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REVIEW

Towards understandings of serine/arginine-rich splicing factors

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Abstract Serine/arginine-rich splicing factors (SRSFs) refer to twelve RNA-binding proteins which regulate splice site recognition and spliceosome assembly during precursor messenger RNA splicing. SRSFs also participate in other RNA metabolic events, such as transcription, translation and nonsense-mediated decay, during their shuttling between nucleus and cytoplasm, making them indispensable for genome diversity and cellular activity. Of note, aberrant SRSF expression and/or mutations elicit fallacies in gene splicing, leading to the generation of pathogenic gene and protein isoforms, which highlights the therapeutic potential of targeting SRSF to treat diseases. In this review, we updated current understanding of SRSF structures and functions in RNA metabolism. Next, we analyzed SRSF-induced aberrant gene expression and their pathogenic outcomes in cancers and non-tumor diseases. The development of some well-characterized SRSF inhibitors was discussed in detail. We hope this review will contribute to future studies of SRSF functions and drug development targeting SRSFs.

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1. Introduction

Precursor messenger RNAs (pre-mRNAs) are immediate products of transcription and composed of non-coding introns residing amongst discreet exons and require modifications to obtain mature mRNAs. In higher eukaryotes, gene splicing, which excises introns and ligates exons, is an indispensable process for mRNA maturation. In contrast to constitutive splicing which removes introns and ligates remaining exons unbiasedly, alternative splicing (AS) selectively determines exon inclusion or exclusion, engendering multiple mRNA isoforms according to one pre-mRNA[1]. It is estimated the expression of over 95% human genes are prone to AS and different AS modes have been identified, including exons skipping (ES), alternative 5′ splice site (A5′ SS), alternative 3′ splice sites (A3′SS), intron retention (IR) and mutually exclusive exons (MXE) (Fig. 1A)[2].

Serine/arginine-rich splicing factors (SRSFs) are important *trans*-acting factors which regulate nearly every step of AS[3,4]. SRSFs are classified as RNA-binding proteins (RBPs) featured by a unique serine/arginine-rich domain (RS domain) in the C-terminus. SRSF1 was the first member discovered in 1990 as an SV40 pre-mRNA splicing regulator[5–7]. To date, twelve SRSFs have been disclosed and most of them comprise one or two N-terminal RNA recognition motifs (RRMs) and a C-terminal RS domain, except for SRSF7 which contains an additional zinc-binding domain in the middle[8].

Functional defects of SRSFs disrupt normal splicing leading to generation of pathogenic gene and protein isoforms[2,9–12]. Strikingly, the relationships between SRSF dysregulation and cancer progressions have been demonstrated in recent years. Most studies pointed out promotive effects on cancer development by SRSF overexpression or hyper-activation. Meanwhile, deficiencies in SRSF functions have been found in autoimmune and neurodegenerative diseases and SRSFs also mediate the transcription and propagation of viral genes. Based on these findings, targeting aberrant SRSF activity to rectify erroneous RNA metabolism can be of significant therapeutic benefits, whereas the development of SRSF modulators is still in the preliminary stage. In this review, we summarize the current researches concerning the structure and biological functions of SRSFs, giving an analysis of up-to-date structural biology studies of SRSFs and their modulations on gene splicing and other RNA metabolism events. We also focused on the implications of SRSF dysregulations in cancer phenotypes, autoimmune diseases, neurodegenerative diseases, viral infections, fatty liver diseases and so no. Finally, therapeutic potentials of targeting SRSFs and development of SRSF modulators were discussed in detail.

2. Structures and functions of SRSFs

SRSFs function as *trans*-acting factors to facilitate pre-mRNA splicing. In general, *cis*-acting factors of pre-mRNAs, including exonic or intronic splicing enhancers (ESEs or ISEs) or silencers (ESSs or ISSs), can be recognized and bound by SRSFs, through which SRSFs facilitate the definition of exon-intron boundaries and the stepwise assembly of the splicing machinery, spliceosome (Fig. 1B). Spliceosome is a multi-subunit RNA/protein complex composed of five small nuclear ribonucleoproteins (U1, U2, U4, U5 and U6 snRNPs) and nearly 150 additional splicing proteins[13]. During the initial stage of spliceosome assembly, SRSFs bind ESEs at the 5′ or 3′ of exon to recruit U1 snRNP and U2AF1, promoting pre-spliceosome (complex E) assembly and exon definition. Then SRSFs recruit U2 snRNP to substitute SF1 at the branch point site (BPS), which forms complex A[1,3,14]. Recent studies also indicated that SRSFs could be recruited to U1 snRNP stem-loop 3 to promote 5′ splice site (5′SS) selection in an exon-independent manner[15]. Subsequent recruitment of U4/U6/U5 tri-snRNP can be promoted by SRSF binding as well[16], which leads to the formation of an activated spliceosome (complex B^{act}) with release of U1 and U4 snRNP. Then, complex B^{act} remodels into a catalytically active spliceosome and the first trans-esterification reaction occurs. The second reaction to ligate exons takes place in the resulting complex C^* that undergoes similar conformational change after the first splicing reaction. Spliced mRNAs coupled with SRSFs are released from the post-catalytic spliceosome that sheds an intron lariat and associated snRNPs[17].

SRSFs target an extensive range of pre-mRNAs. Based on the results of cross-linking immunoprecipitation and high throughout sequencing (CLIP-seq) experiments, 23,635 binding sites, among which mostly were purine-rich consensus motifs, in the transcriptome of human embryonic kidney cells were targeted by SRSF1[19]. SRSFs also inhibit splicing in a sequence-dependent manner. For example, exon-bound SRSFs act as enhancers but intron-bound SRSFs mostly function as suppressors[20]. Phosphorylation states of SRSFs also determines their modes of action. For instance, SRSF10 and SRSF12 act as global repressors of splicing, depending on their phosphorylation states[21]. Compared with protein-coding genes, whose splicing is essential for gene expression, long non-coding RNAs (lncRNAs) undergo less splicing before maturation. An analysis of intergenic lncRNAs demonstrated a positive correlation between the strength of 5′SS and polypyrimidine tract and splicing efficiency. SRSFs bound intergenic lncRNAs to a much lower extent than protein-coding genes due to the lack of major binding motifs in lincRNAs. Therefore, spliceosome components have to identify splice sites without the help of splicing enhancers, underscoring dependence on the optimality of splice sites[22].

2.1. *SRSF structures*

SRSFs are composed of one or two RRMs at the C-terminal and an RS domain at the N-terminal. RRMs serve as surfaces interacting with RNA substrates. Differences in RRM sequences contribute to substrate selectivity of each SRSF. The RS domains serve as protein−protein interacting surfaces. They are also the major sites subjective to post-translational modifications (PTMs), which is essential for well-organized functions of SRSFs.

2.1.1. *RNA recognition motif*

For SRSFs containing two RRMs, the RRMs work in tandem to bind pre-mRNAs, both representing the canonical $\beta_1\alpha_1\beta_2\beta_3\alpha_2\beta_4$ fold[23]. SRSF1 RRM1 showed a binding preference to cytosine *via* the β-sheet surface, and CG dinucleotides exhibited highest binding affinity compared with CT, CA and CC. According to the complex structure of SRSF1 with 5′-AACAAA-3′ RNA (PDB code: 6HPJ), Arg17 and Lys48 were important residues for A_4 binding, and Glu87 interacted with C_3 *via* a hydrogen bond. The stacking of C_3/A_4 on Tyr19/Phe58 was observed as well. MD simulations revealed that Arg8 Asn14 and Phe88 also participated in the recognition of C_3 and A_4 *via* forming hydrogen bonds (Fig. 2A)[24].

RRM2 differs from RRM1 in the sequence and is not conforming to the standard homology search as RNA binding domain. Therefore, it is also named as pseudo-RRM[25]. The binding mode

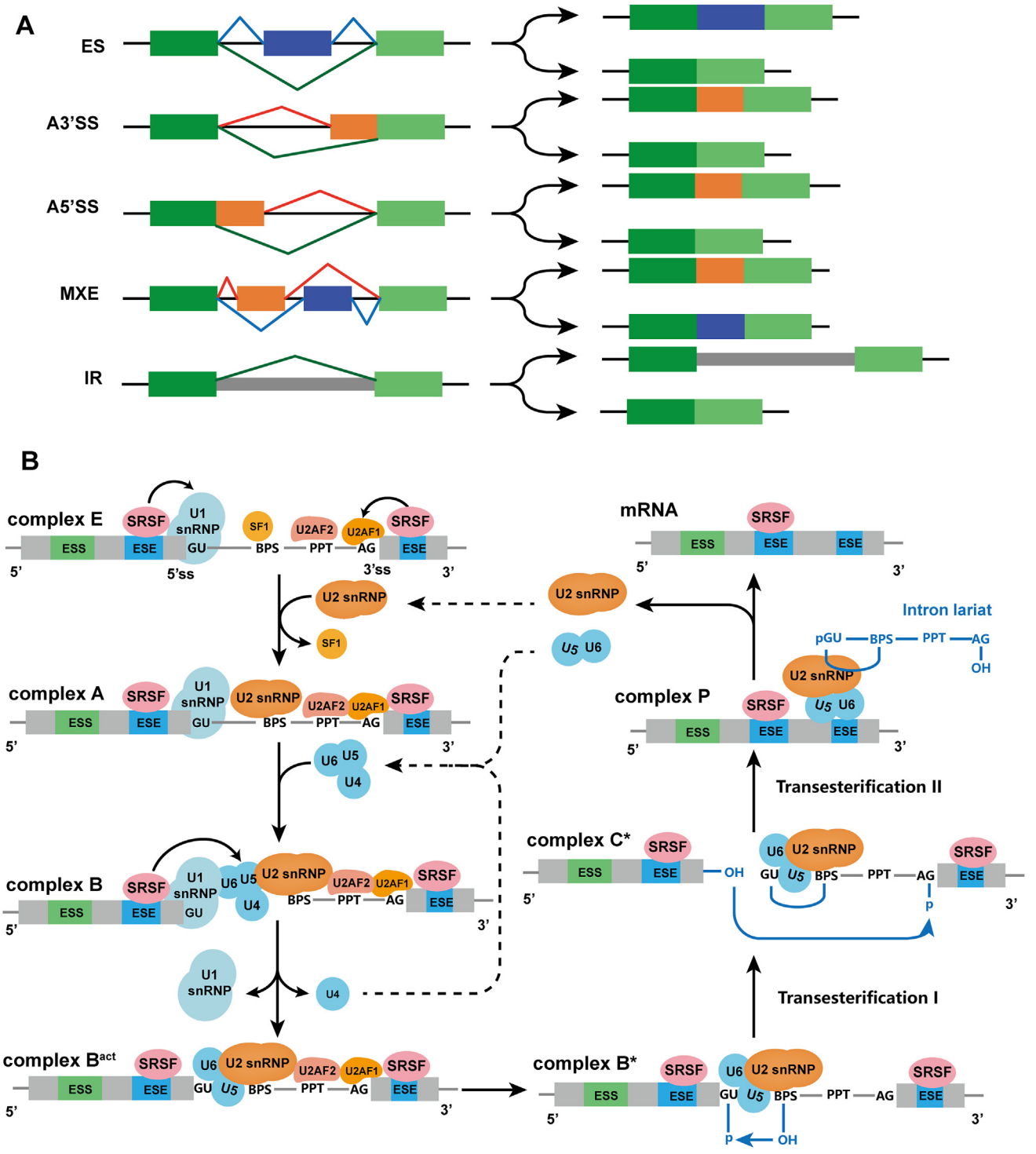


Figure 1 Illustration of the regulatory effects of SRSFs on alternative splicing. (A) Schematic depiction of constitutive splicing and five modes of alternative splicing: exons skipping (ES), alternative 5′ splice site (A5′SS), alternative 3′ splice sites (A3′SS), intron retention (IR) and mutually exclusive exons (MXE)[18]. (B) Illustration of each stage of alternative splicing. SRSFs bind to ESEs to promote the recruitment of U1 snRNP and U2AF1, U2 snRNP and U4/U6/U5 tri-snRNP, which facilitates the formation of complex E, A and B, respectively.

of RRM2 with RNA is unusual because the binding site is centered on α-helix 1 regardless of β-sheet surface. According to the structure of SRSF1 RRM2 and 5′-UGAAGGAC-3′ RNA (PDB code: 2M8D), RRM2 preferentially recognized 5′-GGA-3′ sequence *via* highly conserved S_{133}WQDLKD$_{139}$ sequence within α-helix 1. Of note, Ser133, Trp134, and Gln135 constituted an ideal surface for recognition of the three nucleotides. Ala150 (in the β2-strand) and the sidechains of Lys138 and Asp139 (in the α-helix 1) also contributed to sequence specificity through forming hydrogen bonds with G_5 and G_6, respectively (Fig. 2B)[26]. Further studies showed that SRSF1 RRMs in tandem could utilize a bimodal mode of RNA binding: RRM1 bound pre-mRNAs equally

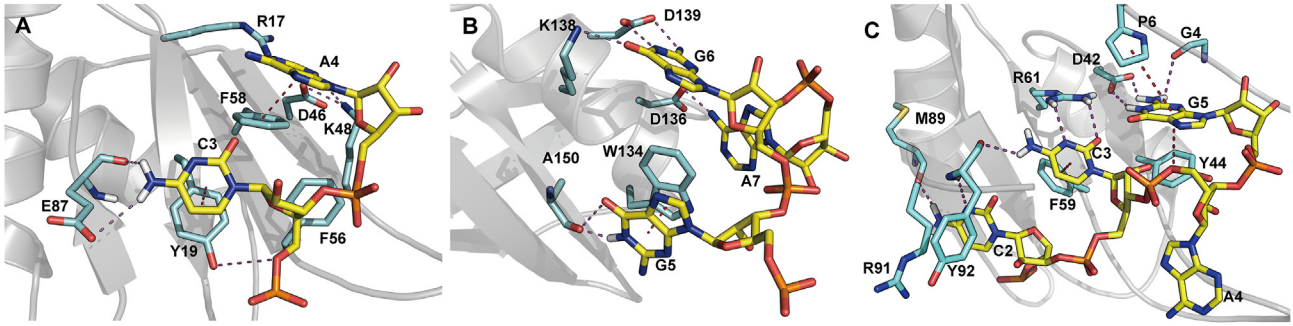


Figure 2 Overview of structures of SRSF RRMs in complex with RNA substrates. (A) represents the mode of interactions between SRSF1 RRM1 and 5′-AACAAA-3′ (PDB code: 6HPJ). The protein backbone is shown in cyan as cartoon and heavy atoms are shown in red (O atoms), blue (N atoms), cyan (C atoms of protein), yellow (C atoms of RNA), orange (P atoms) and H atoms involved in non-bonded interactions are shown in gray. CA dinucleotides and protein residues involved in contacts with RNA are represented as sticks. Intermolecular interactions are represented by magenta and red dashes. (B) depicts the mode of interactions of SRSF1 RRM2 with 5′-UGAAGGAC-3′ (PDB code: 2M8D). Only highly ordered RNA sequences are shown (5′-GGA-3′). Color schemes are as in (A). (C) shows the mode of interaction of SRSF2 RRM with 5′-UCCAGU-3′ (PDB code: 2LEB). Only highly ordered RNA sequences are shown (5′-CCAG-3′). Color schemes are as in (A).

optimal when the cytosine was located in −4 and +6 of the edges of the 5′-GGA-3′ RRM2 binding site[24]. To this end, the two RRMs must function together to realize highest affinity with RNA substrates and optimal substrate specificity, which underscored the flexible nature of the glycine-rich linker between the two RRMs. The flanking basic regions of the linker communicated with each other to bring the RRMs in proximity for an optimal interaction with RNAs. This semi-conservative binding mode between RNAs and SRSFs also contributes to a broader range of substrates[27].

SRSFs with single RRM interact with RNA substrates in a manner distinct from those with double RRMs. For instance, SRSF2 preferentially bound a 5′-SSNG-3′ (S═C/G) motif. According to the structure of SRSF2 RRM with 5′-UCCAGU-3′ (PDB code: 2LEB), C2, C3 and G5 were specifically recognized by the residues on the β-sheet surface, forming hydrogen bonds with Arg61, Met89, Arg91, Tyr92, Asp42 and Gly4 (Fig. 2C). Due to the lack of another RRM and glycine-rich linker that bring about the flexibility of substrate recognition, SRSF2 with a single RRM accommodates altered RNA sequences dependent on the conformation changes of nucleotides. Mutating the first two cytosines to guanines resulted in a higher affinity, whereas the conformations of G2 and G3 turn into *syn* from *cis*[28].

2.1.2. *RS domains*

According to the crystal structure of SRSF1 in complex with transportin-SR2 (PDB code: 4C0O), a major modulator of SRSF mobilization[29], the RS domain (residues 201−211) formed a complicated interacting network with a concave surface comprised of inner helixes of HEAT repeats 15−18 of transportin-SR2. Arg206, Arg208 and Arg210 were three major anchor points in contact with transportin-SR2 *via* hydrogen bonds, while phospho-serine 207/209 were bridged to the arginine side chains on the surface of inner helix of H15 (Fig. 3A and B). Besides, Ser/Arg dipeptides can be phosphorylated by serine/arginine-rich protein kinases (SPRKs) in the cytoplasm. Based on the complex structure of SRSF1 and SRPK1 (PDB code: 3BEG), N′-RS1 (residues 201−210 of SRSF1) was accommodated in the docking groove site of SRPK1. This interaction was oriented electrostatically: Arg204, Arg206 and Arg210 formed ion pairs and/or hydrogen bonds with residues of SRPK1. SRPK1 recognition also included sporadic contacts with residues in RRM2 of SRSF1, indicating that RRMs contributed to protein interaction as well (Fig. 3C and D)[30].

SRSFs were classified as intrinsically disordered proteins according to the prediction from charge-hydropathy measure and the cumulative distribution function of the disorder scores. This result could be largely attributed to the disordered nature of the RS domain where over 80% residues were classified as disorder-promoting residues. The disorder nature was significant considering the broad binding specificity of proteins or RNAs and susceptibility to multiple phosphorylation of the RS domain[31], which also accounted for the modularity and functional dispensability of the RS domains: they were interchangeable and functionally independent to some degrees. For instance, introduction of a second RRM to SRSF2 resulted in altered substrate specificity[32]. The RS domain was dispensable for splicing activity since its deletion, although impaired the overall splicing activity, had no effect on the RRMs-mediated alternative splicing 5′ splice site selection[33]. Nevertheless, some splicing processes, like the *IgM* pre-mRNA splicing, indeed relies on the integrity of RS domain. However, such dependence could be relieved through deletion of 11 N-terminus residues, which increased the splicing activity of SRSF1 proteins deprived of RS domain and enhanced its pre-mRNAs binding potency[34]. SRSFs also had exon-independent functions on gene splicing, since RNA substrates with only one nucleotide of 5′ exon also formed lariat and underwent the first step of splicing in the presence of SRSFs. In this case, the RS domain alone was sufficient to elicit low levels of splicing and likely to promote spliceosome assembly within the intron considering its critical roles in mediating protein−protein interactions. However, the efficiency of lariat formation increased by 50-fold when a functional RRM was appended, indicating RNA binding was essential for efficient splicing activity within the intron[35].

2.1.3. *Post-translational modifications*

SRSFs undergo a series of post-translational modifications (PTMs) including phosphorylation, acetylation, methylation and ubiquitination, which affects the RNA-binding, subcellular distribution and expression levels. In the cytoplasm, SRSFs are phosphorylated by SRPKs and migrate into the nucleus *via* interaction with transportin-SRs[36−38]. In the nucleus, SRSFs can be phosphorylated by Cdc-like kinases (CLKs). SRPK1

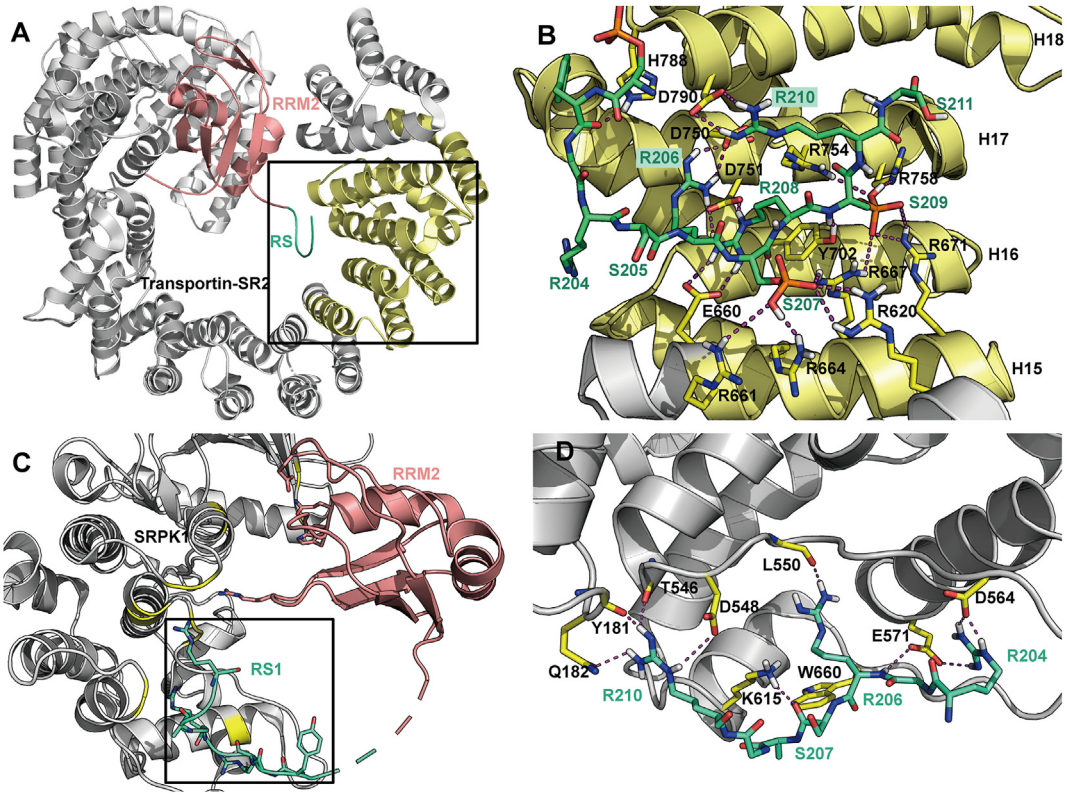


Figure 3 Structural overview of SRSF1 in complex with SRPK1 (PDB code: 3BEG) and transportin-SR2 (PDB code: 4C0O). (A) indicates the overall representation of SRSF1 in complex with transportin-SR2. Protein backbone is shown in cartoon and SRSF1 RRM2 is represented in salmon and RS domain is shown in green cyan. Transportin-SR2 helixes in contact with RS are shown in pale yellow. (B) shows the mode of interactions between RS domain and inner helixes of H15-18 repeats. RS domain (residues 201−210) and important residues in contact with RS are represented as sticks. Heavy atoms are shown in green cyan (C atoms of RS), yellow (C atoms of transportin-SR2), blue (N atoms), red (O atoms), orange (P atoms) and H atoms are shown in white. Non-bonded contacts are shown in magenta dashes. (C) represents the structure of SRSF1 in complex with SRPK1. Key residues of SRSF1 are shown in sticks and the surface of SRPK1 contacts is colored by yellow. (D) suggests the mode of interactions of SRSF1 RS1 and SRPK1. Color schemes are as in (B).

preferentially phosphorylates approximate 10 serines on the N-terminal of RS domain (RS1 domain) in a semi-processive and directional manner[39,40]. This process initiates from the C-terminal of RS1 domain and advances in a C-to-N-terminal direction to phosphorylate the serine/arginine dipeptides in the RS1 domain[40]. SRPK2 shares similar sequence identity, substrate preference and kinase activity with SRPK1 but possesses a unique proline-rich sequence in the N-terminal and different tissue-specific expression pattern[41].

CLK family includes CLK1, CLK2, CLK3 and CLK4, all of which are featured by a C-terminal kinase domain and a disordered N-terminal RS-rich domain[42]. Cytoplasmic CLK1 mobilizes into the nucleus through a ternary complex of SRSF1/transportin-SR/CLK1, interacting with the RS domain of hypo-phosphorylated SRSF1 in a 'piggyback' mechanism[43]. In the nucleus, CLK1 phosphorylated serine/arginine, serine/lysine and serine/proline dipeptides of the whole RS domain unbiasedly in a processive manner to produce hyper-phosphorylated SRSFs[44,45]. Phosphorylation of three proline/serine dipeptides enlarged the conformational ensembles of the RS domain and triggered the disassociation of SRSF1 from the nuclear speckles[46]. Besides, CKL1 could form complex with nuclear SRPK1 *via* the N-terminal sequence, which promoted the dissociation of hyper-phosphorylated SRSFs from CLK1[47,48]. During splicing, SRSF dephosphorylation induced conformation changes, enabling the formation of fully active spliceosome, which is catalyzed by phosphatase PP1 (Fig. 4)[49,50].

Acetyltransferase Tip60 acetylated SRSF2 at Lys52 and led to SRSF2 proteasomal degradation. Tip60 also inhibited SRSF2 phosphorylation by reducing the nuclear accumulation of SRPK1 and SRPK2, while histone deacetylase 6 antagonized the Tip60 activity to induce SRSF2 acetylation[51]. Global proteomic analysis of arginine methylation indicated that SRSF1 could be methylated at Arg93, Arg97 and Arg109, which controlled the subcellular localization of SRSF1[52]. Likewise, arginine methylation induced by protein arginine methyltransferase 4/5 was important for SRSF2 shuttling and functions[53]. Ubiquitination is an important protein degradation mechanism which removes aberrant or misfolded proteins and generates peptides and amino acids for recycling. In particular, T cells of systemic lupus erythematosus (SLE) patients showed increased ubiquitination of SRSF1 when compared with those from heathy individuals, indicating the prompting effects of SRSF1 degradation on SLE progress[54]. SRSF5 could be ubiquitylated by smad ubiquitination regulatory factor 1, which was antagonized by Tip60-mediated acetylation on K125[55].

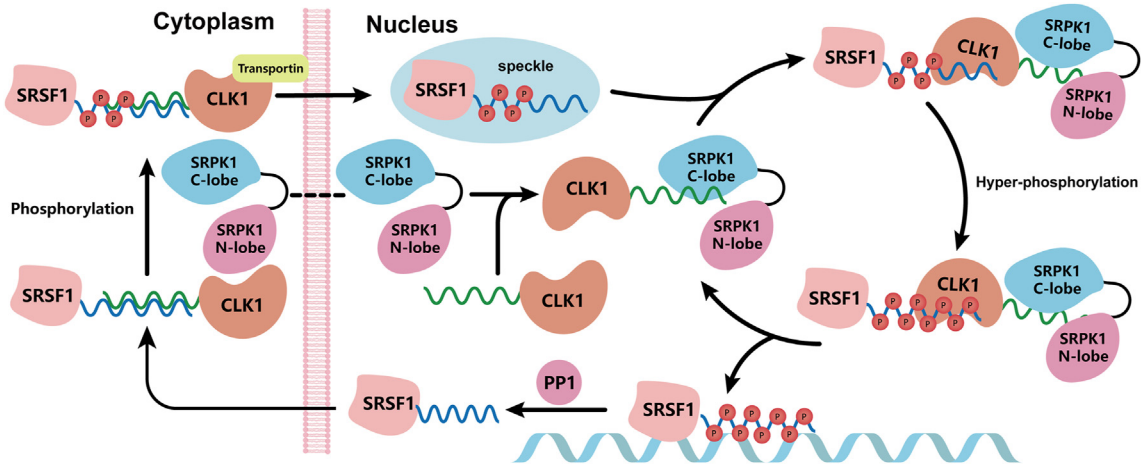


Figure 4 CLKs, SRPKs and PP1 modulate the phosphorylation states of SRSF1, which determines the subcellular distribution and functions of SRSF1.

2.2. *Modulations on RNA metabolism*

Nuclear SRSFs not only serve as splicing regulator but also maintain genome stability, regulate polyadenylation and promote transcription elongation. Besides, some SRSFs shuttle between the nucleus and cytoplasm[56], which confers them extensive involvements in cytoplasmic RNA metabolism (Fig. 5)[57].

2.2.1. *Genome stability*

SRSFs are crucial factors to maintain genome stability, which can be attributed to their direct interactions with nascent RNA transcripts during transcription. For example, the formation of R loops results from rehybridization between nascent transcripts and the template DNA strands, being the hallmark of DNA double-strand breaks. SRSF1 can occupy nascent transcripts with high affinity during transcription to preclude the formation of R loops[58,59]. SRSF3 also inhibits R loop formation during transcription yet with a lower potency compared with SRSF1[59].

SRSFs also modulate the expression of genes related to DNA damage and repair. SRSF3 was involved in homologous recombination-mediated DNA repair pathway in context of neoplastic transformation. Deletion of SRSF3 resulted in the downregulation of homology-dependent recombination repair genes[60]. SRSF6 was revealed to regulate the AS of a set of genes enriched in DNA damage response pathway through transcriptomic analysis[61]. SRSF6 also controlled the inclusion of exon11 of *BRCA1*, another gene responsible for DNA repair, by interacting with a splicing regulatory motif in exon11[62].

2.2.2. *Polyadenylation*

Polyadenylation is an essential step before mRNA maturation, which results in endonucleolytic cleavage and introduces a non-template poly(A) tail of ~150 nt at polyadenylation sites (PASs)[63]. Mammalian genes contain several PASs and differential usage of PASs generating transcript isoforms refers to alternative polyadenylation (APA) which affects the length of 3′ untranslated region (3′-UTR) or the potential of mRNAs to be regulated[64,65]. SRSF3 and SRSF7 recognized the upstream of proximal PASs (pPASs) while exerting opposite effects on polyadenylation. SRSF7 enhanced pPAS usage *via* the recruitment of FIP1. FIP1 in turns escorted PAP and promoted endonucleolytic cleavage and polyadenylation and generation of short 3′-UTRs. SRSF3 similarly bound the upstream of pPAS but could not recruit FIP1, therefore impairing the enhancing effects of SRSF7 on pPAS usage. Besides, SRSF3 was essential to *Cpsf6* splicing and maintained the expression of CFIm that bound the upstream of dPASs to recruit FIP1, favoring the generation of long 3′-UTRs. Therefore, high SRSF3 expression was positive to distal PASs (dPASs) usage. The differences in the regulation of polyadenylation between SRSF3 and SRSF7 could be due to their discrepancies in structural domains[66].

2.2.3. *Transcription elongation*

SRSF2 was involved in transcription elongation *via* triggering the release of positive elongation factor b (P-TEFb). Following transcription initiation, RNA polymerase II (RNAPII) promoter proximal pause occurs near the transcription start site with Ser5 in the heptapeptide repeat of the C-terminal domain being phosphorylated. Productive elongation requires phosphorylation on Ser2 in C-terminal domain[67], which requires P-TEFb. P-TEFb are sequestered in an inhibitory multi-subunit ribonucleoprotein particle 7SK complex[68]. SRSF2 serves as a component of 7SK complex and after transcription pause, it dissociates from the inhibitory complex for RNA binding, which leads to the release of P-TEFb, RNAPII phosphorylation and finally transcription elongation[69]. SRSF2 knockdown induced RNAPII accumulation near the nascent transcripts and impaired transcription elongation, accompanied with defective P-TEFb and reduced Ser2 phosphorylation[70].

2.2.4. *mRNA export*

Nuclear RNA export factor 1 (NXF1) and its adapter proteins cooperate to export mature mRNA from nucleus to the cytoplasm. Shuttling SRSFs can substitute adaptor proteins to mediate mRNA export *via* NXF1 pathway[71]. Through a combination of cellular fractionation and RNA sequencing, over 1000 transcripts were identified as export targets of SRSF1-7, among which SRSF3 presented the most potential NXF1 adaptor[72]. Overexpression of SRSF1 induced nuclear accumulation of NXF1, indicating an increase of mRNA transport activity[73]. LncRNA export was also regulated by SRSFs. SRSF1 and SRSF7 enhanced *NKILA* export by binding to its cytoplasmic accumulation region to facilitate the

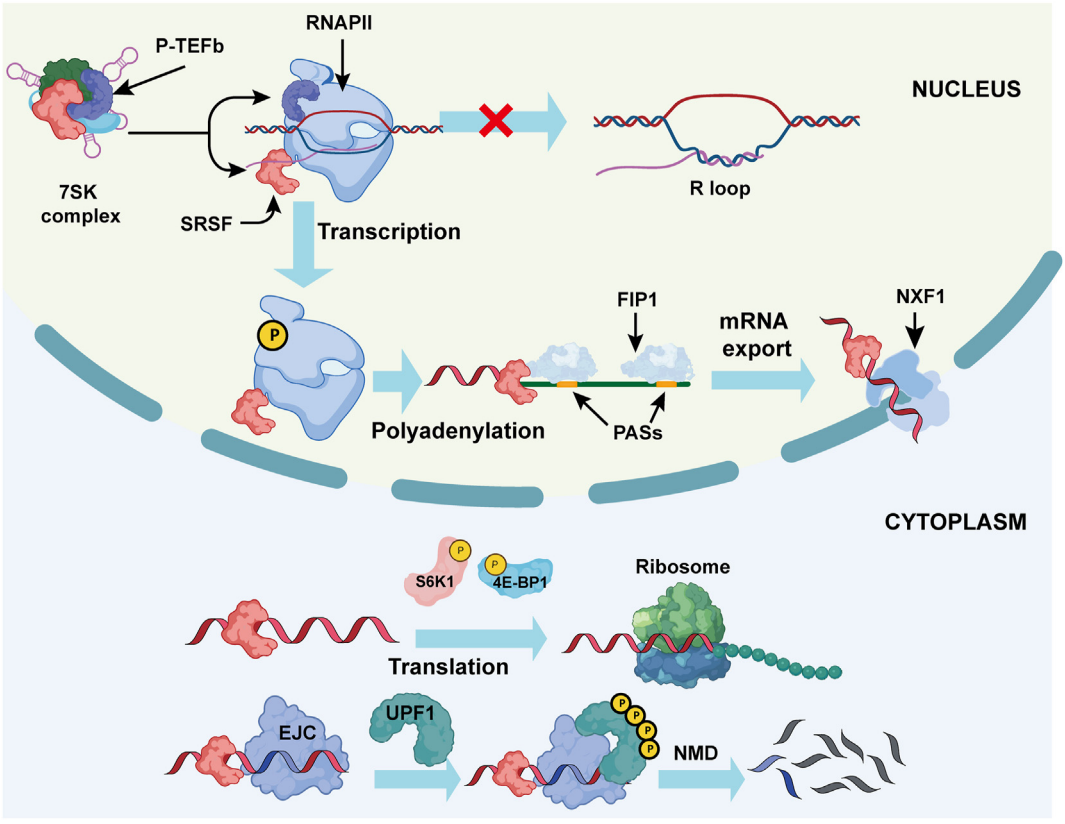


Figure 5 Schematic representation of SRSF-regulated RNA metabolism. SRSFs bind to nascent transcripts to prevent R loop formation, contributing to the maintenance of genome stability. When transcription pause occurs, SRSFs dissociate from 7SK complex to extract P-TEFb into RNA polymerase II (RNAPII). P-TEFb phosphorylates RNAPII to induce transcription elongation. SRSF3 and SRSF7 are involved in polyadenylation. SRSFs are associated with mRNAs after transcription and promote mRNA export *via* nuclear RNA export factor 1 (NXF1). SRSFs promote eIF4E-binding protein 1 (4E-BP1) and S6 kinase 1 (S6K1) phosphorylation to increase translation in an mTOR-dependent manner. Moreover, SRSFs can interact with exon junction complex (EJC) to recruit RNA helicase UPF1, facilitating the degradation of PTC-containing transcripts *via* NMD pathway.

recruitment of NXF1[74]. Otherwise, SRSF7 facilitated mRNA export by enhancing the translation of constitutive transport element-containing RNA[75]. These results suggest shuttling SRSFs can remain coupled with mRNA even when transcription and splicing are completed to promote mRNA export, thereby stretching their modulations on RNA metabolism from nuclear to cytoplasmic events.

2.2.5. *Translation*

SRSFs exert promotive and/or inhibitory effects on mRNA translation. Approximate 1500 mRNAs involved in cell cycle regulation were identified as translation targets of SRSF1[76]. Mechanistically, SRSF1 enhanced mRNA translation by recruiting phosphatase PP2A and mammalian target of rapamycin (mTOR) to phosphorylate eIF4E-binding protein 1 (4E-BP1), which inhibited the suppression of eIF4E-mediated translation initiation[77]. SRSF1 overexpression also enhanced the phosphorylation of another mTOR complex substrate, S6 kinase 1 (S6K1), and activated mTOR signaling pathway to promote translation[78]. In contrast, SRSF3 was shown to suppress translation of programmed cell death 4 (PDCD4) by binding its 5′ untranslated region, which delivered PDCD4 to P-bodies where translationally silenced mRNAs were deposited[79]. SRSF3 regulated the translation of innate immune genes by binding to putative binding sites in 3′-UTRs of these genes. SRSF3 silence led to an increase of expression of immune mediators, suggesting SRSF3-mediated translation regulation was involved in innate immunity[80].

2.2.6. *Nonsense-mediated mRNA decay*

Nonsense-mediated mRNA decay (NMD) is a transcriptome quality surveillance mechanism which eliminates abnormal and nonfunctional mRNAs to preclude the generation of potentially deleterious truncated proteins[81]. Transcripts with premature termination codons (PTCs) are targeted by NMD for degradation[82]. NMD canonically depends on the recruitment of exon junction complexes (EJCs) and NMD factors such as RNA helicase UPF1, UPF2 and UPF3B. Phosphorylated UPF1 precludes further round of translation and signals PTC-containing mRNA degradation carried out by endo- and exo-nucleases[83,84]. SRSF1 interacted with EJC components and enhanced the binding of UPF1 to the PTC-containing transcripts, bypassing the need for UPF2, an essential NMD factor. Besides, SRSF1 promoted the dephosphorylation of p-UPF1, which accelerated UPF1 recycling and improved NMD efficiency[85]. SRSF2 mutants could also enhance NMD efficiency. Mutant SRSF2 with altered substrate consensus motifs enhanced the inclusion PTCs and the generation of NMD targets. Binding of mutant SRSF2 to PTC-containing RNAs also enhanced the deposition of EJCs to a greater extent

than wide type SRSF2, resulting in enhanced association with key NMD factors to augment mRNA decay[86].

3. SRSFs and cancers

Abnormal RNA metabolism, ranging from alternative splicing, transcription and translation to RNA degradation, gives rise to aberrant productions of oncologic genes and proteins which finally promote the progress of cancers. SRSFs comprehensively regulate various aspects of RNA metabolism and balance the expression of different gene isoforms. Aberrant SRSF expression has been observed in cancer cells and clinical samples. Various cancer phenotypes have been found originated from aberrant SRSF functions. Besides, non-coding RNAs (ncRNAs) also exert stimulative or suppressive effects on tumor progression *via* modulating SRSF expression. While some SRSFs have been validated as promising targets for cancer treatment, SRSFs conversely exhibited anti-tumor activity in some cases, suggesting that more studies are required to entangle the relationships between SRSFs and tumor progression.

3.1. Expression and mutations

Overexpression of SRSFs has been observed in a large group of cancer cell lines and clinical samples. In most cases, higher SRSF level preordained tumor progression, short overall survival and poor prognosis. For instance, SRSF3 was highly expressed in colorectal cancer (CRC) and enhanced *B7-H3* exon4 inclusion. High expression of either B7-H3 or SRSF3 was correlated with poor prognosis of the patients[87]. Overexpression of SRSF3 was also associated with relevant clinical and molecular parameters of aggressiveness in prostate cancer samples while SRSF3 down-regulation predicted good prognosis in patients with head and neck cancer (HNC)[88]. SRSF1/2 and SRPK1/2 were overexpressed in lung adenocarcinoma and squamous cell lung carcinoma, and partially responsible for proliferation, metastasis and drug resistance of non-small cell lung cancer (NSCLC)[89]. Upregulation of SRSF6 was observed in colon and colorectal cancer, lung cancer and melanoma[90–92]. The expression level of SRSF5−7 was markedly up-regulated in lung cancer samples especially small cell lung cancer (SCLC)[93]. SRSF10 upregulation was found in HNC patient samples in comparison to paired normal tissues[94]. In contrast, higher expression of SRSFs could be tumor-protective as well. For instance, reasons accounting for SRSF3 overexpression in hepatocellular carcinoma (HCC) may be an attempt to maintain hepatocyte metabolism[95], since SRSF3 could protect from hepatic carcinogenesis *via* suppressing fibrosis, mitogenic splicing and epithelial-mesenchymal transition (EMT). Depletion of SRSF3 predisposed mice to spontaneous HCC with aging[96].

SRSF2 mutations have been extensively identified in chronic myelomonocytic leukemia[97–99], acute myeloid leukemia[100], chronic neutrophilic leukemia[101] and myelodysplasia syndromes[102,103]. Mutant SRSF2 presaged poor prognosis and shorter overall survival than those without the mutation and was correlated with disease progression and clinical outcomes of pharmacological therapies[100,104,105]. Mechanistically, disease-related mutations altered the RNA-binding capacity of SRSF2 with cognate RNA sites in target transcripts, leading to dysfunction of exon inclusion[106]. SRSF2 mutants also elicited the formation of R-loops, enhanced replication stress and activation of ATR−Chk1 pathway[107].

3.2. Cell transformation

Cell transformation describes the changes associated with loss of normal homeostatic control, particularly of cell division, which ultimately results in the development of a neoplastic phenotype. SRSF1 regulated the AS of *S6K1* which coded S6K1, an important substrate of mTORC1 and implicated in the regulation of cell size and transformation. Slight SRSF1 overexpression transformed immortal rodent fibroblasts and promoted sarcoma formation by favoring the expression of short isoform of S6K1, which resulted in mTORC1 activation and 4E-BP1 phosphorylation[108]. Overexpression of SRSF1 in MCF-10A acini also promoted the generation of S6K1 short isoform to transform mammary epithelial cells[109]. Metastasis-associated lung adenocarcinoma transcript 1 (*MALAT1*) transformed hepatocytes *via* up-regulation of SRSF1 and S6K1 short isoform as well[110]. Human epidermal growth factor receptor 2 (HER2) overexpression was associated with many aggressive tumors and a poor prognosis. Δ*16HER2* is a highly tumorigenic isoform of *HER2* increasing malignant transformation of breast cancer cells, whereas *p100* isoform is involved in the inhibition of tumor cell proliferation and oncogenic signals. Overexpression of SRSF3 in tumors switched splicing variants of *HER2* mRNA from *p100* to Δ*16HER2*, leading to tumor progression (Fig. 6)[111].

3.3. Glucose metabolism

Reprogrammed glucose metabolism with enhanced aerobic glycolysis is known as Warburg effect, representing a hallmark of cancer. SRSFs were key modulators tumor glucose metabolism and contributed to enhanced glycolysis in cancers. For instance, *MALAT1* enhanced translation of transcription factor 7-like 2 (TCF7L2) to upregulate the expression of glycolytic genes and inhibit gluconeogenic enzymes in HCC. This effect was mediated by SRSF1 upregulation-induced mTORC1-4EBP1 activation[112]. Besides, c-Myc/SRSF1 axis activation promoted *circMYC* overexpression, which induced higher glycolysis and lactate production *via* sponging *miR-1236* in Mel-CV cells[113]. Pyruvate kinase (PK) is one of the important rate-limiting enzymes in glycolysis. Two isoforms of PK, PKM1 and PKM2, are encoded by the same *PKM* gene while PKM2 dominantly promotes aerobic glycolysis of cancer cells. SRSFs promoted PKM2 expression *via* favoring *PKM* exon9 exclusion, and played a positive role in cancer-specific energy metabolism. For instance, SRSF3 switched *PKM* splicing into *PKM2* isoform. Loss of SRSF3 in human colon cancer cells induced an increasing in the ratio of PKM1/PKM2, leading to a metabolic shift from glycolysis toward oxidative phosphorylation[114]. SRSF5 also facilitated PKM2 expression. Knockdown of SRSF5 repressed glycolysis in NSCLC cells *via* reducing PKM2 level[115]. Tip60-mediated acetylation induced SRSF5 overexpression, which switched the AS of cell division cycle and apoptosis regulator protein 1 (*CCAR1*) to *CCAR1S*. *CCAR1S* enhanced glucose consumption and promoted tumor growth (Fig. 6)[55].

3.4. Proliferation and apoptosis

Unqualified proliferation represents the most predominant characteristic of cancer cells. SRSFs increase proliferation and reduce apoptosis of cancer cells through reprogrammed AS of oncogenic genes. For instance, SRSF1 inhibited the tumor suppressive activity of promoted bridging integrator 1 (BIN1) in NSCLC *via*

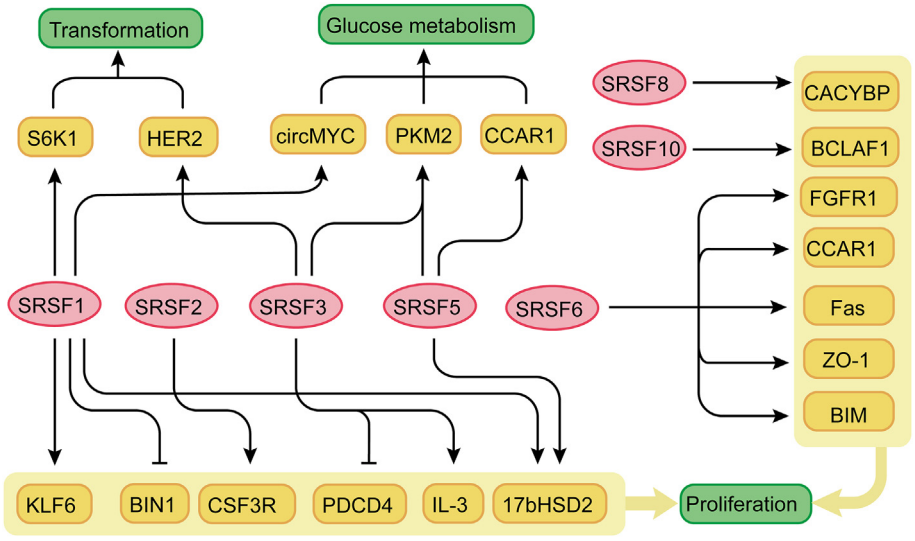


Figure 6 SRSFs are involved in cancer cell transformation, reprogrammed glucose metabolism and proliferation by regulating the functions of diverse genes and proteins.

promoting *BIN* exon12A inclusion which generated *BIN1+12A* isoform[116]. In bladder cancer, 17β-hydroxysteroid dehydrogenase type 2 (HSD17B2) decreased potent androgen production and its overexpression suppressed androgen-induced cell proliferation and xenograft growth. However, its tumor suppressive activity could be abrogated by SRSF1 and SRSF5 that induced the generation of two catalytic-deficient M and S isoforms of HSD17B2[117]. The truncated M/S isoforms could promote wild-type HSD17B2 degradation by forming a heterodimer with the long isoform. Production of the long isoform of Bcl-x, Bcl-xL, was promoted by SRSF1. Bcl-xL disrupted the interaction of beclin−PIK3C3 complex, leading to inhibition of autophagy in lung cancer[118]. SRSF2 mutation targeted colony stimulating factor 3 receptor (*CSF3R*) splicing and promoted the expression of *CSF3R-V3* mRNA which brought about a hypo-proliferative phenotype associated with defective JAK−STAT3 activation in acute myeloid leukemia[119]. Increased SRSF3 expression in cancer cells favored the production of interleukin enhancer binding factor 3 (ILF3) isoform-1 and isoform-2, two critical factors for tumor proliferation and transformation[120]. SRSF3 was also found to repress the export and translation of programmed cell death 4 (*PDCD4*) mRNA and SRSF3 silencing increased the expression of all isoforms of PDCD4 which was a tumor suppressor involved in apoptosis[121].

SRSF6 promoted the exon15−22 skipping of *CCAR1* and engendered CCAR1S protein with anti-apoptosis activity in T-cell acute lymphoblastic leukemia[122]. Overexpression of SRSF6 also reduced the most potent apoptotic isoform of *Bim* gene, *BimS*, by promoting exon3/4 inclusion in HEK293 cells[123]. In addition, SRSF6 regulated zona occludens 1 (*ZO-1*) exon23 splicing and promoted its exclusion, which was associated with proliferation and metastasis of CRC *in vivo*[90]. SRSF10 was found to promote proliferation in colon cancer cells by favoring the Bcl-2-associated transcription factor 1 (*BCLAF1*) exon5a inclusion[124]. SRSF7 was recently revealed to be a novel m^{6}A regulator of tumorigenic genes. SRSF8 promoted multiple myeloma (MM) cell proliferation *via* regulating the AS of calcyclin binding protein (*CACYBP*). *CACYBP* isoform 2 was elevated by SRSF8 and reduced the ubiquitination degradation of β-catenin to promote MM progression. Therefore, SRSF8/CACYBP/β-catenin axis might be a novel target for MM treatment[125].

Ferroptosis is a newly discovered iron-dependent form of cell death, accompanied by the damage of membrane lipid peroxide and repression of lipid repair enzyme glutathione peroxidase 4 (GPX4)[126]. In CRC, SRSF9 inhibited ferroptosis *via* upregulating GPX4 level and SRSF9 overexpression significantly promoted cell viability and colony formation of CRC cells and tumor growth *in vivo*. Consistently, SRSF9 inhibition increased erastin-induced ferroptosis by downregulating GPX4 level[127]. An AS-induced isoform of GPX4, iGPX4, could enhance ferroptosis and deteriorated metabolic-associated fatty liver disease through transforming GPX4 into inactive oligomers[128]. Considering the inhibitory effects of SRSF9 on ferroptosis, it will be interesting to reveal whether SRSF9 regulates AS of *GPX4* and how SRSF9 affects ferroptosis in metabolic-associated fatty liver disease.

Although above-mentioned data correlated SRSF hyperactivation with enhanced proliferation of cancer cells, some studies conversely demonstrated that SRSF defects also contributed to cancer progression. For example, SRSF1 was essential for the splicing of kruppel-like factor 6 (*KLF6*) which suppressed tumor proliferation of HepG2 cell[129]. In bladder cancer cells, SRSF6 mediated the tumor-suppression effects of fibroblast growth factor receptor 1 (FGFR1), and SRSF6 knockdown increased the level of *FGFR1β* that was predominantly expressed in cancer[130]. SRSF6 was also implicated in the splicing of cell surface death receptor *Fas*, promoting cassette exon inclusion to generated a pro-apoptotic isoform (Fig. 6)[131]. These results supported the tumor suppressive effects of SRSF on cancer proliferation and progression, yet these tumor suppressive effects could be overwhelmed by the predominant oncogenic effects of SRSF overexpression.

3.5. *Cell senescence*

Activation of oncogenes activates p53-mediated tumor-suppressive barriers, leading to cell-cycle arrest and the onset of premature cellular senescence[132]. Ubiquitin ligase MDM2 is an

endogenous p53 regulator which promote p53 ubiquitylation and degradation. In particular, the interaction of p53 with MDM2 is modulated by a subset of ribosomal proteins which bind to MDM2 and block its p53 ubiquitylating function. SRSFs are regulators of p53-dependent cell senescence[133]. SRSF1 stabilized the interaction between ubiquitin ligase MDM2 and ribosomal protein L5, which blocked the p53 ubiquitylating function of MDM2 and increased p53 activity[134]. Besides, SRSF1 promoted *MDM2* exon11 skipping, engendering an *MDM2* isoform *MDM2-ALT1*[135]. MDM2-ALT1 impairs the proper localization of full-length MDM2, thereby increasing p53 activity. In contrast, SRSF2 played an antagonistic role in *MDM2* AS by facilitating the inclusion of exon11. SRSF2 inhibition was positive to *MDM2-ALT1* expression and induced p53 activation and apoptosis[136]. Likewise, SRSF3 promoted *MDM4* exon6 inclusion to produce p53-suppressive full-length MDM4. SRSF3 knockdown led to MDM4 downregulation, resulting in the activation of p53-mediated tumor suppression[137]. Downregulation of SRSF3 was also associated with elevated expression of p53β, an alternatively spliced isoform of p53 that promoted p53-mediated senescence[138]. Suppression of SRSF4 and SRSF7 *via* Sp1 depletion also effectively facilitated cell senescence[139]. Targeting the differential effects of SRs on cell senescence could be a novel approach to restore tumor-suppression effect of p53, which has been a challenging target for drug development (Fig. 7).

3.6. *Migration, invasion and EMT*

Aberrant SRSF activities are implicated with enhanced metastasis, migration, invasion and EMT in cancer development. SRSF overexpression was observed in highly metastatic cancer cells and tissues[140,141]. SRSFs regulated the expression of genes and proteins involved in cancer metastasis. In HCC, overexpression of SRSF1 and steroid receptor RNA activator 1 long isoform induced migration and invasion by increasing CD44, p-AKT and p-ERK levels[142]. High expression of defective in cullin neddylation 1 domain containing 5 (DCUN1D5) was identified in metastatic MDA-MB-231 cell and predicted poor overall and relapse-free survival of breast cancer patients. Of note, SRSF1 increased DCUN1D5 expression by NMD inhibition[143]. Truncated isoform of coiled-coil domain containing 50 genes (*CCDC50S*) was highly expressed in HCC patients. SRSF3 stabilized *CCDC50S* mRNA *via* direct interaction, which resulted in HCC growth and metastasis *via* activation of Ras/Foxo4 signaling[144]. In triple negative breast cancer, SRSF3 promoted the expression of α isoform of glucocorticoid receptor (*GR*α) gene, which induced enhanced transcription of receptor for activated C kinase 1 to promote cancer cell migration and invasion[145]. In pancreatic cancer, CLK1 was up-regulated to enhance SRSF5 phosphorylation. Enhanced SRSF5 phosphorylation inhibited methyltransferase-like 14 (*METTL14*) exon10 skipping, which afterwards enhanced metastasis of pancreatic cancer cells *via* increasing m^6A modification[146]. Likewise, overexpression of SRSF9 enhanced m^6A modifications of *DSN1* mRNA and was correlated with lymph node metastasis in CRC[147].

EMT normally occurs in a few physiological conditions such as wound healing and tissue regeneration, during which epithelial cells are deprived of their distinguishing features to acquire typical mesenchymal traits. EMT is also utilized by cancer cells to obtain migratory and invasive properties[148]. SRSF1 overexpression promoted EMT in breast and colon cancer *via* elevating the expression of Δ*Ron*, a constitutively active isoform of *Ron* with exon11 excluded[149]. SRSF1 inhibition counteracted Δ*Ron* generation and the invasive phenotypes of cancer cells[150]. Compared with SRSF1, SRSF2 played an opposite role in *Ron* AS. SRSF2 recognized the CGAG motif in *Ron* exon11 to promote exon inclusion, and SRSF2 knockdown increased the constitutively active ΔRon[151]. Otherwise, SRSF2 inhibited cancer invasion by favoring the anti-metastatic isoform of methyl-CpG binding domain protein 2 (*MBD2*), *MBD2c*. In breast cancer, hypoxia-induced hypoxia-inducible factor 1 (HIF1) activation promoted the MBD2a expression *via* suppressing the effects of SRSF2 on *MBD2*, which

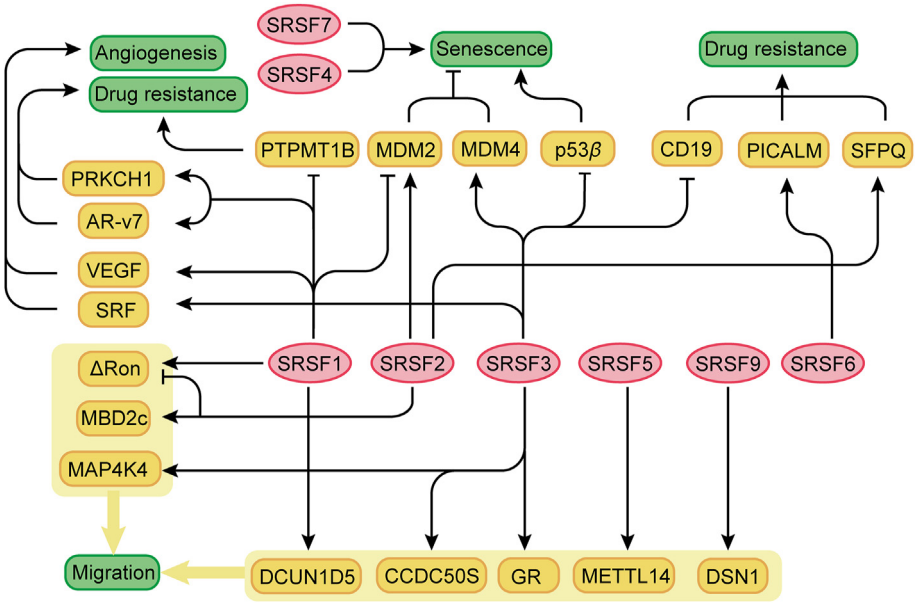


Figure 7 SRSFs are associated with the processing of genes relative to tumor migration, EMT, senescence, angiogenesis and drug resistance.

enhanced metastasis and EMT by activating expression of frizzled class receptor 1[152]. Therefore, SRSF2 could demonstrate a protective role against EMT. In CRC, SRSF3 favored the inclusion of mitogen-activated protein 4 kinase 4 (*MAP4K4*) exon16, inducing yields of *MAP4K4* iso2 and iso5 to promote EMT in HCT-8 cells[153]. The pro-metastasis activity of SRSFs resulting from the modulation of a series genes and proteins related to cell mobility or EMT confers them with great significance of cancer treatment, since metastasis of cancer has been a major cause of death in cancer patients (Fig. 7).

3.7. Angiogenesis

Angiogenesis is a fundamental requirement for embryonic development, wound healing and other physiological processes process during which new vessels form from the existing ones. Tumor growth also requires new vessel formation, which is predominantly driven by vascular endothelial growth factors (VEGFs). VEGFs are the most potent angiogenic molecules and SRSFs have been discovered as major regulators of *VEGF* alternative splicing, which generates two families of isoforms: pro-angiogenic $VEGF_{xxx}$ and anti-angiogenic $VEGF_{xxx}b$[154]. SRSF1 was a promoter of $VEGF_{xxx}$ and SRSF1 up-regulation facilitated angiogenesis in many types of cancer. For instance, the Wilms' tumor suppressor (WT1) is a major regulator of tumor angiogenesis[155]. WT1 mutation resulted in enhanced SRSF1 phosphorylation, which in turn lowered the expression of $VEGF_{165}b$ and rendered the WT1-mutated cancer cells angiogenic[155]. In tumor endothelium, knockdown of *WT1*, *SRPK1* and *SRSF1* promoted the anti-angiogenic $VEGF_{120}$ isoform expression[156]. Analysis of 17 neuroblastoma clinical samples demonstrated that the SRSF1-mediated alternative splicing of *VEGF* was regulated by extracellular matrix (ECM) stiffness. In particular, high ECM stiffness inhibited the expression of YAP and RUNX2, leading to reduced SRSF1 and $VEGF_{165}$ expression[157].

Other angiogenesis-related genes have been found regulated by SRSFs as well, contributing to the angiogenic phenotype of cancer. SRSF3 regulated the splicing pattern of pro-angiogenic gene, serum response factor (*SRF*), by binding to the CAUC motif in exon6 to induce intron exclusion. SRSF3 knockdown inhibited VEGF secretions from CRC cells and induced a significant reduction of tube formation in human umbilical vein endothelial cells[158]. SRSF1 overexpression caused by hypoxia switched the splicing of *HIF-1α* into $HIF\text{-}1\alpha^{-ex14}$, which is more potent in activating vascular endothelial growth factor receptor 2 transcription, thereby enhancing angiogenesis in lung cancer[159]. Conversely, some SRSFs favored the anti-angiogenesis VEGF isoforms and inhibited tumorous neovascularization. SRSF6 could bind a 35-nucleotide motif in exon8 and promoted the usage of distal acceptor site and VEGF165b expression[154]. SRSF2 cooperated with transcription factor E2F1 to increase VEGF165b/VEGF ratio, leading to reduction of neovascularization in lung tumor xenografts (Fig. 7)[160]. Therefore, SRSFs' modulation on angiogenesis can be bivalent and it is necessary to distinguish their unique roles in different cancer types.

3.8. Drug resistance

Resistance against chemical agents and radiotherapy frequently occurs during cancer treatment and presents an intractable obstacle onto anti-cancer drug development. Altered AS events have been implicated in the genesis of multidrug resistance (Fig. 7). For instance, the reduction of CDK6, CDK4 or PI3K 110α/110β level induced high frequency of AS and reduced drug resistance caused by ATP-binding cassette subfamily B member 1[161]. SRSF expression altered upon chemistry or radiation therapy, which in turn changed the expression of genes related to drug resistance and induced the insensitivity of cancer cells. In H1299 and A549 cells, SRSF1 expression was elevated upon ionizing radiation treatment, whereas decreased SRSF1 favored higher rate of apoptosis. Depletion of SRSF1 switched protein tyrosine phosphatase mitochondrial 1 (*PTPMT1*) into the short isoform *PTPMT1B,* which in turn markedly up-regulated p-AMPK level and induced DNA double-strand breaks, leading to sensitization of NSCLC cells[162]. In CML, SRSF1 overexpression increased *PRKCH1* and *PLCH1* levels and decreased the sensitivity to imatinib[163]. In castration-resistance prostate cancer, SRSF1 was responsible for the expression of an androgen receptor splice variant, AR-v7, which led to resistance to androgen deprivation therapy[164]. In epithelial ovarian cancer, splicing factor proline- and glutamine-rich protein (SFPQ) was highly expressed and correlated with platinum resistance. Mechanistically, SFPQ in complex with p54 bond to SRSF2 and decreased SRSF2's binding to caspase-9 mRNA, which promoted the expression of its anti-apoptotic isoform. As a consequence, SFPQ/p54/SRSF2 protected cells from platinum-induced death, leading to chemoresistance[165]. SRSF3 was essential for exon2 retention of *CD19*. SRSF3 downregulation was observed in relapsed B-cell acute lymphoblastic leukemia, which promoted *CD19* exon2 skipping to generate N-terminally truncated CD19 variant. Compared with full-length CD19, the variant failed to trigger cell death by CART-19 in B-ALL cells[166]. In gastric cancer, SRSF6 was related to the resistance of gastric cancer cells to oxaliplatin and 5-FU. Mechanistically, SRSF6 promoted phosphatidylinositol-binding clathrin assembly protein (*PICALM*) exon14 inclusion to produce a full-length PICALM, which caused autophagy-induced resistance of gastric cancer cells to oxaliplatin and 5-FU (Fig. 7)[167]. These results indicate an important aspect of SRSFs in eliciting drug resistance and targeting SRSF-related splicing events could be a promising approach to overcome drug resistance in cancer treatment.

3.9. Cancer immunology

In tumor microenvironment, tumor cells negatively impact cytotoxic T lymphocytes through associating with immunosuppressive cells which secret cytokines to stimulate the expression of immune check point molecules, leading to T cell exhaustion and immune escape. SRSFs promoted the immune escape of cancer by elevating the levels of check point molecules. For instance, SRSF2 was overexpressed in exhausted T cells and involved in exhaustion of tumor-infiltrating lymphocytes (TILs). Transcription of check point immune genes was upregulated by SRSF2 and acetyltransferases P300/CPB complex, which altered the H3K27Ac level near these genes and recruitment of signal transducer and activator of transcription 3. Therefore, SRSF2 could be a target for reversing the exhaustion of TILs[168]. Other researches also indicated involvements of aberrant SRSF activation in cancer-specific immune environment. In pan-cancer SRSF3 expression was positively correlated with immune cell infiltration, tumor mutation burden, microsatellite instability[169]. Dysfunction in T-cell anti-tumor activity resulting from high expression of programmed cell death-1 contributes to progression of clear cell renal carcinoma. Mechanistically, TGFβ1 promoted the expression of SRSF3 which

coordinated with NXF1 to induce *PD-1* mRNA extranuclear transport, inhibiting the antitumor activity of T cells[170].

3.10. Non-coding RNAs

SRSFs are targeted by ncRNAs and mediate the carcinogenetic or anti-cancer effects of ncRNAs. Major findings concerning the interplays between lncRNAs, miRNAs, circRNAs and SRSFs are summarized (Table 1). Interactions between lncRNAs and SRSFs affected the stability and expression of SRSFs, which could result in altered RNA metabolism and altered tumorous progression. For instance, *LINC01123* upregulation was associated with enhanced proliferation, invasion and migration of CRC cells due to its inhibition on SRSF7 expression[171]. *AC091729.7* was upregulated in sinonasal squamous cell carcinoma tissues and promoted proliferation and invasion. Mechanistically, *AC091729.7* bound SRSF2 directly and was positive to the expression level of SRSF2 protein. SRSF2 silencing significantly reduced the cancer cell viability and migration caused by *AC091729.7* overexpression[172].

MicroRNAs regulate mRNA translation *via* binding to the 3′-UTR and leading to mRNA silence or degradation. Elevation of *miR-28* and *miR-505* levels enhanced apoptosis and senescence of mouse embryonic fibroblasts by decreasing SRSF1 translation[173]. In renal cell carcinoma, *miR-766-3p* inhibited SRSF1 translation to decrease downstream p-AKT and p-ERK levels, which suppressed A498 and 786-O cell proliferation[174]. *MiR-193a-5p* could interact with *SRSF6* mRNA leading to its downregulation. SRSF6 inhibited pancreatic cancer cell migration and invasion, while

Table 1 NcRNAs regulate the expression level of SRSFs and generate tumor promotive or inhibitory effects.

Name	Class	Target	Cancer type	Effects on tumor development	Associated genes or proteins	Ref.
DGCR5	lncRNA	SRSF1	ESCC	Increase SRSF1 stability. Enhance proliferation, migration, and invasion	DGCR5/SRSF1/Mcl-1	178
MALAT1	lncRNA	SRSF1	HCC	Increase SRSF1 expression. Enhance glycolysis, and inhibit gluconeogenesis	MALAT1/SRSF1/TCF7L2	112
LINC01210	lncRNA	SRSF3	CRC	Increase SRSF1 expression. Promote proliferation, migration, and invasion	/	179
CircSMARCA5	circRNA	SRSF1, SRSF3	GBM	Decrease SRSF3 expression. Inhibit glioma cell migration	CircSMARCA5-SRSF1, SRSF3-PTB	180
circPLCE1	circRNA	SRSF2	CRC	Inhibit SRSF2-dependent splicing. Promote tumor growth *in vivo*	circPLCE1/SRSF2/PLCE1	181
miR-193a-3p	miRNA	SRSF2	BCa	Inhibit SRSF2 expression. Induce BCa growth and chemoresistance	miR-193a-3p/SRSF2, PLAU, HIC2	182
			NPC	Inhibit SRSF2 expression. Induce NPC radio-resistance	/	183
NRON	lncRNA	SRSF2	BRC	Downregulate SRSF2. Inhibit proliferation and invasion	NRON/miR-302b/SRSF2	184
MRUL	lncRNA	SRSF2	NSCLC	Increase SRSF2 expression. Promote NSCLC proliferation, invasion and migration	MRUL/miR-17-5p/SRSF2	185
CRNDE	LncRNA	SRSF6	GC	Reduce SRSF6 stability. Inhibit autophagy-mediated chemoresistance of GC	CRNDE/SRSF6/PICALM	167
miR-193a-5p	miRNA	SRSF6	PC	Contributed to the metastasis of pancreatic cancer cells both*in vitro* and *in vivo*	miR-193a-5p/SRSF6/ECM1/OGDHL	175
PANDAR	LncRNA	SRSF7	OSCC	Downregulate SRSF7. Promote proliferation and inhibit apoptosis in OSCC	PANDAR/SRSF7/PIM1	186
circ_0006006	circRNA	SRSF7	NSCLC	Increase SRSF7 expression. Repress proliferation, cell migration and invasion, angiogenesis and promot cell apoptosis	circ_0006006/miR-924/SRSF7	187
TUG1	LncRNA	SRSF9	HCC	Increase SRSF9 expression. Promote HCC cell proliferation and migration. Enhance aggressive progression of HCC	TUG1/miR-328-3p/SRSF9	177
miR-802	miRNA	SRSF9	Cervical cancer	Suppress SRSF9 expression. Inhibit proliferation and induce apoptosis of cervical cancer cells	/	188
circ-ATXN1	circRNA	SRSF10	GBM	SRSF10 promotes circ-ATXN1 expression, which enhances cell viability, migration and tube formation of GECs	SRSF10/circ-ATXN1/miR-526b-3p	189

miR-193a-5p overexpression facilitated cancer mobility through SRSF6 depletion[175]. However, lncRNAs could act as miRNA sponges to ameliorate the translation inhibition effects of miRNAs on SRSFs. In CRC patient tissues, *ZNF561-AS1* and SRSF6 were upregulated, which promote cancer cell proliferation and survival. *ZNF561-AS1* elevated SRSF6 expression *via* sponging *miR-26a-3p* and *miR-128-5p*, whereas SRSF6 overexpression reversed the antitumor activity of *ZNF561-AS1* depletion *in vivo*[176]. LncRNA *TUG1* augmented SRSF9 expression *via* sponging *miR-328-3p*, which was associated with aggressive progression and prognosis of HCC patients[177].

4. SRSFs and non-tumorous diseases

4.1. Autoimmune diseases

SRSFs regulate the AS of a series of immune-related genes responsible for controlled systemic autoimmunity. Impaired SRSF function caused aberrant cytokine production, hyperactive effector T cells and regulatory T cell dysfunction, finally leading to autoimmune disease development.

4.1.1. Systemic lupus erythematosus

Impaired SRSF1 function contributes to the development of SLE (Fig. 8). SRSF1 binds to the 3′-UTR of *CD3ζ* to promote wild type CD3ζ protein expression, whose down-regulation was recognized in T cells from SLE patients[190]. Of note, reduced SRSF1 expression in SLE was caused by *has-miR-10b-5p* that reduced 3′-UTR activity of SRSF1[191]. Higher proteasomal degradation or estrogen-mediated post-transcriptional suppression

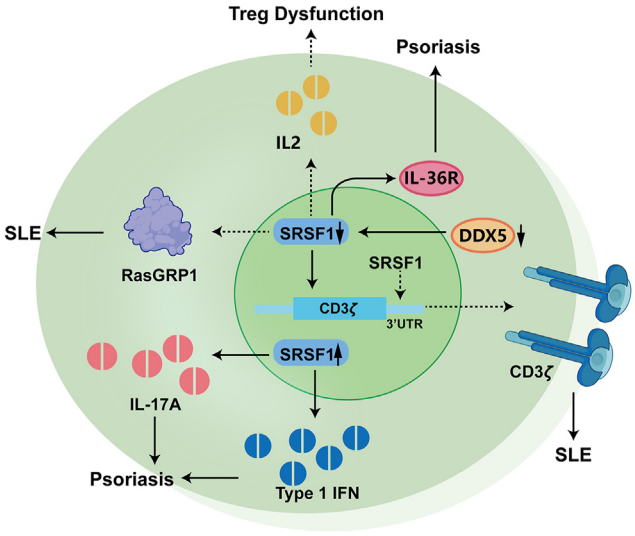


Figure 8 SRSF1 dysregulation was associated with autoimmune diseases. Decreased SRSF1 failed to bind the 3′-UTR of *CD3ζ*, reducing the expression of wide-type CD3ζ whose insufficiency was the hallmark of SLE. Decreased RasGRP1 and IL2 expression in SLE patient T cells were also caused by SRSF1 down-regulation. DDX5 downregulation resulted in decreased expression of SRSF1, leading to IL-36R at the expense of sIL-36R, which promoted inflammation in psoriasis and atopic dermatitis. In contrast, SRSF1 overexpression was positive to increased IL-17A and immune response in psoriasis. SRSF1 also formed a complex with RIG-1 to induce cytosolic DNA-triggered type-1 IFN generation and immune response in psoriasis.

of SRSF1 contributed to CD3ζ insufficiency. Reduced cytokine IL-2 level is also a hallmark in SLE T cells and leads to regulatory T cells (Tregs) dysfunction and inefficiency to attenuate immune response. SRSF1 down-regulation contributed to reduced IL-2 due to its critical role to maintain normal IL-2 expression and transcription[192]. In another study, SRSF1 also regulated the alternative splicing of RAS guanyl releasing protein 1 (*RasGRP1*) in T cells. SRSF1 inefficiency led to the generation of *RasGRP1-AS* isoform which was predominant in SLE T cells[193]. Additionally, SRSF1 deletion resulted in T cell lymphopenia, a frequent clinical manifestation and risk factor for infections in SLE, with decreased expression of anti-apoptotic Bcl-xL[194]. SRSF1 also played a significant role in Treg metabolism since its deletion led to Treg cell dysfunction *via* inducing glycolytic metabolism and pro-inflammatory cytokine productions[195]. A recent study demonstrated that SRSF1 targeted the AS of 189 and 582 genes, most of which were involved in autoimmune diseases, unique to Tregs and effector T cells, respectively, further confirming the important role of SRSF1 in protecting from attack on healthy cells and tissues by the immune system[196].

4.1.2. Psoriasis

Meta-analysis from psoriasis patients demonstrated a positive correlation between SRSF1 and IL-17A production, yet *SRSF1* cKO mice showed increased *Il-17a* and *Il-17f* mRNA level. The discrepancy of IL-17A and IL-17F expression on the protein and mRNA level might suggest SRSF1 regulated IL-17A production in a splicing-independent manner, such as inhibition of myeloid-derived suppressor cells or stimulating translation[197]. Viral or bacterial infections are closely related with psoriasis and accompanied with release of cytosolic DNAs which triggers the generation of type-1 interferons (IFNs) and immune response. SRSF1 facilitated cytosolic DNA-triggered type I IFN production *via* directly interacting with RIG-1/RNA polymerase III complex that served as nucleic acid sensor and was responsible for IFNs production[198]. Downregulation of RNA helicase DDX5 was induced by IL-17D in keratinocytes of atopic dermatitis and psoriasis. Due to the direct interaction between DDX5 and SRSF1, expression of SRSF1 was also reduced, which caused the production of IL-36R at the expense of sIL-36R. Strikingly, restoration of sIL-36R inhibited cutaneous inflammation and overexpression of SRSF1 elevated sIL-36R level (Fig. 8)[199]. These results highlight the significance of SRSF1 in immune response and T cell metabolism. Particularly, impaired SRSF1 function is a feature of autoimmune diseases, which offers a novel approach to the treatment of these diseases.

4.2. Tauopathy

Intracellular accumulation of microtubule-associated protein tau (MAPT) is a common feature of a heterogeneous subset of neurodegenerative diseases, such as Alzheimer's disease (AD) and Huntington disease (HD) (Fig. 9A)[200]. *Tau* exon10 exclusion or inclusion generates tau with three or four microtubule-binding repeats (3R-tau or 4R-tau). In healthy brains the ratio of 3R-tau/4R-tau is about 1:1, while perturbations to this ratio are implicated with neurodegenerative diseases. SRSFs play important roles in AS of *tau* exon10. SRSF1, SRSF2 and SRSF6 promoted the inclusion of exon10 leading to 4R-tau expression, whereas SRSF4 bound to the proximal downstream intron and inhibited exon10 inclusion[201–204]. Dysregulation of *tau* exon10 resulted from abnormal SRSF phosphorylation caused 3R/4R imbalance.

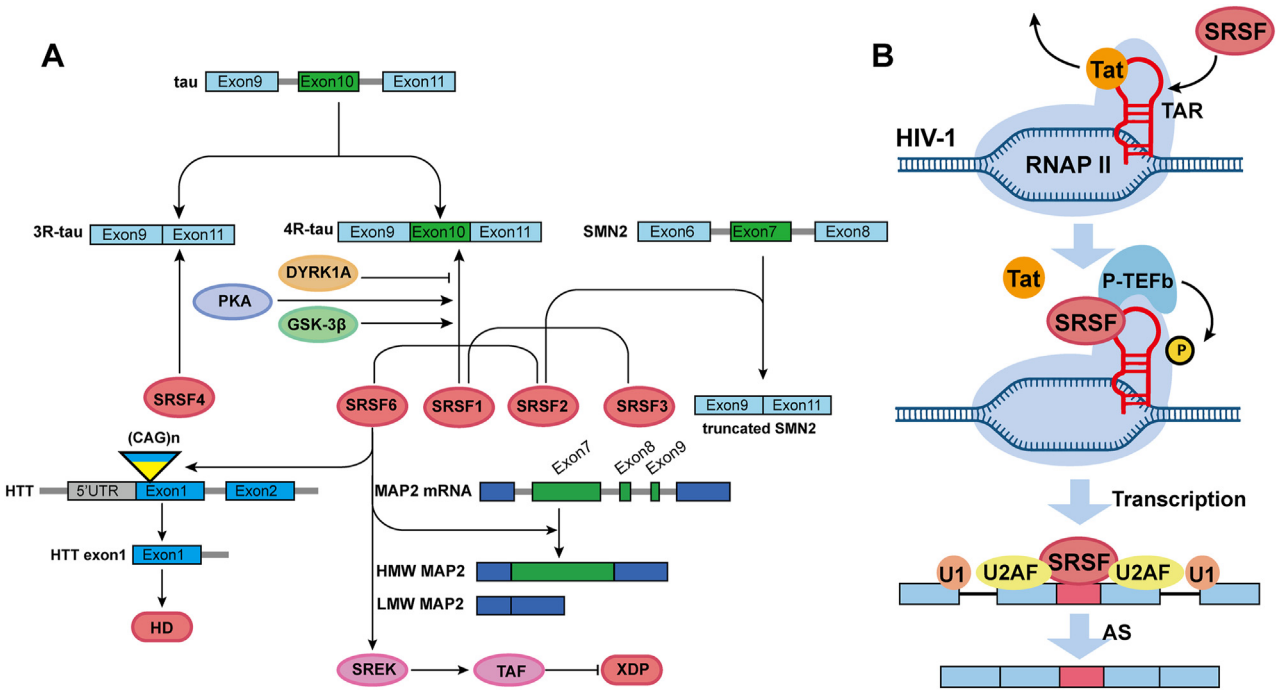


Figure 9 (A) SRSFs were implicated in neurodegenerative disorders due to their modulation on the AS of *Tau*, *HTT* and *MAP2*. Imbalanced tau exon10 splicing was vital to tauopathy, such as HD, AD and Down Syndrome. Abnormal activities of kinases including PKA, DYRK1A and GSK-3β increased SRSF phosphorylation, which resulted in overexpression of 3R-tau or 4R-tau. In HD, SRSF6 bond the CAG repeat on *HTT* exon1 and promoted the pathogenetic HTT exon1 isoform. SRSF6 was also found to regulate the AS of *MAP2* exon7-9 and the dysregulated expression of *HMW* and *LMW MAP2* was relative to the development of HD. In XDP. SRSF6 reduction was relative to the diminishment of SERK that caused low expression of TAF. Transgenic expression of SREK1 could attenuate TAF1 deficiency and striatal atrophy and motor phenotype of HD. SRSF2 and SRSF3 potently inhibited exon7 inclusion of *SMN2*, which decreased functional SMN proteins and exacerbated SMA. (B) SRSF1 competes with Tat in TAR binding and recruits P-TEFb for RNAP II phosphorylation and transcription elongation. SRSFs also participate in the AS of HIV-1 transcripts, which offered an anti-HIV approach by inhibiting SRSF function.

For instance, dual-specificity tyrosine phosphorylation-regulated kinase 1A (DYRK1A) was upregulated in Down syndrome and interfered with *tau* exon10 splicing by phosphorylating SRSFs. Ser227, Ser234 and Ser238 of SRSF1 was phosphorylated by DYRK1A, which precluded SRSF1 from promoting exon10 inclusion and refrained SRSF1 within the nuclear speckles[205]. Increased DYRK1A expression also inhibited exon10 inclusion by increasing SRSF2 and SRSF6 phosphorylation[203,206]. Cyclic AMP-dependent protein kinase, PKA, induced *tau* exon10 inclusion *via* SRSF phosphorylation. Downregulation of PKA pathway in Alzheimer's brain led to neurofibrillary degeneration[207]. GSK-3β activation induced by amyloid-beta peptide also promoted *tau* exon10 inclusion by SRSF2 phosphorylation[208]. SRSF2 could stabilize *tau* mRNA and promote tau expression[209]. In this case, aberrant expression or activation of kinases caused altered SRSF phosphorylating levels, which led to the dysregulation of *tau* exon10 splicing and development of neurodegenerative diseases. Targeting abnormal SRSF phosphorylation can be a novel approach to correct imbalanced tau 3R/4R ratio in tauopathy treatment.

HD is an autosomal dominant neurodegenerative disorder caused by an expanded CAG repeat in exon1 of the *HTT* gene. CAG repeat length-dependent aberrant splicing of exon1 results in a short polyadenylated mRNA that is translated into an exon1 HTT protein, which is consistently pathogenic in HD[210]. SRSF6 was initially found to bind the CAG repeats in mouse *Htt* transcripts and inhibited the splicing of exon1 to exon2. Overexpression of human *SRSF6* increased the levels of incomplete splicing but lowered levels of SRSF6 led to significantly reduced levels of *HTT* exon1[211]. Although SRSF6 has been bioinformatically predicted and biochemically confirmed to bind CAG repeats and promoted incomplete splicing, recent studies failed to observe the effects of SRSF6 knockdown on incomplete splicing in mouse model[212]. Therefore, more researches are still needed to verify the roles in the aberrant splicing of *HTT*. The imbalance of tau isoforms was also discovered with rod-like tau deposits in the brains of HD patient. The attenuated motor phenotype of *HTT* transgenic mice with genetic tau reduction demonstrated a role of tau in HD pathogenesis. Alterations in SRSF6 activity led to imbalance of tau isoforms since SRSF6 could increase the inclusion of *tau* exon 10 leading to pathogenic increase in 4R-tau isoforms[213]. SRSF6 dysregulation was also correlated with aberrant splicing of microtubule-associated protein 2 (MAP2) exon7−9 in HD[214]. Splicing regulatory glutamine/lysine-rich protein 1 (SREK1) was reduced in the HD mouse models upon SRSF6 downregulation. Decrease of SREK1 in turn caused decreased level of TATA-box binding protein associated factor 1 (TAF1), whose loss of function was related to X-linked dystonia parkinsonism (XDP). In particular, neuronal transgenic expression of SREK1 could attenuate TAF1 deficiency and striatal atrophy and motor phenotype of HD mice[215].

4.3. Spinal muscular atrophy

Spinal muscular atrophy (SMA) is an inherited neurodegenerative disorder that results in weakness of voluntary muscles in the limbs and affects motor neurons, causing pediatric mortality worldwide. SMA results from mutation or deletion of *SMN1* gene, which generates insufficient SMN proteins. The patients also possess the centromeric *SMN2* gene which, due to a C > T in the exon7, mostly codes an alternatively spliced transcript without exon7 and the truncated unstable isoform of SMN protein. Increasing the inclusion of *SMN2* exon7 is of therapeutic significance to increase the abundance of functional SMN proteins. SRSFs inhibited exon7 inclusion. In particular, knockdown of SRSF2 and SRSF3, two most potent inhibitor of exon7 inclusion, increased SMN protein abundance in SMA patient-derived fibroblasts (Fig. 9A)[216].

4.4. Viral infections

The transcription and splicing of viral genes require modulations from splicing factors. For instance, SRSFs are important modulators of the splicing and transcription of human papilloma virus 16 (HPV-16). SA3358 is a major splice site of HPV-16 genome and utilized to produce the majority of HPV-16 mRNAs including E4, E6 and E7 mRNAs. An SRSF1 binding site was found on the exonic sequence downstream SA3358 and was essential for SA3358-related alternative splicing and HPV-16 propagation[217,218]. HPV-16 infection led to SRSF1/2/3 accumulation and enhanced SRSF1 phosphorylation, and siRNA-mediated SRSF1 knockdown induced a significant reduction in spliced HPV-16 mRNA[219,220]. Besides, upon HPV-16 infection, SRPK1 upregulation was observed to enhance SRSF1 phosphorylation[219]. SRSF1, SRSF3 and SRSF9 could likewise regulate HPV-16 late L1 mRNA splicing, an essential step to establish persistent infections in cervical cancer development[221].

The transcription of HIV-1 genome required the binding of Tat to TAR, an RNA stem-loop structure on the nascent viral transcripts. Such interaction recruited P-TEFb and other nucleoplasmic complexes to phosphorylate RNAPII, which was required for transcription elongation. Besides, approximately half the HIV-1 transcripts entering the cytoplasm undergo complex splicing processes to generate over 40 mRNA isoforms[222]. SRSF1 could impact both transcription and splicing processes of HIV-1 propagation. SRSF1 activated transcription in the early stage of virus infection through recruiting P-TEFb to TAR from the 7SK complex to phosphorylate RAPII, which bypassed the need of Tat for transcription elongation yet in a much lower efficiency[223]. However, at high concentration, SRSF1 competed with Tat in TAR binding to inhibit viral transcription (Fig. 9B)[224]. During splicing, overexpression of SRSF1 increased *vpr* mRNA level by activating the usage of splicing site A2 and altered the splicing patterns of viral RNAs[225,226]. Intriguingly, depleting the RS domain of SRSF1 uplifted the inhibitory potency towards HIV-1 replication by over 2000-fold and reduced the impacts on cell viability, which provided a novel approach to treat AIDS with truncated SRSF1 protein[224]. SRSF2 and SRSF7 were found to inhibit Tat mRNA production[226] while SRSF4 and SRSF6 inhibited splicing from the 5′ splice site of exon3 inducing an accumulation of 13a7 *vpr* mRNA. In contrast, SRSF5 induced splicing from exon3 to exon4, promoting the production of the 1347 tat mRNA. SRSF6 stimulated *vpr* mRNA production by interacting with HIV-1 splicing enhancer GAR to inhibit its function[227,228]. Depletion of SRSF10 affected HIV splicing and decreased the expression of Tat, Gag and Env[229]. The above studies pointed out the essential roles of SRSFs in regulating the gene expression of viruses, indicating the RNA regulation functions of SRSFs are not limited to endogenous genetic materials. These results also revealed the specific interaction of SRSFs with viral genes and proteins, implying the prospects to target SRSFs to treat virus infection diseases.

4.5. Fatty liver diseases

SRSF3 is essential for hepatic maturation and metabolic functions. Hepatic-specific deletion of SRSF3 in a mouse model resulted in disturbed hepatic architecture and dysregulations in glucose and lipid metabolism, leading to prolonged expression of fetal markers, decreased expression of adult markers and persistence of hepatic hematopoiesis. The missplicing of genes required for hepatocyte maturation, hepatic lipogenesis and cholesterol synthesis was altered upon the loss of SRSF3[230].

Another research correlated SRSF3 degradation to the genesis of nonalcoholic fatty liver disease (NAFLD), nonalcoholic steatohepatitis (NASH) and cirrhosis. Loss of SRSF3 expression was observed in both human NAFLD/NASH liver samples and mouse NAFLD/NASH models. Mechanistically, degradation of SRSF3 *via* neddylation on lysine 11 occurred in response to lipid-induced oxidative stress. Mutation of lysine 11 or prevention of SRSF3 neddylation inhibited the deleterious changes in liver metabolism and provided a new pathway to treat fatty liver diseases[231]. DRAK2, a serine/threonine kinase belonging to death-associated protein kinase family, could exacerbate hepatic steatosis and inflammation in a high-fat diet induced mouse NAFLD model *via* reducing SRSF6 phosphorylation. DRAK2 directly interacted with SRSF6 thus preventing its phosphorylation by SRPK1. In particular, DRAK2-SRPK1-SRSF6 axis played an important role in the AS of mitochondrial function-related genes in the progression of NAFLD. The DRAK2-SRSF6 signaling cascade may offer a potentially novel therapeutic pathway for the treatment of NAFLD[232]. SRSF10 regulated alternative polyadenylation in the liver and decreased SRSF10 was also associated with NAFLD progression. SRSF10 interacted with a number of the polyadenylation factors, prevented their binding to RNA and inhibited cryptic intronic polyadenylation. However, in the liver diseases, SRSF10 inactivation induced the usages of intronic polyadenylation sites, downregulated liver PPARα signaling that regulated lipid accumulation, and increased susceptibility to NAFLD and obesity-induced metabolic dysfunctions[233].

4.6. Fibrosis

Fibrosis is defined as an irreversible and progressive progress with continuous accumulation of extracellular matrix components that result in destruction of normal tissues and comprised functions[234]. SRSFs have been implicated in the progression of fibrosis. For instance, SRSF6 was overexpressed and promoted synthesis of main fibrotic protein COL1A2 and pleural mesothelial cell *in vitro*. The involvements in SRSF6/wnt5A and SRSF6/SMAD1/5/9 signaling induced pleural fibrosis progression and SRSF6 inhibition prevented pleural fibrosis in a mouse model[235]. SRSF1 and SRSF9 activated wnt/β-catenin signaling by enhancing β-catenin translation in an mTOR-dependent manner[236]. Accumulation of β-catenin was also involved in the pathogenesis of pulmonary fibrosis. SRSF1 downregulation attenuated collagen deposition and differentiation of mouse lung fibroblasts and

reversed established lung fibrosis through reduction of β-catenin[237]. Collectively, aberrant SRSF functions contributed to the progression of fibrosis and, accounting for the limited therapeutic approach to treat fibrosis, SRSFs could be novel targets for fibrosis diseases.

5. Targeting the therapeutic potential of SRSFs

Targeting aberrantly activated or overexpressed SRSFs is a promising approach to treat diseases. SRSF inhibition with siRNAs or small molecules exhibited reduction of cancer proliferation or metastasis and HIV-1 replication. Otherwise, restoring impaired SRSF expression helps to maintain the functions of immune system and balance of tau isoforms, thereby inhibiting the genesis of SLE and tauopathy. Small molecules are characterized by optimal oral bioavailability and blood brain barrier penetrance, particularly with the wealth of knowledge from medicinal chemistry whereby physicochemical properties can be systematically optimized to improve pharmacokinetics and potency. In this work, we focused on the discovery of small molecules with SRSF modulating activities.

5.1. *Indole derivatives*

NB-506, a glycosylated indolocarbazole derivative, was initially identified as a topoisomerase I inhibitor. It turned out NB-506 selectively inhibited the SRSF phosphorylating activity of topoisomerase I, which reduced SRSF1 phosphorylation (IC_{50} = 1.5 μmol/L) and spliceosome assembly at 25 μmol/L. NB-506 affected SRSF1 phosphorylation of murine P388 leukemia cells but not topoisomerase I mutant P388CPT5 cells (Fig. 9)[238]. Inspired by the indole moiety of NB-506, a series of indole derivatives were screened for inhibition against spliceosome assembly *via* blocking the kinase activity of topoisomerase I. Pyrido-carbazole derivatives were formerly found as DNA-interacting agents with antitumor activity. However, binding affinity assays based on the intrinsic fluorescence of pyrido-carbazole motif verified their DNA-independent interactions with SRSFs. Compound C76 with a pyrido-pyrrolo-isoquinoline motif bond SRSF1 with a K_d value of 0.19 μmol/L. Heat denaturation assay revealed that the domain integrity was indispensable for binding. The inhibition was possibly attributed to the binding to RS domain, which blocked the interaction between SRSFs and topoisomerase I. The inhibition of ESE-dependent splicing events of these indole-derivatives were determined in *in vitro* splicing assay, which showed that these compounds had biased binding preference to SRSFs. For instance, the inhibitory effect of C22 on SRSF6 was much higher than SRSF1 (ID_{50} = 1 and 25 μmol/L, respectively). In particular, C77 and C83, which had a 9-methoxy-pyrido[4,3-*b*]carbazole moiety, selectively inhibited SRSF2-induced splicing events. These results revealed that slight structural difference affected the selectivity of indole-derivatives. Using a cell-based HIV-1 splicing model, compounds C5, C6, C16 and C20 showed inhibition against HIV-1 genome splicing at 5 μmol/L (Fig. 10)[239]. Importantly, drug treatment neither affected the global splicing events nor cell viability.

Another screen of a library of 220 indole derivatives led to IDC16 which shared a similar pyrido-pyrrolo-isoquinoline moiety with C76 but differed in a Cl-substituent instead of amine on the pyridine ring (Fig. 11A). IDC16 also inhibited ESE-dependent splicing events of SRSF1. Significantly, IDC16 inhibited the splicing of HIV-1 pre-mRNA *via* specifically inhibiting 3′SS selection and completely blocked the synthesis of splicing isoforms at 2.5 μmol/L. IDC16 also inhibited HIV-1 replication in primary cells at 1 μmol/L while having no effects on cell proliferation and apoptosis. Compared with zidovudine, an antiretroviral agent

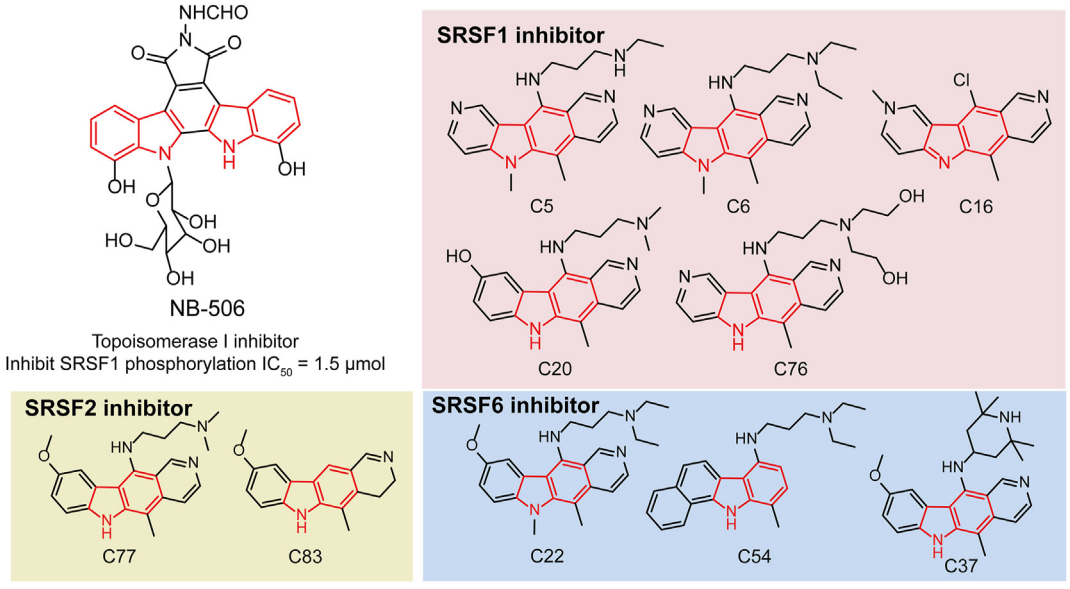


Figure 10 Indole derivatives were discovered as SRSF inhibitors and exhibited different inhibitory selectivity against SRSFs. NB-506 inhibited the kinase activity of topoisomerase I and reduced SRSF1 phosphorylation. Screening of indole derivatives led to the discovery of a series of compounds with selective inhibition against SRSF-induced splicing. C5, C6, C16, C20 and C76 inhibited SRSF1 while C22, C54 and C37 inhibited SRSF6 in the *in vitro* splicing assay. C77 and C88 were specific inhibitors of SRSF2-mediated AS.

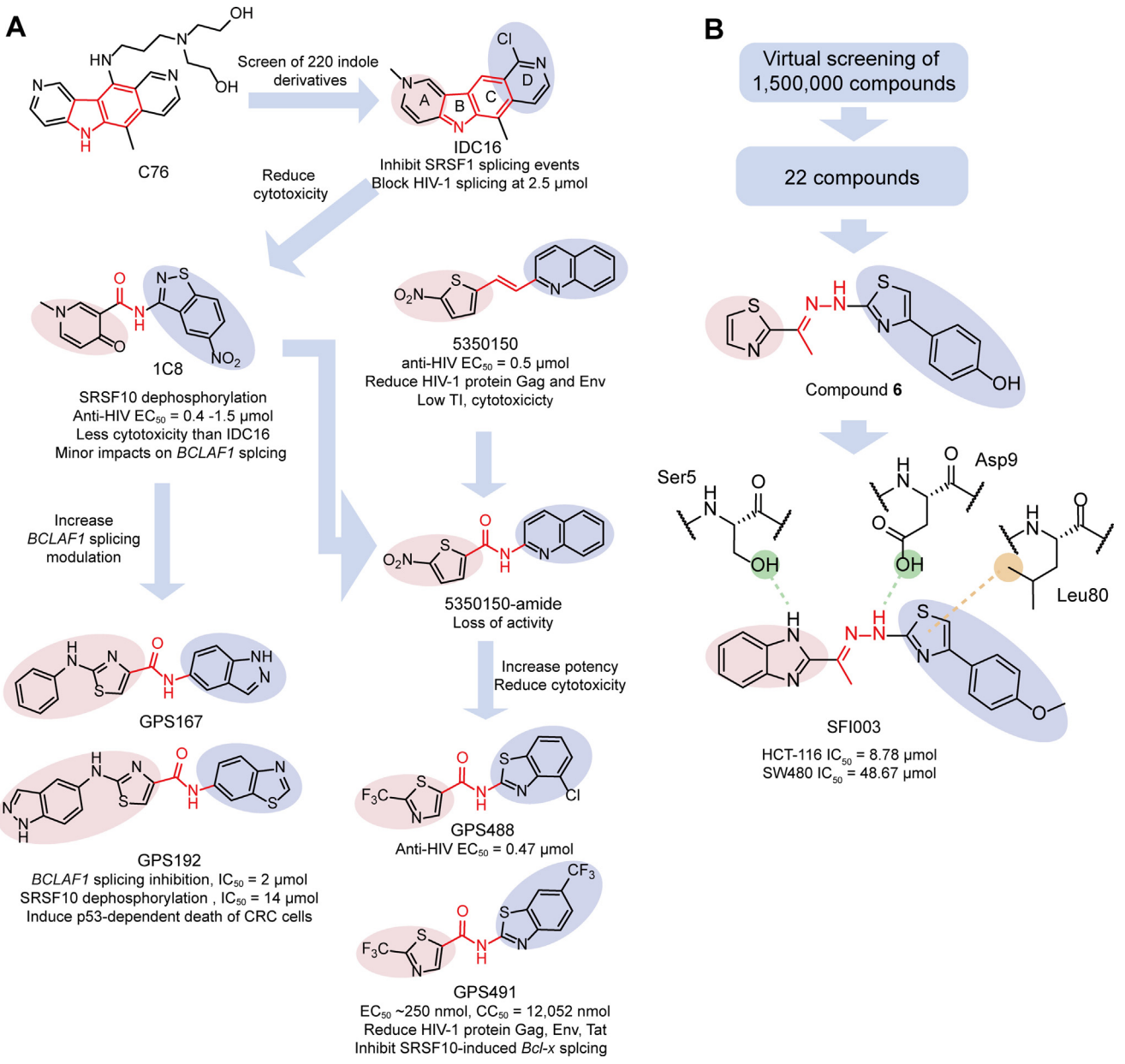


Figure 11 (A) Structure optimization and activities of indole derivatives as SRSF inhibitors. IDC16 was discovered in the screening of 220 indole derivatives and was similar to C76, showing SRSF1 inhibition and HIV-1 splicing inhibition. To reduce the cytotoxicity of IDC16, the B/C ring motif was replaced with amide spacer and further optimization led to 1C8 that showed reduced impacts on cell viability and induced SRSF10 dephosphorylation. Bases on the structure 1C8, G167 and GPS192 were designed and synthesized with enhanced modulation on *BCLAF1* splicing and showing inhibitory effects on CRC cells. Compound 5350150 was initially discovered as anti-HIV agent. Subsequent optimization led to the discovery of GPS491 that showed increased anti-HIV activity and reduced cytotoxicity. Of note, GPS491 selectively inhibited SRSF10-induced *Bcl-x* splicing. (B) Discovery, activity and binding mode of SFI003.

which altered the expression of apoptotic genes, IDC16 impacted little on the splicing of endogenous genes[240]. The pharmaceutical effects of SRSF inhibitors were further expanded by the discovery of IDC92, which showed anti-cancer effects by SRSF1 down-regulation. Mechanistically, IDC92 treatment altered the splicing pattern of *Ron via* inhibiting exon11 inclusion, reducing the invasiveness of Cl.SF2 cells. Interestingly, IDC92 treatment had no interference with the AS pattern of caspase 9 and *Rac1* genes, another two targets of SRSF1. It remains elusive how IDC92 achieved such specificity[150].

The DNA-intercalating ability of IDC16 endowed by the polyaromatic structure made it unsuitable for long-term use as anti-HIV drug due to potential cytotoxicity, although it blocked HIV-1 replication at a lower concentration than the threshold of its cytotoxic effect. In this regard, a structure derivation campaign was conducted to reduce the cytotoxicity by disrupting the planar tetracyclic motif and retaining structural resemblance to IDC16. A series of molecules were designed and synthesized *via* replacing the B and/or C rings with spacer elements, or fusing A/B rings (Fig. 11A). This led to the discovery of several diheteroarylamide

compounds featured by a 5-nitro substituted benzo[*d*]-isothiazole motif as the D-ring and an amide linker which replaced B/C rings. SAR analysis showed that 5-nitro-isobenzoisothiazole was important to the activity, since reducing the size or changing the spatial orientation of this subunit resulted in activity loss. However, the A-ring motif was dispensable between methoxy or methoxyethanol-substituted pyridine/pyridinone, but removing the oxygen-substituted side chain led to activity loss. Compound 1C8 was the most potent one and inhibited different wild-type and drug-resistant HIV stains (EC_{50} = 0.4−1.5 μmol/L) (Fig. 11A). 1C8 also showed optimal therapeutic index ranging from 66 to 100[229]. Compared with IDC16, 1C8 had equivalent anti-HIV-1 potency but significantly reduced effects on cell viability of CEM-GXR cells at higher concentrations (for IDC16, 33.2% at 4 μmol/L, 19.9% at 8 μmol/L, and 11.9% at 16 μmol/L; for 1C8, 81.5% at 4 μmol/L, 80.9% at 8 μmol/L, and 78.7% at 16 μmol/L). At the concentration of 1 μmol/L, 1C8 elicited a 40%−60% decrease in HIV-1 RNA accumulation and reduced the production of splice variants including Nef, Vpr1 and Tat1 by ∼60%. Instead of targeting SRSF1, 1C8 was confirmed to specifically modulate the activity of SRSF10 by dephosphorylating Ser133, which altered the interaction of SRSF10 with HIV-1 transcripts and other splicing factors such as hnRNP F/H and hTRA2β. Further studies showed that the impacts of 1C8 on SRSF10-targeted gene splicing, including *BCLAF1*, *GLYR1*, *CHEK1* and *SMN2,* and global splicing events were only moderate at 10 μmol/L, which was consistent with the minimal cytotoxic effect of 1C8[229].

Based on the moderate effect on *BCLAF1* AS of 1C8, over 400 IDC16 mimics with a variety of linkers replacing the central benzene and pyrrole rings were screened to find SRSF10 modulators. This led to GPS167 and GPS192, which possessed an aminothiazole carboxamide core and an amide linker (Fig. 11A). GPS167/GPS192 induced 50% of *BCLAF1* splicing response at 2 μmol/L and altered the splicing events of other SRSF10-targeted genes, including *MDM4*, *WTAP*, *CLK1* and *SLK1*. Mechanistically, treatment of GPS167/GPS192 produced higher proportion of dephosphorylated SRSF10 *via* inhibition of CLKs, leading to activity shift and improper subcellular localization of SRSF10. GPS167 impaired cell and organoid growth, inhibited migration and anchorage-independent colony formation of CRC cells. In particular, GPS167 treatment altered the splicing patterns of *MDM4* and *MDM2*, which led to p53-dependent cell death of CRC cells. Of note, GPS167 disrupted organoid-like structures in the CRC organoid line CCOL-01 but not in the normal organoid line CNOL-11. Although GPS167 turned out to be an inhibitor of CLKs, its inhibitory activity was prominent only when SRSF10 was used as substrate, indicating the preferential modulation of GPS167 on SRSF10[241].

Compound 5350150 was initially identified active in the screening for modulators of *SMN2* alternative splicing, but later revealed to show anti-HIV-1 activity (EC_{50} = 0.5 μmol/L)[242]. Due to limited therapeutic index, structure optimization of this compound was conducted to reduce the cytotoxic effects. Firstly, the double bond was considered to largely account for the cytotoxicity and inspired by the amide spacer of 1C8, a series of compound with amide replacing the C=C double bond and diverse functionalized mono- and bicyclic aromatic/heteroaromatic motifs on the right-side were screened for anti-HIV-1 activity, which finally led to the discovery of GPS488 and GPS491 that achieved higher potency (EC_{50} = 0.47 and 1.66 μmol/L, respectively) and were nontoxic at 10 μmol/L (Fig. 11A). SAR analysis showed that the functionalized benzothiazole motif was essential for anti-HIV activity and substituents with other cyclic fragments, including quinoline, pyridine, thiazole, isoxazole and 1,3,4-thiadiazole, caused activity loss. Of note, compounds with 4-Cl, 6-NO_2 or 6-CF_3 group on the benzothiazole ring showed activity−viability profiles with a clear separation between activity and cell viability (toxicity) relative to 5350150 at 1 μmol/L. Optimization on the 5-nitro-thiophene ring revealed that CF_3 group was an effective alternative to the nitro function and the polar neutral functionality at C-5 of the thiophene ring played an essential role in target binding. Lastly, changing the left-side thiophene ring by a thiazole ring led to marked potency increase from EC_{50} value of 5.5 to 0.47 μmol/L[243]. GPS491 had potent inhibition on the replication of wild-type HIV-1 strains as well as variants resistant to antiretroviral drugs (EC_{50} ∼250 nmol/L, CC_{50} = 12,052 nmol/L) in the CEM-GXR cell line and peripheral blood mononuclear cells. GSP491 treatment induced over 90% reduction of HIV-1 protein Gag, Env and Tat at low μmol/L dose *via* reducing unspliced and single spliced HIV-1 RNA accumulation. GPS491 also shifted the abundance of multiple spliced HIV-1 RNA such as Rev and Tat, which was similar to the effects of 5350150. Mechanism studies discovered that GPS491 selectively altered the abundance of SRSFs, increasing levels of SRSF5 and SRSF9 by 1.5-fold, while reducing SRSF6 level by 50%, respectively. GPS491 also increased the phosphorylation extent of SRSF4. In the assay of Bcl-x RNA splicing, GPS491 decreased the ability of SRSF10 to promote *Bcl-xS* 5′ splice site usage, preventing the SRSF10-mediated switch towards more endogenous *Bcl-xS* RNA. In addition, GPS491 also reduced adenovirus infectious yield ∼1000 fold and inhibited viral structural protein expression and the formation of virus particles of multiple coronaviruses (229E, OC43, SARS-CoV-2). These results implied a broad application range of GPS491 in the treatment of virus infections[244].

The discovery of indole derivatives gives insights into the design of small molecules to target the splicing activities of SRSFs. They showed promising therapeutic effects on HIV-1 infections and cancers with minimal cytotoxicity and impacts on global gene expression. However, it remains unsolved how these molecules interact with SRSFs and how they achieved target selectivity. They were initially revealed to bind to RS domains, which interfered with SRSF phosphorylation and protein interaction. Later it was discovered that they showed inhibitory activity to SRSF phosphorylation and GPS167 preferentially inhibited CLK-mediated SRSF10 phosphorylation, indicating that indole derivatives could specifically target the interaction between SRSFs and SRSF kinases such as CLKs and SRPKs. Researches elucidating the mechanisms of indole derivatives were significant since they could illuminate further rounds of structural optimization. Meanwhile, studies of the *in vivo* effects of indole derivatives are also desired to further verify their therapeutic effects.

5.2. *SFI003*

Virtual screening of over 1,500,000 compounds based on the homology model of SRSF3 led to 22 compounds with moderate inhibition to growth of HCT-116 cells. The optimal lead compound **6** was featured by a hydrazone linker bridging two thiazole fragments. Structure optimization of compound **6** *via* replacing the 2,3-dihydrothiazole motif with a benzimidazole and methylation of the hydroxyl led to the discovery of SFI003, which was the most potent SRSF3 inhibitor in this category (Fig. 11B). SFI003 showed potent antitumor efficacy both *in vitro* and *in vivo*. *In*

silico binding prediction revealed that SFI003 occupied the pocket comprised of Ser5, Asp9, and Leu80, distant from the pre-mRNA binding site of SRSF3. SFI003 did not disrupt the binding of SRSF3 to target genes but inhibited SRSF3 expression *via* neddylation-dependent SRSF3 degradation. SFI003 inhibited proliferation and migration of HCT-116 and SW480 cells with IC_{50} values of 8.78 and 48.67 μmol/L, respectively. Orally administered SFI003 at 100 mg/kg and above efficiently suppressed the growth of HCT-116 and SW480 xenografts. SFI003 attenuated DHCR24 expression *via* SRSF3 inhibition, leading to induced ROS generation and cell apoptosis. Since mTOR pathway is a downstream signaling pathway of ROS, SFI003 showed mTOR inhibition-based antitumor activity and synergetic effects with rapamycin with CI values less than 1. In HCT-116 and SW480 cells, the IC_{50} values of SFI003 in combination with rapamycin decreased from 8.78 to 2.39 μmol/L and 48.67 to 1.45 μmol/L, respectively, when compared with SFI003 alone. Besides, SFI003 possessed optimal pharmacokinetic properties, oral bioavailability, and tumor distribution, making SFI003 a promising candidate for CRC treatment[245].

5.3. *Indacaterol*

There has been a growing interest in repurposing pharmaceuticals from drugs that have been approved by the US Food and Drug Administration (FDA). Due to the well-characterized pharmacological effects and ensured safety, these drugs are readily for clinical application once their second indication is confirmed. Some clinical compounds approved by FDA have been discovered as SRSF modulators, which broadens the chemical space and offers optimal lead compounds for the development of potent and selective SRSF modulator. Based on the finding that SRSF6 RRM2 was indispensable for AS of *ZO-1* in CRC progression and SW620 cell migration and invasion, homology modeling of the RRM2 was constructed to screen 4855 FDA-approved drugs. Indacaterol, a β2-adrenergic receptor agonist approved to treat chronic obstructive pulmonary disease, was discovered as SRSF6 inhibitor which suppressed CRC cell proliferation and migration with IC_{50} = 19.01 μmol/L in SW620 cell. Indacaterol also exhibited similar cytotoxic effect in RKO. HCT116 and HCT8 cells. Intraperitoneally injection of indacaterol inhibited tumor formation in a colitis-associated CRC model in a dose-dependent manner and abrogated tumor formation at the dose of 2.5 mg/kg. *In vitro* splicing assay revealed that indacaterol shifted the AS of *ZO-1* gene and promoted exon23 inclusion, which reduced the proliferation and migration of CRC cells. Prediction of binding mode with molecular docking showed that indacaterol formed two hydrogen bonds with the backbone residue LEU58 and another two hydrogen bonds with ASP59 and GLU72. This result suggested that the RRMs of SRSFs could be promising binding sites for small molecule binding. Although the binding mode of indacaterol with SRSF6 has not been confirmed by complex crystal structure, indacaterol serves as an ideal lead compound for future SRSF6 inhibitor development[90]. $Zinc^{2+}$ also functioned as a specific SRSF6 inhibitor through inducing SRSF6 hyperphosphorylation but impacted little on the phosphorylation states of other SRSFs, which inhibited the RNA-binding ability of SRSF6. Zn^{2+} treatment affected the alternative splicing pattern of *Bim via* SRSF6 inhibition, promoting the generation of potent apoptosis inducer *BimS*[123].

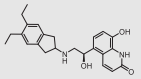
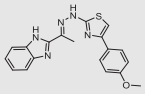
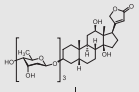
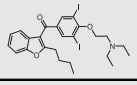
5.4. *Methylxanthines*

Xanthines and methylxanthines were assayed for antitumor activity *via p53* AS regulation, which discovered that caffeine and theophylline switched the splicing pattern of *p53* from α to β isoform at 5 mmol/L through downregulation of SRSF3. These compounds synergistically suppressed cell survival and colony formation of HeLa cells and induced apoptosis in MCF-7 cells. Besides, theophylline and caffeine also induced EMT, p53-dependent senescence and G2/M arrest in MCF-7 cells at 5−10 mmol/L but had no effect on normal epithelial cells at 10 mmol/L. SAR data showed the importance of methyl groups on the xanthine backbone, since compounds with different methyl substituent positions, theobromine (3,7-dimethylxanthine), xanthine and hypoxanthine, had no effects on SRSF3 and *p53* AS. Although the efficacy of these compounds are not sufficient for systemic treatment considering their clinical therapeutic serum concentration (5−15 μg/mL), methylxanthine derivatives can be promising lead compounds to target SRSF3[246]. Interestingly, caffeine had been previously revealed to alter the splicing pattern of *KLF6* and promoted an *SpKLF6* isoform that antagonized the tumor suppression of KLF6. In particular, this effect was found mediated by induction of SRSF2[247]. Therefore, caffeine and theophylline might have more complicated effects on SRSF expression and the splicing patterns of downstream genes.

5.5. *Cardiotonic steroids*

Cardiotonic steroids have been widely used in the treatment of congestive heart failure and these molecules were recently discovered as SRSF modulators. Digitoxin depleted SRSF3 protein level by 58% and Tra2-β by 36% at 50 nmol/L in HEK293 cells. Alterations in a large set of splicing events were observed upon digitoxin treatment, most of which were rich in binding sites of SRSF3 and Tra2-β. Re-expression of SRSF3 or Tra2-β restored normal splicing patterns after digitoxin treatment, which indicated that digoxin altered global AS *via* reducing SRSF3 and Tra2-β[248]. Other researches verified the effects on AS of digoxin in neuronal cells. For example, digoxin promoted the inclusion of *MAPT* exon10 with EC_{50} = 50 nmol/L in a two-color fluorescent reporter splicing assay[249]. Facilitating the production of exon20-containing *IKBKAP* was of therapeutic significance to reverse familial dysautonomia (FD), an autosomal recessive disorder. Of note, digoxin also induced reduction in SRSF3 level and impacted the AS pattern of *IKBKAP* in neuronal cells. At the concentration of 100 ng/mL, digoxin resulted in almost exclusive production of exon20-containing *IKBKAP* transcripts and this response depended on an SRSF3 binding site located in the intron 5′ of the alternatively spliced exon. At therapeutic concentration of 0.5−2.0 ng/mL, digoxin still achieved responsiveness, suggesting its promising application to treat FD[250]. Another study revealed that during HIV-1 viral processing, digoxin altered expression of CLKs and enhanced SRSF phosphorylation, which inhibited HIV-1 gene processing in HIV-1 transduced HeLa cells (inhibition of Gag expression with an IC_{50} of 45 nmol/L) and human $CD4^+$ PBMCs (IC_{90} = 2 nmol/L). Digoxin significantly reduced incomplete spliced HIV mRNAs that encoded structural protein Gag, Gagpol and Env, and affected the usage of splicing site in multiple spliced HIV mRNAs, leading to specific loss of

Table 2 Selected SRSF, SRPK and CLK modulators and their pharmacological effects.

Name	Structure	Target	Pharmacological effect	Ref.
Indacaterol		SRSF6	Inhibition of *ZO-1* splicing and CRC cell proliferation and migration (IC_{50} = 19.01 μmol/L). Reduce tumor growth at 2.5 mg/kg in CRC model	90
Zinc ion	Zn^{2+}	SRSF6	Induction of potent apoptosis inducer *BimS*	123
SFI003		SRSF3	Induce neddylation-dependent SRSF3 degradation and inhibit HCT-116 and SW480 cell proliferation (IC_{50} = 8.78 and 48.67 μmol/L). Induce ROS *via* SRSF3/DHCR24 axis. Inhibit tumor formation at 100 mg/kg *in vivo*	245
Caffeine		SRSF3	Reduce expression level of SRSF3 and promote *p53β* isoform production. Inhibit MFC-7 cell and HeLa cell proliferation at > 5 mmol/L	246
Theophylline				
Digoxin		SRSF3	Reduce SRSF3 expression and affect AS of p53, IKBKAP, MAPT. Inhibit HIV-1 RNA processing in HeLa cells and PBMCs. Induce apoptosis and EMT and in HeLa cells	251
Amiodarone		SRSF3	SRSF3 degradation and SLU7 reduction. Inhibition of HeLa cell growth	253

Rev. Besides, the export of incompletely-spliced HIV transcripts to cytoplasm was also blocked by digoxin treatment. Digoxin enhanced SRSF3 function *via* CLK-induced phosphorylation, since SRSF3 overexpression mostly mimicked its impact on HIV-1 gene processing[251]. Interestingly, similar to caffeine, digoxin switched the AS of *p53* to the production of *p53β* isoform through reducing the expression of SRSF3 in HeLa cells at 1 μmol/L. Digoxin-treated HeLa cells underwent cellular apoptosis, EMT and an increase in S, G2/M and sub G1 populations. The effect of digoxin on *p53* AS was also observed in glioblastoma multiforme cell U87 and GBM8401. Besides, downregulation of *HIF-1α* proteins was observed upon digoxin treatment[252].

Amiodarone, a class III antiarrhythmic drug, induced the PTC-containing isoform of *SRSF3* (*SRSF3-PTC*) at 30 μmol/L and reduced SRSF3 protein level in a dose-dependent manner in HeLa cells. SLU7, which was essential for normal SRSF3 transcription, was reduced under amiodarone treatment. Amiodarone induced G1 arrest and apoptosis, and inhibited colony formation and anchorage-independent growth of HeLa cells. Further studies revealed that amiodarone synergized with caffeine and digoxin to regulate *p53* AS and promote reactive oxygen species generation[253].

Reposition of approved drugs or published bioactive molecules has successfully discovered a series of compounds interfering with SRSF functions (Table 2). Nevertheless, the binding modes and/or pharmacological mechanisms of these compounds still remained unsolved. Future studies should aim to further optimize their potency and selectivity, and clarify the modes of action of these SRSF modulators.

5.6. *Targeting SRSF phosphorylation*

Phosphorylation states are closely related with SRSF cellular mobilization and interactions with genes and proteins. Therefore, inhibition of kinases such as SRPKs and CLKs has significant impacts on SRSFs, leading to impaired SRSF functions. Many studies have demonstrated that abnormal SRSFs phosphorylation caused by SRPK or CLK dysfunction were involved in disease progression. High expression of CLKs was observed in gliomas and renal tumors and correlated with poor survival of renal cancer patients[254]. SRPK inhibition or silence can be protective against many types of cancer and angiogenesis diseases[255]. Detailed analysis concerning SRPK/CLK inhibitors was reviewed elsewhere, with several molecules being investigated in clinical trials for the treatment of cancers, inflammatory diseases and angiogenetic diseases[42,255,256]. Targeting SRSF phosphorylation, the central modification process for their RNA processing activity, indirectly rectifies abnormal AS events and represent a promising approach for the treatment of cancers and other diseases.

6. Conclusions and perspectives

AS determines the exclusion and inclusion of exons, thus generating mRNA and protein isoforms with quite different functions based on one transcript. It is conceivable that AS is indispensable to the maintenance of overall cellular activities and, significantly, many diseases have been demonstrated caused by defects of AS. Mutations of splicing factor genes including *SF3B1*, *U2AF1*, *SRSF2* have been detected in solid tumors and a wide range of hematopoietic malignancies[2]. In this regard, many small molecule inhibitors of SF3B1 were disclosed with further studies revealing a synthetic lethality between SF3B1 inhibition and spliceosome mutations[257]. Quite different from traditional small molecule drugs that directly bind to proteins for functional inhibition, molecules targeting splicing factors decrease the abundance of pathological proteins *via* interfering with their upstream gene splicing, which serves as a promising approach to target undruggable proteins. As important modulators of alternative splicing, SRSFs bind to pre-mRNAs and facilitate splice site recognitions and spliceosome assembly. The significant roles of SRSF overactivation in cancer development and other diseases imply unignorable therapeutic potentials of SRSF inhibition. Based on above

analysis, some critical issues and challenges require more efforts to be resolved.

First, some SRSFs have been found to regulate other RNA metabolism processes apart from alternative splicing, such as inhibition of R loops and promotion of transcription elongation. While for the other members of SRSFs, their unique regulations on RNA processing are rarely studied. Since SRSFs share functional redundancy in alternative splicing, untangling their discrepancies in splicing-independent physiology can depict the unique function pattern. Secondly, cancer cells with *SF3B1*, *U2AF1* and *SRSF2* mutations show higher sensitivity to splicing perturbations, partly due to increased genome instability and DNA damage caused by spliceosome mutations, which rationalizes the treatment of spliceosome-mutant malignancies with SF3B1 inhibitors. We wonder if there is an enhanced sensitivity of spliceosome-mutant cancer cells toward SRSF inhibitors given that SRSFs also function to maintain genome stability. Thirdly, as RNA-binding proteins, SRSFs contain structurally similar RNA binding motifs, making it challenging for selective inhibition and to prevent off-target effects of small molecule inhibitors. Indeed, RNA binding proteins have been considered as undruggable due to lack of ideal drug binding sites and potential off-target effects. It will be beneficial to clarify the binding modes and mechanisms of published SRSF modulators in the future.

In conclusion, current studies of SRSFs have demonstrated their critical functions in AS and other RNA metabolic events. SRSF dysregulations are associated with cancers, autoimmune diseases, fatty liver diseases, tauopathy and viral gene propagation and so on. Although current studies of SRSFs give rise to plenty unsolved questions, we believe these challenges will finally be overcome with deeper understandings of the physiological and pathological roles of SRSFs in the future.

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Author contributions

Maode Lai and Wenying Yu conceived this review. Dianyang Li wrote the manuscript with the guidance of Maode Lai and Wenying Yu. Maode Lai and Wenying Yu revised the manuscript. All of the authors have read and approved the final manuscript.

Conflicts of interest

The authors declare no conflicts of interest.

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