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Review article

RNA splicing alterations in lung cancer pathogenesis and therapy

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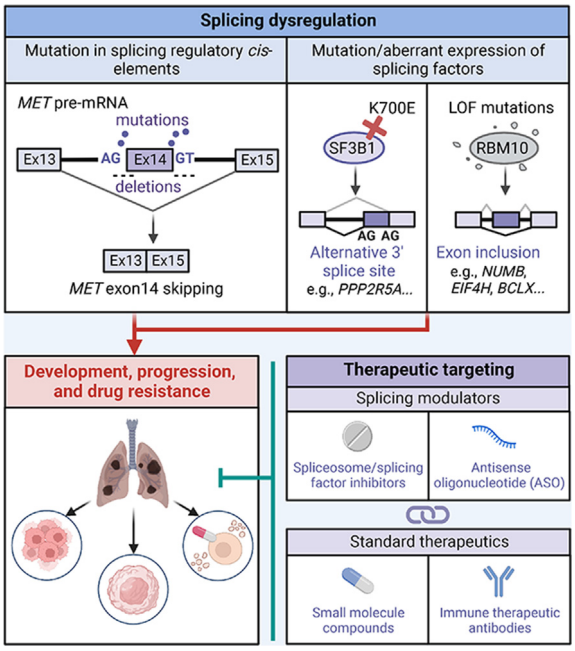
HIGHLIGHTS

- Splicing dysregulation is an emerging molecular feature of lung cancer.
- Splicing alterations are critical for lung cancer pathogenesis.
- Targeting dysregulated splicing holds great potential for lung cancer treatment.

GRAPHICAL ABSTRACT



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ABSTRACT

RNA splicing alterations are widespread and play critical roles in cancer pathogenesis and therapy. Lung cancer is highly heterogeneous and causes the most cancer-related deaths worldwide. Large-scale multi-omics studies have not only characterized the mutational landscapes but also discovered a plethora of transcriptional and post-transcriptional changes in lung cancer. Such resources have greatly facilitated the development of new diagnostic markers and therapeutic options over the past two decades. Intriguingly, altered RNA splicing has emerged as an important molecular feature and therapeutic target of lung cancer. In this review, we provide a brief

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Splicing alterations
Lung cancer

overview of splicing dysregulation in lung cancer and summarize the recent progress on key splicing events and splicing factors that contribute to lung cancer pathogenesis. Moreover, we describe the general strategies targeting splicing alterations in lung cancer and highlight the potential of combining splicing modulation with currently approved therapies to combat this deadly disease. This review provides new mechanistic and therapeutic insights into splicing dysregulation in cancer.

Introduction

Lung cancer is the most prevalent cancer type and the leading cause of cancer-related death worldwide, with an estimated 2 million new cases and 1.8 million deaths every year.[1] Histologically, it is classified as non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC), and NSCLC mainly comprises lung adenocarcinoma (LUAD) and lung squamous cell carcinoma (LUSC).[2] With continuous advances in detection and treatment approaches, clinical outcomes have been markedly improved for patients with lung cancer. However, lung cancer management is still very challenging because of its tremendous complexity and heterogeneity in terms of clinicopathological and molecular features.[3–6] Over the past decade, genomic and functional studies have identified oncogenic molecular changes that greatly facilitate the development of targeted therapies for lung cancer. Intriguingly, the dysregulation of ribonucleic acid (RNA) splicing has been proven to be widespread and plays a critical role in NSCLC pathogenesis and treatment.[7–10]

RNA splicing is the process of excising introns and ligating exons in precursor messenger RNA (pre-mRNA) to generate mature mRNA in eukaryotic cells. The spliceosome, a huge RNA-protein complex, catalyzes the two-step transesterification chemical reactions involved in RNA splicing. RNA splicing is highly dynamic and extensively regulated,[11,12] and this has been described well in previous reviews.[9,13–15] The interactions between *cis*-regulatory elements in pre-mRNA and various *trans*-acting factors (i.e., splicing factors) largely determine splicing outcomes through the modulation of basal spliceosome activity [Figure 1A]. Alternative splicing (AS), the selection and usage of different splice sites, frequently occurs due to splicing regulation [Figure 1B]. Almost all multi-exon genes in humans are regulated by AS, greatly expanding the complexity and diversity of the transcriptome and proteome.[16] According to the pattern of splice-site usage, AS can be divided into five major basic types as follows: skipped exon, alternative 3′ or 5′ splice site, mutually exclusive exons, and intron retention [Figure 1B].

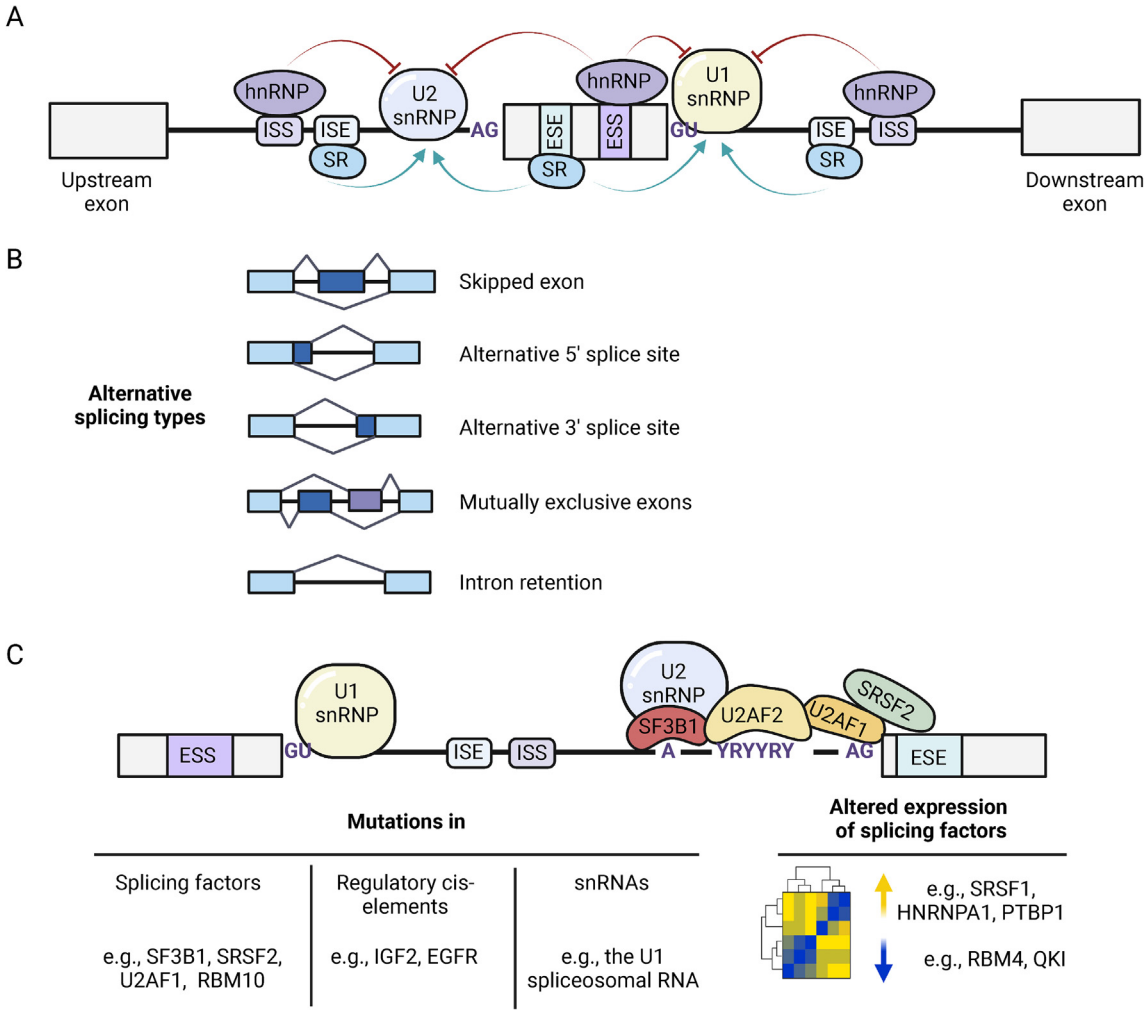

Figure 1. Dysregulation of RNA splicing in cancer. (A) General mechanisms of alternative splicing regulation. (B) Categories of alternative splicing events. (C) Mechanisms by which splicing is dysregulated in cancer. Splicing alterations mainly arise from mutations in splicing regulatory *cis*-elements, *trans*-acting splicing factors, and snRNAs, as well as the altered expression of splicing factors in cancer. ESE/S: Exonic splicing enhancer/silencer; hnRNP: Heterogeneous nuclear ribonucleoprotein; ISE/S: Intronic splicing enhancer/silencer; snRNP: Small nuclear ribonucleoprotein; SR: Serine/arginine-rich protein.

Splicing dysregulation is a major cause of human diseases and has been proposed as an emerging molecular hallmark of cancer. Splicing alterations, arising from mutations in splicing *cis*-regulatory elements and mutations in or changes in the expression of splicing factors, have crucial functions in cancer development, progression, and therapy [Figure 1C; more details are provided in previous excellent reviews[17–21]]. To date, the role of aberrant splicing has been best characterized in hematopoietic malignancies, but this has also been increasingly investigated in solid tumors. Exciting progress has been made in understanding splicing alterations in lung cancer, making it one of the most representative examples of the role of this dysregulated process in solid tumors. Nevertheless, an updated summary of this research direction is currently lacking. In this review, we highlight key oncogenic splicing events and frequently mutated splicing factors in lung cancer, summarize the strategies used to target such splicing alterations, and discuss several potential challenges in the mechanistic understanding and clinical translation of splicing dysregulation in lung cancer.

Oncogenic splicing events in lung cancer

Based on the systematic identification and functional characterization, it is currently well-recognized that splicing alterations exert oncogenic effects and can serve as potential prognostic markers and therapeutic targets in lung cancer.[22,23] As a representative example of global identification, a recent study systematically identified AS changes and investigated their biological implications via multi-omics analyses in NSCLC. Splicing changes in cancer-related genes, such as epidermal growth factor receptor (*EGFR*), fibroblast growth factor receptor 2 (*FGFR2*), and cluster of differentiation 44 (*CD44*), were found to be associated with prognosis in both LUAD and LUSC.[22] In addition to global identification, a rapidly increasing number of aberrant AS events has been characterized in lung cancer. Several key examples are emphasized as follows.

Mesenchymal–epithelial transition (MET) exon 14 skipping

MET gene encodes a receptor tyrosine kinase whose over-activation promotes lung cancer by activating downstream oncogenic signaling pathways, encompassing mitogen-activated protein kinase (MAPK), phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K)–AKT serine/threonine kinase (AKT), and signal transducer and activator of transcription (STAT).[24] Genomic alterations in *MET,* including amplification and mutations, lead to constitutively active *MET* signaling.[25–30] Notably, *MET* exon 14 skipping (*MET-ΔE14*) has been identified as one of the paradigmatic aberrant splicing events, with an oncogenic function and clear clinical significance, in LUAD [Figure 2A].[6,31,32]

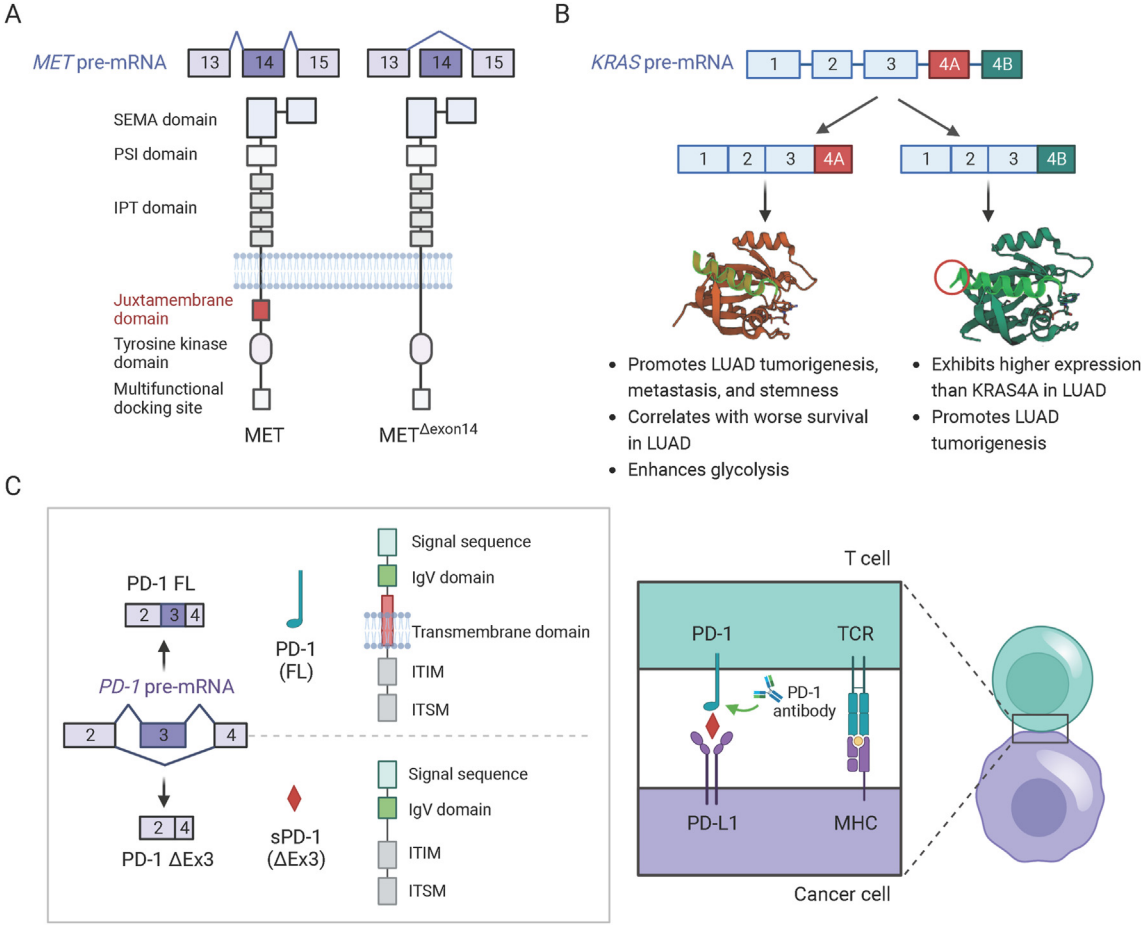

Figure 2. Key oncogenic splicing events in lung cancer. (A) Exon 14 skipping in *MET* is caused by mutations/deletions that disrupt splice donor and acceptor sites, leading to an in-frame deletion of 47 amino acids in the juxtamembrane domain of MET (MET-ΔE14). This deletion inhibits MET degradation and internalization, resulting in increased downstream signaling. (B) The 4th exon of *KRAS* is alternatively spliced, generating two splicing isoforms designated as *KRAS4A* and *KRAS4B*. KRAS4A and KRAS4B share common GTP/GDP-binding domains but differ in hypervariable regions (red). (C) Exon 3 skipping in PD-1 produces a soluble isoform, PD-1ΔEx3, which lacks the transmembrane domain. PD-1ΔEx3 might enhance anti-tumor immunity by interfering with the PD-1/PD-L1 signaling axis. Ex: Exon; FL: Full-length; IgV: Immunoglobulin variable domain; IPT: Immunoglobulins-plexins-transcription factors; ITIM: Immunoreceptor tyrosine-based inhibitory motif; ITSM: Immunoreceptor tyrosine-based switch motif; LUAD: Lung adenocarcinoma; MHC: Major histocompatibility complex; PSI: Plexins–semaphorins-integrins; SEMA: Semaphorin domain; TCR: T cell receptor.

MET-ΔE14 occurs in approximately 4% of NSCLC patients, based on the genomics data from multiple large-scale cohorts (The Cancer Genome Atlas Program (TCGA), Memorial Sloan Kettering Cancer Center (MSKCC), and Singapore Oncology Data Portal (OncoSG)). The genomic mutations in *MET* causing this change are heterogeneous, encompassing deletions and substitutions, and these mostly reside in or across from the splice donor and acceptor sites of exon 14 [Figure 2A]. Exon 14 skipping results in an in-frame deletion of 47 amino acids in the juxtamembrane domain of MET, which inhibits its degradation and internalization [Figure 2A], and thus, this is regarded as a gain-of-function alteration.[33] The juxtamembrane domain is the key negative regulatory region of *MET*. This domain contains a caspase-cleavage sequence (ESVD1002) and a tyrosine-binding site (Y1003) for the E3 ubiquitin ligase Casitas B lineage lymphoma (c-CBL), which mediates the ubiquitination and degradation of *MET*.[34] Compared to the wild type, *MET-ΔE14* markedly slows down ligand-induced ubiquitination but has no significant effect on the phosphorylation of *MET*.[35] In addition, exon 14 skipping causes a much more prominent association between *MET* and the p85 subunit of PI3K, which enhances the activation of downstream oncogenic signaling.[36] Accordingly, mouse NIH3T3 fibroblasts were demonstrated to be tumorigenic *in vivo* when expressing *MET-ΔE14*.[35]

Multi-institutional studies have identified *MET-ΔE14* as an independent oncogenic driver and a biomarker that is significantly associated with poorer survival in NSCLC.[35] Clinical evidence has proven that patients harboring *MET-ΔE14* could benefit from *MET* tyrosine kinase inhibitors (TKIs), including crizotinib, tepotinib, and capmatinib.[37–39] Prior to the approval of new-generation *MET* inhibitors, crizotinib was recommended for NSCLC patients with *MET-ΔE14*, according to the National Comprehensive Cancer Network (NCCN) guidelines. The objective response rate (ORR) of crizotinib treatment in patients with *MET-ΔE14* was determined to be 32.3%, based on the PROFILE 1001 clinical trial. However, the poor blood–brain barrier permeability of crizotinib has resulted in limited therapeutic efficacy in lung cancer patients with brain metastases.[40] Recently, capmatinib, a highly potent, selective type 1b inhibitor of *MET*, was approved in the United States for the treatment of patients with advanced NSCLC.[41–43] Moreover, multiple pre-clinical studies have provided strong evidence that it is more potent than previous *MET* TKIs (crizotinib and tepotinib) for the treatment of NSCLC with *MET-ΔE14*.[41,42,44] In addition to the pre-clinical data, a phase I clinical trial supported the safety of the clinical application of capmatinib.[45] Furthermore, a multi-institutional phase II clinical trial showed that the ORR of capmatinib was 68% and 41% in treatment-naive and pre-treated advanced NSCLC patients with *MET-ΔE14*, respectively.[45–47]

Kirsten rat sarcoma viral oncogene (KRAS) 4A and 4B

KRAS gene is the most frequently mutated oncogene in cancer, including LUAD.[48,49] In Caucasian populations, *KRAS* mutations are found in approximately 30% of LUAD patients.[6] Currently, novel compounds targeting the *KRAS* Gly12Cys mutation, such as sotorasib and adagrasib, have been developed and approved for clinical applications.[50–52] Unfortunately, both are only effective against the *KRAS* G12C mutation, with limited effects on other *KRAS* driver mutations, such as G12D and G12V. Moreover, resistance to these inhibitors often occurs rapidly.[51] Therefore, therapy targeting *KRAS* mutations remains a challenge in lung cancer.[50,53,54]

The 4th exon of *KRAS* is alternatively spliced, generating two splice variants designated as *KRAS4A* and *KRAS4B* [Figure 2B].[55,56] Since the oncogenic mutation in *KRAS* is predominantly located in the 2nd and 3rd exons and leads to constitutively active oncoproteins, both isoforms were demonstrated to promote tumorigenesis in LUAD[57] [Figure 2B]. Although *KRAS4A* was identified from the Kirsten rat sarcoma virus by Shimizu, early in 1983,[48] over the past decades, the vast majority of studies have focused on *KRAS4B* largely owing to its higher expression (than *KRAS4A*) in lung cancer. *KRAS4A* and *KRAS4B* share the first 165 amino acids encoding G domains but differ substantially in their hypervariable regions that mediate membrane association and subcellular trafficking [Figure 2B]. The distinct landscape of interactomes for *KRAS4A* and *KRAS4B* was identified via affinity-purification mass spectrometry.[58] For example, the v-ATPase A2 was shown to specifically interact with *KRAS4B*, but not *KRAS4A*, whereas the RAF-1 proto--oncogene serine/threonine kinase (RAF1) preferentially interacts with *KRAS4A*.[58] In addition, *KRAS4A* was shown to profoundly enhance glycolysis by directly associating with hexokinase 1 on the outer mitochondrial membrane in cancer cells.[59]

KRAS4A is widely expressed in various types of cancer, especially in lung cancer and colon cancer.[60,61] Recently, a multi-institutional study examined the expression of *KRAS4A* and *KRAS4B* in advanced-stage NSCLC patients and found that *KRAS4A* expression was elevated in most patients.[56] Another study, based on the genomic and transcriptomic data of TCGA LUAD cohort, revealed that *KRAS4A* expression is positively correlated with genomic alterations in *KRAS* and significantly worse survival in LUAD.[61] Further, an *in vivo* study consistently showed that *KRAS4A* alone could induce metastasis in LUAD in the absence of *KRAS4B*.[62] Together, these findings indicate that *KRAS4A* plays critical roles, likely distinct from those of *KRAS4B*, in the development and progression of LUAD. Intriguingly, targeting *KRAS4A* splicing through degradation of the RNA-binding protein RBM39 was shown to inhibit cell stemness in lung cancer,[63] providing a potential strategy to modulate *KRAS* splicing in cancer therapy. However, the regulatory mechanisms of *KRAS* splicing in cancer remain poorly understood and require further investigation.

Splicing alterations of programmed cell death protein 1/programmed cell death ligand 1 (PD-1/PD-L1)

Targeting the immune checkpoint molecules PD-1/PD-L1, known as immune checkpoint blockade (ICB), has resulted in remarkable clinical responses in various types of cancer, such as melanoma, NSCLC, and colon cancer.[64–66] However, only a fraction of patients respond to ICB, calling for a deeper understanding of immune-escape mechanisms.[67,68] Emerging evidence demonstrates that the AS of specific immune checkpoint molecules has significant effects on ICB. For example, exon 3 skipping in the PD-1-encoding gene *PDCD1* produces a soluble form of the protein (designated as *PD-1ΔEx3*), which was shown to suppress the PD-1/PD-L1 signaling axis, thereby enhancing anti-tumor immunity [Figure 2C].[69] Moreover, a clinical study in Denmark revealed that the upregulation of *PD-1ΔEx3* expression correlates with improved survival in patients with *EGFR*-mutant NSCLC treated with TKIs.[70] In addition, the expression of *PD-1ΔEx3* was reported to enhance the response rate to immunotherapies, such as anti-PD-1 (a-PD-1) and anti-CTLA4 therapy, in NSCLC.[71] Antisense oligonucleotides (ASOs) can shift *PDCD1* splicing toward *PD-1ΔEx3*, providing an alternative approach when targeting *PD-1* in lung cancer. Recently, a novel *PD-L1* splice variant lacking the transmembrane domain has been identified.[72] Compared with the canonical isoform expressed on the surfaces of cancer cells, this PD-L1 isoform appears to be secreted into the tumor immune microenvironment, conferring resistance to anti-PD-L1 immunotherapy in NSCLC.[72,73]

Dysregulation of splicing factors in lung cancer

Splicing factors are frequently mutated in hematologic malignancies, as well as in solid tumors.[18,74] In addition to mutations, the abnormal expression or activity of splicing factors is also commonly observed in cancer.[19,75] Accordingly, an increasing number of studies have demonstrated the oncogenic and tumor-suppressive functions of various splicing factors. Mechanistically, the dysregulation of splicing factors generally causes splicing alterations in target genes, consequently affecting cancer development, progression, and drug resistance. The modulation of dysregulated splicing factors and/or their target genes has started to show great potential for cancer therapy. However, despite this exciting progress, a large proportion of splicing factors dysregulated

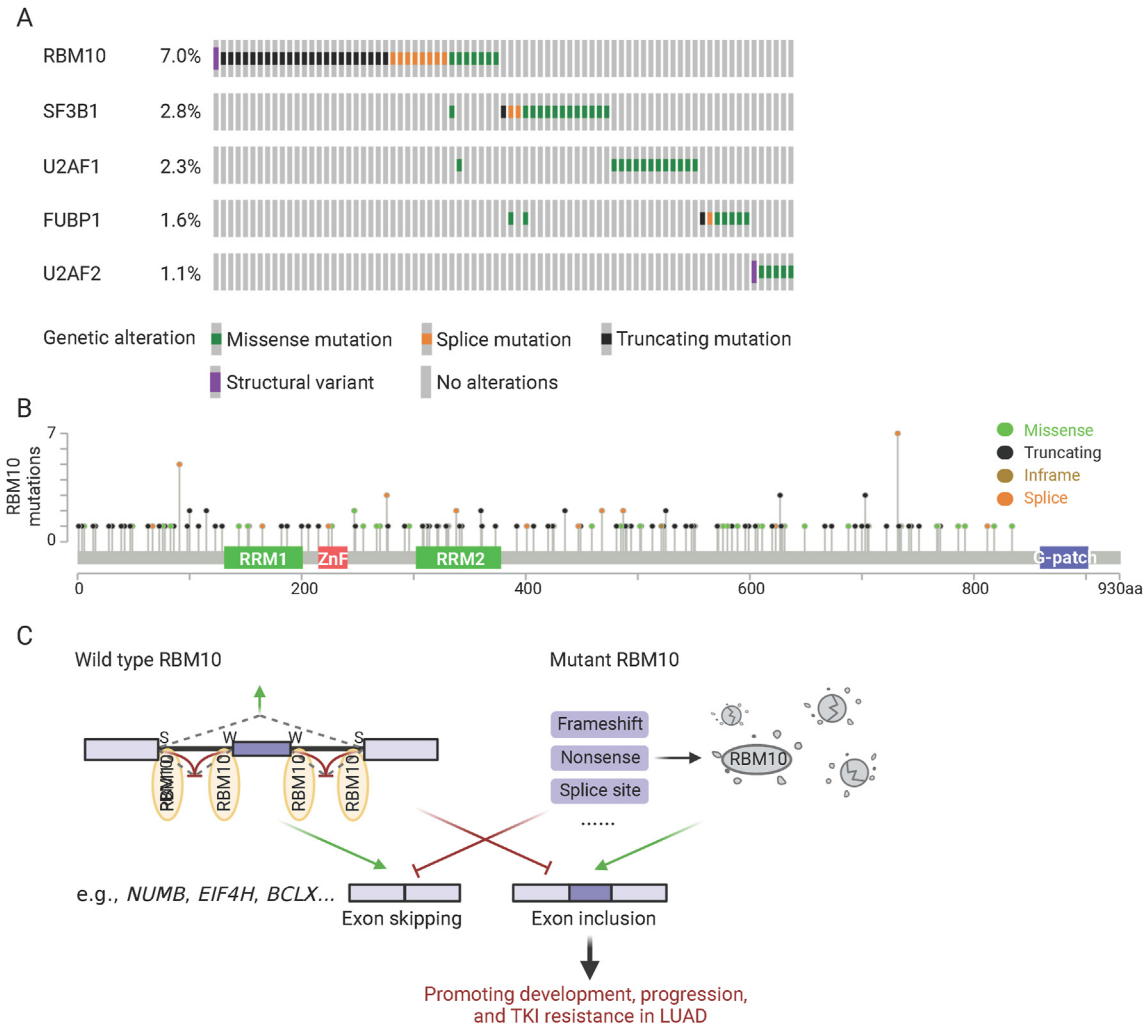

Figure 3. Splicing factors frequently mutated in lung cancer. (A) Spectrum of splicing factors frequently mutated in LUAD. Data are from the cBioPortal TCGA pan-cancer LUAD cohort. (B) Distribution of *RBM10* mutations in LUAD along the RBM10 protein sequence. These mutations are primarily frameshift, nonsense, and splice-site mutations, leading to *RBM10* loss-of-function alterations. Data are from the cBioPortal TCGA pan-cancer LUAD cohort. (C) *RBM10* deficiency promotes lung cancer development, progression, and TKI resistance by regulating the alternative splicing of key target genes, such as *NUMB*, *EIF4H*, and *BCLX*. LUAD: Lung adenocarcinoma; RRM: RNA recognition motif; TKI: Tyrosine kinase inhibitor; ZnF: Zinc finger.

in cancer remain to be investigated. The spectrum of splicing factors exhibiting recurring mutations in lung cancer is shown in Figure 3A, and their functional roles and clinical implications are highlighted in the following sections.

RNA binding motif protein 10 (RBM10)

RBM10 encodes an RNA-binding protein that has been identified as a component of U2 snRNP.[76,77] We and other researchers revealed that RBM10 enhances exon skipping by binding to flanking intronic regions near splice sites[78,79] or to exonic regions.[80] RBM10 exhibits high mutation rates in multiple cancers, such as LUAD, colorectal carcinoma, pancreatic ductal adenocarcinoma, and bladder cancer.[81–83] Moreover, RBM10 is the most frequently mutated splicing factor in lung cancers (e.g., 8.9% in a cohort of Chinese LUAD patients, 7.3% in the TCGA LUAD patient cohort,[84] and even more frequently in early-stage or non-smoking LUAD patients[85,86]). *RBM10* mutations are primarily loss-of-function [Figure 3B] and co-occur with known driver mutations, mostly *EGFR* and *KRAS* mutations, in lung cancer.[84,87,88]

Functional studies have demonstrated the tumor-suppressor functions of RBM10 in LUAD. Specifically, it was shown to repress Notch signaling via the AS-mediated regulation of *NUMB* exon 9[79] [Figure 3C]. We also found that RBM10 represses lung cancer progression by controlling the AS of eukaryotic translation initiation factor 4H (*EIF4H*) exon 5 [Figure 3C]. In particular, *RBM10* loss was shown to enhance the inclusion of exon 5 in *EIF4H*. Importantly, expression of the long isoform of *EIF4H* containing exon 5 (*EIF4H-L*) is specifically upregulated in LUAD, is critical for LUAD cell proliferation and survival, and correlates with unfavorable prognosis, which makes it a promising therapeutic target.[84] In addition, RBM10 was reported to suppress LUAD progression by inhibiting the Wnt/β-catenin and RAP1/AKT/CREB signaling pathways[89,90] and inhibiting the invasion and metastasis of NSCLC cells by recruiting methyltransferase-like 3 (METTL3) to modulate the m6A methylation of its target long non-coding RNA metastasis-associated lung adenocarcinoma transcript 1 (*MALAT1*).[91] Moreover, *RBM10* deficiency in LUAD was demonstrated to confer high sensitivity to spliceosome inhibition,[88] while compromising the efficacy of *EGFR* TKI therapy partially by regulating AS of the anti-apoptotic gene *Bcl-x* [Figure 3C].[92] Notably, *RBM10*-deficient LUADs were linked to higher expression of human leukocyte antigen (HLA) and immune checkpoint molecules and increased immune cell infiltration compared to *RBM10*-wild-type LUADs.[93,94] Additionally, RBM10 overexpression was found to significantly decrease the protein expression of PD-L1, whereas *RBM10* silencing was determined to increase it.[95] Although most studies support

the tumor-suppressive functions of RBM10 in lung cancer,[96] controversial oncogenic activities of RBM10 have been reported.[97,98] Further in-depth studies, particularly those using *in vivo* mouse models combined with clinical samples, are needed to corroborate the functions and therapeutic value of RBM10 in lung cancer.

In addition to that in lung cancer, it was reported that RBM10 physically interacts with p53 in colon cancer and that its overexpression disrupts the mouse double minute 2 homolog (MDM2)-p53 interaction, subsequently repressing p53 ubiquitination.[99] Further, *RBM10* loss enhances sensitivity to BCL2 inhibitors partially through the mis-splicing of X-linked inhibitor of apoptosis (*XIAP*) in acute myeloid leukemia.[100] Given the mutations and altered expression of *RBM10* in multiple cancers, it will be important to elucidate its roles in different cancer types.

U2 small nuclear RNA auxiliary factor 1 (U2AF1)

U2AF1, an essential protein component of the splicing machinery, forms a heterodimer with U2AF2. U2AF1 interacts with the AG dinucleotide of the 3′ splice site through its RNA recognition motif and interacts with serine- and arginine-rich proteins, such as serine/arginine-rich splicing factor 2 (SRSF2), through its arginine–serine-rich domain.[101] *U2AF1* is frequently mutated in myelodysplastic syndromes (MDSs) and chronic myelomonocytic leukemia, as well as in several solid tumors, including LUAD.[74,102] The S34F mutation, located in the first zinc finger domain of U2AF1, is the most pervasive hotspot in lung cancer and predicts worse survival.[103] In general, the S34F mutation influences splicing by affecting the U2AF1-binding preference to the 3′ splice site, and it has been characterized as a change-of-function alteration.[104] Intriguingly, the U2AF1 S34F mutation was shown to perturb mRNA translation by directly binding the mRNA 5′ untranslated region in the cytoplasm to promote cancer progression, implying a non-canonical role of splicing factors in cancer.[105] Specifically, the overexpression of $U2AF1^{S34F}$ was found to lead to the elevated translation of genes associated with the epithelial–mesenchymal transition in lung cancer.[105]

Splicing factor 3b subunit 1 (SF3B1)

SF3B1 is a core component of U2 snRNP that is essential for the recognition and selection of the branch-point sequence. *SF3B1* mutations have been intensively investigated in hematologic malignancies and also explored in several solid tumors, including uveal melanoma and LUAD.[106,107] The hotspot missense mutations of *SF3B1* K700 occur within the C-terminal HEAT repeat domains, and these result in the usage of cryptic 3′ splice sites and aberrant AS.[108] Recent TCGA data analysis suggests that splicing changes induced by *SF3B1* mutations share a similar pattern with that caused by SURP and G-patch domain containing 1 (*SUGP1*) deficiency in lung cancer.[109] Moreover, the SF3B1 K700E mutation or a *SUGP1* mutation disrupts the interaction between *SUGP1* and SF3B1, leading to common splicing changes.[110]

Far upstream element (FUSE) binding protein 1 (FUBP1)

FUBP1 is involved in the regulation of transcription, splicing, and mRNA stabilization by binding to a single strand of deoxyribonucleic acid (DNA) or RNA.[111,112] *FUBP1* expression was found to be upregulated and correlated with poor prognosis in several cancers, including hepatocellular carcinoma, glioma, gastric cancer, ovarian cancer, and nasopharyngeal carcinoma. As such, it was regarded as an oncogene.[113–117] In support of this notion, FUBP1 was shown to be required for efficient splicing of the oncogene *MDM2* in MCF7 breast cancer cells.[118] Conversely, loss-of-function mutations in *FUBP1* have been identified in neuroblastoma, indicating a tumor-suppressive role.[119] Interestingly, the *FUBP1 S11Lfs*43* mutation frequently occurs in LUAD (data from cBioPortal), but its functional significance remains to be determined. *In vitro* experiments showed that *FUBP1* knockdown inhibits the proliferation and migration of lung cancer cells, suggesting its oncogenic functions in lung cancer.[120] Mechanistically, *FUBP1* was shown to be recruited by a novel long non-coding RNA, lung cancer-associated transcript 3 (*LCAT3*), and then bind the FUSE sequence to activate *MYC* transcription and promote cell proliferation.[120] Another recent study indicated that FUBP1 knockdown decreases the expression of PD-L1 and inhibits LUSC tumor growth *in vivo*.[73] Further investigations are required to reconcile the complex functions and underlying mechanisms of FUBP1 in lung cancer and other cancers.

Abnormal expression of splicing factors

The best-known example of splicing factors with altered expression in cancer is the proto-oncogene *SRSF1*. The expression of this protein was found to be upregulated and promote tumorigenesis in several cancer types, including lung cancer.[121–123] Another representative example is *SRSF2*, whose P95 mutations occur frequently in MDSs but not in lung cancer.[124,125] Previous studies showed that the levels of *SRSF2* and phospho-*SRSF2* proteins are overexpressed in LUAD, LUSC, and neuroendocrine lung tumors.[126,127] Moreover, *SRSF2* was shown to interact with E2F transcription factor 1 (E2F1) and positively regulate transcription to control the expression of cell cycle genes in neuroendocrine lung cancer.[127] A recent study further revealed that *SRSF2* expression is transcriptionally upregulated by SRY-box transcription factor 2 (SOX2), leading to a splicing change in vascular endothelial growth factor receptor (*VEGFR*) in LUSC.[128] In addition, *SRSF6* expression was found to be upregulated and induce the transformation of epithelial cells in lung cancer.[129] Various splicing factors have also been found to be deregulated in lung cancer,[19,75] but they still need to be functionally characterized.

Strategies to target splicing alterations in lung cancer

Owing to the fundamental roles of splicing alterations in cancer and technological advancements in the manipulation of splicing, targeting aberrant splicing has been considered an attractive cancer therapeutic approach. Currently, general strategies to modulate splicing mainly encompass targeting the spliceosome, splicing factors, and splicing isoforms using small-molecule inhibitors and ASOs, among others [Figure 4A and B]. Accumulating evidence has demonstrated the potent effects of targeting splicing in various cancers.[7,9,10,17,130,131] Recently, pre-clinical and clinical studies have provided promising results regarding this strategy in lung cancer. Additionally, targeting splicing in combination with current standard treatment options for lung cancer can produce exciting results.

Targeting the spliceosome and splicing factors

Various small-molecule inhibitors have been designed to inhibit the spliceosome and splicing factors, which have been tested in cancer. The classical small-molecule inhibitors of the spliceosome, namely the natural compound pladienolide B and its derivatives, E7107 and H3B-8800, were designed to target the SF3B1 complex.[132–134] These small-molecule inhibitors bind to the branch-point-binding pocket of the SF3B complex, thereby preventing splicing [Figure 4A]. They also exhibited potent anti-tumor effects on lung cancer in pre-clinical studies;[135,136] however, clinical evidence supporting their effectiveness is lacking. Two phase I clinical trials reported ocular complications caused by E7107 with unclear mechanisms, which hindered its further clinical application for advanced solid tumors.[137,138]

In addition to spliceosome inhibitors, small-molecule inhibitors targeting splicing factors, such as protein arginine methyltransferase 5 (*PRMT5*) and *RBM39* have been developed, and their anti-tumor effects on lung cancer were tested *in vitro* and *in vivo* [Figure 4A].[63,139–142] For example, indisulam, a sulfonamide agent, can bind and bridge the splicing factor *RBM39* with the CUL4–DDB1–DDA1–DCAF15 E3 ubiquitin ligase complex, leading to the polyubiquitination and proteasomal

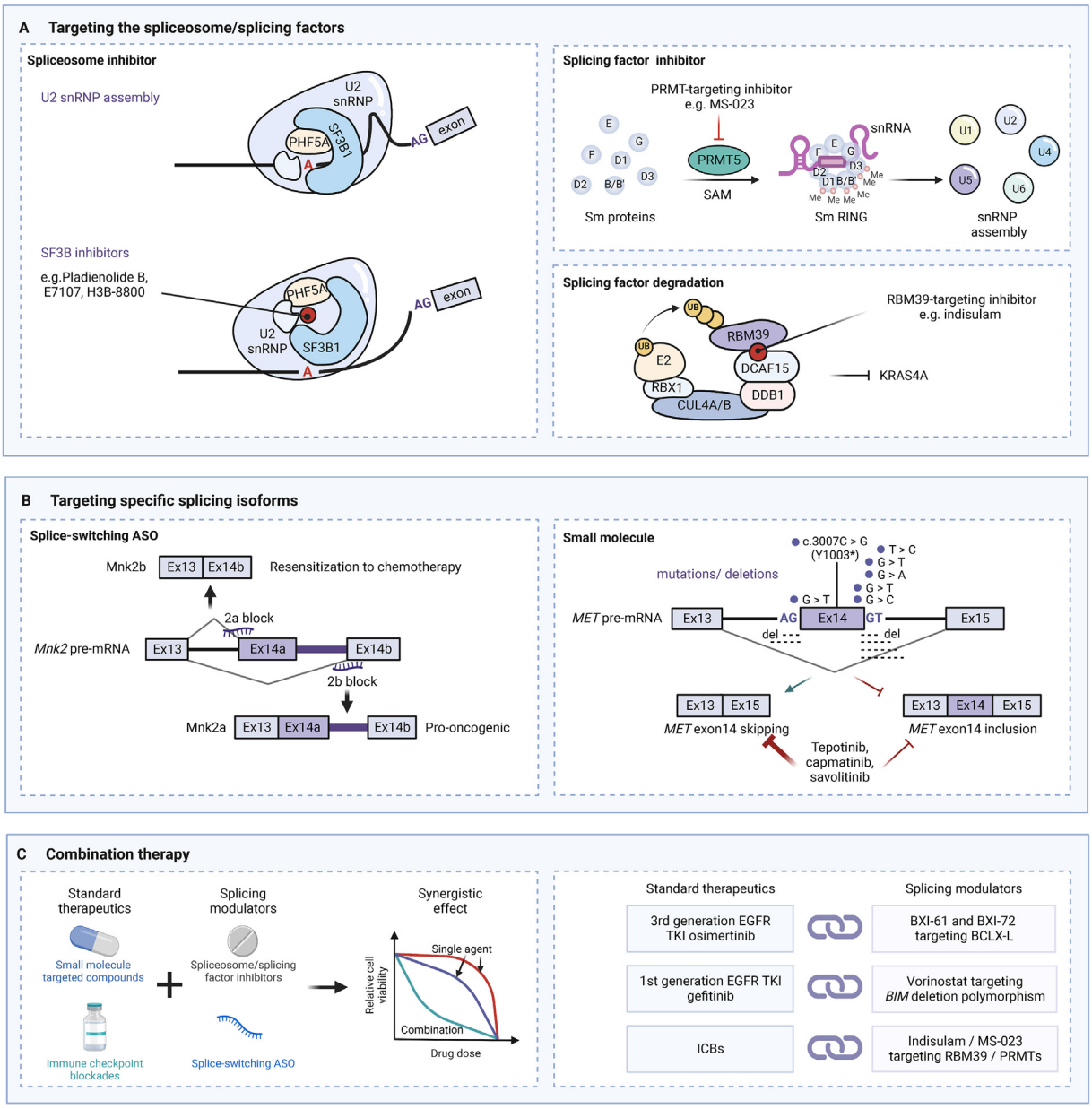

Figure 4. Strategies to target RNA splicing alterations in lung cancer. (A) Approaches targeting spliceosomes and splicing factors mainly include small-molecule inhibitors and the PROTAC system. (B) Approaches to target aberrant splicing events in lung cancer, mainly including splice-switching ASOs and small molecules. (C) Schematic of targeting splicing in combination with standard treatments for lung cancer. ASO: Antisense oligonucleotide; ICB: Immune checkpoint blockade; PROTAC: Proteolysis-targeting chimeric molecule; TKI: Tyrosine kinase inhibitor.

degradation of *RBM39* and the inhibition of tumorigenesis in lung cancer [Figure 4A].[63,143] In addition, proteolysis-targeting chimeric molecules, heterobifunctional compounds that utilize the ubiquitin-proteasome system to achieve specific protein degradation, can be applied to degrade abnormal splicing factors and have great potential for cancer therapy.[142]

Targeting aberrant splicing events

ASOs are short, artificially synthesized single-stranded nucleic acids with different modifications, which can directly bind to the splicing regulatory element in precursor mRNA to regulate splicing or pair with target mRNA to induce its degradation or repress translation. Further, they comprise an effective means to directly interfere with splicing abnormalities.[18,144,145] The efficacy of ASOs targeting splicing alterations has been demonstrated in clinical trials for various cancers but not yet for lung cancer.[146] Nonetheless, pre-clinical studies showed the significant anti-tumor effects of several ASOs targeting specific AS events in cancer-associated genes, such as *EIF4H*, *BCLX*, and MAPK interacting serine/threonine kinase 2 (*MNK2*), in lung cancer [Figure 4B].[92,147,148] In addition to the splicing switch, it is worth noting that ASO-based RNA therapy has broad prospects in lung cancer. AZD9150, an ASO targeting *STAT3*, was shown to directly decrease the expression of *STAT3* and exert anti-tumor effects on lymphoma and lung cancer in a phase I clinical trial.[149] Further, AZD4785, a high-affinity ASO targeting *KRAS*, was found to exert prominent anti-tumor effects on *KRAS*-mutant NSCLC patient-derived xenografts by inhibiting *KRAS* expression.[150] These studies provide foundations for the application of ASOs targeting aberrant splicing events in lung cancer.

Small-molecule inhibitors have also been pursued to modulate specific splicing isoforms. The most representative example is the *MET* TKIs mentioned previously herein. Capmatinib was tested in multiple clinical

trials and resulted in optimistic outcomes for NSCLC patients with *MET* exon 14 skipping [Figure 4B].[33,46,47] Salazosulfapyridine is a small-molecule compound that directly inhibits the splicing of isoforms of *CD44* and prolongs progression-free survival in lung cancer.[151] Another study showed that vorinostat could effectively target the oncogenic *BIM* splicing isoforms resulting from a deletion polymorphism.[152,153]

Targeting splicing in combination with standard treatment

According to NCCN guidelines, the standard treatment for NSCLC mainly consists of chemotherapy, radiotherapy, targeted therapy, and immunotherapy, beyond surgical approaches. However, therapy resistance is a major challenge encountered with these treatment options. Aberrant RNA splicing has been linked to therapy resistance in lung cancer. For example, the AS of the gene encoding caspase 9 was found to cause chemotherapy resistance in NSCLC.[154] Further, SGOL1-B, a splice variant of shugoshin-like 1 (SGOL1), induces aberrant mitosis and resistance to taxane in LUAD.[155] In addition, dysregulation of the splicing factor small nuclear ribonucleoprotein polypeptides B and B1 (SNRPB) was found to lead to platinum-based chemotherapy resistance in NSCLC.[156]

Currently, targeted therapies based on driver mutations, particularly TKIs, have brought about great benefits for patients with NSCLC, but resistance is almost inevitable. The mechanisms of resistance to TKIs include genomic alterations and other molecular and cellular changes.[157] As such, the aberrant splicing of cancer-associated genes, such as *HER2*, *BIM*, and *ATG16*, was found to contribute to TKI resistance.[158–160] In addition, deficiency of the splicing factor RBM10 was recently reported to limit the response to osimertinib in *EGFR*-mutant LUAD partially due to a splicing alteration in *Bcl-x*.[92] Importantly, the combination of a *Bcl-x* inhibitor with osimertinib was found to synergistically inhibit LUAD [Figure 4C].[92] Moreover, a phase I clinical trial led to the approval of the combination of vorinostat and gefitinib in *BIM*-deletion polymorphism/*EGFR* mutation-double positive LUAD [Figure 4C].[152,153] Hence, targeting aberrant splicing in combination with conventional treatment options could be a very promising strategy to improve therapy efficacy and overcome resistance in lung cancer [Figure 4C].

The development of immunotherapy has revolutionized the treatment of lung cancer. The a-PD1 antibodies, represented by nivolumab and pembrolizumab, are a standard treatment strategy for advanced-stage NSCLC, especially those lacking driver mutations for targeted therapy. However, the ORR of a-PD1 was found to be approximately 20% in NSCLC due to primary or acquired resistance. Aberrant splicing has also been shown to affect the tumor's immune microenvironment.[161–163] On one hand, the aberrant splicing of genes encoding immune checkpoint molecules could interfere with their normal functions, which in turn confers resistance or sensitivity to ICBs.[69,72] Accordingly, ASOs designed to target those aberrant splicing events should be able to enhance the effects of ICBs in lung cancer. On the other hand, AS can generate neoantigens that reprogram the tumor immune microenvironment, similar to the tumor mutation burden, which correlates with the ORR of a-PD1 immunotherapy.[164,165] The modulation of exon skipping and intron retention was predicted to generate numerous aberrant peptides, four times more than mutation-derived neoantigens,[166,167] highlighting their important roles in anti-tumor immune responses. Interestingly, the splicing factor inhibitors indisulam and MS-023, targeting *RBM39* and *PRMT*s, respectively, were found to significantly enhance sensitivity to ICBs in a pre-clinical study [Figure 4C].[166] These studies provide direct evidence for combining the targeted modulation of splicing with ICBs as a promising therapeutic option for lung cancer [Figure 4C].

Conclusion and perspective

In this review, we summarized recent progress on the key splicing events and splicing factors that are altered in lung cancer. We also described the general strategies used to target splicing alterations in lung cancer and proposed a combination of splicing modulation with currently existing therapeutics as a promising direction to improve treatment outcomes. This review highlights the critical roles of RNA splicing alterations in the pathogenesis and treatment of lung cancer, providing new insights into cancer-related splicing dysregulation.

Despite encouraging advancements, there are pressing challenges that need to be addressed. First, many splicing events and splicing factors that are altered in lung cancer have not been functionally elucidated. Since splicing factors often regulate many RNA splicing events, it is difficult to determine whether a few key splicing alterations or many changes in combination are responsible for splicing factor dysregulation in cancer. Hence, efficient functional screening methods are important for elucidating splicing aberrations in lung cancer. Currently, several high-throughput screening libraries based on clustered regularly interspaced short palindromic repeats (CRISPR)-associated (Cas) systems have been developed and used to systematically interrogate cancer-related splicing factors and events.[168–170] Such screening strategies have been applied to identify functional splicing alterations in lung cancer.[171–174] Second, splicing changes can be used as invaluable diagnostic biomarkers and therapeutic targets for lung cancer patients, yet these have not been translated to the clinic. Therefore, it is urgent to implement rationally designed clinical trials to test the efficacy of various splicing-modulating drugs, including large-scale, multi-institutional trials combining splicing modulation with targeted or immune therapy. It is also critical to develop sensitive, specific, and low-cost technologies to detect splicing changes in clinical samples, improve the delivery efficiency of ASOs to tumor sites, and limit the potential toxicity of spliceosome inhibitors. Such efforts will provide a foundation for the clinical application of splicing modulators in lung cancer treatment. Third, in stark contrast to knowledge on NSCLC, few studies have focused on splicing alterations in SCLC, for which more investigations are needed.

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Author contribution

Yongbo Wang,Yueren Yan, Yunpeng Ren, and Yufang Bao Bao conceived the review and wrote the manuscript. Yufang Bao, and Yueren Yan created the figures. Yongbo Wang edited the manuscript.

Ethics statement

None.

Data availability statement

The datasets used in the current study are available from the corresponding author on reasonable request.

Conflict of interest

None.

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