REVIEW

Serine-arginine protein kinases and their targets in viral infection and their inhibition

Kai Zheng[1] · Zhe Ren[2] · Yifei Wang[2]

Abstract

Accumulating evidence has consolidated the interaction between viral infection and host alternative splicing. Serine-arginine (SR) proteins are a class of highly conserved splicing factors critical for the spliceosome maturation, alternative splicing and RNA metabolism. Serine-arginine protein kinases (SRPKs) are important kinases that specifically phosphorylate SR proteins to regulate their distribution and activities in the central pre-mRNA splicing and other cellular processes. In addition to the predominant SR proteins, other cytoplasmic proteins containing a serine-arginine repeat domain, including viral proteins, have been identified as substrates of SRPKs. Viral infection triggers a myriad of cellular events in the host and it is therefore not surprising that viruses explore SRPKs-mediated phosphorylation as an important regulatory node in virus–host interactions. In this review, we briefly summarize the regulation and biological function of SRPKs, highlighting their involvement in the infection process of several viruses, such as viral replication, transcription and capsid assembly. In addition, we review the structure–function relationships of currently available inhibitors of SRPKs and discuss their putative use as antivirals against well-characterized viruses or newly emerging viruses. We also highlight the viral proteins and cellular substrates targeted by SRPKs as potential antiviral therapeutic candidates.

Keywords Serine-arginine protein kinases · Antiviral · SR proteins · Alternative splicing · Virus–host interaction

Introduction

A multitude of studies have consolidated the interaction between virus infection and host alternative splicing [1, 2]. Serine-arginine (SR) proteins are a class of highly conserved splicing factors with one or two RNA recognition motifs (RRMs) at the N-terminal and a serine-arginine dipeptide highly repetitive domain (RS domain) at the C-terminal (Fig. 1A) [3]. As RNA binding proteins, SR proteins are critical for the spliceosome maturation, splice site selection, alternative splicing and RNA metabolism, including mRNA export, translation, localization and nonsense-mediated RNA decay [4–8]. Notably, distribution and activities of SR proteins are mainly regulated by the reversible phosphorylation within the RS domain [9], and two protein kinase families, cdc2-like kinases (CLKs) [10] and serine-arginine protein kinases (SRPKs) [11, 12], are predominantly involved in the sequential phosphorylation modification process. SRPKs phosphorylate SR proteins in the cytoplasm to promote their nuclear import and storage in interchromatin speckles, whereas CLKs further phosphorylate SR proteins to stimulate their translocation and participation in RNA splicing (Fig. 1B). Under stress stimuli, SRPKs also translocate to the nucleus to regulate the hyperphosphorylation of SR proteins. Therefore, CLKs and SRPKs-mediated phosphorylation cooperate to precisely mobilize the splicing functions of SR proteins.

SRPKs belong to the serine-arginine protein kinase family that specifically phosphorylate SR splicing factors (SRSFs) and regulate the crucial pre-mRNA splicing process [11, 12]. To date, three members of SRPKs have been characterized, including the well-investigated SRPK1 (or its yeast analogue Sky1p) [13, 14], SRPK2 [15] and the less understood SRPK3 [16]. Apart from the prevailed canonical

✉ Kai Zheng
  zhengk@szu.edu.cn

1 School of Pharmacy, Shenzhen University Medical School, Shenzhen 518055, China

2 Institute of Biomedicine, College of Life Science and Technology, Guangdong Province Key Laboratory of Bioengineering Medicine, Key Laboratory of Innovative Technology Research On Natural Products and Cosmetics Raw Materials, Jinan University, Guangzhou 510632, China
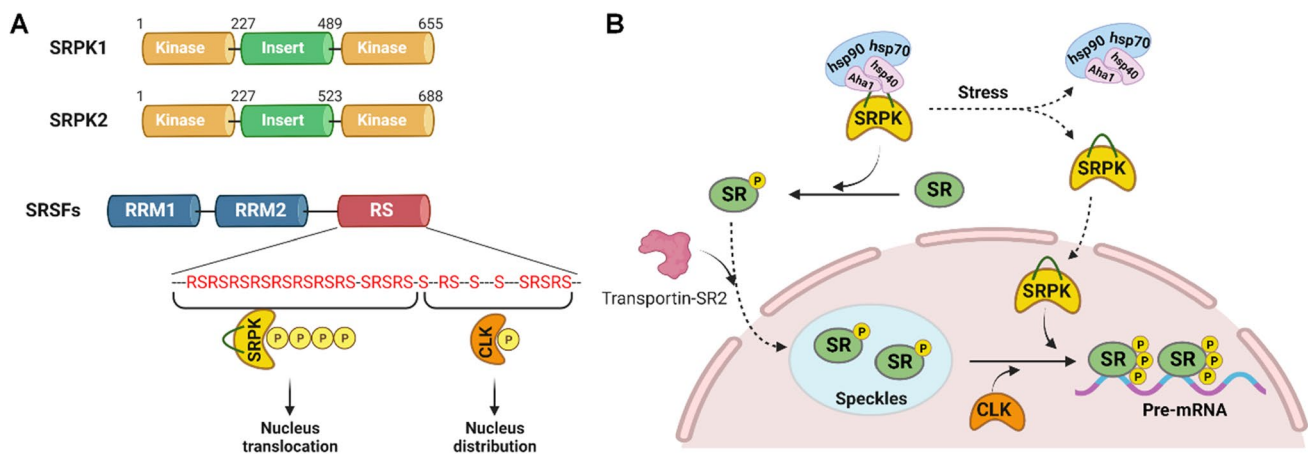
Fig. 1 SRPKs phosphorylate SR proteins. **A** Structures of SRPKs and SR proteins. SRPKs contain two well-conserved catalytic kinase domains, which is separated by a non-conserved insert domain. SR splicing factors (SRSFs) consist of one or two RNA recognition motifs (RRMs) and a C-terminal RS domain, which is sequentially phosphorylated by SRPKs and CLKs for nuclear transport and distribution. **B** Cytoplasmic SRPKs interact with the cochaperones hsp40/Aha1 and the hsp90/hsp70 chaperon complex through the insert domain (green curve) and phosphorylate SR proteins to promote their nuclear translocation and nuclear speckles formation with the help of transportin-SR2. In the nucleus, CLKs further phosphorylate SR proteins to facilitate their participation in RNA splicing. Under different stresses, SRPKs dissociate from their chaperones and translocate to the nucleus to regulate the hyperphosphorylation of SR proteins and consequently alternative splicing. P phosphorylation

SRSFs (named SRSF1–12 according to their chronological order of discovery), other cytoplasmic proteins containing the RS domain have been identified as substrates of SRPKs, including viral proteins. Phosphorylation is an important regulatory mechanism leveraged by viruses to modulate the subcellular location, stability and activity of viral proteins and their interactions with host cellular proteins, which in turn facilitates viral infection and pathogenesis. It is therefore reasonable that viruses explore SRPKs-mediated phosphorylation of viral proteins or cellular substrates, such as SRSFs, to manipulate the alternative splicing of viral and host cell mRNA transcripts and to establish an appropriate microenvironment for viral replication and assembly. In this review, we highlight the complex contributions of SRPKs in viral infection and discuss the potential of SRPKs, viral proteins targeted by SRPKs, and SRSFs as therapeutic antiviral candidates.
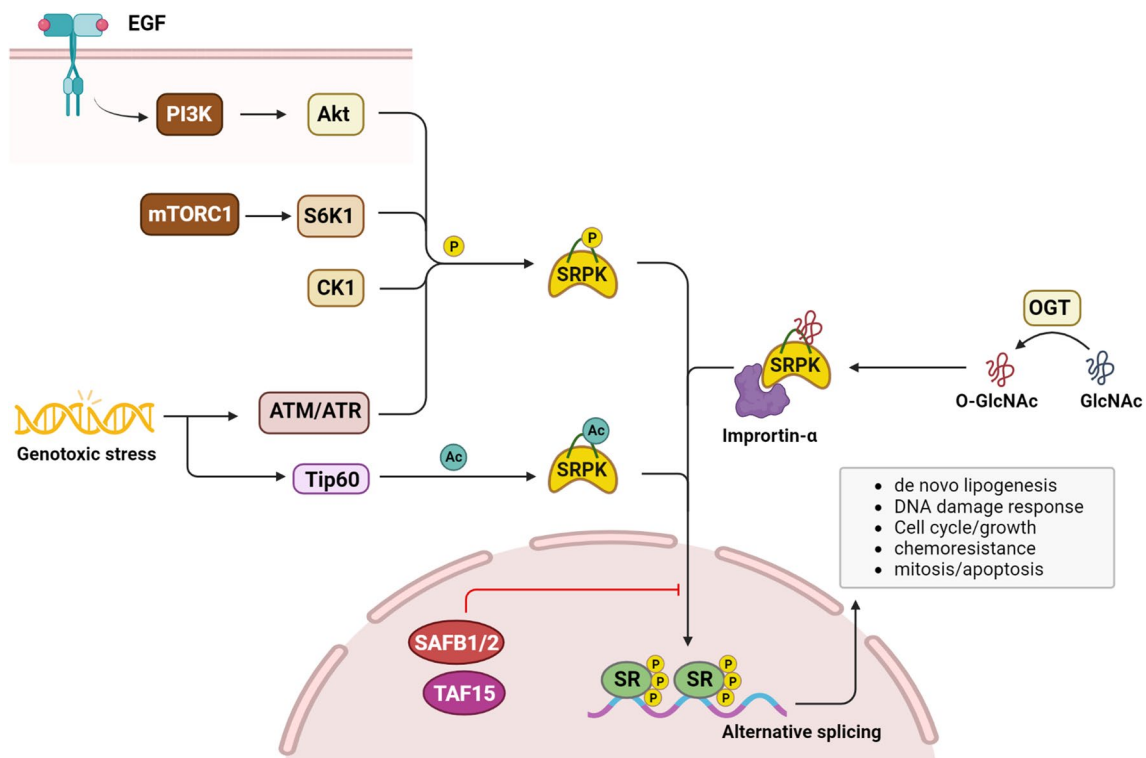
Regulation and function of SRPKs

SRPKs consist of two conserved catalytic domains and a large non-conserved insert domain that separates the bipartite kinase domains [17]. The insert domain binds directly to the cochaperones Aha1 and Hsp40, mediating the dynamic interaction between SRPKs and the major heat-shock complex Hsp70/Hsp90 and anchoring SRPKs in the cytoplasm (Fig. 1B) [18–20]. Accordingly, cytoplasmically localized SRPKs predominantly phosphorylate newly synthesized SR proteins and other cytoplasmic RS-like domain-containing

proteins (e.g., ZO-2, RNF12) [21, 22], and facilitate their nuclear import via transportin-SR2 [23, 24]. On the other hand, nuclear SRPKs collaborate with CLKs to tightly control the phosphorylation levels of SR proteins and modulate the efficient splicing pattern of several genes [25]. Under various external signals, such as osmotic stress [19], growth factors [20], cell cycle signals [18] and genotoxic agents [26], SRPKs are released from the Hsp70/Hsp90 chaperone complex and translocate to the nucleus to stimulate the expression of splicing isoforms conducive to cell growth via phosphorylation of SR proteins [20, 27], or to regulate lamin B receptor (LBR)-mediated chromatin reorganization [28, 29]. However, the unbalanced and aberrant increase in nuclear SRPKs is generally believed to cause the hyperphosphorylation of SR proteins and LBR, leading to splicing factor aggregation, splicing inhibition and chromatin segregation from the nuclear envelope [18, 29]. Notably, the transient interaction of nuclear matrix-localized SRPK1 with scaffold attachment factors (SAFB1/2) [30, 31], or the RNA binding protein TAF15 [32], in the nucleus impairs its enzymatic activity. When exposed to hypoxia, SRPK1 rapidly dissociates from SAFB1/2 and transfers to the cytoplasm, resulting in the dephosphorylation of SRSFs and the production of splicing variants.

In addition to these interacting proteins, post translational modifications, such as phosphorylation, acetylation and O-GlcNAcylation, have also been shown to regulate the kinase activity and nuclear translocation of SRPKs (Fig. 2). For example, casein kinase 2 (CK2) can phosphorylate SRPK1 at Ser51 to enhance its catalytic activity

Fig. 2 Regulation of SRPKs. Stimuli-responsive kinases phosphorylate/acetylate/*O*-GlcNAcylate SRPKs at different amino acids, mainly within the insert domain, leading to the dissociation of SRPKs from the Hsp90/hsp70 complex and subsequent cytoplasm-nucleus translocation, which in turn modulate alternative splicing to achieve differ-ent outcomes. Transient interactions of SRPKs with nuclear SAFB1/2 and TAF15 further suppress SRPKs activity. Under stress, SRPKs can be released from these inhibitory interactions into the cytoplasm. P phosphorylation, Ac acetylation

approximately sixfold in vitro [33]. Akt phosphorylates SRPK2 at Thr492 to promote its nuclear import and regulate cell cycle and cell death [34], whereas SRPK1 is phosphorylated at Thr326 and Ser587 in response to epidermal growth factor signaling [20]. Ribosomal S6 kinase 1 (S6K1) phosphorylates SRPK2 at Ser494 and cooperates with CK1-mediated phosphorylation at Ser497 to promote lipid biosynthesis [27]. In addition, the DNA damage response kinases ATM/ATR phosphorylate SRPK1 at Thr326 and Ser408 to adapt genotoxic stress [35]. Furthermore, the acetyltransferase Tip60 acetylates SRPK1 at Lys215/258/265/301/318/585/588 to promote its nuclear translocation and regulate cisplatin resistance [36, 37]. Finally, the O-GlcNAc transferase O-GlcNAcylates SRPK2 at Ser490, Thr492 and Thr498, triggering its binding to importin α and promoting the splicing of lipogenic pre-mRNAs [38]. Taken together, the cytoplasmic-nuclear shuttling of SRPKs mediated by post-transcriptional modifications represents a key approach for cells to respond to different stimuli.

Remarkably, SRPKs, initially considered to be phosphorylation factors of SR proteins during mitosis, are nowadays gradually being recognized as multifunctional proteins involved in a variety of cellular processes [12, 39]. Most SR proteins, including typical SRSFs and atypical SR splicing factors, have been confirmed to be substrates of SRPKs, allowing SRPKs to accomplish various mRNA splicing events. In addition, there are more than 100 RS or RS-like domain-containing proteins [40], suggesting that SRPKs may phosphorylate these potential targets to exert diverse cellular functions, such as mRNA maturation, spermiogenesis, cell cycle progression, chromatin reorganization and innate immunity [12, 41]. For example, SRPKs phosphorylate P1 protamine and LBR to regulate spermiogenesis [42]. SRPKs may modulate energy homeostasis and glucose/lipid metabolism through phosphorylating PPARgamma coactivator-1alpha (PGC-1α) [12]. Notably, there is growing amount of evidence that SRPKs are involved in various human diseases, including neurodegeneration [43], arthritis [44], atherosclerosis [45, 46], and cancer [47, 48]. A prominent example is that SRPKs-mediated alternative splicing facilitates the transition of vascular endothelial growth factor (VEGF) from anti-angiogenic to pro-angiogenic isoforms, resulting in neovascularization and angiogenesis [45, 46]. Additionally, SRPKs are highly expressed in numerous epithelial

cancers and regulate several signaling pathways, such as NF-κB, TGF-β and PI3K/AKT pathways, to affect tumor cell growth and chemoresistance, and are considered as prognostic biomarkers and potential therapeutic targets [35, 49–51].

Viral manipulation of SRPKs-mediated phosphorylation

There is increasing evidence that, in addition to host cellular proteins, viral proteins containing RS or RS-like domains are also phosphorylated by SRPKs. Phosphorylation regulates various properties of viral proteins, such as activity, stability, subcellular location and interaction with protein partners or nucleic acids, and is essential for several critical steps of viral life cycle [52–54]. Substantial evidence suggests that different viruses interact with and hijack SRPKs to favor their replication, transcription and assembly (Table 1). Depending on the specific characteristics of the viral life cycle, SRPKs-mediated phosphorylation either induces the dissociation of viral proteins from the transcription complex/nucleic acids and their subsequent nuclear-cytoplasmic transport for transcription, or provides a suitable environment for viral capsid assembly and replication (Fig. 3). SRPKs also phosphorylate several SRSFs involved in the alternative splicing of viral and host cellular mRNAs.

## Roles of SRPKs in dsDNA virus infection

Herpesviridae

Herpes simplex virus 1 (HSV-1) is a widely-spread double-stranded (ds) DNA virus and a member of the alpha-herpesvirus sub-family that infects and establishes latency in sensory neurons. The viral ICP27 protein, an mRNA export factor, plays a central role in regulating HSV-1 replication and triggering alternative splicing of host and viral transcripts [55], and its interaction with SRPK1 is one of the major mechanisms preventing host pre-mRNA maturation (Fig. 3A) [56]. ICP27 recruits SRPK1 to the nucleus, where it is directly phosphorylated by SRPK1.

Table 1 Manipulation of SRPKs by virus

Genome	Virus family	Virus	SRPK	Viral targets of SRPKs	Biological effect
dsDNA	*Herpesviridae*	HSV-1	SRPK1	ICP27	Relocalization of SRPK1 into the nucleus by ICP27 interaction to promote viral replication
		VZV	SRPK1	IE4	Phosphorylates IE4 to promote its cytoplasm-to-nucleus shuttling to regulate viral gene expression
		HCMV	SRPK1	/	Relocalization of SRPK1 into the cytoplasm to promote egress of viral nucleocapsid and spliced RNA
		EBV	SRPK2	BLRF2	Phosphorylates BLRF2 to promote viral replication
	Papillomaviridae	HPV1	SRPK1	E1^E4	Inactivation of SRPK1 by E1^E4 to promote viral alternative splicing
				E2	Facilitates nucleus-to-cytoplasm translocation of E2
		HPV5	SRPK1	E2	Facilitates nucleus-to-cytoplasm translocation of E2
		HPV8	SRPK1	E2	Binds to E2 with unclear effect
		HPV16	SRPK1	E2	Upregulation of SRPK1 by E2 to promote viral replication
(+)ssRNA	*Coronaviridae*	SARS-CoV-1	SRPK1	N	Phosphorylates N protein to regulate its translational suppressive activity, prevents its aggregation and promote viral assembly
		SARS-CoV-2	SRPK1 SRPK2	N	Phosphorylate N protein to promote viral replication
	Flaviviridae	HCV	SRPK1	/	Colocalizes with core and NS5A to facilitate viral replication
			SRPK2	/	Colocalizes with core and NS5A to facilitate viral replication; enhances de-novo lipogenesis
(-)ssRNA	*Filoviridae*	Ebola	SRPK1 SRPK2	VP30	Phosphorylate VP30 to promote viral repetitive transcription
ssRNA-RT	*Retroviridae*	HIV	SRPK1 SRPK2	/	Activate SRSF4 to ensure viral replication; regulate SRSF10 to modulate viral RNA splicing
dsDNA-RT	*Hepadnaviridae*	HBV	SRPK1 SRPK2	HBc	Phosphorylate HBc to promote viral DNA synthesis, and to regulate pgRNA encapsidation
		DHBV	SRPK1	DHBc	Phosphorylates DHBc with unclear effect

RT reverse transcribe, / not determined
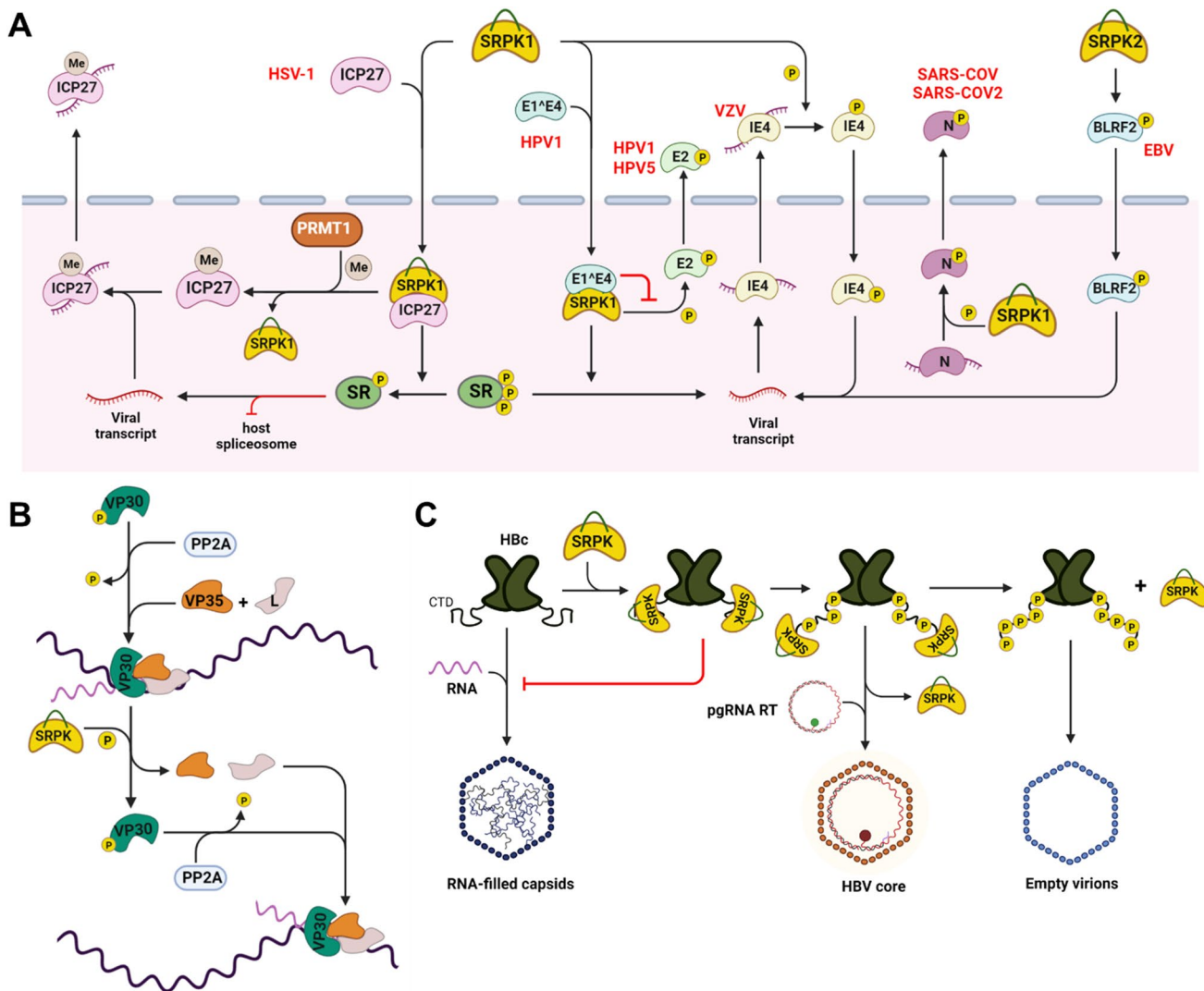
Fig. 3 Viral manipulation of SRPKs-mediated phosphorylation. **A** SRPKs-mediated phosphorylation regulates cytoplasmic-nuclear translocation of viral proteins, including HPV E2, VZV IE4, EBV BLRF2 and SARS-CoV N proteins. SRPKs also co-operate with viral proteins (HSV ICP27 and HPV1 E1^E4) to shut-off host splicing and promote viral transcription and replication. **B** SRPKs regulate VP30-mediated Ebola virus transcription. The transcription factor VP30 is dephosphorylated by PP2A and sequentially forms a complex with VP35 and polymerase L to initiate the primary transcription. VP30 is then phosphorylated by SRPK and dissociates from the RNA and polymerase complex to re-initiate the secondary transcription at the next transcription start site. **C** SRPKs regulate HBV capsid assembly. SRPKs bind to the CTD of the HBV core protein (HBc) to prevent its spontaneous assembly into RNA-filled capsids. The transient binding and appropriate phosphorylation by SRPKs allows the HBc-mediated adequate and organized HBV core formation, whereas hyperphosphorylation of HBc leads to the assembly of empty virions. Other viruses that interact with and modulate SRPKs function to favor their infection are not shown, including HCMV, HIV and HCV. CTD, C-terminal domain; P, phosphorylation; Me, methylation

Concurrently, ICP27 inactivates SRPK1 and induces its nuclear accumulation, which in turn causes the hypophosphorylation of SRSFs (SRSF1 and SRSF5) and stalls host spliceosome assembly, favoring HSV-1 transcripts and viral replication [56]. Subsequently, ICP27 dissociates from the RNA and acts as a chaperone to promote the cytoplasmic export of viral RNA, a process that requiries arginine methylation. The arginine residue within the RGG motif (RS-like domain) is methylated by protein arginine methyltransferase 1 (PRMT1), the inhibition of which causes the hypomethylation of ICP27 and its earlier and more rapidly nuclear export, resulting in defective viral replication [57]. Notably, PRMT1-mediated methylation within the RGG motif also significantly reduces the SRPK1-ICP27 interaction and prevents the nuclear recruitment of SRPK1 [58, 59]. Mechanistically, the RGG motif exhibits a higher binding affinity to SRPK1 than to SR proteins, possibly explaining the function of ICP27 in preventing nuclear SRPK1 contact with hypophosphorylated SRSF1 and ensuring accurate splicing of viral genes

with functional coding sequences [60, 61]. Therefore, the intricate interactions between ICP27, SRPK1 and PRMT1 occur sequentially in a specific order to ensure adequate viral replication and shut-off of host cellular splicing.

In addition to HSV-1 ICP27, SRPK1 has also been shown to interact with viral IE4 protein, an ICP27 homologue encoded by another alpha-herpesvirus, varicella-zoster virus (VZV) [62]. VZV IE4 protein is a chaperone that exports viral mRNA transcripts and interacts with SRSFs (SRSF1, SRSF3 and SRSF7) to act as a transcriptional activator. In the cytoplasm, SRPK1 binds to and phosphorylates IE4, triggering its dissociation from the exported mRNA and its cytoplasm-to-nucleus reshuttling (Fig. 3A). Furthermore, SRPK1 is involved in the infection of beta-herpesvirus human cytomegalovirus (HCMV) [63]. HCMV infection progressively triggers the accumulation of cytoplasmic SRPK1, which may allow efficient egress of viral nucleocapsids and continued RNA splicing during late HCMV infection. Interestingly, SRPK2 has been shown to interact with BLRF2, a tegument protein of the gamma-herpesvirus Epstein-Barr virus (EBV) [64]. BLRF2 either serves as a platform for tegument organization and capsid formation, or is essential for EBV replication by an undefined mechanism. SRPK2 phosphorylates BLRF2 within the RS motif (Ser148 and Ser150) to promote its nuclear localization, and depletion of this phosphorylation abrogates the critical and supportive function of BLRF2 in EBV replication. Finally, EBV EB2 protein, an ICP27 homologue belonging to the mRNA export factor, also interacts with SRSF1, SRSF3, and SRSF7 to stimulate cytoplasmic viral RNA accumulation and alternative splicing [65, 66]. Whether EB2 cooperates with SRPKs to modulate SRSF activity and viral transcription is unclear.

Papillomaviridae

Human papillomaviruses (HPVs) are non-enveloped dsDNA viruses that infect the oropharynx and anogenital tract to cause hyperproliferative warts and neoplasia. Although the pathogenesis of different HPV genotypes (*Alpha*, *Beta*, and *Mu*) is heterogeneous, the molecular mechanism of the viral life cycle is conservative, which is dependent on keratinocyte differentiation and requires the intervention of SRSFs-mediated HPV RNA splicing to balance the production of viral early (in undifferentiated cells) and late (in differentiated keratinocytes) proteins [67, 68]. Notably, phosphorylation of SRSFs by SRPK1 is usurped by some HPVs, and the interaction between SRPKs and HPV viral proteins is more complicated (Fig. 3A) [69]. During the virion-producing (late) phase of HPV, viral protein E4 is highly expressed to perform multiple roles, such as inducing cell cycle arrest and reorganizing the keratin network. E4 is derived from an E1^E4 fusion protein by proteolysis and sequesters various

kinases, including SRPK1, to regulate late phase viral infection. It has been showed that HPV1 E1^E4 protein directly binds to and inhibits SRPK1 activity, reducing the phosphorylation of SRSFs (e.g., SRSF1, SRSF3, SRSF4 and SRSF7) and promoting the production of alternative viral RNA isoforms [70, 71]. Besides, SRPK1 facilitates the nuclear-to-cytoplasmic translocation of the HPV1 E2 protein, a viral DNA binding factor regulating viral replication and transcription partially through upregulating SRSFs. SRPK1 phosphorylates the RS domain within the hinge region of HPV1 E2, whereas E1^E4 inhibits such phosphorylation and retains E2 in the nucleolus to modulate post transcriptional expression of viral gene [70]. Likewise, SRPK1-mediated phosphorylation also transfers HPV5 E2 protein from the nucleus to the cytoplasm [23, 72], or binds to HPV8 E2 with unclear consequences [69]. In contrast, HPV16 E2 is able to enhance the expression of SRPK1 [73], which in turn phosphorylates and stabilizes the splicing factor SRSF1 to promote the production of viral late proteins and the completion of the HPV infection cycle in differentiated keratinocytes. Further works are required to prove whether E2 binds directly to the SRPK1 promoter or interacts indirectly with other transcription factors, such as WT1, to activate SRPK1 expression [74].

Roles of SRPKs in (+)ssRNA virus infection

Coronaviridae

The outbreak of the emerged severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has caused global pandemic with huge economic losses and human casualties. The nucleocapsid (N) protein of coronavirus (CoV) is a highly conserved, multiple functional and abundant RNA-binding protein responsible for genomic RNA encapsidation [75]. Besides, the N protein undergoes liquid–liquid phase separation to facilitate capsid assembly and to cluster the RNA polymerase complex for viral transcription [76, 77]. In addition, the N protein suppresses the cell-intrinsic antiviral immune defense mechanism RNAi and RIG-I-mediated innate immune response [78, 79]. Importantly, the above functions of N protein are affected by phosphorylation, particularly of its RS domain, which is located at the N-terminal domain (NTD) and determines its binding affinity to RNA and DNA [80]. During infection with the beta-coronavirus SARS-CoV-1, several kinases, such as CDK, GSK, CK2 and SRPK1, have been demonstrated to phosphorylate the N protein, at least in vitro [81]. SRPK1-mediated phosphorylation within the RS motif mobilizes N protein translocation to the cytoplasm, prevents its aggregation in stress granules and regulates its translational suppressive activity against host cells in the cytoplasm (Fig. 3A), whereas phosphorylation of the N protein by other kinases stimulates its

cytoplasmic transport with unclear functional significance [82]. Besides, SRPK1-mediated extensive phosphorylation of newly synthesized N proteins prevents their aggregation and oligomerization in stress granules, leading to the soluble dimers favored for viral assembly [83]. In addition, SRPK1 and SRPK2 also phosphorylate the SARS-CoV-2 N protein at Ser188 and Ser206 to promote viral replication [84]. Through high-throughput screening, Alectinib, an FDA-approved inhibitor of anaplastic lymphoma kinase (ALK), is confirmed to inhibit SRPKs, resulting in decreased viral propagation and N protein phosphorylation [84]. Alectinib also dramatically inhibits the infection of alpha-coronavirus HCoV-229E, implying the extensively requirement of SRPK1/2 activity in both alpha- and beta-coronavirus life cycle. How insufficient phosphorylation of N protein compromises viral replication remains unclear, and one possibility is that N protein suppresses the host antiviral immune response [85]. Interestingly, non-COVID-19 patients show lower expression of SRPK2 when compared to COVID-19 patients, further suggesting the necessity of SRPKs for SARS-CoV-2 infection [86].

Flaviviridae

Hepatitis C virus (HCV) is an enveloped single-stranded RNA virus of the *Flaviviridae* family and is known for its pathogenic role in chronic liver diseases. In the case of HCV infection, alternative splicing has been demonstrated to shape the host antiviral immune response [87, 88]. Splicing factors generate different isoforms of interferon stimulated genes and human leukocyte antigen to significantly influence HCV infection and HCV-related carcinogenesis. On the other hand, HCV core protein, which is critical for nucleocapsid formation, viral genome encapsidation and interference with host cellular machinery, interacts with splicing factor DDX3 [89, 90], a DEAD-box RNA helicase involved in mRNA splicing and translation, to cooperatively activate viral polyprotein translation and RNA replication [91]. In addition to DDX3, SRPK1 and SRPK2 have also been shown to colocalize with HCV core protein and NS5A (an essential component of viral replication) in the perinuclear area, and inhibition of SRPKs by the chemical inhibitor SRPIN340 or by siRNA significantly reduces HCV replication [92]. Since phosphorylation of the core protein and NS5A regulates their subcellular localization and translational or replicative activity [93–95], it is unclear whether SRPKs directly phosphorylate core and NS5A protein to facilitate HCV infection. SRPKs may also phosphorylate other splicing factors (e.g., DDX3, DDX17) that contribute to HCV replication and pathogenesis of [96]. Furthermore, a recent work reported that HCV infection upregulates SRPK2 expression by downregulating the expression of a long non-coding RNA, Linc-Pint, which interacts with SRPK2

and suppresses its protein level. SRPK2 further promotes the phosphorylation of SRSFs (SRSF1, SRS4, SRSF5, and SRSF6) for the efficient splicing of several key genes involved in lipogenesis, such as ATP citrate lyase (ACLY) and fatty acid synthase (FASN), which in turn enhances *de novo* lipogenesis to favor virus replication and liver disease [97]. Linc-Pint is regulated by p53 [98], and it is certainly conceivable that HCV core protein or NS5A may interfere with p53 signaling to inhibit Linc-Pint expression [99, 100].

Roles of SRPKs in (−)ssRNA virus infection

Filoviridae

Ebola virus is a filovirus that causes severe haemorrhagic fever, with a fatality rate of 34–81%. Once Ebola virus enters the cytoplasm via micropinocytosis, the release of the viral ribonucleoprotein (RNP) complex (consisting of the viral genome, transcription factor VP30, polymerase L, cofactor VP35, and nucleoprotein NP) promotes the production of all viral mRNAs (primary transcription) [101]. The newly produced viral mRNAs are then translated into various viral proteins, further facilitating the secondary transcription of viral mRNAs, genome replication, RNP complex formation as well as genomic RNA encapsidation in inclusion bodies. In particular, VP30 is indispensable for viral repetitive transcription, and reversible phosphorylation/dephosphorylation circuit of VP30 [102], mainly controlled by the cellular kinase phosphatase PP2A and SRPKs, modulates its subcellular location and binding affinity to the RNP complex [103–105]. VP30 is initially recruited and dephosphorylated by the cellular phosphatase PP2A interacting with NP, which in turn facilitates the replicative complex formation of VP30 with polymerase L and cofactor VP35 to initiate the primary transcription [106] (Fig. 3B). Upon completion, SRPK1 and SRPK2 phosphorylate VP30 at Ser29 within the 'RXXS' motif, and sequentially disperse it from the polymerase complex and RNA, allowing PP2A-mediated rapidly dephosphorylation of VP30 and ultimately redirection of VP30 to the secondary transcription start site to reinitiate the secondary transcription within inclusion bodies [105]. Therefore, SRPKs dynamically maintain the appropriate phosphorylation status of VP30 and ensure VP30-mediated effective transcription. Furthermore, VP30 is associated with the nucleocapsid [107], and it is uncertain whether SRPKs phosphorylate the nucleocapsid to promote capsid assembly.

Roles of SRPKs in reverse transcribing virus infection

Retroviridae (+ ssRNA-RT)

Human immunodeficiency virus (HIV) is a member of the *Retroviridae* family and its dynamic propagation and infectivity are dramatically affected by SR protein-mediated alternative splicing of viral RNAs [108]. Different forms of viral transcripts are produced to generate the nine HIV proteins: unspliced gRNA encodes Gag and Pol; fully spliced viral mRNA encodes Tat, Rev, and Nef; and the singly spliced mRNA (containing functional intron) generates Vif, Vpr, Vpu, and Env. In addition, more than 70 viral transcripts generated by suboptimal splicing have been identified to produce the viral proteins described above, in particularly Tat and Rev, two proteins critical for HIV replication. SR proteins regulate HIV gene expression at multiple steps, including transcription capping, splicing, 3′end formation, nuclear export and translation [109]. Consequently, modulation of SR protein expression and activity dramatically alters the extent of HIV RNA splicing and affects viral replication. For example, overexpression of the SR proteins SRSF1 (ASF/SF2), SRSF2 (SC35) or SRFS7 (9G8) suppresses HIV production, whereas overexpression of SRSF4 (SRp75), SRSF5 (SRp40) or SRSF6 (SRp55) exerts conversely functions that enhance HIV production and infectivity [110, 111]. Accordingly, HIV infection causes the dephosphorylation/abundance of most SR proteins, with the exception of SRSF4. HIV mobilizes SRPK2, and possibly SRPK1, to stabilize and phosphorylate SRSF4 to guarantee HIV-1 efficient replication [112]. In addition, depletion of SRPK1 has been shown to suppress HIV gene expression and the production of the viral structural proteins Gag and Env [113]. SRPK1 maintains HIV RNA levels and affects the transcriptional initiation of viral RNAs possibly by regulating the phosphorylation status of SRSF10 to modulate RNA splicing. Furthermore, a quantitative phosphorylation proteomics analysis indicates that the HIV accessory protein Vpr can phosphorylate and inhibit SRPK activity [114]. Future experiments are required to confirm the Vpr-SRPK interaction and to examine whether SRPK is involved in Vpr-mediated cell cycle regulation.

Hepadnaviridae (dsDNA-RT)

Hepatitis B virus (HBV), a small DNA virus of the *Hepadnaviridae* family, causes hepatitis and is causally associated with hepatocellular carcinoma. Unlike HIV, HBV does not require alternative splicing to produce viral proteins, although spliced viral transcripts exist [115]. HBV pregenomic RNA (pgRNA) is reverse transcribed into a circular and partially double-stranded DNA molecule after being packaged into the newly assembled capsid, a process predominantly regulated by the HBV core protein (HBc). Similar to the capsid proteins (N or core protein) of other viruses, HBc controls viral capsid assembly and persistent and effective HBV infection [116]. HBc activity is also regulated by phosphorylation, particularly phosphorylation within the RS domain located in the C terminal domain (CTD) of HBc [117, 118]. Notably, several host cellular kinases have been implicated in the phosphorylation of HBc, such as Cdc2, Cdk2, PKC and SRPKs, of which SRPKs are mainly responsible for the regulation of HBc phosphorylation and its function in vitro [118–120]. It is postulated that the encapsidation of HBV pgRNA requires an appropriate level of HBc phosphorylation, and SRPK1 and SRPK2 interact with and phosphorylate several serine residues within the CTD of HBc [120], acting as chaperones or neutralizers to ensure successful HBV genome packaging (Fig. 3C) [121–123]. Non-phosphorylated HBc has a higher binding affinity to RNA and readily self-assembles into RNA-filled capsids, which is inhibited by SRPKs-mediated phosphorylation [121]. In contrast, high phosphorylation of HBc by SRPKs leads to low RNA binding capacity and empty capsids/virions [122]. It is, therefore, speculated that after transient binding and phosphorylation of HBc, SRPKs dissociate from HBc to allow for slow HBc-mediated capsid formation and pgRNA incorporation [122, 123].

In addition to capsid assembly, the nucleic acid binding affinity of the CTD accredits HBc to participate in HBV DNA replication, and phosphorylation of different serine residues within the RS domain exhibits inconsistent but essential effects [124]. Interestingly, overexpression of both SRPK1 and SRPK2 has also been shown to suppress HBV replication by reducing pgRNA packaging without impact on core particle formation, and such suppressive effects of SRPKs do not depend on their phosphorylation activities on HBc [125]. Possible explanations are that SRPKs interfere with the interaction between HBc/pgRNA and HBc/viral DNA polymerase, or SRPKs are not the major kinases for HBc phosphorylation during HBV replication [126]. In addition, it remains to be determined whether SRPKs regulate the phosphorylation of splicing factor SRSFs to affect the binding of HBc to viral DNA/RNA and viral replication, as one recent work has shown that nuclear HBc interacts with several SR proteins and that inhibition of SRSF10, one of the substrates of SRPKs, regulates the amount of nascent HBV RNA [127]. Therefore, these results indicate the multiple and complex roles of SRPKs in the HBV life cycle. Finally, preliminary data from a recombinant co-expression system indicate that the core protein of the related duck hepatitis B virus (DHBV) is also highly phosphorylated by human SRPK1 [128]. Whether SRPK1 plays a key role in the virus-specific differential rates of nucleocapsid uncoating and pgRNA encapsidation remains to be determined.

SRPKs and SRPKs-associated viral or cellular proteins as emerging antiviral targets

It is clear that viruses hijack SRPKs-mediated phosphorylation of viral proteins and/or cellular substrates, mainly SRSFs, to manipulate multiple steps of the viral life cycle, such as alternative splicing, transcription, capsid assembly, viral pathogenesis, and even the host antiviral immune response. Therefore, SRPKs and SRPKs-targeted viral or cellular proteins are potent antiviral targets.

SRPKs as antiviral targets

Based on the essential role of SRPKs in pre-mRNA processing, inhibition of SRPKs has been suggested as a potential therapeutic opportunity against cancer and other diseases [11, 47, 48]. For example, EXN-107, a small molecule inhibitor of SRPK1 synthesized by the company Exonate (structure undisclosed), is currently in clinical trials (phase I/II, NCT04565756) to investigate its therapeutic efficacy against diabetic retinopathy. SCO-101, a potent inhibitor of SRPK1 and the drug efflux pump ABCG2 [129, 130], is currently in phase I/II clinical trials for the treatment of colorectal cancer (NCT04247256) and pancreatic ductal adenocarcinoma (NCT04652206).

To date, several small molecule inhibitors of SRPK (SRPKi), mainly derived from three scaffolds, have been developed, which bind irreversibly or reversibly to the ATP pocket. As presented in Fig. 4, the backbone of SRPKi, mainly the imine-nitrogen of benzimidazole [131] or the carbonyl group [132, 133], forms a crucial hydrogen (H)-bond with the backbone amide of Leu168 (SRPK1) in the hinge region, which contributes significantly to the activity of SRPKi and requires the flipping of the backbone carbonyl of Leu168. The presence of the hinge residue Gly169 further increases the flexibility and facilitates the flipping of the hinge region [134]. In addition, the interaction of residues Lys109 and Glu124 within the active site with the conserved Asp-Leu-Gly (DLG) motif at the adjacent 'allosteric' site is critical for SRPK catalytic activity [132]. Accordingly, H-bond formation between Glu124 and the S1 substituent
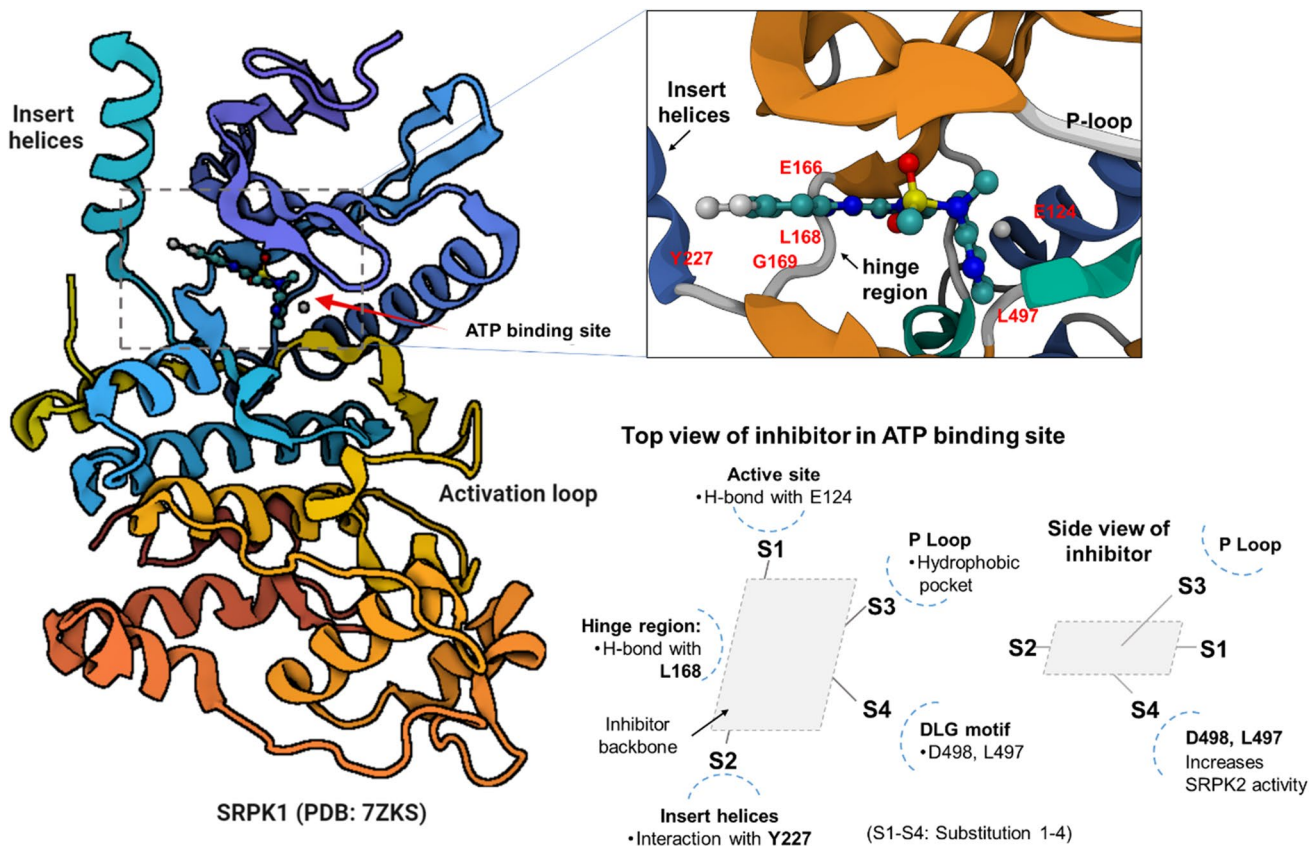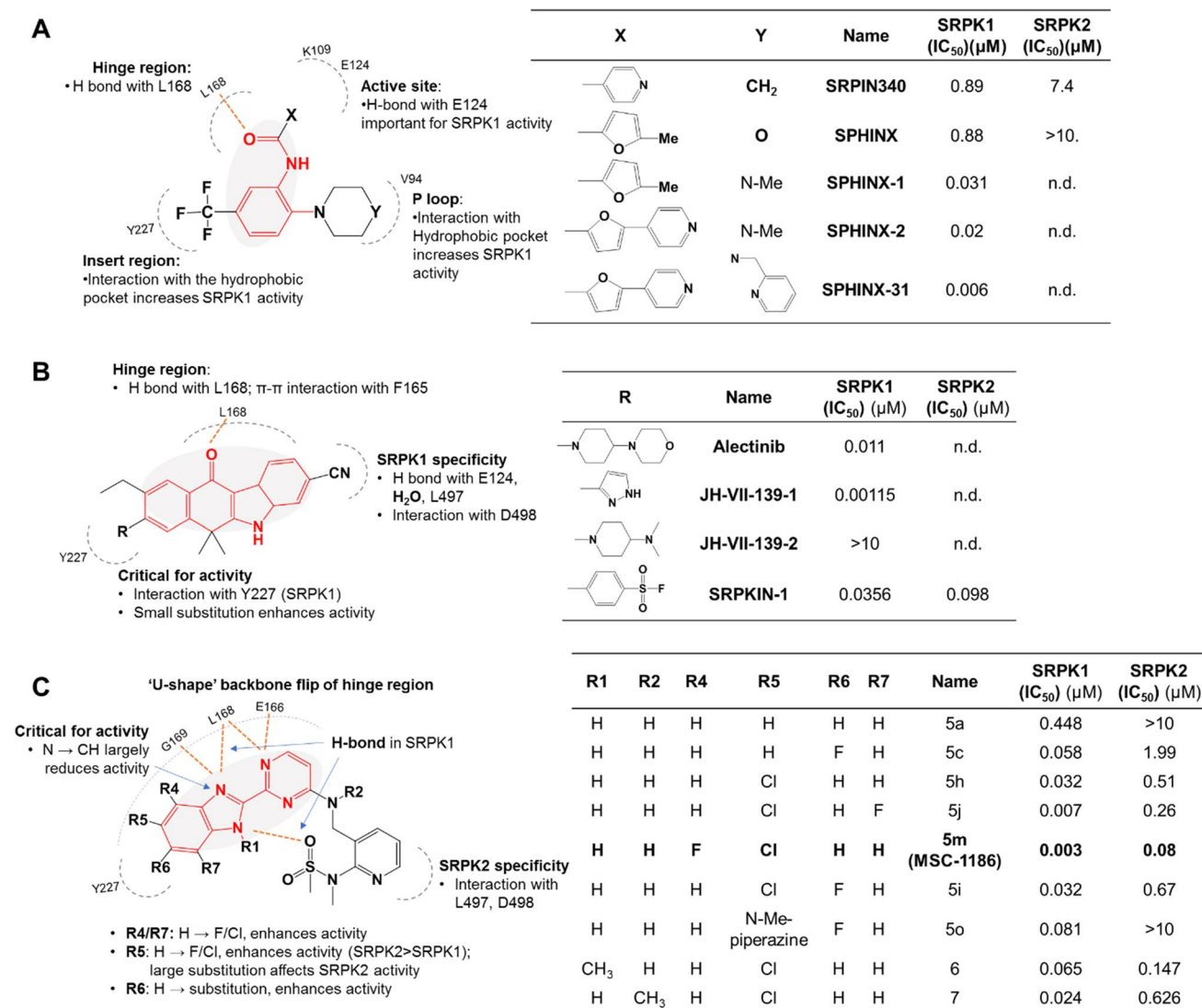

Fig. 4 Illustration of SRPK inhibitor binding in the ATP pocket. The crystal structure of SRPK1 in complex with inhibitor (PDB: 7ZKS) and key domains and relative residues around the substrate binding site are shown (SRPK1). Top and side views of the SRPK inhibitor in the ATP binding site are also presented. The grey parallelogram represents the backbone of the inhibitor, and the S1–S4 represent different substituents. Their interactive residues or domains of SRPK1 are shown

at one end of the backbone of SRPKi, as well as water molecule-mediated interaction between the S1 and Asp497/Leu498 of the DLG motif, significantly affects the inhibitory activity against SRPK1 [133]. At the opposite terminal end of SRPKi, key residues Tyr227, Ile228 and Leu231 of SRPK1 (Tyr239, Val240 and Met243 of SRPK2) within the insert helices adjacent to the ATP binding pocket influence both the activity and selectivity of SRPKi [132, 133]. The side chain of Tyr227 provides a steric constraint on the size of the S2 substitution. Alternatively, a covalent bond formation between Tyr227 and the S2 substituent (e.g., sulfonyl fluoride group) converts SRPKi into an irreversible inhibitor (e.g., **SRPKIN-1**) [133]. In addition, the larger side chains of Val240 and Met243 in SRPK2 create a smaller hydrophobic pocket and slightly change the binding orientation of

SRPKi, resulting in reduced SRPK2 activity [132]. Interestingly, the S3 and S4 substituents may have opposite spatial positioning, with S3 interacting with the P-loop spatially in an upward direction and S4 partially interfacing with the DLG motif in a downward direction. Residues Ser92, Val94 and the P-loop backbone form a hydrophobic cavity oriented towards S3, and the improved contact between S3 and the P-loop, especially through aromatic stacking, significantly enhances inhibitor potency against SRPK1 [132]. Finally, a possible link between the S4 substituent and the DLG motif (Asp497/Leu498) or the lipophilic back pocket shaped by the side chains of Val145, Phe165, and Ala496 (SRPK1) is thought to increase the inhibitory activity of SRPKi on SRPK2 (to be determined).

A

X	Y	Name	SRPK1 (IC$_{50}$)(µM)	SRPK2 (IC$_{50}$)(µM)
pyridine	CH$_2$	SRPIN340	0.89	7.4
furan-Me	O	SPHINX	0.88	>10.
furan-Me	N-Me	SPHINX-1	0.031	n.d.
furan-pyridine	N-Me	SPHINX-2	0.02	n.d.
furan-pyridine	—	SPHINX-31	0.006	n.d.

B

R	Name	SRPK1 (IC$_{50}$) (µM)	SRPK2 (IC$_{50}$) (µM)
piperazine-morpholine	Alectinib	0.011	n.d.
pyrazole-NH	JH-VII-139-1	0.00115	n.d.
piperidine-N	JH-VII-139-2	>10	n.d.
phenyl-SO$_2$F	SRPKIN-1	0.0356	0.098

C

R1	R2	R4	R5	R6	R7	Name	SRPK1 (IC$_{50}$) (µM)	SRPK2 (IC$_{50}$) (µM)
H	H	H	H	H	H	5a	0.448	>10
H	H	H	H	F	H	5c	0.058	1.99
H	H	H	Cl	H	H	5h	0.032	0.51
H	H	H	Cl	H	F	5j	0.007	0.26
H	H	F	Cl	H	H	5m (MSC-1186)	0.003	0.08
H	H	H	Cl	F	H	5i	0.032	0.67
H	H	H	N-Me-piperazine	F	H	5o	0.081	>10
CH$_3$	H	H	Cl	H	H	6	0.065	0.147
H	CH$_3$	H	Cl	H	H	7	0.024	0.626

Fig. 5 Structure–function relationships of SRPK inhibitors. The chemical structures and corresponding SRPK-targeting activities of small molecule inhibitors and their derivatives, mainly derived from three scaffolds (**A–C**), are shown. The grey cycle represents the structural backbone and the orange dashed line represents the H-bond. IC$_{50}$ half maximal inhibitory concentration. n.d. not determined

Table 2 Antiviral effects of SRPKi

SRPK inhibitor	Virus	Concentration used (μM)	Host cell	Cytotoxicity (CC$_{50}$) (μM)
SRPIN340	HCV 1b	EC$_{50}$: 4.7	Huh7/Rep-Feo-1b	>30
	HCV 2a	EC$_{50}$: 15.8	Huh7/Rep-Feo-2a	>30
SRPIN340	Sindbis Virus	EC$_{50}$: 60	BHK	>100
SRPIN340	SARS-CoV-2	53.3	ACE2-A549	>53.3
		53.3	Calu-3	>53.3
SPHINX31	SARS-CoV-2	12	ACE2-A549	>12
Alectinib	SARS-CoV-2	10	ACE2-A549	>10
		10	Calu-3	>10
	HCoV-229E	0.12	Huh7	>0.12
SRPIN340	Ebola virus	30	HEK293	>100

EC$_{50}$ 50% effective concentration, CC$_{50}$ 50% cytotoxic concentration

The structure–function relationships of SRPKi are summarized in Fig. 5, and their potent and efficient antiviral activities are shown in Table 2. The isonicotinamide compound **SRPIN340** was firstly identified as an ATP-competitive inhibitor that covalently binds to the ATP binding pocket to inhibit SRPKs activities [112] (Fig. 5A). SRPIN340 exhibits high specificity for SRPK1/2 and does not significantly inhibit other SR protein-related kinases (e.g., CLK1 and CLK4) and Ser/Thr kinases. The half maximal inhibitory concentrations (IC$_{50}$) of SRPIN340 against SRPK1 and SRPK2 are 0.89 μM and 7.4 μM, respectively [132]. SRPIN340 has also been very early shown to efficiently inhibit viral replication and production of HCV, Sindbis virus, CoVs, and Ebola virus (Table 2) [84, 92, 105, 112]. **SPHINX** and its derivatives **SPHINX-1**, **SPHINX-2** and **SPHINX-31**, are other ATP-competitive inhibitors that share the same scaffold with SRPIN340 [132, 135]. Due to the interactions of different S3 substituent moieties with the P-loop of SRPK1, the **SPHINX** compounds exhibit higher activity and selectivity for SRPK1 over SRPK2 with a much lower IC$_{50}$ value than that of SRPIN340 [135]. As a result, SPHINX-31 has a higher antiviral activity against SARS-CoV-2 than SRPIN340 (Table 2) [84]. SPHINX derivatives are also currently in preclinical studies for the treatment of leukemia, solid tumors and eye diseases [136–138]. However, the SPHINXs also exhibit possible effects on kinases of the CMGC family (CDK, MAPK, GSK, and CLK) [135].

In addition, **Alectinib**, an FDA-approved primary anaplastic lymphoma kinase (ALK) inhibitor for the treatment of non-small-cell lung cancer, also displays potent inhibitory capacity to target SRPK1 (IC$_{50}$ = 11 nM) [133] (Fig. 5B). Recently, Alectinib has been shown to inhibit the infection of coronavirus HCoV-229E and SARS-CoV-2 and reduce the SRPK1/2-mediated N protein phosphorylation [84], which may partly contribute to the favorable outcome of COVID-19 patients [139, 140]. Notably, replacing the 4-morpholinopiperidine moiety in Alectinib with a smaller pyrazole ring (compound **JH-VII-139-1**) results in a tenfold increase in SRPK1-targeting activity (IC$_{50}$ = 1.1 nM). However, the inhibitory activity is reduced by ~ 1000-fold when substituted with a dimethyl amino-piperidine ring (compound **JH-VII-206-2**). Apart from SRPKs, Alectinib and JH-VII-139-1 also show broad interactions with numerous additional targets and inhibit the catalytic activity of other kinases, such as ALK and CHK2, to a similar extent [133]. To improve selectivity, the pyrazole ring at the S2 substitution in JH-VII-139–1 is further replaced by a 3-benzene-sulfonyl fluoride to generate a more specific SRPK-targeting analogue, **SRPKIN-1**. The sulfonyl fluoride group covalently interacts with Tyr227 of SRPK1 (or Tyr239 of SRPK2), making SRPKIN-1 an irreversible SRPK inhibitor with an IC$_{50}$ of 35.6 nM for SRPK1 and an IC$_{50}$ of 98 nM for SRPK2 [133]. Notably, SRPKIN-1 substantially reduces ALK activity (IC$_{50}$ = 195 nM) and has little effect on CLK1 (IC$_{50}$ > 10 μM) in a cell-based KiNativ profiling assay. In Ba/F3 cells transduced with EML4-ALK, a widely used model for testing ALK inhibitors, SRPKIN-1 shows 50-fold lower activity while JH-VII-206-2 and Alectinib show potent inhibitory effects, suggesting that the applicable concentrations of SRPKIN-1 at the cellular level have no influence on ALK. In addition, SRPKIN-1 is more than 50-fold more potent than SRPIN340 at phosphorylating SRSFs in a cellular context (200 nM vs. 10 μM) [133]. Future work is encouraged to investigate whether SRPKIN-1 has more potent and higher antiviral activity.

Recently, a series of compounds sharing a common benzimidazole-pyrimidine scaffold have been shown to be potent and reversible SRPKi (Fig. 5C), of which **MSC-1186** (compound **5 m**) is the most efficient and highly selective inhibitor with an IC$_{50}$ of 3 nM for SRPK1 and IC$_{50}$ of 80 nM for SRPK2 [131]. The benzimidazole-pyrimidine backbone acts as a hinge-binding moiety that interacts with the hinge region and flips it to form the essential H-bond with the Leu168 backbone amide (SRPK1). There is also a specific

intramolecular H-bond exists between the protonated benzimidazole 3N-atom and one sulfonamide O-atom, which allows the S4 substitution to adopt a conformation capable of interacting with the lipophilic back pocket and the DLG motif. Disruption of such an intramolecular H-bond results in a twofold loss of SRPK1 inhibitory activity, while SRPK2 activity is enhanced (compound **5h** vs **6**). Notably, MSC-1186 shows good selectivity (rarely off-target) than other SRPK inhibitors SRPIN340, SPHINX-31, and SRPKIN-1 in in vitro kinase screening assay [131]. Surprisingly, MSC-1186 has very low to negligible activity as a single agent, but shows a robust additive effect in combination with CLK inhibitor in phosphorylating SRSFs in a cellular context. Future work is encouraged to investigate its antiviral activity and potential clinical application.

SRPKs-targeted viral proteins as antiviral targets

In addition to SRPKs, SRPKs-targeted viral proteins also represent attractive therapeutic targets for the control of viral infections and virus-related diseases. SRPKs-mediated phosphorylation of the RS motif within viral capsid proteins (the NTD of CoV N protein and EBV BLRF2, or the CTD of HBV core protein), significantly affects their binding affinity to RNA/DNA, which is pivotal for viral RNP formation, genome encapsidation and replication. Accordingly, blocking the nucleic acid binding ability of viral capsid proteins may be a potent antiviral strategy [141]. Indeed, several compounds, such as *N*-(6-oxo-5,6-dihydrophenanthridin-2-yl)(*N*,*N*-dimethylamino) acetamide hydrochloride (**PJ34**) and 6-chloro-7-(2-morpholin-4-ylethylamino) quinoxaline-5,8-dione (**H3**), have been shown to target the RNA binding site within the NTD of the N protein to inhibit CoV replication [142, 143]. Alternatively, the compound **5-benzyloxy-gramine** induces abnormal dimerization and aggregation of N protein to suppress RNP formation and MERS-CoV infection [144]. In addition, inhibition of the oligomerization of N protein by competing peptides is also presumed to be an effective approach in the fight against HIV infection [145]. Similarly, core proteins of HBV and HCV are involved in multiple steps of the viral cycle through their dimerization and interaction with other viral or cellular proteins, and their specific inhibitors have been developed as novel antiviral agents. For example, an NTD-derived peptide inhibitor dramatically inhibits the dimerization of HCV core protein and blocks HCV infectious production [146]. In the context of HBV, different chemotypes of core protein allosteric modulators (**CpAMs**) have been discovered to inhibit HBV replication and to treat chronic hepatitis B in clinical trials (reviewed in ref. [147]). All the CpAMs bind to the hydrophobic pocket between the interface of core protein dimers, and type I CpAMs accelerate core protein assembly into degradative polymers or aberrant capsids, whereas type II CpAMs promote the formation of empty capsids without pgRNA. CpAMs also disrupt the de novo cccDNA synthesis and DNA replication with unclear mode of action. Notably, several CpAMs, such as RO9389 (type I) [148] and JNJ-6379 (type II) [149], are under clinical investigation for chronic HBV infection and have shown beneficial effects in patients. It will be interesting to investigate whether these above described inhibitors regulate SRPKs to exert their antiviral effects.

In addition, SRPKs-mediated phosphorylation of the Ebola virus transcription factor VP30 from is essential for repetitive viral transcription [150], and compounds targeting VP30 phosphorylation status, such as **okadaic acid** [151], have been shown to impair Ebola virus infection. In addition, VP30 interacts with NP protein to modulate viral RNA synthesis and disruption of the VP30-NP interaction is also a potential antiviral treatment, as illustrated by the antiviral activity of an NP-competitive peptide mimic [152].

Furthermore, SRPKs-mediated phosphorylation of herpesviral mRNA export proteins (HSV ICP27 and VSV IE4) or HPV E2 protein regulates their nucleus-to-cytoplasm reshuttling and viral gene expression, which may also be considered as novel therapeutic candidates [153, 154]. One example is that a peptide-conjugated phosphorodiamidate morpholino oligomer (**PPMO**) has been shown to inhibit HSV-1 replication by targeting ICP27 [155]. Further structure-based high-throughput screening and experimental validation may identify more potential inhibitors of these viral proteins.

Substrates of SRPKs as antiviral targets

As discussed above, SRSFs are mainly substrates of SRPKs and their appropriate phosphorylation levels are critical for viral infection, suggesting them as alternative candidates for antiviral therapy. For example, SRSF10 interacts with HBV core protein and the small molecule compound **1C8**, which specifically inhibits SRSF10 phosphorylation, significantly reduces HBV nascent RNA production [127]. SRSF10 also affects HIV pre-mRNA splicing and **1C8** treatment reduces HIV replication [156, 157]. Cardiotonic steroids, such as digoxin and digitoxin, deplete the expression of SRSF3 and SRSF10 to alter viral alternative splicing and suppress HIV replication [158, 159]. In addition, SRSF5 directly enhances the pre-mRNA splicing of the M protein to promote influenza virus replication, which is further suppressed by **anidulafungin**, an FDA-approved antifungal drug that has been identified as an inhibitor of SRSF5 [160]. Anidulafungin also exhibits a broad-spectrum antiviral activity that effectively blocks the infection of Zika virus, HSV-1, SARS-CoV-2 and other viruses [161, 162], suggesting the functional involvement of SRSF5 in different viruses. Recently,

several new inhibitors of SRSFs have gradually emerged, making it possible to target specific SRSFs in viral infections. For example, through high-throughput screening and modification, **SFI003** is confirmed to be a specific inhibitor of SRSF3 that binds to SRSF3 and induces its neddylation-dependent degradation [163]. **Indacaterol**, an approved β2-adrenergic receptor agonist, is identified as an inhibitor of SRSF6 [164], and has been implicated in disrupting the interaction between ACE2 and the S protein of SARS-CoV-2 by virtual screening [165]. Future investigations into the understanding of the biological functions of SRSFs and SRPKs in the viral life cycle, and the development of novel specific inhibitors of SRSFs (e.g., SRSF2, SRSF4, SRSF7-9) may pave the way for clinical application of SRSFs inhibitors. Alternatively, antisense oligonucleotide-mediated downregulation of SRSFs is another exciting therapeutic avenue to be further explored.

Perspectives

Nowadays, the emergence of a variety of highly infectious and pathogenic viruses poses a serious threat to human health. How to find the effective antiviral drugs quickly and accurately is a major challenge, making drug repurposing an optimal alternative strategy. SRPKs are one of the key regulators of mRNA maturation and splicing, and their role in viral infection is receiving increasing attention. In this review, we briefly summarize the regulation and biological function of SRPKs, highlight their involvement in the infection process of several viruses and discuss the putative use of inhibitors targeting SRPKs and SRPKs-associated viral proteins or cellular substrates as potential antiviral strategies.

Although the cellular biological functions of SRPKs in RNA splicing have been recognized for many years, several questions remain unanswered. For example, whether other post-transcriptional modifications or interacting proteins regulate the activity and cellular location of SRPKs. Besides, SRPKs-mediated phosphorylation represents as a prevalent regulatory mechanism to influence the cellular location and transcriptional activity of RS domain-containing viral proteins. Different functional types of viral proteins, including capsid protein (e.g., CoV N protein, EBV BLRF2, HBV core protein), mRNA export factor (e.g., HSV ICP27, VSV IE4, HPV E2), and transcription factor (e.g., Ebola virus VP30), are phosphorylated and regulated by SRPKs. More interactive connections between viral proteins (e.g., capsids and transcriptional activators of other undisclosed viruses) and SRPKs may be found by analyzing phosphorylation proteomics. It is also unclear whether SRPKs influence viral infection by modulating the antiviral immune response [41, 166]. In addition to the above-mentioned viruses that have been clearly shown to interact with SRPKs, other viruses that usurp the cellular

machinery mediated by SRSFs for their replication are emerging as being able to control SRPKs-mediated phosphorylation. For example, several SRSFs (e.g., SRSF2, SRSF3, and SRSF5) are essential for viral replication of influenza virus, a small RNA virus hijacking cellular splicing components to produce its M and NS proteins [160, 167–169]. It is, therefore, of interest to investigate whether influenza virus modulates the activity of SRPKs to maintain appropriate phosphorylation levels of SRSFs and to explore the possible antiviral activities of SRPK inhibitors. In addition, considering that CLKs and SRPKs jointly regulate the phosphorylation of SR proteins in the nucleus and a growing body of evidence shows the interplay between different viruses and CLKs [113, 169–172], inhibition of CLKs by inhibitors, or synergistically in combination with SRPKs inhibition, is novel robust therapeutic option for viral infections.

Furthermore, SRPKs and SRPKs-targeted viral proteins or cellular proteins (e.g., SRSFs) are potential antiviral targets. The feasibility of developing SRPKs inhibitors that specifically target unique virus–host interactions, as SRPKs interact directly with different viral proteins, needs to be validated. Targeting SRSFs with inhibitors, competing peptides, or antisense-oligonucleotides may also be an alternative and interesting strategy to counteract viral infection. Given the abnormally high expression of SRPKs and SRSFs in some cancers [49, 173], inhibitors of SRPKs and SRSFs may have both anticancer and antiviral effects in the case of oncogenic viruses (e.g., HPV, HBV). Future work is also warranted to verify the potential of SRPKs and SRSFs inhibitors in relevant anti-infective clinical applications. However, the existing SRPKs inhibitors developed in cancer therapy are both insufficiently potent and selective, with unclear selectivity towards other kinases. Although the inhibitor MSC-1186 exhibits high selectivity and activity towards SRPKs, its cellular potency and efficacy are limited [131]. Therefore, the development of more efficient and specific SRPKs inhibitors will enable more reliable investigation of the undiscovered biological functions of SRPKs and provide more options for antiviral treatment.

Declarations

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