Acta Pharmaceutica Sinica B 2025;15(5):2301−2322

Chinese Pharmaceutical Association
Institute of Materia Medica, Chinese Academy of Medical Sciences

Acta Pharmaceutica Sinica B

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REVIEW

RNA splicing: Novel star in pulmonary diseases with a treatment perspective

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Received 5 December 2024; received in revised form 23 February 2025; accepted 3 March 2025

KEY WORDS

Lung diseases;
RNA splicing;
Therapeutic targets;
Biomarkers;
RNA-binding proteins;
Splicing variants;
Analysis method;
Splicing factor

Abstract Alternative splicing (AS) serves as a fundamental regulatory mechanism in gene expression, contributing to proteomic diversity by generating an array of mRNA isoforms from precursor mRNA *via* distinct splice site combinations. In light of the limited therapeutic options currently available, the exploration of AS as a target for drug development is of paramount importance. This review offers an exhaustive analysis of the biological functions and underlying molecular mechanisms associated with various AS-induced splice variants, RNA-binding proteins, and *cis*-elements, highlighting their significance as clinical biomarkers. We place particular emphasis on the current therapeutic applications of AS in an array of lung diseases, including but not limited to lung cancer, cystic fibrosis, silicosis, acute respiratory distress syndrome, pneumonia, asthma, chronic obstructive pulmonary diseases, pulmonary arterial hypertension, and idiopathic pulmonary fibrosis. The review delves into the role of AS events in the diagnosis and treatment of lung diseases, focusing on the regulatory influence of splicing factors and RNA-binding proteins, while also enumerating the mutated components implicated in AS misregulation. Consequently, a comprehensive understanding of the intricate mechanisms governing these splicing events could potentially offer novel avenues for the development of splicing-targeted therapeutics and diagnostic tools for the prevention and treatment of lung diseases.

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Peer review under the responsibility of Chinese Pharmaceutical Association and Institute of Materia Medica, Chinese Academy of Medical Sciences.

https://doi.org/10.1016/j.apsb.2025.03.023

1. Introduction

The lung is the main organ of the respiratory system and serves additional roles in immune defense, endocrine, and metabolic functions. Common clinical lung diseases include cancer, chronic bronchitis, emphysema, tuberculosis, asthma, cystic fibrosis, pneumonia, etc. Epidemiological studies show that the prevalence rate of lung disease is increasing year by year[1]. Over the past decade, there has been a comprehensive understanding of DNA mutations, abnormal gene expression profiles, epigenomics, and proteomics, leading to the successful application of targeted drugs in lung disease therapy. Despite these advances, there is little known about its aberrant alternative splicing (AS) profiles and without suitable targeted therapeutic options. Therefore, it's urgently necessary to improve the understanding of lung disease biology and develop more novel therapeutic strategies to tackle AS-associated lung disease. AS is an essential mechanism for the post-transcriptional regulation of gene expression. Up until 1977, AS was first discovered, detail described, and extensively studied[2]. Alternative splicing is the process by which precursor messenger mRNA (pre-mRNA) generates different mRNA splicing isoforms through different splicing methods[3,4]. Murray and Holliday[5] subsequently proposed three main modes of RNA−RNA splicing. So far, common different types of alternative splicing events have been identified, including cassette exons, mutually exclusive exons, alternative 5′ splice sites, alternative 3′ splice sites, alternative promoters, alternative 3′ exons, retained intron, and alternative polyadenylation as shown in Fig. 1.

It has been reported that more than 90% of mammal genes can produce different isoforms through alternative splicing, thereby facilitating cell functions such as cell proliferation and differentiation[6]. AS not only has massive effects on physiological processes, such as development and aging[7,8], but also results in gene product diversification to mediate various pathological processes such as prostate cancer, gastric cancer, breast cancer, and lung cancer, etc[9-11]. Recently, it has been increasingly recognized that applying splicing modulatory strategies improves therapeutic efficacies. These strategies aim to correct the erroneous utilization of splice sites, which leads to splicing dysregulation and important physiological protein function disruption[12]. For instance, previous studies have reported that interfere with RNA splicing drugs as EPZ015666, PRMT5 inhibitor, can interfere with tumor cells to produce new antigens, which are presented as new epitope through MHC 1, stimulate the endogenous anti-tumor immune response, and increase the inhibitory effect of immune checkpoints[13]. While aberrant splicing is commonly observed in lung cancer, other critical lung diseases also have been linked with mutations or variations in splicing sites, expression of splicing factors, upstream signaling pathways, and nucleosome localization[14,15].

This paper surveys recent empirical studies on the connection between lung disease and alternative splicing, emphasizing molecular activities that are therapies and diagnosis clinical applications. Through the perspective of several typical splicing variations, element mutation, single nucleotide polymorphism, and the ratios of alternative protein isoforms as a diagnostic biomarker of the lung disease phenotype, we thoroughly emphasize the main phenomenon and mechanism of RNA splicing in every kind of lung disease and classification. In addition, to detail and illustrate the connection between lung disease and alternative splicing, we also list some typical influencing factors of splicing to lead or therapy lung disease such as splicing factor serine/arginine splicing factor 1 (SRSF1), quaking (QKI), fibroblast growth factor receptor2 (FGFR2), etc. However, due to the limitations of analytical materials and means, the importance of RNA splicing in lung diseases and the role of RNA-related indirect factors in lung diseases have not attracted enough attention. Consequently, there is an urgent need to deeply understand the function of alternative splicing and discover the mechanism of lung disease and splicing. This study not only provides new insights into the opportunities to develop novel therapies that target splicing-driven lung diseases

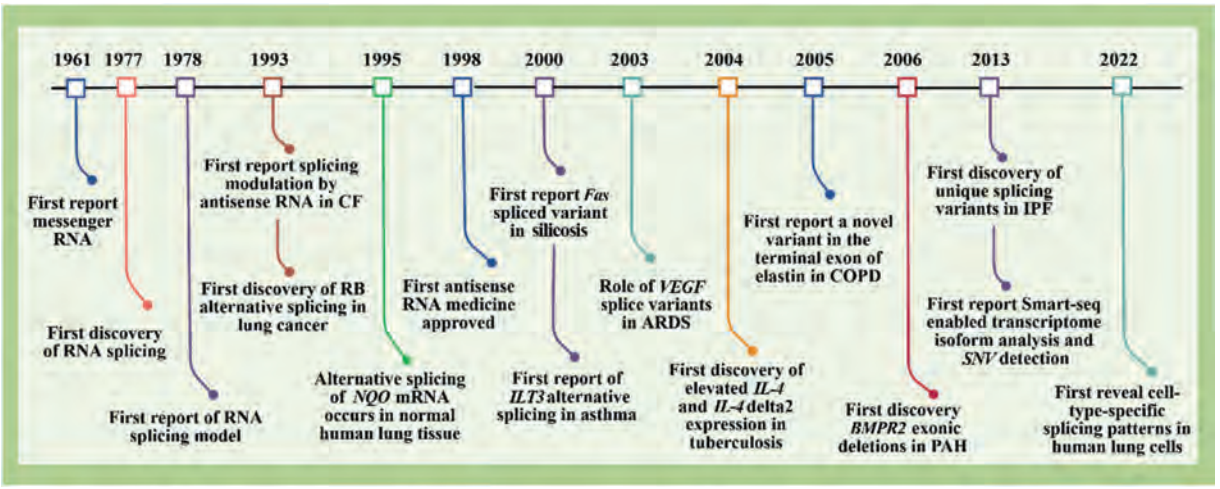

Figure 1 The inaugural documentation and evolutionary history of RNA alternative splicing in the context of pulmonary diseases.

by revealing the characteristics of splicing dysregulation patterns, but also lays the foundation for prospects with high organ-specific, tissue-specific, or cell-type-specific in lung disease diagnosis and treatment, which a major focal point of research studies on human diseases and the normal development of organs.

2. The process of RNA splicing

Prasanth et al.[16] believed that the size of the transcriptome can measure levels of biological complexity. RNA splicing is a central component in the process of transcription and also an important cellular mechanism leading to different protein isoforms, through removing the intron of the pre-mRNA by spliceosome and splicing regulatory elements to produce mature RNA and regulating different functions such as growth and development[17].

In the normal physiological state, many biological processes in cells are regulated by alternative splicing of upstream genes[18]. Already in 1995, Gasdaska et al.[19] demonstrated the presence of an alternatively spliced form of *NQO* mRNA in human normal lung tissues. This form lacks exon 4 and is present in nearly equal amounts to the full-length *NQO* mRNA. For instance, in the process of regulating cell apoptosis, the alternative splicing of ligands, receptors, apoptotic regulatory proteins, and apoptotic protease genes that encode apoptotic signals can all play a regulatory role in apoptosis. Some of these genes, such as *BCL-X* and *MCL-1* are found to be switched from their pro-apoptotic into anti-apoptotic splicing isoforms[20]. Besides, epithelial splicing regulatory proteins (ESRPs) can regulate epithelial−mesenchymal transition (EMT), cell migration, and connection-associated gens alternative splicing. Its targets are very extensive, including proteins from the FGFR, mitogen-activated protein kinase (MAPK), and RAPGEF families[21]. RNA splicing methods mainly include intron-splicing (including 1-type and 2-type), nuclear mRNA spliceosome splicing, and nuclear precursor tRNA enzyme-catalyzed splicing. However, the introns will still be removed, and the exons will remain in sequence. During alternative splicing, sequences are selectively excluded or included. Alternative splicing can be divided into different events: exon skipping, mutually exclusive exons, alternative 3′ and 5′ splice site selection, and intron retention[22]. The type of alternative mRNA processing contains alternative splicing and alternative poly(A) sites, and the method of alternative splicing always uses different promoters, different poly(A) sites, removing or retention intron, and exon inclusion or skipping[23].

Although some introns can be self-spliced, most of the time also require the involvement of a spliceosome. The spliceosome is a compound that consists of five snRNPs and hundreds of multifunctional peptides, assembled through a series of interactions between RNA−RNA, RNA−proteins, and protein−protein[24]. The more stable the spliceosome binds to the pre-RNA, the more likely take place alternative splicing. The recognition part of the spliceosome is composed of some conservative sequence elements at the exon−intron junction, which are represented as 5′ and 3′ splice sites[6]. The occurrence of alternative splicing begins with the identification of splice sites. Following this, the spliceosome, upon recognizing these sites, starts to assemble into a spliceosome complex. Under the function of this complex, introns are excised and exons are joined together to form a continuous RNA molecule. After the splicing process, a mature mRNA is produced, which is ultimately translated into different proteins[25,26] (Fig. 2).

RNA binding proteins (RBPs) regulate alternative splicing by interacting with pre-mRNA, and its regulatory function exhibits positional specificity, meaning that binding to different regions may have opposite functions[27]. Nevertheless, there are hundreds of RBPs in humans, and not all RBPs will directly participate in the regulation of alternative splicing. They can also interact with each other in a cooperative or antagonistic manner, affecting splice-site choices. RNA splicing can correct frameshift mutations in some genes and is a means for organisms to cope with harmful mutations. Secondly, some gene transcripts can be constructed to delete the start codon or stop codon, control gene translation, increase the complexity of higher organisms, and improve the diversity of proteins. At the same time, RNA splicing is controlled by external signals, has high tissue and stage specificity, and plays an important role in the process of cell differentiation and ontogeny. There are also interactions between AS-related factors. These interactions are complex and involve a variety of mechanisms. For instance, splicing factors like serine/arginine-rich (SR) proteins and heterogeneous nuclear ribonucleoproteins (hnRNPs) can interact with the spliceosome. SR proteins generally act as splicing activators by binding to exonic/intronic splicing enhancers (E/ISEs) to facilitate exon formation, while heterogeneous nuclear ribonucleoproteins are splicing inhibitors by binding to exonic/intronic splicing silencers (E/ISSs) to interfere with splice site recognition. Therefore, RNA splicing is significant in the regulation of complementary mechanisms in gene expression.

3. RNA splicing in respiratory diseases

Splicing dysregulation may occur as a consequence of the disease or serve as its underlying cause. It has been demonstrated to impact various lung diseases significantly, including but not limited to tumor metastasis, hyperoxia-induced lung injury, bronchial asthma, and pulmonary fibrosis. As a result, this area is garnering increasing attention and is summarized as follows (Fig. 3).

3.1. RNA splicing in lung cancer

Lung cancer remains one of the most common cancers, boasting the highest fatality rate and standing as a leading cause of cancer death[28,29]. At present, the primary treatment strategies are surgery, radiotherapy, and chemotherapy. Histologically, lung cancer is categorized into two main types: small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC), with NSCLC comprising 85% of cases[30]. NSCLC further subdivides into various histological subtypes, with the most common being lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC), and large cell carcinoma. Various factors contribute to the onset of lung cancer, including but not limited to smoking mainly, age, mis-splicing, and excessive alcohol consumption[31]. For instance, airborne particulate matter can promote lung carcinoma cell proliferation through the effect of RNA splicing[32].

In 1990, Mori et al.[33] first identified an alternative splicing variant of the RB gene in lung cancer cells, which resulted in the functional loss of the tumor suppressor protein pRB. Subsequent research revealed alternative splicing events in genes such as tumor protein P53 (*TP53*), fibroblast growth factor receptor 1 (*FGFR1*), and vascular endothelial growth factor (*VEGF*) within lung cancer cells. A significant advancement came in 2013 when Zhang et al.[34] discovered that RNA binding motif protein 10 (RBM10), which plays a role in splicing regulation, exhibited a high frequency of mutations across various tumor types, with a particularly high mutation rate of 5%−20% in lung adenocarcinoma. By

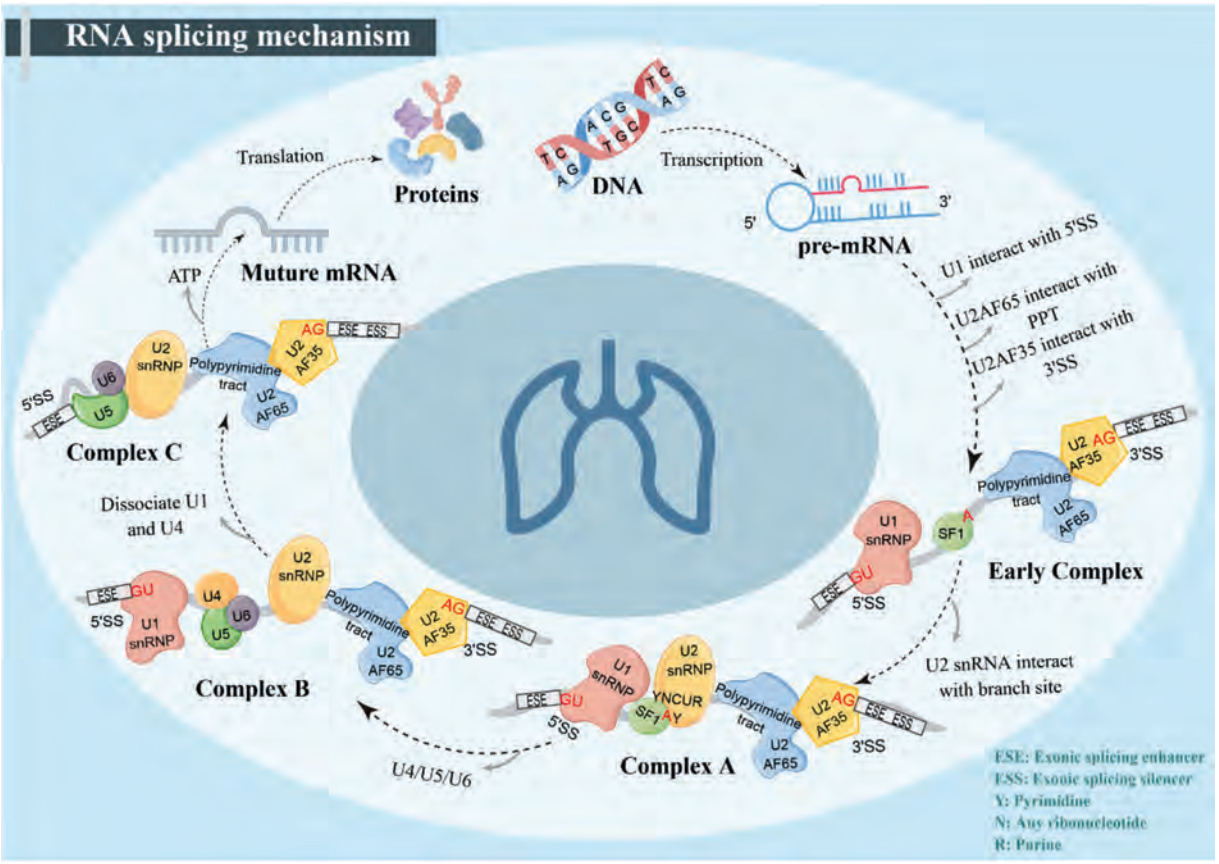

Figure 2 An overview of the major spliceosome's role in pre-mRNA splicing. At the beginning of splicing, the 5′ splicing site interacts with U1snRNP, U2AF65 interacts with the polypyrimidine region, and U2AF35 binds to the 3′ splicing site. Subsequently, U2AF65 mediated the binding of Branchpoint Binding Protein (BBP) to branching sites, forming early complexes. Subsequently, U2AF mediates U2 to replace BBP and bind to the branching site, complementing and pairing with nucleotide bases near the branching site. However, due to the inability to pair adenosine at the branching site, protrusions will form, making it easy to undergo ester transfer reactions with the 5′ splicing site. Afterward, U4 and U6 are paired through base complementarity, while U5 is added to the complex through protein interactions. After releasing U1, U6 pairs with the 5′ splicing site. Then U4 is released, in which base complementary pairing occurs between U2 and U6, generating a catalytic active site, making the 5′ splice site close to the branching site, and completing the first transesterification reaction. U5 promotes the proximity of 5′ and 3′ junction sites, connecting the two exons, and finally releasing mature mRNA and snRNPs.

2024, numerous targets for tyrosine kinase inhibitors in lung cancer treatment had been identified, including *EGFR*, *RET*, *NTRK*, and *MET*. Consequently, RNA splicing has emerged as a critical factor influencing biological functions, immune infiltration, and therapeutic strategies for lung cancer. Simultaneously, RNA splicing can predict tumor type and stage, and be used as biomarkers and treatment targets because some splicing products are specifically expressed in cancerous tissues. In the following sections, we will classify the events based on the alternative splicing molecular mechanisms (Table 1[35–59]).

3.1.1. Chromatin and transcriptome dependence

Gene-related mechanisms are primarily orchestrated through transcription and mRNA precursor processing, with co-transcriptional splicing playing a crucial role in enhancing splicing efficiency. A wealth of research has established a link between alternative splicing regulation and RNA polymerase II activity. This process is regulated by promoter activity, chromatin state, and splicing factors recruited by RNA polymerase II[4]. Consequently, alterations in transcription and chromatin states associated with cancer can significantly affect alternative splicing. In LUAD, a typical example is the direct activation of *SRSF1* transcription by MYC, which occurs through two noncanonical E-boxes in its promoter. This activation increases SRSF1 protein levels, leading to SRSF1-mediated alternative splicing of signaling kinase *MKNK2* at the 3′ exons and transcription factor *TEAD1* at exon 5[60]. It is also known that VEGF, crucial for angiogenesis, is overexpressed in tumors and produces angiogenic isoforms *via* alternative splicing. Activation of SRSF1 by *Wt1* and *PKC* enhances pro-angiogenic VEGF expression in tumor endothelium. The LUAD-specific *VEGFxxxb* variant boosts tumor growth and invasion through the VEGFR pathway, and its inhibition can slow tumor progression[61].

Furthermore, methylation, a type of chromatin modification, has been demonstrated to enhance the binding capacity of hnRNPC. This enhancement impedes the binding of SRSF1, thereby influencing intron processing. Xie et al.[62] discovered that METTL16, an N^6-methyladenosine (m^6A) RNA methyltransferase, facilitates the development of lung cancer and Cr (VI) carcinogenesis *via* glutamine biosynthesis and the expression of glutamate-ammonia ligase (GLUL). Additionally, TARBP2, the RNA binding protein controls RNA stability within nuclei and

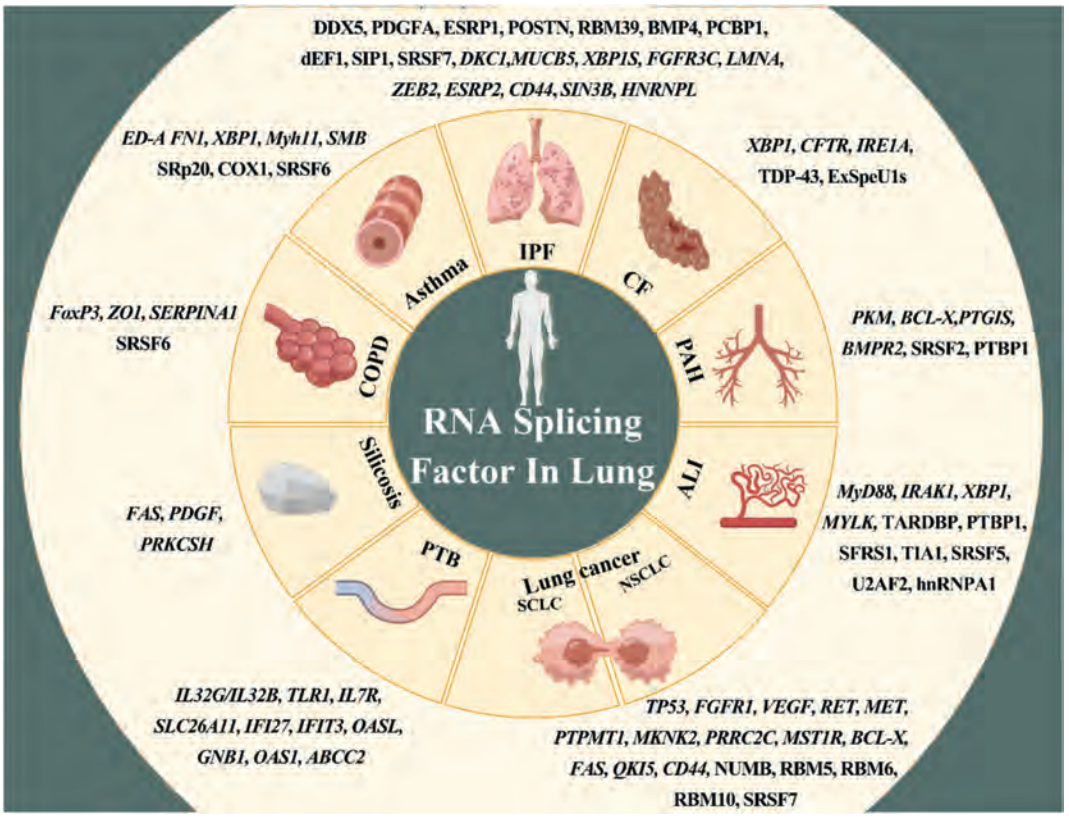

Figure 3 AS-related splicing factors and RBPs in lung diseases. AS, or alternative splicing, is a crucial process that takes place after transcription in gene expression. It allows for the production of multiple mRNA variants from a single gene, resulting in a wider range of protein diversity. RNA-binding proteins (RBPs) associated with AS play a significant role in determining the fate of mRNAs through various functional mechanisms. In particular, selective splicing and RBPs have been found to play important roles in a variety of lung diseases, including IPF, CF, PAH, ALI, lung cancer, PTB, COPD, asthma, and silicosis.

affects lung tumor growth, and this is dependent on TARBP2-mediated the expression of ABCA3 and FOXN3. TARBP2 binding to pre-mRNAs leads to intron retention increasing and recruits m^6A methylation machinery to decrease transcript stability[63]. These epigenetic changes can have profound effects on gene expression and alternative splicing patterns, which are often disrupted in cancer. Moreover, the splicing factor neuro-oncological ventral antigen 1 (NOVA1), which is specifically expressed in various human cancer cells, enhances *hTERT* splicing by binding to the DR8 region, thereby including exons 7 and 8 that code for the RT domain in NSCLC. Silencing *NOVA1* results in telomere shortening, decreased telomerase activity, shifts in *hTERT* splicing to non-catalytic forms, and affects *hTERT* transcript production[64]. The regulation of the lung cancer transcriptome is heavily influenced by RNA splicing. PRPF8 abundance limits the splicing of human genes with weak 5′ splice sites, suggesting a tight coupling between RNA splicing and transcription. *PRPF8* depletion may partially decouple these processes, providing additional time for intron removal at weak sites[65].

3.1.2. Mutations in splicing-related regulatory sequences and single nucleotide polymorphism

Mutations in single nucleotide polymorphisms (SNPs) do not directly affect the structure or function of proteins. However, mutations of SNP that alter *cis*-acting sites of exons and adjacent introns are among the multiple factors influencing AS, thereby affecting protein expression[66]. Notably, many tumor suppressors, including *TP53*, *ARID1A*, *PTEN*, *CHD1*, *MLL2*, and *PTCH1*, are impacted by mutations at the exon-intron boundary[67]. For instance, *TP53* mutations have a significantly negative effect on the prognosis in LUAD cases[68]. *TP53* encodes the p53 protein, whose primary function is to regulate the cell cycle in response to DNA damage. The loss of *p53* function serves as a mechanism for tumor cells to evade apoptosis and promote cellular proliferation[69,70]. *Mdm2* and *MdmX* are two key repressors of p53. A decrease in the MdmX protein level contributes to p53 activation in response to targeting the spliceosome[71].

According to research by Mironov et al.[72], chromosome 3p21.3, where the *RBM5* and *RBM6* genes are located, is frequently deleted in lung cancers. Furthermore, the sequence of the *RBM10* gene revealed a T-to-A substitution at position 1062, which replaces valine 354 with glutamic acid in A549 cells. Previous studies have indicated that changing methionine to isoleucine at residue 276 (M276I) of MORC2 enhances the combination of MORC2 and heterogeneous nuclear ribonucleoprotein M, a spliceosome, therefore, promotes cell migration, invasion, and lung metastasis. Moreover, this progress facilitated the switch of *CD44* from epithelial isoform (*CD44v*) to mesenchymal isoforms (*CD44s*), thereby accelerating EMT[73]. When zinc finger E-box binding homeobox 1 (ZEB1) expression in HBECs was inhibited, epithelial splicing regulatory protein 1 (ESRP1) was directly repressed, resulting in an increase in the expression of the mesenchymal splice variant of *CD44* and a more invasive behavior[74].

Table 1 Splicing events in lung cancer.

Gene	Splicing factor	Mechanism	Type or role in lung cancer	Ref.
PTPMT1	SRSF1	SRSF1 modulates *PTBP1* exon3 skipping	Non-small cell lung cancer	35
lncRNA ABHD11-AS1	CD44	Increase *CD44v6* level	Cell malignant transformation	36
RBP	QKI	*rs156697-G* variant	Non-small cell lung cancer cells proliferation	37
CD44	TRA2β	Promote exon 4 and exon 5 inclusion of gene *CD44*	Tumor progression	38
NUMB	QKI	Represses the splicing of *ADD3* exon 14	Non-small cell lung cancer	39
FN1	QKI	*ED-B* upregulate	Non-small cell lung cancer	40
NUMB	RBM10	Exon 11 contains increased	Non-small cell lung cancer	41
TP53	RBM5	Deletion at chromosome 3p21.3	Apoptosis and cell cycle arrest	42
BCL2L1	RBM4	Shift *BCL-XL* into *BCL-XS*	Non-small cell lung cancer	43
BCL2L1	SRSF1	SRSF1 binds to E2P just downstream of *PTBP1* in *BCLX*	Repress apoptosis	44
C-FLIP	RBM5	Enhances chemosensitivity	Apoptosis	45
MENA	PTBP1	Exon11a skipping	Lung cancer migration	46
FBXO5	PTBP1	Silencing of *PTBP1* enhanced the skipping of exon 3 in *FBXO5*	Lung adenocarcinoma	47
hnRNPM	PARP4	Dysregulated intronic splicing	Lung adenocarcinoma	48
KAT2A	SF3B4	An alternative splice site occurred at the 5′-UTR of *KAT2A*	Lung adenocarcinoma	49
hnRNPL	MYO1B	The inclusion of *MYO1B* exon 23	Lung adenocarcinoma	50
LncRNA BC	IMPAD1	Regulated the assembly of the splicing-regulator complex	Lung adenocarcinoma	51
MDM2	SRSF1	Induces exclusion of *MDM2* exon11	Non-small cell lung cancer	52
PARP1	USP15	Gefitinib resistance	Non-small cell lung cancer	53
TRA2B	SRSF1	Promote exon 2 inclusion of *TRA2β1* gene	Non-small cell lung cancer	54
FGFR2	hnRNP A1	Repress exon8 or *FGFR3b* inclusion	Non-small cell lung cancer	55
VEGFA	SRSF2	Promote the use of *VEDF165*	Non-small cell lung cancer	56
MKNK2	SRSF1	Modulate exon 14 to form *MKNK2a/b* isoform	Non-small cell lung cancer	57
FAS	RBM5	Promotes the skipping of exon6 of *CD95*	Non-small cell lung cancer	58
MST1R	SRSF1 /hnRNP H	Prevent the skipping of *RON* exon 11 and inhibit the production of RON	Non-small cell lung cancer/Small cell lung cancer	59

3.1.3. DNA damage

DNA damage, such as that caused by radiation and drugs, triggers alternative splicing of genes associated with cellular processes such as apoptosis, cell cycle regulation, and DNA repair[75]. Upon DNA damage, splicing undergoes various modifications, including post-transcriptional changes, which in turn influence interactions with other proteins or RNAs. For instance, DNA damage can lead to dephosphorylation of SRSF10, inhibit the interaction of hnRNP F/H, change the splicing of *BCL-X*, and enhance the ability to promote apoptosis[76]. Moreover, splicing modulator E7107 induces selective apoptosis and high expression of *MCL1* or *BCL-2A1* and combined inhibit splicing and BCL-XL induces synergistic cytotoxicity in cancer cells[77]. In NSCLC cells treated with MDA7/IL24, the ratio of *BCL-XL/BCL-XS* mRNA and protein expression is decreased, reducing the viability and resulting in increased cell death[78]. Additionally, Fan et al.[79] discovered that *SNRPA* knockout induces *ERCC1* exon 8 skipping and diminishes ERCC1−XPF complex formation, thereby reversing cisplatin resistance and enhancing DNA damage repair in LUAD. Given that splicing is crucial to DNA damage repair, splicing-related changes can lead to genomic instability and mutation accumulation.

3.1.4. Mutated spliceosome components and altered expression of splicing factors

Mutations in spliceosomes or dysregulated RNA splicing in cancer genes are increasingly recognized as cancer biomarkers[80]. Many hallmarks of cancer are associated with splicing events, which explains lung cancer's high complexity of aberrant alternative splicing. Several mutations are commonly observed in non-small cell lung cancer, including *SF3B1*, *U2AF1*, *SRSF2*, *ZRSR2*, and *RBM10*. A recent analysis found that *RBM10* mutants were associated with alternatively splicing protein NUMB isoforms in cancer cell lines. The *RBM10* mutation diminishes the ability to regulate *NUMB* exon 9 skipping, whereas the overexpression of RBM5, RBM6, and RBM10 enhances *NUMB* 11 exon alternative splicing and fosters tumor proliferation in NSCLC[72,81]. Moreover, *PRMT5* deletion can activate the *p53* transcriptional response, but this function could be rescued by reintroducing full-length *MDM4* or inhibiting the expression of the *MDM4* splicing exon 6 skipping variant[82].

Contrary to mutations, changes in the expression levels of splicing factors are more often the culprits behind splicing events. Such altered expression is primarily responsible for causing alternative splicing, which in turn leads to lung diseases[43]. Specifically, SR proteins, RNA binding motif (RBM) proteins, hnRNP, and QKI stand out as four well-established RNA splicing factors.

The SR protein family, known for its role in regulating proto-oncogenes and tumor suppressors, is often overexpressed in malignant tumors and plays a pivotal role in RNA metabolism and splicing[83]. For instance, SRSF5 facilitates the splicing of *CCAR1* into *CCAR1S*, enhancing glucose uptake and acetyl-CoA synthesis, which promotes tumor growth[84]. Moreover, there is evidence to suggest that SRSF1 modulates the splicing of proline-rich coiled-coil 2C (*PRRC2C*), increasing its proliferative capacity and resistance to apoptosis in NSCLC[85,86]. SRSF1 has also been implicated in binding to Bridging Integrator 1 with exon 12A inclusions, promoting tumor progression[87]. Conversely, reduced SRSF1 levels elevate AMPK phosphorylation, modulate protein

tyrosine phosphatase, mitochondrial 1 (*PTPMT1*) splicing, and sensitize cancer cells to irradiation[82]. Additionally, hnRNP A1 counteracts SRSF1, preventing the production of an active isoform that activates the EMT antagonist[88]. hnRNP E1 also interacts with *PNUTS* pre-RNA, regulating its alternative splicing and EMT progression[89]. Under glucose stress, *circZFR* protects hnRNPLL from degradation, leading to the inclusion of *MYO1B* exon 23 and enhancing OXPHOS *via* AKT−mTOR signaling to promote tumor growth[50]. Notably, RBM4 specifically promotes the production of the *BCL-XS* isoform by competing with SRSF1, thereby inhibiting cancer cell proliferation, migration, and inducing apoptosis[43].

QKI, a splicing factor that is notably downregulated in lung cancer, has three main isoforms: *QKI-5*, *QKI-6*, and *QKI-7*[90]. QKI serves an inhibitory function in lung cancer by constraining the splicing of *Nume11* exons and modulating the splicing of fibronectin into three distinct isoforms[91]. Specifically, the *ED-B FN1* isoform is predominantly regulated in NSCLC[92]. As previously reported, QKI-5 specifically downregulates *ADD3* exon 14, enhancing lung cancer survival[39]. Qin et al.[93] in 2018 discovered that low *circUBR5* expression in NSCLC correlates with poor tumor differentiation. *CircUBR5* interacts with QKI, NOVA1, and U1 small nuclear RNA (snRNA), participating in the RNA splicing regulatory process.

Additionally, other splicing factors like polypyrimidine tract-binding protein 1 (PTBP1) play a crucial role in lung cancer by modulating alternative splicing events. PTBP1 activity can lead to the skipping of *Mena* exon 11a, enhancing lung cancer cell migration, invasion, and EMT progression[46]. In its absence, PTBP1 causes the exclusion of F-box protein 5 (*FBXO5*) exon 3, resulting in the formation of the *FBXO5-S* variant, which reduces FBXO5 expression and is associated with LUAD senescence[47]. Additionally, the poly(U)-binding splicing factor PUF60 promotes lung cancer progression by inhibiting the exon 3 skip of *CDC25C*, while SFPQ up-regulates *PD-L1* expression by binding to its 3′UTR, inhibiting NSCLC cell cytotoxicity[94,95]. Furthermore, the absence of pre-mRNA splicing factor 4 kinase (*PRP4K*) disrupts *EGFR* gene trafficking, reducing cancer cell growth[96]. Moreover, fascin actin-bundling protein 1 (FSCN1) modulates the alternative splicing of *NME4*, *NCOR2*, and *EEF1D*, affecting the expression of ACTG1, KRT7, and PDE3A, which are highly abundant in NSCLC[97].

These examples not only enrich our understanding of the upstream mechanisms driving tumorigenesis in lung cancer but also shed light on the intricate pathways involved in the development of the disease, thereby underscoring the pivotal role of RNA splicing.

3.2. *RNA splicing in cystic fibrosis*

Cystic fibrosis (CF) is a genetic disorder primarily caused by mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene. These mutations compromise the structure of the CFTR protein, leading to imbalances in airway fluid and subsequent lung function impairment. The majority of CF-related fatalities arise from bacterial infections, predominantly caused by *P. aeruginosa,* which lead to chronic respiratory tract infections and ultimately, respiratory failure[98]. Research has indicated that CF is associated with mutations or variations in either *trans*-acting factors or *cis*-acting elements, resulting in abnormal protein production and splicing patterns. Modulating mRNA splicing has emerged as a promising anti-inflammatory treatment strategy for airway disease in CF. For instance, a study by Buratti et al.[99] in 2001 demonstrated that overexpression of TDP-43 led to the skipping of exon 9 in the *CFTR* gene while inhibiting endogenous *TDP-43* expression corrected this aberrant splicing in CF patients. Moreover, the *CFTR* c.2657+5G > A splicing mutation, primarily located near intron 16, results in transcripts lacking exon 16 compared to their wild-type counterparts. The antisense oligonucleotide (ASO) approach has been employed to prevent exon 16 skipping, thereby modulating splicing to restore both normal *CFTR* transcript levels and functional CFTR protein expression[100].

Another noteworthy *CFTR* splicing mutation introduces a new 5′ splice site, leading to splicing into a recessive exon that contains an early stop codon. However, the application of ASO and splice-switching oligonucleotides (SSOs) has been shown to correct this abnormal splicing in primary bronchial epithelial cells, thereby improving CFTR activity and restoring chloride secretion[101]. Furthermore, CFTR modulators such as Kalydeco® (Ivacaftor/VX-770) and Symdeco® (Tezacaftor/Ivacaftor), which are ASO-based drugs, have received approval for treating CF patients carrying the 3849 + 10 kb C-to-T splicing mutation[102]. In the context of inflammation in CF, a study highlighted the role of inositol-requiring enzyme 1α (IRE1α) kinase and RNase activity in the mRNA splicing of X-box binding protein-1 (*XBP-1s*). This splicing event subsequently promotes the production of inflammatory cytokines in CF airway epithelial cells. The compound KIRA6 has been shown to dose-dependently inhibit *XBP1* splicing by targeting IRE1α kinase and RNase activities, offering another avenue for therapeutic intervention[103].

The alternative splicing switch involving *Zip2/SLC39A2* interacts with CFTR, ENaC, and ZIP2 transporters, and triggers the expression of ENaC and MUC5AC, contributing to CF-associated mucus hypersecretion phenotype[104]. Studies have shown that the introduction of premature termination codons (PTCs) can impact the synthesis of the CFTR protein[105]. Moreover, the use of *AsCas12a* for allele-specific genome editing has been demonstrated to correct splicing defects in various cell types. Specifically, it has been effective in correcting aberrant CFTR splicing mutations such as 3272−26A > G and 3849 + 10 kb C > T in primary airway epithelial cells and intestinal organoids derived from CF patients[106]. Changes in simple repeat length, such as UG duplication, can also influence the splicing pattern of genes, leading to abnormal splicing of exon 9 in the *CFTR* gene, thereby contributing to the pathology of CF[99]. Gene therapy approaches have also been employed to correct abnormal splicing. For instance, the use of exon-specific U1 small nuclear RNAs (ExSpeU1s) in conjunction with the intron sequence downstream of each defective exon has been shown to effectively correct diseases caused by *CFTR* mutations after exon skipping, restoring the function of the CFTR protein[107]. These examples underscore the importance of understanding genetic mutations and their impact on splicing regulation, as they offer valuable insights into the pathophysiology of cystic fibrosis.

3.3. *RNA splicing in airflow restriction disease*

3.3.1. *RNA splicing in chronic obstructive pulmonary disease*

Chronic obstructive pulmonary disease (COPD) is a complex respiratory condition that encompasses chronic bronchitis and emphysema, characterized by irreversible airflow obstruction[108]. It holds the grim distinction of being the fourth leading cause of death globally, with a high prevalence and morbidity rate[109]. In addition, statistical tests revealed that COPD was notably the only disease in the United States that continued to rise from 1965 to 1988[110]. Smoking, including passive exposure, is a primary risk

factor for COPD, with the risk escalating in proportion to the duration of smoking[111]. Genome-wide association studies have revealed that genes associated with COPD exhibit a high level of transcriptional diversity and complexity compared to genes implicated in other diseases[112]. RNA alternative splicing can modulate the expression of genes involved in inflammatory responses, thereby influencing the chronic inflammation that characterizes COPD, affecting the airways, lung parenchyma, alveoli, and pulmonary blood vessels. Parker et al.[113] have shown through RNA sequencing that smoking can alter isoform expression and modify the number of exons, thereby affecting the transcriptome. Moreover, smoking exerts anti-inflammatory effects by downregulating the expression of the pro-inflammatory gene AGE and upregulating the relative abundance of the anti-inflammatory isoform known as endogenous secretory receptors for advanced glycation end products (esRAGE). Patients with COPD often exhibit reduced levels of alveolar replacement fluid and blood esRAGE, indicating that esRAGE signaling plays a crucial role in the pathophysiology of COPD[114].

In the realm of genomics, SNP has traditionally been employed for genomic variation analysis, including nucleic acid quantification and the identification of alternative splicing events[115]. With the advent of RNA sequencing, new insights have emerged, such as the identification of splicing sites and susceptibility factors in the *FBXO38* gene concerning COPD. The F-box protein FBXO38 interacts with substrates to fulfill its function and co-activates the kruppel-like transcription factor 7 (*KLF7*), which plays a role in regulating splicing[116]. The KLF family itself is implicated in various cellular processes, including cell growth, EMT, differentiation, activation, and airway inflammation[117].

High expression of FoxP3-E2, a variant containing exon 2 of the *FoxP3* gene, has been associated with the progression of COPD, leading to lung inflammation and compromised lung function[118]. Chen et al.[119] demonstrated that *miR-133a* promotes airway EMT *via* alternative splicing progression, with ESRP1 serving as a central coordinator for these events. Additionally, A-1-antitrypsin deficiency, caused by abnormal serpin family a member 1 (*SERPINA1*) transcript isoforms, has been implicated in COPD[120,121]. Emerging evidence also suggests that alternative splicing of extracellular viral matrix proteins, such as nephronectin (NPNT), may play a role in the pathogenesis of COPD[122]. Indacaterol, a splicing-associated drug with low side effects, has shown promise in COPD treatment. It is believed to inhibit SRSF6 by binding to the RRM2 domain, thereby promoting the formation of the *ZO-1* exon23 inclusion isoform and inhibiting the *ZO-1* exon23 skipping isoform in alternative splicing events[123]. These findings open new avenues for the treatment of cigarette smoke-related lung diseases, including COPD.

3.3.2. RNA splicing in asthma

Asthma is a chronic inflammatory respiratory disorder primarily regulated by T helper 2 (TH2) cells in the immune system[124]. The initial identification of immunoglobulin-like-transcript 3 (ILT3) splicing isoforms in bronchial asthma was reported in 2000[125], and the regulatory role of SFRS8 in *CD45* alternative splicing, impacting T cell activation and asthma pathogenesis, was first described by Brasch-Andersen et al.[126] in 2006. Emerging evidence suggests that isoforms generated through alternative splicing of inflammation-related genes can play a pivotal role in asthma pathogenesis. For instance, an isoform of *IL33* produced by alternative splicing has been shown to activate basophils and mast cells, leading to the production of type 2 inflammatory factors and the initiation of chronic asthma[127]. The interplay between bronchial epithelial cells and immune responses has emerged as a promising avenue for identifying novel therapeutic targets in asthma. According to research by Carlini et al.[128], damage to human leukocyte antigen G (*HLA-G*) isoforms can lead to inflammation and structural degradation of bronchial epithelial cells. Moreover, endoplasmic reticulum stress has been implicated in the aberrant splicing of the *XBP1* gene in mild asthma, and this aberrant splicing is associated with the asthma-active gene interferon-stimulated gene (*ISG*)[129]. Asthma occurrence is also regulated by the splicing of SR proteins. A study in 2018 found the upregulation of SRSF6 and SRSF1 in asthmatic airway smooth muscle (ASM) cells and demonstrated that SRSF6 induces the inclusion of exon 5b in the myosin heavy chain 11 (*Myh11*) self-multilating behavior (*SMB*) gene, which enhances smooth muscle contraction velocity and provides relief in asthma symptoms[130]. The *SFRS8* gene also regulates the splicing of *CD45* in asthma and atopy, while *CD45* is a crucial molecule in T cell activation[131].

In clinical studies examining fibroblasts in the airway lumen, elevated expression levels of smooth muscle actin (SMA), serine−arginine splicing factor 20 (SRp20), and EDA fibronectin (an isoform of FN1) have been observed[132]. In our research, we identified that the long non-coding RNA (lncRNA) *PFI* plays a regulatory role in pulmonary fibrosis. Specifically, *PFI* binds to SRSF1, inhibiting both its expression and activity, and consequently suppressing the production of *EDA + Fn1* splicing isoforms[133].

Cyclooxygenase-1 (COX-1) is a critical enzyme in the biosynthesis of prostaglandins, and its activity is inhibited by aspirin, leading to reduced prostaglandin synthesis. A groundbreaking discovery in 2007 revealed that individuals with aspirin-sensitive asthma are more likely to exhibit a 111-nucleotide deletion in exon 9 of the cyclooxygenase-1 gene in their airway epithelial cells[134]. These findings collectively indicate the significant role of alternative splicing in the pathogenesis of asthma and highlight its potential as a target for novel therapeutic interventions.

3.4. RNA splicing in interstitial lung disease

3.4.1. RNA splicing in silicosis

Silicosis is a severe, irreversible, and rapidly progressing pulmonary disease primarily induced by chronic inhalation of substantial amounts of free silica dust, leading to extensive nodular fibrosis in the lungs[135]. In 2000, Otsuki et al.[136] first discovered that the serum levels of alternative splicing variant soluble Fas (*sFas*) were elevated in silicosis patients and that *sFas* played a significant role in the immune dysfunction observed in these patients. Recent studies have begun to elucidate the role of circular RNAs (circRNAs) in silicosis. CircRNAs are primarily formed due to splicing errors that connect the 3′ end of an upstream exon to the 5′ end of a downstream exon *via* back-splicing[137]. For instance, Fang et al.[138] discovered that *circHECTD1* competes with its pre-mRNA to regulate HECTD1 protein levels, thereby promoting EMT *in vitro* following exposure to silicon dioxide (SiO_2). This suggests a potential mechanism for fibrosis in pulmonary silicosis and offers a new avenue for therapeutic intervention. Patients with silicosis often exhibit abnormalities in their immune systems. Research has indicated that alternative splicing isoforms of the *FAS* gene in the serum of silicosis patients can impact alveolar macrophage apoptosis and immune regulation[136]. Specifically, in the FAS gene isoform that lacks exon 6, competition between membrane-bound Fas (*mFas*) and Fas ligand (*FasL*) inhibits apoptosis, thereby influencing autoimmunity[139].

Zhao et al.[140] used the spliced transcripts alignment to a reference (STAR) method to analyze alternative splicing events in rats treated with saline or silica. The study revealed that exon skipping was the predominant form of alternative splicing, accounting for the majority of events, while other types of splicing isoforms constituted less than 20% of the total alternative splicing events.

Furthermore, the complexity and diversity of splicing alterations are reported to be closely related to silica exposure. Several splicing factors have been implicated in the pathogenesis of silicosis. For example, *PDGF* is a critical growth factor that stimulates myofibroblast replication and proliferation during tissue injury. Elevated serum levels of tumor necrosis factor alpha (TNFα) and PDGF-D have been shown to correlate with the severity of pneumoconiosis. This is particularly relevant as, post-exposure to inorganic dust, chemokines like TNFα induce the aggregation of chemotactic neutrophils (PMN), which in turn cause endothelial cell damage and stimulate fibroblast growth and collagen secretion. In another study, Zhang et al.[141] identified *Mdig*, a dust-induced gene, and found alternatively spliced transcripts of this gene in cells from the A549 lung cancer model. Huang et al.[142] discovered two alternatively spliced transcripts of protein kinase C substrate 80K-H (*PRKCSH*), namely *PRKCSH-1* and *PRKCSH-2*, which exhibited distinct expression patterns and functions in silica-induced EMT. These findings collectively highlight the pivotal role of upstream signaling pathways and variations in gene splicing in the development and progression of silicosis. Advances in genetic detection techniques offer the potential for identifying individuals at elevated risk for developing pneumoconiosis, thereby enabling early intervention and management.

3.4.2. RNA splicing in idiopathic pulmonary fibrosis

Idiopathic pulmonary fibrosis (IPF) is a complex, diffuse fibrotic lung disease that causes a wide range of health problems and impacts certain proteins and their structure[143]. The etiology and treatment modalities for IPF remain largely elusive. Alternative splicing is crucial for alveolar regeneration and treatment in IPF. Research has found that after bleomycin-induced lung injury, the alternative splicing variant *ΔNp63* of *P63* is activated, initiating a cytokeratin 5 remodeling program to promote alveolar regeneration[144]. Liu et al.[145] recently identified that DDX5 plays a pivotal role in curbing fibroplasia by regulating the content of *Fn1* exon 25-containing variants and *Plod2* exon 14-containing variants in pre-mRNA, highlighting the intricate link between AS events and fibrotic processes. One of the genetic risk factors implicated in IPF is the polymorphism in the promoter region of the *MUC5B* gene, which has previously been identified as a critical gene enriched in the bronchial epithelium across various lung diseases. Intriguingly, spliced *XBP1S* mRNA has been shown to selectively bind to the *MUC5B* promoter region containing rs35705950 minor allele "T", thereby inducing *MUC5B* expression in distal bronchiolar epithelial cells. However, this binding does not lead to the induction of MUC5AC expression[146]. Zhang et al.[147] further explored the role of alternative splicing in IPF and found that inhibiting the alternative splicing or protein *N*-glycosylation of *XBP1* could suppress the secretion of extracellular matrix (ECM) proteins during mesenchymal transitions, thereby mitigating airway remodeling.

In the progression of IPF, the role of ECM splicing is increasingly recognized as pivotal. Tracy Nance et al.[148] employed RNA sequencing to identify splicing alterations in periostin and collagen 6α3, implicating these changes in the progression of IPF. Moreover, a specific splice variant lamin A/C (*LMNA*) has been found highly expressed in IPF, which has a new *LMNA* splice variant that includes retained introns 10 and 11 and exons 11 and 12. Yin et al.[149] found that stiff ECM environments induce the generation of this novel isoform. Inhibition of pulmonary fibrosis by hnRNPL is caused by its activation of circular RNAs ankyrin repeats domain 42 (*ANKRD42*) back-splicing and through biochemical signaling and mechanical stiffness[150].

Additionally, secreted proteins like FGF, PDGFA, and POSTN are subject to alternative splicing, switching to subtypes with enhanced profibrotic functions. These spliced variants can release additional growth factors or bind to fibroblast surface receptors, leading to abnormal ECM deposition, lung tissue remodeling, and ultimately, the onset of pulmonary fibrosis. For instance, Chen et al.[151] provide evidence that BLM inhibits ESRP1 expression, resulting in *FGFR* alternative splicing, producing more profibrotic isoforms of *FGFR3c*.

In the alveolar epithelium, splicing can regulate the occurrence of fibrotic processes, particularly in the context of cellular damage and repair mechanisms. One noteworthy example is the homozygous mutations in surfactant-associated protein (*SFTPA1*), which have been reported to induce the death of type 2 alveolar epithelial cells through JNK-mediated inhibition of RIPK3 expression, ultimately leading to the onset of IPF[152]. In the realm of tissue repair mechanisms, the ratio of *VEGF-Axxxa* to *VEGF-Axxxb* from AT2 cells has been suggested to be a key indicator. Barratt et al.[153] demonstrated that overexpression of *VEGF-A165b* could inhibit the development of pulmonary fibrosis, highlighting the potential therapeutic role of *VEGF* isoforms in fibrotic lung diseases. Another intriguing aspect is the role of decreased BMP signaling in the etiology of fibrosis. Specifically, the knockdown of RBM39 has been shown to inhibit BMP4-induced shifts in *SIN3B* expression towards its long isoform. This is significant because the short isoform of *SIN3B* is unable to recruit histone deacetylases (HDACs)[154]. In the context of damaged alveolar epithelium, HDACs are known to play a role in the repair process by modulating the expression of fibrosis suppressor genes.

Gene polymorphisms are a primary driver of differential gene expression, and their impact extends to the regulation of alternative splicing pathways that are critical in diseases like IPF. For instance, a specific polymorphism involving the substitution of base G with C at position +915 changes codon 25 from encoding arginine (Arg) to proline, leading to decreased synthesis of TGFβ. Tripathi et al.[155] found that TGFβ through a phosphorylated T179 mediated by SMAD3, interacts with RNA-binding protein PCBP1 to directly regulate *CD44* alternative splicing. This SMAD3-promoted alternative splicing pathway is implicated in EMT and fosters TGFβ-driven tumor growth. The ERK/EGR1 signaling pathway also plays a role in TGFβ induced lung fibroblasts by initiating transcriptional activation that regulates the splicing of *CD44v6*, a *CD44* isoform containing variable exon 6[156]. Moreover, the *ZEB2/ESRPs* axis can be modulated by transmembrane 4 L six family member 5 (TM4SF5) to induce and regulate *CD44v8-10* splicing variants, offering a potential target for IPF treatment[157]. Beyond these well-known genes, mutations in *DKC1* also have a significant impact on splicing. These mutations create competition for newborn exon-splicing enhancers, leading to the degradation of mis-spliced products through nonsense-mediated decay. This, in turn, affects dyskerin levels and results in telomerase RNA deficiency, contributing to familial pulmonary fibrosis[158].

Additionally, EMT in pneumocytes has been identified as a significant contributor to the progression of IPF, and targeted therapies against EMT are emerging as potential treatment modalities[159]. The TGFβ/Smads signaling pathway is particularly noteworthy in this context, as it has been linked to lung fibrosis. Individuals with higher levels of TGFβ expression are more susceptible to developing pulmonary fibrosis, underscoring the variability in TGFβ expression among different people. Additionally, Horiguchi et al.[160] provided further insights into the role of TGFβ in EMT. They found that TGFβ upregulates the expression of dEF1 and SIP1 proteins from the dEF1 family. These proteins, in turn, significantly inhibit the transcription of *ESRP2* by binding to its promoter in NMuMG cells. The downregulation of ESRP leads to alterations in splicing patterns, which subsequently modulate TGFβ-induced EMT. These findings collectively emphasize the importance of EMT and its associated post-transcriptional regulation, particularly TGFβ-mediated alternative splicing, in the pathogenesis and potential treatment of IPF.

3.5. RNA splicing in pulmonary arterial hypertension

Pulmonary arterial hypertension (PAH) is a specialized form of pulmonary vascular disease characterized by hemodynamic abnormalities and pathophysiological alterations. It is often accompanied by severe lung inflammation and can co-occur with other complex diseases. One of the key mechanisms underlying the vascular remodeling observed in PAH is the hyperproliferation and resistance to apoptosis of pulmonary artery smooth muscle cells (PASMCs) and endothelial cells (PAECs). AS contributes to pulmonary vascular remodeling and vasoconstriction, impacting the progression of pulmonary vascular disease. *MiR-124* has been identified as a critical microRNA that is downregulated in both pulmonary vascular cells and circulating progenitor endothelium in PAH patients[161]. This downregulation has significant implications for cellular metabolism, proliferation, and inflammation. Specifically, *miR-124* modulates the *PKM2/PKM1* ratio through its interaction with the alternative splicing factor PTBP1[162]. *PKM1* includes exon 9, whereas *PKM2* includes exon 10, with the difference depending on the specific splicing of pyruvate kinase muscle (*PKM*) pre-mRNA. *CircSMOC1* also has been shown to alleviate pulmonary vascular remodeling by directly interacting with PTBP1, which competitively inhibits alternative splicing of *PKM* pre-mRNA and results in elevated PKM2 expression[163]. In addition to microRNA or circular RNA-based regulation, alternative splicing of ion channels also offers a promising avenue for modulating PAH. For instance, Galectin-1 has been shown to regulate the function of the CaV1.2 calcium channel in the cardiovascular system through alternative splicing, thereby influencing arterial contractile function[164].

Additionally, changing the proportion of protein subtypes produced by alternative splicing is another way to regulate PAH. One such example involves the bone morphogenetic protein receptor 2 (BMPR2), which can be selectively spliced to produce various isoforms. The splicing factor SRSF2, when upregulated, inhibits the production of a *BMPR2* isoform that lacks exon 12, thereby influencing the isoform ratio and contributing to heritable PAH[165]. Moreover, the inhibition of BMPR2 expression has been shown to regulate the alternative splicing of the apoptosis regulator *BCL-X* (B-cell lymphoma X) transcript, specifically promoting the expression of the anti-apoptotic *BCL-XL* isoform in PASMCs, which in turn impacts apoptosis pathways in PAH[166]. Another layer of complexity is added by m^{6}A RNA modifications, which have been significantly correlated with the onset of pulmonary hypertension. RNA-binding proteins like hnRNP interact with RNA polymerase II and bind to m^{6}A-modified RNA, thereby accelerating alternative splicing processes[167]. Rare variants in the gene encoding prostacyclin synthase (*PTGIS*) have been implicated in reduced prostacyclin production and increased cell death in pulmonary microvascular endothelial cells, contributing to pulmonary hypertension[168]. These findings underscore the importance of understanding the role of alternative splicing and its regulatory factors in PAH. By analyzing changes or deletions in the expression of splicing factors and identifying genetic mutations, researchers can pave the way for more effective treatments for PAH.

3.6. RNA splicing in pulmonary tuberculosis

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (MTB), remains a significant global health concern, affecting nearly a quarter of the world's population. Accurate and timely diagnosis is crucial for effective management of the disease[169]. The mechanism of RNA alternative splicing in pulmonary tuberculosis encompasses regulation of the host immune response, effects on MTB-infected macrophage behavior, and its role as potential biomarkers. One notable example is the differential alternative splicing of Interleukin7 (*IL7*) in tissues infected with MTB[170]. Specifically, soluble IL7R (*SIL7R*), a receptor variant lacking exon 6, has been found dominantly expressed in MTB-infected lung tissues. Interestingly, this spliced variant is exclusive to lung tissues, suggesting a tissue-specific role in the disease process. The presence of *SIL7R* could serve as a potential biomarker for TB diagnosis or as a target for therapeutic interventions. Furthermore, the ratio of splicing subtypes of *IL32*, namely *IL32G* to *IL32B*, has been shown to influence disease outcomes. A higher *IL32G*/*IL32B* ratio is associated with increased production of interferon-gamma, a cytokine crucial for the immune response against TB. Conversely, a lower ratio correlates with elevated levels of other cytokines such as IL1Ra, IL6, and IL17, which are implicated in the disease's progression. The production of IL32 itself has been shown to confer resistance against MTB infection[171].

The role of MTB in modulating alternative splicing events within host cells adds another layer of complexity to the pathophysiology of TB. Mvubu et al.[172] have reported that MTB infection in lung epithelial cells can specifically regulate the alternative splicing of several genes, including Sodium-independent sulfate anion transporters (*SLC26A11*), interferon alpha-inducible protein 27 (*IFI27*), interferon-induced protein with tetratricopeptide repeats 3 (*IFIT3*), oligoadenylate synthetase like protein (*OASL*), Guanine nucleotide-binding protein G subunit beta-1 (*GNB1*), oligoadenylate synthetase 1 (*OAS1*), and ATP-binding cassette sub-family C member 2 (*ABCC2*). These splicing events result in the formation of specific isoforms that may contribute to the pathogenesis of TB. This suggests that MTB not only survives within host cells but also manipulates host cellular machinery to create a more favorable environment for its survival and proliferation.

Another study has identified that MTB induces a human splice variant of *TLR1* that lacks exon 2 in TB patients[173]. This variant could potentially modulate the host immune response, further complicating the disease's progression and treatment. Collectively, these findings indicate that alternative splicing events triggered by MTB infection are not random but are highly specific

and may have significant implications for disease progression and treatment. These splicing events could serve as potential biomarkers for TB diagnosis or as targets for novel therapeutic interventions.

3.7. RNA splicing in acute respiratory distress syndrome

Acute respiratory distress syndrome (ARDS), a severe stage or type of acute lung injury (ALI), is characterized by refractory arterial hypoxemia and respiratory failure and results in high morbidity and mortality of ARDS/ALI. RNA alternative splicing modulates the pathogenesis of ARDS by regulating gene expression involved in critical pathological processes, including inflammation, signal transduction, vascular remodeling, biomarker expression, and cell death and apoptosis. One of the key pathways implicated in the pathogenesis of ARDS is the Toll-like receptor (TLR) signaling pathway, which plays a dual role in both combating infection and promoting pathological inflammation.

In ARDS patients, alternative splicing events have been observed in TLR signaling-related genes, specifically *MyD88* and *IRAK1*. These splicing events result in the production of long pro-inflammatory mRNAs (*MyD88L* and *IRAK1*) and shorter anti-inflammatory mRNAs (*MyD88S* and *IRAK1c*)[174]. This differential splicing may have significant implications for the inflammatory response in ARDS and could serve as a potential target for therapeutic intervention or as a prognostic marker. Another intriguing aspect of ARDS pathophysiology is the role of endoplasmic reticulum (ER) stress and the splicing of *XBP-1*. Inhibition of ER stress and induced *XBP-1* splicing have been shown to inhibit M1 polarization and ameliorate LPS-induced ALI. This suggests that modulating ER stress and *XBP-1* splicing could be a viable therapeutic strategy for ARDS. Additionally, both the class B scavenger receptor B1 (SR-B1) and its splice variant *SR-B2* have been found to protect against LPS-induced lung injury and sepsis. This highlights the potential for targeting specific splice variants in the treatment of ARDS and related conditions. In summary, the alternative splicing events in genes related to TLR signaling, ER stress, and scavenger receptors offer promising avenues for the development of new prognostic markers and therapeutic strategies for ARDS.

ARDS in children with pneumonia presents a unique set of challenges, and understanding the genetic variations that contribute to this condition is crucial for developing targeted therapies. Several splicing factors, including TDP-43 (TARDBP), PTB1 (PTBP1), SRp40 (SFRS1), TIA-1 (TIA1), SR2/ASF (SFRS5), and U2AF 65 (U2AF2), have been implicated in ARDS in pediatric pneumonia patients. These splicing factors are known to regulate exon 9 skipping and may contribute to the disease's pathogenesis[175]. Wang et al.[176] were pioneers in identifying dysregulated circRNAs in the lungs of mice with hypoxia-induced pulmonary hypertension (PH). CircRNAs, which are formed by the ligation of exons and introns, were found to play a significant role in hypoxia-induced PH and could serve as potential therapeutic targets. SNPs in the splicing factor CELF2 have also been associated with an increased risk of developing ARDS[175]. Fredericks et al.[177] utilized RNA sequencing data to identify 11 genes, including *FAS*, *ARL3*, and *GJA1*, that exhibited changes in alternative splicing and alternative transcription in ARDS patients.

Additionally, the splicing factor hnRNPA1 has also been reported to regulate the *MYLK* gene, leading to the production of two non-muscle isoforms of myosin light chain kinase. These isoforms differ in the presence of exon 11 and are implicated in lung vascular integrity during acute respiratory distress or mechanical stress[178]. Corona Virus Disease 2019 (COVID-19) is a kind of acute respiratory infectious disease. Nakanishi et al.[179] found COVID-19 severity is associated with alternative splicing in the lung, rather than the total expression of OAS1, ATP11A, DPP9, and NPNT, while COVID-19 susceptibility is related to *MUC1* and *PMF1* splicing. These examples nicely illustrate the importance of splicing factors and alternative splicing in the pathogenesis of ARDS and offer potential targets for therapeutic intervention. Understanding how these splicing factors and genetic variations contribute to ARDS could lead to more effective and personalized treatment strategies for this severe respiratory condition.

4. Diagnosis and therapy of pulmonary diseases by RNA splicing

The intricate relationship between aberrant gene splicing and a myriad of diseases, including cancer and genetic disorders, is increasingly recognized. While splicing mutations can directly cause diseases, they can also modulate the severity of phenotypes and influence susceptibility to various conditions by affecting splicing efficiency. However, it is still difficult to determine the physiological function of alternative splicing phenomena, primarily because few experimental approaches exist to regulate endogenous RNA splicing. This creates an imperative for the development of innovative gene-editing techniques that can precisely modulate RNA splicing, both for academic research and clinical applications (Fig. 4).

One promising avenue is the application of genome editing technologies that target genomic DNA for permanent correction. For instance, CRISPR-Cas9 technology has shown promise in this regard. Ifuku et al.[180] utilized CRISPR-Cas9 to restore dystrophin expression in myoblasts derived from patients with Duchenne muscular dystrophy by facilitating the skipping of single or multiple exons. Such advancements are not just academically intriguing but also have profound clinical implications. For example, gene re-editing methods are gradually being introduced into the clinical setting for the treatment of genetic diseases like cystic fibrosis.

4.1. Application of RNA splicing

It is possible that abnormal RNA splicing precedes and contributes to carcinogenesis, and that changes in RNA splicing may be a precursor in cancer initiation and warrant further investigation. RNA splicing can be applied to the following applications.

4.1.1. RNA splicing as a biomarker

Many variable splicing events occur at different phenotypic characteristics in cancer and normal tissues, and it is valuable to detect splicing isoforms generated by variable splicing events as molecular biomarkers. For instance, the downregulation of SRSF1 has been shown to lead to the skipping of an exon in the *PRRC2C* gene, which is overexpressed in primary lung tumors. This differential splicing compared to normal lung tissue could serve as a potential biomarker for lung cancer[86]. Similarly, Tan et al.[181] identify F-*circEA-2a*, a circRNA generated from the splicing of EML4-ALK variant 3b (*EML4-ALK v3b*), as a significant diagnostic tool for patients with NSCLC who are positive for the *EML4-ALK* fusion gene. This circRNA was found in tumor tissues

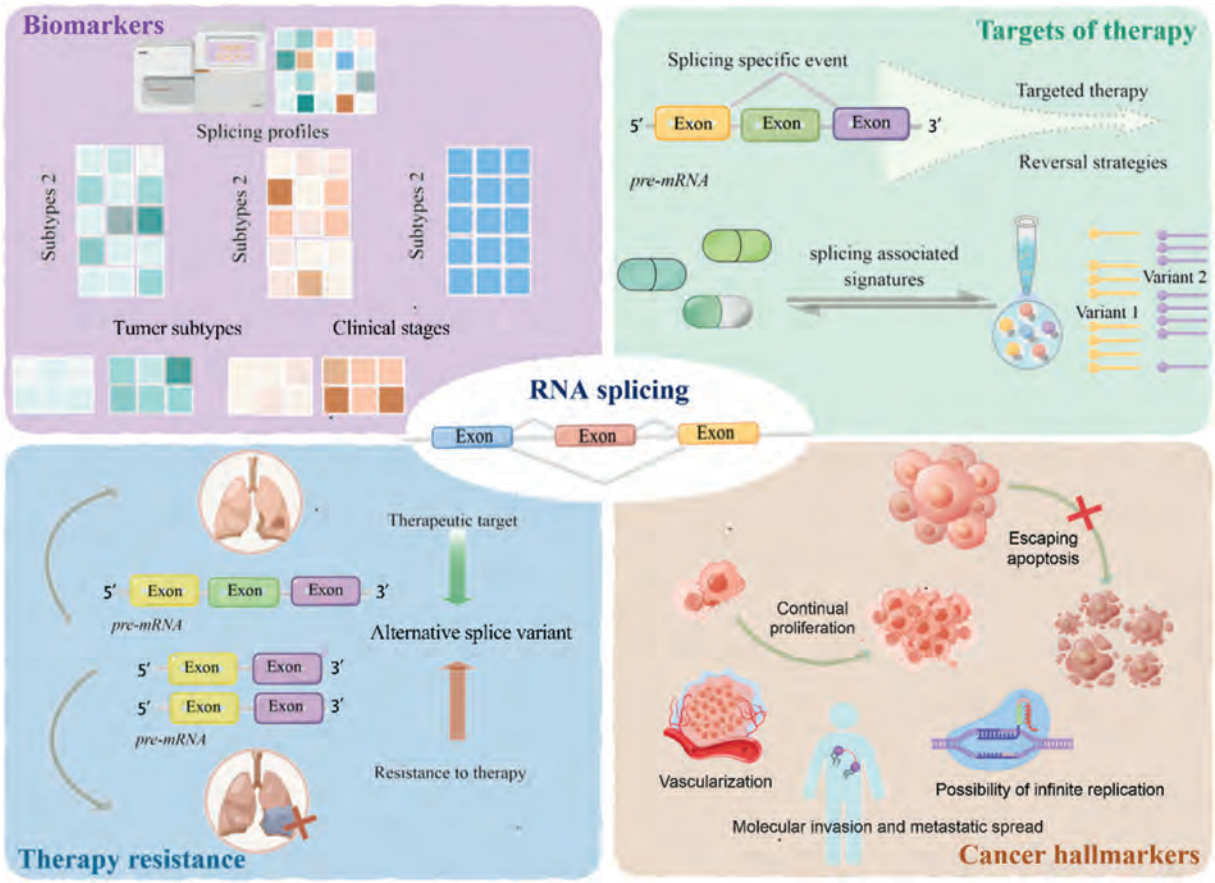

Figure 4 Application of RNA splicing in pulmonary diseases. (A) AS as a biomarker, can be used to differentiate between different types of tumors as well as subtypes of tumors and are associated with tumor staging and patient prognosis. Therefore, variable splicing has the potential to be used as a biomarker for specific clinical conditions. (B) AS and Drug Resistance: AS has been shown to play a role in tumor drug resistance. For instance, when the RNA-binding protein PRPF8 is specifically reduced, it can lead to the skipping of exon 3 in *ERCC1*, which in turn reduces the ability to repair DNA damage. This can result in increased resistance to cisplatin in lung cancer and also activate the cGAS−STING natural immune pathway. (C) As a therapeutic target, antisense nucleotides can be designed to target specific splicing events, thereby restoring the normal phenotype of cells. This approach is currently being evaluated in clinical trials. Additionally, small molecule complexes can be used to modulate splicing factors, potentially leading to varying effects depending on the type of tumor and the specific mutation of the splicing factor. (D) The spliceosome can serve as both a direct target for drugs and a tumor marker. For example, in some lung cancer patients, exon 14 jumps of the *MET* gene have been detected in the somatic cells. These patients are sensitive to *MET*-targeted therapies, making exon 14 jumps in the *MET* gene a potential secondary diagnostic marker.

but not in the plasma, further emphasizing its potential as a tissue-specific marker for NSCLC. Blazquez-Encinas et al.[182] identified alterations in the splicing machinery of lung carcinoids, highlighting NOVA1, PRPF8, and SRSF10 as novel candidates for advancing the understanding of tumor biology and enhancing biomarker discovery.

Beyond the aforementioned splicing regulatory factors, certain splicing isoforms may serve as diagnostic markers for pulmonary diseases. The *Ehm2* gene, a member of the NF2/ERM/4.1 superfamily, produces transcript variants *Ehm2/1* and *Ehm2/2* through alternative splicing. These isoforms have opposing effects on the invasiveness and migration of human lung adenocarcinoma cells, thereby highlighting the functional significance of splicing isoforms in cancer[183]. The transcriptional loss of the oncogenic driver *MET* exon 14 is observed in 3%−4% of patients diagnosed with NSCLC. Tipotinib, a highly selective MET inhibitor, demonstrates a moderate efficacy and safety profile within this patient cohort. The *MET* exon 14 skippings could serve as a potential biomarker for therapeutic intervention in these patients. Consequently, the intricacies of RNA splicing offer valuable insights into the early diagnosis of diseases. The subtle alterations in gene expression facilitated by alternative splicing can serve as indicators of the initial phases of disease progression.

4.1.2. RNA splicing as a therapy target

The manipulation of alternative splicing events offers a promising avenue for targeted therapies in various diseases, including cancer. Numerous clinically approved pharmaceuticals can modulate RNA splicing *via* a variety of mechanisms. Here, we summarized the molecules that could be potential targets of splicing factors or events for pulmonary disease treatment (Table 2[184−218]).

A diverse array of methodologies, encompassing the inhibition of essential spliceosomal proteins or regulatory splicing factors as well as the modulation of specific alternative splicing events, is currently undergoing preclinical and clinical development. Focusing on splicing factors presents novel opportunities for

Table 2 Alternative splicing medicine related to lung disease.

Medical	Disease	Mechanism/Target	Ref.
Crizotinib	Non-small cell lung cancer	*ALK* mutation, inhibitor target of *MET*	184, 185
Larotrectinib	Non-small cell lung cancer	*NTRK* gene fusion	186
Bevacizumab	Non-small cell lung cancer	Targeting the *VEGF*	187
Entrectinib	Non-small cell lung cancer	*NTRK* gene fusion	188
Capmatinib	Non-small cell lung cancer	*MET* exon14 skipping	189
Foretinib	Non-small cell lung cancer	*MET* exon14 skipping	190
Savolitinib	Non-small cell lung cancer	*MET* exon14 skipping	191
Tepotinib	Non-small cell lung cancer	*MET* exon14 skipping	189
Gemcitabine	Non-small cell lung cancer	Targeting *BCL-XL* and *BCL-XS*	192
Pladienolide B	Non-small cell lung cancer	Targeting the *SF3B1*	193
IndisuPLalam (E7070)	Non-small cell lung cancer	Recruit *DCAF15* to induce RBM39 degradation	194
Pembrolizumab	Non-small cell lung cancer	Targeting the *PD-1*	195
Nivolumab	Non-small cell lung cancer	*PD-1* exon 3 splicing	195
Gefitinib	Non-small cell lung cancer	*EGFR*	196
Cabozantinib	Non-small cell lung cancer	*RET* gene fusion, *KIF5B-RET*, *TRIM33*-RET	197
Amivantamab	Non-small cell lung cancer	*EGFR* exon20 insert	198
Vandetanib	Non-small cell lung cancer	*EGFR*, *VEGFR* and *RET* gene fusion	199
Selpercatinib	Non-small cell lung cancer	*RET* gene fusion or mutation	200
Afatinib	Non-small cell lung cancer	*EGFR*	201
Lenvatinib	Non-small cell lung cancer	*cKit, Ret, PDGFR, FGFR1, VEGFR-1, VEGFR-2, VEGFR-3*	202
Agerafenib	Non-small cell lung cancer	*RET* gene fusion	203
Alectinib	Non-small cell lung cancer	*ALK* and *RET* mutation	204, 205
Pralsetinib	Non-small cell lung cancer	*RET* gene mutation	206
Erlotinib	Non-small cell lung cancer	*EGFR*, *caspase 9* exon 4 skipping	207
Ceritinib	Non-small cell lung cancer	*ALK*	208
Topotecan	Small cell lung cancer	*NHP2L1*	209
Cisplatin	Small cell lung cancer	*hPPARg1*	210
Carboplatin	Small cell lung cancer	*SRSF2*, *hPPAR* gamma1(tr)	211, 210
Nintedanib	Idiopathic pulmonary fibrosis	*VEGFR*, *FGFR*	212, 213
Lumacaftor	Cystic fibrosis	*CFTR* modulators	214
VX-661	Cystic fibrosis	*CFTR* modulators	215
Symdeco® (Tezacaftor/Ivacaftor)	Cystic fibrosis	CF patients carrying the 3849 + 10 kb C-to-T splicing mutation, *CFTR* modulators	216
Indacaterol	Chronic obstructive pulmonary disease	Binding RRM2 to interrupt *SRSF6*	217
Kalydeco® (Ivacaftor/VX-770)	Cystic fibrosis	CF patients carrying the 3849 + 10 kb C-to-T splicing mutation, *G551D* mutation, *CFTR* modulators	218

therapy methods, consequently, targeting protein degradation (TPD) emerges as a highly promising avenue in the field of drug discovery. Gosavi et al.[219] developed a novel proteolysis targeting chimeras (PROTAC)-O4I2 compound which selectively targets and degrade SF3B1, a splicing factor associated with a variety of cancers. More than 20 proteolysis targeting chimeras models are now in clinical trials, showcasing the rapid advancement of this innovative therapeutic approach[220].

In addition, antisense oligonucleotides (ASOs) and gene-editing technologies like CRISPR/Cas9 could be promising treatment strategies for further investigation by blocking or altering negative splicing-regulatory elements to fix abnormal splicing. Among the ten ASO drugs currently available on the market, four utilize the exon inclusion/jump mechanism, primarily targeting muscle-related diseases. These drugs include Nosinersen sodium for the treatment of spinal muscular atrophy (SMA), which involves exon 7[221]. Additionally, three drugs are indicated for the treatment of Duchenne Muscular Dystrophy (DMD): Eteplirsen, Golodirsen, and Casimersen[222]. Cas9/gRNA targeted excision has been shown to restore normal splicing in all three mutations of *CFTR* mRNA, which is implicated in cystic fibrosis. This approach can also be extended to correct abnormal splicing signals or remove disruptive transcription regulatory motifs, offering a promising therapeutic strategy for a range of genetic diseases[223]. Nevertheless, the primary challenge in the *in vivo* application of CRISPR/Cas9 remains the off-target effects. Furthermore, the optimization of CRISPR/Cas9 delivery systems, including viral vectors such as adeno-associated virus (AAV) and the advancement of non-viral vectors, is imperative for its successful implementation.

4.1.3. RNA splicing in drug-resistant

The role of abnormal RNA splicing in tumorigenesis and drug resistance is increasingly being recognized as a critical factor. One such example is the overexpression of metastasis-associated lung adenocarcinoma transcript 1 (*MALAT1*) has been shown to promote the expression of anti-apoptotic and EMT-related genes. Inhibition of *MALAT1*, on the other hand, leads to reduced expression of the RNA-binding Fox-1 homolog 2 (RBFOX2). This, in turn, prioritizes the splicing of the pro-apoptotic isoform

of KIF1B (*KIF1B-beta*), thereby increasing anoikis, a form of programmed cell death that occurs in anchorage-dependent cells when they detach from the surrounding extracellular matrix[224].

Additionally, the deletion of *MALAT1* also changed the phosphorylation status of SR proteins, including SRSF1, SRSF2, and SRSF3[225]. Wang et al.[226] reported that lung cancer cells can respond to chemotherapy by decreasing alternative splicing events. This is achieved through specific splicing factors, including SRSF7, SRSF3, PRPF8, and HNRNPC, as well as RNA deconjugating enzymes such as EIF4A3, DDX39A, DDX11, and BRIP1. As a result, there is a decrease in gene transcription. Ouyang et al.[227] also found that *Circ_0001786* increases gefitinib tolerance and malignant development in NSCLC through *miR-34b-5p*/SRSF1 signaling. These SR proteins are essential splicing factors, and their altered phosphorylation status could have significant implications for RNA splicing and, consequently, for cellular functions and responses to therapies. The destruction of splicing factor activity can indeed contribute to drug resistance, making it a challenging obstacle in cancer treatment. It is suggested that the splicing changes may have a specific promoting effect on the tumor, and targeting these processes may offer a novel approach to overcome drug resistance and improve treatment outcomes.

4.2. Analysis methods of RNA splicing

High-throughput transcriptome sequencing, known as RNA-seq, has offered extensive data on splicing events and splicing factors across entire genomes. For instance, Di Modugno et al.[228] utilized RNA sequencing to determine tumoral and stromal *hMENA* isoforms in lung cancer, which impacted tertiary lymphoid structure localization and predicted immune checkpoint blockade response. SRSF6 has been identified as a potential therapeutic target for small-cell lung cancer based on RNA sequencing. This has significant implications for precision medicine, particularly in the diagnosis and treatment of genetic diseases and various forms of cancer (Table 3[9,152,229–233]).

Recently, the third-generation sequencing technology single-molecule real-time sequencing (SMRT) has been of great significance in the understanding and development of RNA splicing. Single-molecule real-time sequencing offers the advantage of long-read sequencing, which allows researchers to more comprehensively analyze RNA structure and function, especially in identifying and annotating splice variants. Oka et al.[234] identified aberrant splicing isoforms as transcripts of potential neoantigens through full-length transcriptome sequencing in NSCLC. With advancements in the accuracy and throughput of long-read sequencing technologies, their integration into single-cell sequencing methodologies is accelerating. Long-read sequencing not only elucidates intricate patterns of cell type-specific splicing but also delivers unparalleled insights into the origins of cellular complexity, thereby presenting novel opportunities for drug development[235].

In terms of analytical tools, several software programs and models have been developed to study alternative splicing. ASTALAVISTA is a visualization tool that can identify splicing sites within databases[236]. The MISO (Mixture-of-Isoforms) model has been designed to estimate the expression of alternative splicing exons and isoforms[237]. The ENCORI database and correlation analysis can be utilized to identify key splicing factors[226]. Cufflinks is an open-source software that allows for the quantitative and differential expression analysis of transcripts[238]. Another innovative approach is Targeted AID-induced mutagenesis, which has been used to analyze mutations related to tumor drug resistance. This method allows for the random mutation of the G base of sgRNA to other bases on the DNA targeted by CCMG, providing insights into the mechanisms of drug resistance in cancer[239].

A significant limitation of existing sequencing methods is their inability to accurately quantify splicing changes in low-expression genes. This poses a challenge for the comprehensive understanding of alternative splicing in the context of disease. Therefore, to improve the accuracy of splicing quantification, there is an urgent need to overcome these obstacles and realize the full potential of RNA splicing in the diagnosis and treatment of diseases.

5. Conclusion and perspective

RNA splicing serves as a pivotal mechanism in regulating gene expression, both in pathological and physiological contexts across various tissues and organs, including muscle, lung, and heart development[240]. AS in lung diseases has a unique genetic mechanism compared to alternative splicing in other diseases. Among them, lung diseases are usually caused by a complex interplay between genetic susceptibility and environmental exposure (such as smoking and pollutants), which are not as prevalent in other diseases. For instance, the severity of COVID-19 is associated with alternative splicing in the lung, rather than the overall expression of genes like *OAS1*, *ATP11A*, *DPP9*, and *NPNT*[179]. This indicates that the regulation of alternative splicing in lung tissue is a key factor in disease severity, which may not be as evident in other disease contexts. AS provides a mechanism through which genetic variations can regulate susceptibility to environmental damage, thereby affecting disease risk and severity. Meanwhile, ATP11A, DPP9, NPNT, and MUC1 are specifically enriched in alveolar epithelial cells, highlighting their tissue-specific roles. This tissue-specific expression pattern emphasizes

Table 3 Common splicing therapy targets.

Gene	Class	Model	Phenotype	Ref.
SFTPA1	C-type lectins subfamily	Homozygous missense mutations	Idiopathic pulmonary fibrosis, SP-A failure secretion,	152
SRSF1	SR protein family	mRNA stability	Idiopathic pulmonary fibrosis, non-small cell lung cancer	9, 229
MUC5B	MUCIN protein family	Promoter region variant, Promoter polymorphism	Idiopathic pulmonary fibrosis, non-small cell lung cancer	230
EGF	EGF family	EGF 61A/G-polymorphism	Lung cancer	231
MET	Receptor tyrosine kinase family	*MET* exon14 skipping	Non-small cell lung cancer	232
CFTR	ATP-binding cassette transporters family	Homozygous mutations *ORKAMBI*	Cystic fibrosis	233

the importance of AS in lung function and disease. Ultimately, AS also holds significant potential in the treatment of lung diseases. Previous research has mentioned a fundamental principle, which is to explore the use of splicing switch oligonucleotides and other therapeutic methods to target lung AS for respiratory diseases. This method is particularly relevant to lung diseases because of the possibility of delivering drugs directly to the lungs through inhalation, which may not be feasible for other organs[241].

During lung development and injury, mRNA transcript levels of specific genes, such as *CD44*, undergo significant changes. *CD44* exists in two isoforms-*CD44s* and *CD44v*-generated through alternative splicing. In normal lung tissue, *CD44s* is predominantly expressed on the cell surface of alveolar macrophages, some interstitial cells, and epithelial cells. During lung development, the *CD44v9* isoform appears at the pseudo glandular stage, while exon v6-encoded isoforms are observed at the saccular stage[242]. In the realm of cancer genomics, alterations in RNA splicing often reflect broader changes in gene expression. Recent research in lung diseases has unearthed invaluable insights into previously unidentified RNA targets, mis-spliced RNAs, and the regulation of aberrant splicing events. Investigating these RNA editing events can lead to the discovery of novel disease biomarkers and facilitate more personalized therapeutic interventions. For instance, the gene *ESYT2* has two primary splicing variants distinguished by the presence or absence of exon 13B. The short splice variant (*ESYT2-S*) is downregulated in tumors, while the long splice variant (*ESYT2-L*) is upregulated[243].

Targeting cancer-specific alternative splicing events or the splicing factors that regulate them offers a promising avenue for therapeutic intervention. This review also reviewed more current literature on the role of alternative splicing as biomarkers, therapeutic targets, and therapeutic resistance in various lung diseases, and described the functions and mechanisms of multiple splicing variants in detail. Drugs like Indisulam inhibit tumor growth by inducing RBM39 degradation through DCAF15 recruitment. Another example is the oral small-molecule drug H3B-8800, which targets spliceosome mutations and inhibits the SF3b complex containing either wild-type or mutant RNA splicing factor SF3B1, thereby suppressing tumor growth[244]. Gene-editing techniques like CRISPR have shown promise in correcting splicing mutations, such as those in the *MET* gene. However, challenges like the presence of multiple mutations and the precision required for CRISPR targeting may limit its immediate applicability as a first-line treatment option.

Research on alternative splicing in lung diseases has made significant strides, especially with the advent of advanced analytical methods. For instance, Olivieri et al.[245] succeeded in revealing cell-type-specific splicing patterns in human lung cells through a novel statistical method in 2022. These have led to the identification of new splicing events and splicing signatures that could serve as predictors for various lung diseases. Furthermore, existing single-cell long-read sequencing technologies enable more accurate analysis of tissue-specific alternative splicing events, as exemplified by studies such as Yang et al.[246] employed long-read sequencing to identify coordinated splicing and cell type-specific intron retention events. They found that autism patients had significantly more de novo mutations in cell type-specific exons than their siblings. Joglekar et al.[247] analyzed single-cell RNA isoforms and discovered significant cell type-specific differences in splicing, transcription initiation, and polyadenylation sites. These differences impact protein structure and correlate with disease-related variations. Additionally, neurotransmitter transport and synaptic transition genes show cell type specificity across various anatomical regions. However, there is currently a lack of single-cell long-read sequencing research related to lung diseases.

Moreover, the effectiveness of many chemotherapy drugs could be compromised by abnormal splicing events in lung tissues. Research in this area has the potential to further define various lung diseases and their subtypes and provide more personalized treatment options. Therefore, further exploration into the molecular and biological mechanisms of alternative splicing is imperative. Identifying functional roles and developing techniques to target specific splicing events are crucial areas for future research. This holds significant promise for elucidating the pathogenesis of lung diseases and offers new avenues for their prevention and treatment.

Many cancer-related RNA transcripts are not due to point mutations in RNA itself but to the expression or functional changes of splicing factors, which regulate the orderly splicing of primary gene transcription, resulting in abnormal expression of carcinoembryonic subtypes with greater proliferative ability. Taken together, RNA splicing events have emerged as a major research focus in the study of human diseases, particularly lung diseases. They offer high specificity for organ, tissue, or cell type in diagnosis, treatment, and prognosis[248]. This review article effectively summarizes previously unknown novel RNA splicing biomarkers that are specifically associated with the progression of pulmonary diseases. For instance, mutations in *RBM10* can serve as a potential negative prognostic/predictive biomarker for the treatment of non-small cell lung cancer[249]. These markers provide a more nuanced understanding of the pathology of the disease. The study also provides a comprehensive summary of new bioinformatics technologies for detecting alternative splicing, enabling a more holistic analysis of RNA splicing patterns across different stages of pulmonary diseases. Mechanistically, the article lists the functions of newly discovered splicing factors in the progression of lung diseases, which have never been summarized before. It also correlates splicing events with clinical relevance, summarizing that specific splicing events can serve as predictive markers for disease severity and treatment response. Finally, we have also listed medicines that target splicing pathways for the clinical treatment of lung diseases. Given the current state of research, the development of splicing-related drugs could be revolutionary, emphasizing the need for further studies on the function and mechanisms of splicing in lung diseases.

Acknowledgments

This study was supported by the National Natural Science Foundation of China (Grant Nos. 82200070, 32171127, and U24A20645), the Scientific Fund Project of Heilongjiang Province (JQ2022H001, China), the Harbin Medical University Graduate Research and Practice Innovation Project (Grant No. YJSCX2023-37HYD, China), the Innovation and Development Joint Fund of the Natural Science Foundation of Chongqing (Grant No. CSTB2023NSCQ-LMX0037, China), and the CAMS Innovation Fund for Medical Sciences (CIFMS, 2019-I2M-5-078, China).

Author contributions

Haihai Liang and Zhihui Niu conceived the manuscript and outlined it. Jian Sun and Bingqian Xu conducted the literature search

and wrote the draft. Wei Li reviewed and edited the draft. All authors have approved the final review and the submission.

Conflicts of interest

All the authors declare no competing interests.

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