sousa, A.A., Koblan, L.W., Levy, J.M., Chen, P.J., Wilson, C., Newby, G.A., Raguram, A., et al. (2019). Search-and-replace genome editing without double-strand breaks or donor DNA. Nature *576*, 149–157.

202. Zhou, Q., Derti, A., Ruddy, D., Rakiec, D., Kao, I., Lira, M., Gibaja, V., Chan, H., Yang, Y., Min, J., et al. (2015). A chemical genetics approach for the functional assessment of novel cancer genes. Cancer Res. *75*, 1949–1958.

203. Slansky, J.E., and Spellman, P.T. (2019). Alternative splicing in tumors - a path to immunogenicity? N. Engl. J. Med. *380*, 877–880.

204. Vauchy, C., Gamonet, C., Ferrand, C., Daguindau, E., Galaine, J., Beziaud, L., Chauchet, A., Henry Dunand, C.J., Deschamps, M., Rohrlich, P.S., et al. (2015). CD20 alternative splicing isoform generates immunogenic CD4 helper T epitopes. Int. J. Cancer *137*, 116–126.

205. Sotillo, E., Barrett, D.M., Black, K.L., Bagashev, A., Oldridge, D., Wu, G., Sussman, R., Lanauze, C., Ruella, M., Gazzara, M.R., et al. (2015). Convergence of acquired mutations and alternative splicing of CD19 enables resistance to CART-19 immunotherapy. Cancer Discov. *5*, 1282–1295.

206. Gong, B., Kiyotani, K., Sakata, S., Nagano, S., Kumehara, S., Baba, S., Besse, B., Yanagitani, N., Friboulet, L., Nishio, M., et al. (2019). Secreted PD-L1 variants mediate resistance to PD-L1 blockade therapy in non-small cell lung cancer. J. Exp. Med. *216*, 982–1000.

207. Lu, S.X., De Neef, E., Thomas, J.D., Sabio, E., Rousseau, B., Gigoux, M., Knorr, D.A., Greenbaum, B., Elhanati, Y., Hogg, S.J., et al. (2021). Pharmacologic modulation of RNA splicing enhances anti-tumor immunity. Cell *184*, 4032–4047.e1.

208. Hanahan, D., and Weinberg, R.A. (2011). Hallmarks of cancer: the next generation. Cell *144*, 646–674.