REVIEW

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Connecting the speckles: Splicing kinases and their role in tumorigenesis and treatment response

Dale P Corkery[1], Alice C Holly[2], Sara Lahsaee[2], and Graham Dellaire[1,2,3,*]

[1]Department of Biochemistry & Molecular Biology; Dalhousie University; Halifax, Nova Scotia, Canada; [2]Department of Pathology; Dalhousie University; Halifax, Nova Scotia, Canada; [3]Beatrice Hunter Cancer Research Institute; Halifax, Nova Scotia, Canada

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Abbreviations: SR, serine arginine; SRPK, serine arginine protein kinase; CLK, CDC-like kinase; PRP4K, pre-mRNA processing factor 4 kinase; snRNP, small nuclear ribonucleic protein.

Alternative pre-mRNA splicing in higher eukaryotes enhances transcriptome complexity and proteome diversity. Its regulation is mediated by a complex RNA-protein network that is essential for the maintenance of cellular and tissue homeostasis. Disruptions to this regulatory network underlie a host of human diseases and contribute to cancer development and progression. The splicing kinases are an important family of pre-mRNA splicing regulators, , which includes the CDC-like kinases (CLKs), the SRSF protein kinases (SRPKs) and pre-mRNA splicing 4 kinase (PRP4K/PRPF4B). These splicing kinases regulate pre-mRNA splicing via phosphorylation of spliceosomal components and serine-arginine (SR) proteins, affecting both their nuclear localization within nuclear speckle domains as well as their nucleo-cytoplasmic shuttling. Here we summarize the emerging evidence that splicing kinases are dysregulated in cancer and play important roles in both tumorigenesis as well as therapeutic response to radiation and chemotherapy.

Introduction

Increased complexity of alternative splicing during evolution and the expansion of the SR-protein kinases

When comparing single-cell eukaryotes like the yeast *S. cerevisae* (~6,000 genes),[1] to metazoans of increasing complexity, there appears to be a general relationship between increased complexity of the organism and the number of genes. After the human genome was sequenced in 2001, it was found that our genome contains approximately 23,000 genes, a much lower number than expected.[2] The human genome is larger than the genome of the fly *D. melanogaster* (~14,000 genes) and comparable to the genome of the worm *C. elegans* (~20,000 genes).[3,4] At the same time, it was discovered that genes containing introns encode many possible transcripts, which arise by alternative mRNA splicing and allow organisms with a similar number of genes to have more complex and diverse proteomes as a result of mRNA splicing. The potential of alternative mRNA splicing to increase protein diversity is most clearly illustrated by the extreme example of the fly axonal guidance gene Down syndrome cell adhesion molecule 1 (Dscam1), which is predicted to produce up to ~38,000 possible alternative transcripts.[5]

Pre-mRNA splicing allows increased protein diversity and cellular complexity between species and also provides the plasticity for one cell to alter its protein complement dynamically in response to cellular stress or developmental cues. As one would expect, the mechanisms of pre-mRNA splicing are tightly regulated to maintain cellular and tissue homeostasis, and errors in splicing underlie a host of genetic diseases and can contribute to cancer development and progression. In fact, it is estimated that 22% of disease causing mutations affect splicing[6] (for review of splicing defects resulting in disease, see:[7-9]). Although there are many dozens of splicing factors, many of which are serine arginine (SR)-rich, ostensibly their functions in splicing are regulated by several serine/threonine kinases. These kinases share a general preference for phosphorylating SR-rich proteins and collectively are referred to as SR protein specific kinases, or simply splicing kinases. Therefore, it is perhaps not surprising to note that during evolution there appears to be a concomitant increase in the diversity and number of isoforms of these kinases. This occurs in lockstep with increasing gene complexity in terms of alternative splicing between single-cell eukaryotes like *S. cerevisiae*, which encode very few intron-containing genes and a single *bona fide* SR-protein kinase *Sky1*, to complex metazoans like humans, whose genome encodes many intron containing genes and multiple paralogs of at least 3 classes of splicing kinases (**Fig. 1**). In humans, the 3 classes of splicing kinases include the serine-arginine protein kinases (SRPK1/2/3), the CDC-like kinases

*Correspondence to: Graham Dellaire; Email: dellaire@dal.ca
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	CLK-like	PRP4K-like	SRPK1-like
Species			
Baker's Yeast *(S. cerevisae)*	**Kns1***	**N/A**	**Sky1**
Fission Yeast *(S. pombe)*	**Lkh1**	**Prp4**	**Dsk1**
Fly *(D. melanogaster)*	**Doa**	**CG7028**	**Srpk**
Worm *(C. elegans)*	**tag-172**	**prpf-4**	**spk1**
Human	**CLK1**	**PRPF4B**	**SRPK1**
	CLK2		**SRPK2**
	CLK3		**SPRK3**
	CLK4		

*Putative SR protein kinase based on amino acid similarity to human CLK1; N/A = no homolog identified

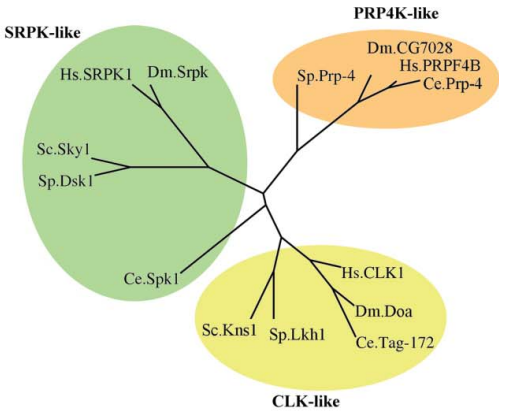

Figure 1. Splicing kinase diversity across species. A table of the known and putative splicing kinases in baker's yeast (S. cerevisiae (Sc)), fission yeast (S. pombe (Sb), the worm *C. elegans* (Ce), the fly *D. melanogaster* (Dm) and humans (Hs), is shown on the left. On the right, a phylogenetic tree showing the evolutionary relationships between the various splicing kinase families and their homologs in yeast, worms, flies and in humans. The phylogenetic tree was created based on amino acid composition of the splicing homologs using the web resource: phylogeny.limrr.fr.

(CLK1/2/3/4), and the pre-mRNA processing factor 4 kinase (PRP4K). Each class of splicing kinase has a distinct cellular localization, which may be based in part on their different roles in splicing regulation (**Fig. 2**).

One of the first splicing kinases to be described in the literature is the SRSF protein kinase 1 (SRPK1), which was identified by Gui *et al.* in 1994 when the authors purified and cloned a cell cycle regulated kinase which was responsible for redistribution of SR proteins from a nuclear speckle localization in interphase cells, to a more ubiquitous nucleoplasm localization in mitotic cells.[10,11] SRPK2 and SRPK3 were later identified based on sequence homology with SRPK1.[12,13] SRPK2, much like SRPK1, was shown to regulate splicing through SR protein phosphorylation[12] while SRPK3 was identified for its role in normal muscle growth and homeostasis.[13]

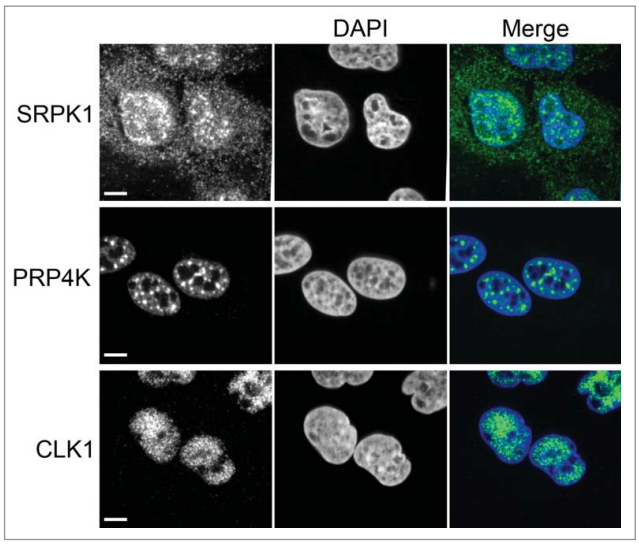

Figure 2. Splicing kinase cellular localization. Human osteosarcoma U2OS cells were analyzed by immunofluorescence confocal microscopy using an anti-SRPK1, anti-PRP4K or anti-CLK antibody (green). Nuclei were stained with DAPI (blue). Scale bar = 5 microns.

CDC-like kinase 1 (CLK1) was identified as a splicing kinase in 1996 when a yeast 2 hybrid screen using Clk/sty (Clk1) kinase as bait identified 5 SR proteins as binding partners.[14] The authors went on to show that one of the interacting SR proteins, ASF/SF2 (SRSF1), was phosphorylated within its RS domain by Clk/sty, and that overexpression of Clk/sty, much like SRPK1, caused a redistribution of SR proteins from nuclear speckles, to a ubiquitous nucleoplasm localization.[14]

Pre-mRNA processing factor 4 kinase (PRP4K)(also known as PRPF4B), a lesser-known splicing kinase, was first linked to splicing in 1991 when a temperature sensitive library of *Schizosaccharomyces pombe* mutants were screened for splicing defects.[15] At the restrictive temperature, yeast carrying a temperature sensitive mutation in *prp4* accumulated un-spliced pre-mRNA. Subsequent characterization of the *prp4* gene revealed that the splicing factor encoded by the gene contained the characteristic sequence that defines a serine/threonine protein kinase, making *prp4* the first kinase shown to play a role in splicing.[16] The mammalian homolog of *prp4* (PRP4K) has been shown to interact with pre-mRNA splicing factors PRP6 and Suppressor-of-White-Apricot (SFSWAP/SRSF8) and copurify with the U5 snRNP.[17] Furthermore, PRP4K has been shown to be a key regulator of U4/U6-U5 tri-snRNP assembly through the phosphorylation of PRP6 and PRP31.[18]

In humans, pre-mRNA splicing and the expression of the splicing kinases are perturbed in cancer. In this review, we will discuss the conserved roles of these kinases in pre-mRNA splicing and their emerging roles in tumorigenesis and treatment response.

Splicing kinases in spliceosomal assembly and mRNA splicing

Transcribed pre-mRNA must be spliced to remove introns prior to nuclear export and translation. This process is carried out by the spliceosome; a large macromolecular machine composed of 5 small nuclear ribonucleic proteins (snRNPs) and numerous protein cofactors.[19] Spliceosome assembly is a complex, multistep process as illustrated in **Figure 3**. The first step involves recognition of the 5′ and 3′ splice sites located on adjacent exons by U1 and U2 snRNP respectively; a process which is mediated by the C-terminal domain of polymerase II. Binding of the U1 snRNP to the 5′ splice site is mediated by SRSF1, but only when the RS-domain of SRSF1 is hyper-phosphorylated by CLK1 and SRPK1.[20,21] Once U1 and U2 snRNP have bound their target splice site, they interact with each other to form the pre-spliceosome (complex A). The next step in assembly involves the binding of pre-assembled U4/U6-U5 tri-snRNP to the pre-spliceosome to form complex B; a reaction catalyzed by pre-mRNA processing factors PRP28, PRP6 and PRP31, among others. PRP28 association with the tri-snRNP is dependent on its phosphorylation by SRPK2[22] while PRP6 and PRP31 association is dependent on their phosphorylation by PRP4K.[17,18] Loss of these phosphorylation events have been shown to inhibit association of the pre-mRNA processing factor with the tri-snRNP and, ultimately, tri-snRNP association with complex A.[18] Complex B next undergoes a series of rearrangements resulting in the release of U1 and U4 snRNPs, creating a catalytically active complex B. Once catalytically active, the complex carries out the first of 2 splicing reactions, to form complex C containing free exon from the 5′ splice site, and the intron-exon lariat intermediate from the 3′ splice site. Complex C, after a series of rearrangements, carries out the second splicing reaction resulting in a post-spliceosomal complex containing the 2 spliced exons and the lariat intron. Finally, the remaining U2, U5 and U6 snRNPs are released from the transcript to be re-used in additional rounds of splicing.

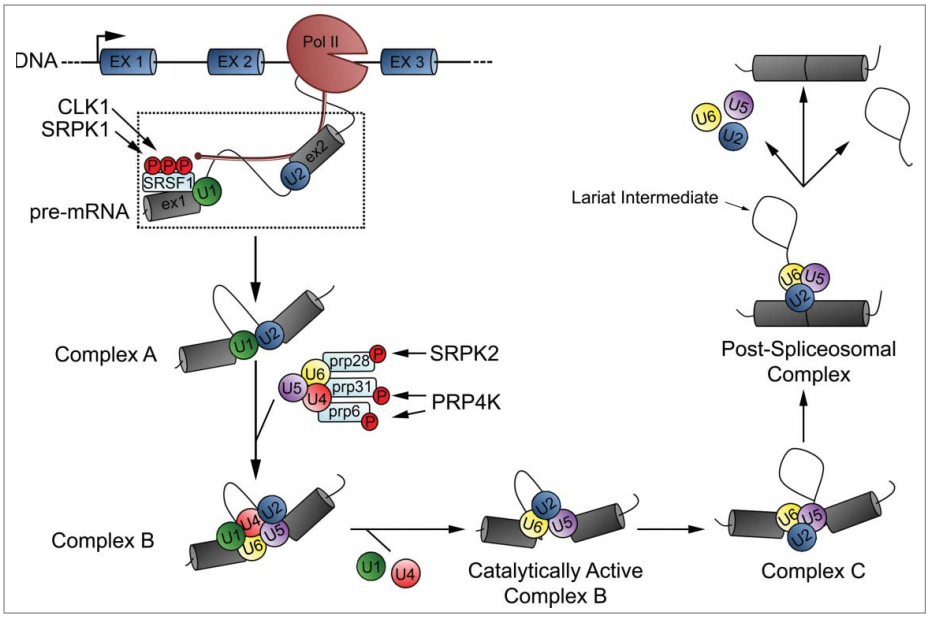

Figure 3. The role of splicing kinases in spliceosome assembly and pre-mRNA splicing. Splicing kinases are involved in 2 distinct steps in spliceosome assembly. First, CLK1 and SRPK1 are required to hyper-phosphorylate SRSF1. This is essential for SRSF1 binding to the 5′ splice site which, in turn, recruits the U1 snRNP. Second, SRPK2 and PRP4K are required to phosphorylate PRP28 and PRP31/PRP6 respectively. These phosphorylation events are required to mediate tri-snRNP association with complex A.

Splicing kinases in SR protein shuttling

SR proteins are a family of RNA binding proteins containing a characteristic arginine/serine rich domain (RS domain).[23,24] The first SR proteins (SRSF1 and SRSF2) were identified as essential regulators of constitutive splicing for their ability to promote U1 and U2 snRNP binding to the 5′ and 3′ splice site, respectively.[25-29] In addition to their role in constitutive splicing, SR-proteins play an essential role in the regulation of alternative splicing by promoting splice site selection through the binding of *cis*-acting splicing regulatory elements (SREs). SREs are classified as exonic splicing enhancers (ESEs), exonic splicing silencers (ESSs), intronic splicing enhancers (ISEs) or intronic splicing silencers (ISSs). These designations depend on where in the pre-mRNA the SR protein is binding (exon or intron), and what the net effect of binding is on splicing (enhance or inhibit) (reviewed in [30-32]). Adding even further to the complexity of splicing regulation, binding of SR proteins to SREs can be antagonized by the heterogeneous nuclear ribonucleoparticles (hnRNP) A/B family of proteins.[33] As a result, disruptions in the molar ratio of SR protein to hnRNP antagonist in the nucleus can have profound effects on splicing.[34-36]

In addition to regulating U1 snRNP binding to the 5′ splice site and tri-snRNP assembly, splicing kinases are able to regulate splicing through the phosphorylation dependent shuttling of SR-proteins (**Fig. 4**). Unphosphorylated SR proteins in the cytoplasm are phosphorylated by SRPK1 and targeted for nuclear import via the SR protein import receptor, transportin-SR (TRN-SR; also known as transportin-SR2 (TRN-SR2) and transportin 3 (TNPO3)).[37,38] Once in the nucleus phosphorylated SR proteins become enriched in interchromatin granules called splicing speckle domains. To be recruited from speckles to nascent pre-mRNA, where they act to regulate splicing, SR proteins require additional phosphorylation events[39] which are mediated by the splicing kinase CLK1.[40] Studies suggest that the extent to which SR proteins are phosphorylated can impact their cellular function. For example, hypo-phosphorylation of the RS domain of SRSF1 promotes interaction with its own RNA recognition domain (RRM), while hyper-phosphorylation

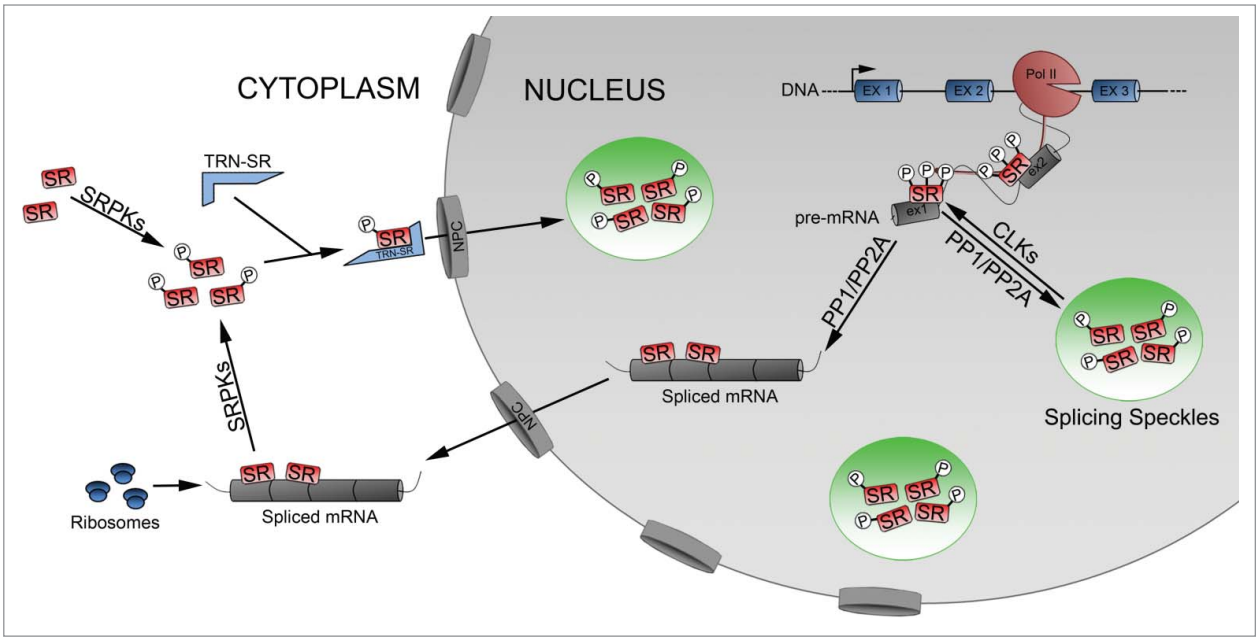

Figure 4. The role of splicing kinases in SR protein shuttling. Unphosphorylated SR proteins in the cytoplasm are phosphorylated by SRPK1 which promotes shuttling into the nucleus. Additional phosphorylation events mediated by CLK1 promote translocation from splicing speckles to nascent pre-mRNA.

releases the RS domain and promotes RRM binding with the U1snRNP, promoting spliceosomal assembly.[21] Therefore, stepwise phosphorylation mediated by SRPK1 and CLK1 is essential in the regulation of splicing. Once splicing is complete hyper-phosphorylated SR proteins bound to mRNA are dephosphorylated by nuclear phosphatases (protein phosphatase 1 (PP1) and 2A (PP2A)) and either recycled to the cytoplasm as a chaperone for mRNA export[41,42] where they also play a role in regulating translation of specific transcripts[43] or re-phosphorylated and returned to speckles to await the next round of splicing. Thus, the splicing kinases connect the cell biology of splicing speckles domains to the biochemistry of pre-mRNA splicing by providing a mechanism for the shuttling of splicing factors to and from these domains to sites of splicing through reversible phosphorylation events.

Regulation of SR Protein Phosphorylation

SR-protein phosphorylation by the splicing kinases plays an essential role in splice site selection, subcellular localization, mRNA transport and translation, as described above. Regulation of these phosphorylation events is mediated in part by the distinct cellular localization and substrate specificity of the splicing kinases. For example, the RS domain of SR-protein SRSF1 contains an N-terminal stretch of Arg-Ser repeats (termed "RS1") and a C-terminal stretch of Ser-Pro repeats (termed "RS2"). Cytoplasmic SRPK1 phosphorylates RS1 at multiple sites using a directional and processive mechanism. An acidic docking grove distal to the active site of SRPK1 binds RS1 leading to the "priming" phosphorylation of a single site in RS2. This site then binds a basic site within SRPK1 which serves to advance the RS repeat sequence through the docking grove and toward the kinase domain resulting in sequential phosphorylation events.[44] The phosphorylation of SRSF1 enhances the interaction with TRN-SR allowing it to transport SRSF1 into the nucleus where it assembles into nuclear speckles.[40,44] Nuclear CLK1 then phosphorylates RS2 causing SRSF1 dispersion from speckles and changes in alternative splicing.[14,45] CLK1 substrate specificity has been shown to be mediated, at least in part, by nature and extent of CLK1 autophosphorylation. It has been shown that CLK1 specificity for SRSF1 phosphorylation is sensitive to Tyr, but not Ser/Thr autophospharylation whereas its specificity for SC35 displays the opposite pattern.[46] In this example, sequential phosphorylation of SRSF1 by SRPK1 and CLK1 is regulated both by the cellular localization of the splicing kinases and by the unique specificity of SRPK1 for structural features within SRSF1 and of CLK1 for Ser-Pro dipeptide phosphorylation.[47] Interestingly, PRP4K has also been shown to phosphorylate SRSF1 *in vitro*,[48] is nuclear and resides predominately in splicing speckle domains (**Fig. 2**), suggesting PRP4K may too be involved in the sequential phosphorylation of SRSF1.

Alternative splicing in tumorigenesis

Alternative splicing occurs in an estimated 95% of human gene transcripts enhancing transcriptome complexity and proteome diversity in higher eukaryotes.[49] The most frequent alternative splicing event is the choice to include or skip an exon, termed a cassette exon. Other events involve the inclusion of one of 2 mutually exclusive exons, the use of alternative 3′ and 5′ splice sites, intron retention and the use of alternative promoters or poly(A) sites.[50] Alternative splicing also occurs in the 3′ and 5′ untranslated regions (UTR) of mRNA which can alter mRNA

stability and/or translation efficiency.[51] Dysregulation of alternative splicing can render an mRNA transcript inactive by introducing a stop codon, alter the protein-coding function or even result in the transcript encoding a protein of opposing function. Given the striking ability through which alternative splicing can alter the proteome, it is perhaps not surprising that this system is frequently manipulated throughout the process of tumorigenesis.[52-54] In fact, changes in alternative splicing have been found to affect nearly every aspect of tumor biology including metabolism, apoptosis, cell cycle control, invasion, metastasis and angiogenesis (reviewed in [55-58]).

While the molecular mechanism of alternative splicing has been shown to play a prominent role in tumorigenesis, there is limited knowledge of the regulation of these alternative splicing events observed in human cancers. One emerging mechanism of regulation is through altered expression of the splicing kinases, stemming from the observation that several of these kinases are overexpressed in various human cancers (SRPK1: breast,[59] colon,[59] pancreatic,[59] lung,[60] melanoma,[61] prostate.[62] SRPK2: acute myelogenous leukemia,[63] lung.[60] CLK1-4: erythroleukemia.[64] CLK2: breast[65]). The remainder of this review will focus on the 3 families of splicing kinases and the specific roles they have been identified to play in tumorigenesis and the response to chemotherapy.

Splicing kinases and their role in tumorigenesis and therapeutic response to chemotherapy *SRPK Family*

SRPK1, perhaps the most widely studied splicing kinase, has been shown to directly regulate pathways essential to the development, growth and dissemination of cancer. A number of proteins have been shown to be directly phosphorylated by SRPK1 including SC35,[11] SRp20,[11] SRp55,[11] SRSF1,[11] Tra2β1[66] and RBM4.[67] Of these, the most well characterized with respect to tumorigenesis are SRSF1 and RBM4. SRSF1 is a prototypical member of the SR protein family which, in addition to its role in splicing regulation, has also been shown to regulate nuclear export,[42] mRNA stability,[68] miRNA processing,[69] translation,[43] and nonsense-mediated mRNA decay.[70] While the oncogenic potential of SRSF1 is likely due to a combination of the above mentioned functions, it is the splicing function which has been most extensively studied. Several SRPK1-SRSF1 mediated alternative splice events have been linked to tumorigenesis (reviewed in [71]), but for the purpose of this review, we will focus on 3 splice events each effecting different aspects of tumorigenesis.

Rac1, a member of the Rho GTPase family, is involved in the regulation of cytoskeletal reorganization and cell motility.[72] An alternative splice variant of Rac1 (Rac1b) was identified in colorectal tumors that contain an alternative exon (3b) and is thought to maintain the GTPase in an active GTP-bound state.[73] Expression of Rac1b has been shown to induce epithelial-to-mesenchimal transition in cultured cells,[74] induce cell cycle progression and promote survival under conditions of serum-starvation.[75] Importantly, SRSF1 has been shown to promote inclusion of exon 3b in *RAC1* pre-mRNA[76]; an event which requires SRPK1 mediated phosphorylation to shuttle SRSF1 to the nucleus. Consequently, knockdown of SRPK1 has been shown to decrease SRSF1 shuttling to the nucleus and reduce expression of the oncogenic Rac1b splice variant.[77]

SRPK1-SRSF1 mediated alternative splicing has also been shown to regulate angiogenesis, the formation of new blood vessels, via vascular endothelial growth factor (VEGF) splicing control. VEGF alternative splicing leads to anti-angiogenic and pro-angiogenic mRNA isoforms which differ in the 3′ splice site of exon 8. Splicing at the proximal 3′ splice site results in pro-angiogenic splice isoforms (including $VEGF_{165}$)[78] while splicing at the distal 3′ splice site gives rise to a family of isoforms with anti-angiogenic properties (including $VEGF_{165}$b).[79] SRSF1 binds VEGF pre-mRNA in a region near the proximal 3′ splice site of exon 8, promoting use of this splice site, resulting in increased production of the pro-angiogenic VEGF splice isoforms.[80,81] Much like in Rac1 alternative splicing, SRSF1 binding to VEGF pre-mRNA is dependent on its nuclear localization, mediated by SRPK1 phosphorylation. Interestingly, recent studies have taken this model one step further to show that in Denys Drash Syndrome podocytes, mutations in the *WT1* tumors suppressor gene prevents WT1-mediated transcriptional repression of *SRPK1*. Up-regulation of SRPK1 led to increased hyper-phosphorylation, and nuclear translocation, of SRSF1 which pushed the $VEGF_{165}$/$VEGF_{165}$b ratio in favor of the pro-angiogenic $VEGF_{165}$, resulting in the formation of highly vascularized tumors using a colorectal tumor xenograft model.[82] Importantly, inhibition of SRPK1 using the small molecule inhibitors SRPIN340 or SPHINX increased expression of the anti-angiogenic $VEGF_{165}$b isoform in colorectal[82] and prostate[62] cancer cell lines *in vitro*, while SPHINX treatment in a mouse model of prostate cancer led to smaller tumors with decreased microvessel density.[62] These studies indicate that small molecule inhibition of SRPK1 may prove to be an effective anti-angiogenic therapy for some cancers.

The Myeloid Cell Leukemia 1 (*MCL1*) gene is a member of the *BCL2* family of apoptosis regulating genes and was first identified in differentiating myeloid leukemia cells.[83] MCL1 has 2 alternatively spliced variants, the full-length $MCL1_L$ which is anti-apoptotic, and an exon 2 skipped variant, $MCL1_S$, which can form a dimer with, and antagonize, the anti-apoptotic effects of $MCL1_L$.[84] In breast cancer, SRSF1 expression has been shown to increase stability and translational efficiency of the anti-apoptotic $MCL1_L$ isoform.[85] While it is likely that SRPK1 is mediating the nuclear localization of SRSF1and thus promoting splicing of the anti-apoptotic $MCL1_L$ in a manner similar to that seen during *RAC1* and *VEGF* splicing regulation, another mechanism of SRPK1 regulation has also been proposed. A recent study has shown that phosphorylation of RBM4 by SRPK1 targets RNA binding protein RBM4 to the cytoplasm, inhibiting its binding to MCL1 and preventing an exon 2 skipping event.[86] Therefore, in the case of MCL1, SRPK1 may promote splicing of the anti-apoptotic isoform though at least 2 distinct mechanisms.

In addition to playing several roles in tumorigenesis, SRPK1 is also implicated in the therapeutic response to cisplatin. Cisplatin is a platinum-based chemotherapy drug, among the most commonly used to target human cancers. Intrinsic or acquired cellular resistance to cisplatin is common, limiting the therapeutic efficacy and requiring increasing doses of drug to treat recurring

cancers. Cisplatin resistance is correlated with down-regulated SRPK1 expression in testicular germ cell tumors and ovarian cancers.[87,88] Furthermore, silencing of SRPK1 induces cisplatin sensitivity in multiple epithelial cell types including colon, breast, pancreatic and ovarian cancers and is accompanied by increased apoptosis, reduced cell proliferation, slower cell cycle progression and decreased anchorage-dependent growth *in vitro*.[59,89]

While SRPK1 has received the most attention for its role in tumorigenesis, SRPK3 has recently been described to promote tumorigenicity in rhabdomyosarcoma (RMS) as a regulator of MEF2C alternative splicing.[90] MEF2C, a member of the myocyte enhancer factor 2 (MEF2) family of proteins, plays a key role in synaptic formation and muscle differentiation.[91] *MEF2C* has 3 alternative splice variants which appear to perform distinct functions in myogenesis and neurogenesis.[92-94] In particular, MEF2Cα2, the isoform containing the alternative α2 exon, has been shown to be required for differentiation of skeletal muscle cells and is frequently downregulated in RMS cells.[95] It has recently been demonstrated that SRPK3, which has been shown to be upregulated during myogenesis,[13] is required for the isoform switch between MEF2Cα1 and MEF2Cα2. In RMS, SRPK3 is down-regulated preventing the isoform switch and failure of myogenic precursors to differentiate into normal muscle.[90]

The body of evidence surrounding the SRPK family of splicing kinases in tumorigenesis has made it clear that alterations in SR protein phosphorylation can have a significant impact on cancer development. As a result, recent studies have begun to focus on other splicing kinases to determine their possible roles in tumourigenesis and/or therapeutic response.

CLK Family

The splicing factor 45 (SPF45), first identified as a member of the spliceosome complex,[96] is known to promote exon 6 skipping in *Fas* pre-mRNA.[97] This exon encodes the transmembrane domain of the Fas death receptor, and its deletion results in the formation of a soluble Fas protein molecule.[98] Interestingly, expression of the soluble Fas molecule has been shown to prevent Fas mediated cell death, presumably by binding to Fas ligand (FasL), preventing FasL from binding to membrane-bound Fas and activating the apoptotic pathway. Given that evasion of apoptosis is a hallmark of cancer, it is not surprising that elevated levels of soluble Fas have been found in a variety of cancers.[99,100] A recent study has shown that CLK1 directly phosphorylates SPF45 on 8 serine residues, and that this phosphorylation led to the stabilization of SPF45 protein levels, and regulated exon 6 skipping in *Fas* pre-mRNA.[101] Furthermore, SPF45 overexpression induced cell migration and invasion in ovarian cancer cells,[101] suggesting CLK1 mediated stabilization of SPF45 could impact multiple aspects of tumor progression.

CLK2, a member of the CLK family of splicing kinases, has recently been shown to function as an oncogene in breast cancer.[65] A lentiviral shRNA cell viability screen was carried out that targeted 26 genes which the authors found to be commonly overexpressed or amplified in breast tumors or breast cancer cell lines based on SNP array analysis.[65] Of the 26 targets, knock-down of CLK2 was identified for its ability to inhibit breast cancer cell growth *in vitro*, and later confirmed to inhibit tumorigenesis in a mouse xenograft model. However, the knock-down of CLK2 was also shown to promote metastasis and invasion of breast cancer cells *in vivo* by inducing alternative splice patterns characteristic of the epithelial-to-mesenchymal transition (EMT). Specifically, knockdown of CLK2 was shown to regulate the alternative splicing of *ENAH*, an actin cytoskeletal protein, which contains a small coding exon (11a) which is included in the mRNA of epithelial cells, but excluded in mesenchymal cells.[65] Loss of CLK2 promoted the exclusion of exon 11a. Thus, CLK2 expression levels could represent an important marker for EMT during breast cancer progression.

PRP4K

PRP4K, the least studied of the 3 splicing kinases, has recently begun to emerge as an important regulator of therapeutic response. In addition to its role in splicing, PRP4K has been implicated in the regulation of mitosis as expression of a dominant truncated form of PRP4K in *S. pombe* was shown to induce mitotic aberrations.[102] Consequently, mammalian PRP4K was shown to be a regulator of the mitotic spindle assembly checkpoint (SAC) through its ability to recruit checkpoint proteins MPS1, MAD1 and MAD2 to the kinetochore.[103] This finding has important implications for therapeutic response to taxanes, a family of anti-cancer agents that depend on SAC activity for cell killing.[104] The taxanes (docetaxel, paclitaxel and cabazitaxel) function by binding to and stabilizing mictrotubules, resulting in a disruption of microtubule dynamics. As a cell progresses through mitosis, taxanes inhibit the ability of sister chromatids to properly segregate which triggers activation of the SAC and arrests the cell in prometaphase.[105] If the checkpoint cannot be satisfied, its prolonged activation will result in mitotic cell death.[106] Not surprisingly, disruption of SAC function has been has been shown to increase cellular resistance to taxanes in a number of different cancer models.[107-112] Consistent with these models, we have shown that PRP4K functions downstream of the receptor tyrosine kinase HER2 to regulate paclitaxel response in breast and ovarian cancer, presumably by altering SAC activity, and that its expression is decreased in ovarian cancer patients that have relapsed from taxane treatment.[113] Importantly, we also demonstrated that, among ovarian cancer patients with low HER2 expressing tumors, PRP4K expression can be used as a predictive marker to identify patients likely to benefit from taxane therapy.[113]

In addition to promoting resistance to microtubule targeting chemotherapeutics, disruptions in the SAC has been suggested to facilitate tumorigenesis by inducing chromosomal instability (CIN), a hallmark of human neoplasia.[114,115] Under normal conditions, the SAC becomes activated in response to one or more unattached chromosomes, blocking progression to anaphase. When checkpoint components are mutated, or expression levels decreased, un-attached chromosomes are unable to activate the SAC and become mis-segregated, leading to increased aneuploidy.[116-118] While it is still unclear as to whether or not a weakened checkpoint is sufficient to drive tumorigenesis, there is significant evidence suggesting it can facilitate tumorigenesis,

especially in collaboration with a weakened tumor suppressor such as a BRCA2 deficiency.[119,120] Therefore, it is tempting to speculate that loss of PRP4K expression would not only increase cellular resistance to taxanes, but could also facilitate tumorigenesis by inducing CIN.

PRP4K, in addition to its role in regulating taxane response, has also been shown to regulate the cellular response to ionizing radiation (IR) and the phytochemical curcumin.[121,122] In the human colorectal carcinoma cell line HCT-15, treatment with curcumin or IR led to a decrease in PRP4K expression and an increase in reactive oxygen species (ROS) production; a well-known mediator of apoptosis (reviewed in [123]). Importantly, overexpression of PRP4K was shown to prevent ROS production and provide cellular protection from apoptosis in response to both treatments, possibly through the activation of an anti-oxidant enzyme system.[121,122] This data suggests that tumors with high PRP4K expression may show increased resistance to radiation therapy due to increased ROS scavenging. With both increased and decreased PRP4K expression shown to be associated with resistance to different anti-cancer therapies, knowing PRP4K expression levels at the time of diagnosis may prove to be useful in choosing an appropriate treatment modality.

Regulation of tumorigenic alternative splice events by other kinases

While this review focuses on the classic SR-protein kinases, it is important to note that they are not the only kinases involved in the regulation of alternative splicing with implications in tumorigenesis. For example, the RNA binding protein SAM68 (Src-associated in mitosis, with a molecular weight of 68 k_D) has been shown to regulate a number of alternative splice events with implications in tumor progression and metastasis. This includes the splicing of cyclin D1 (CCND1), a proto-oncogene frequently deregulated in human cancers.[124,125] Alternative splicing of the CCND1 gene produces a variant transcript (*CyclinD1b*) which retains intron 4.[126] CyclinD1b displays increased oncogenic potential[126,127] and its upregulation correlates with poor prognosis in multiple types of tumors.[128] Retention of intron 4 has been shown to be mediated by SAM68 which binds the proximal region of intron 4, inhibiting U1snRNP recruitment.[129] Importantly, the phosphorylation of SAM68 by Erk1/2 increases its binding affinity to the CCND1 transcript enhancing intron 4 retention.[129] SAM68 has also been implicated in the regulation of EMT through its ability to repress alternative-splicing activated nonsense-mediated mRNA decay of SRSF1.[130] SAM68 promotes the retention of intron 4 in *SRSF1* pre-mRNA which inhibits its degradation by nonsense-mediated decay and increases SRSF1 protein levels. Increased SRSF1 impacts a number of tumorigenic alternative splice events, including stimulating the skipping of exon 11 in the proto-onocgene RON, producing a constitutively active isoform that promotes an invasive cellular phenotype.[131] While SAM68 phosphorylation by Erk1/2 provides an example of tumorigenic alternative splicing regulated by non-classical SR-protein specific kinases, numerous other kinases have been shown to phosphorylate splice factors with implications in cancer (reviewed in [132]).

Concluding Remarks

In light of recent evidence implicating the splicing kinases as major regulators of tumorigenic alternative splice events, drugs which modulate splicing kinase activity are actively being studied as potential anti-cancer agents. As an example, SRPK1 small molecule inhibitors have already been shown to promote the splicing of the anti-angiogenic VEGF isoform *in vitro* and *in vivo* in prostate cancer cells which have elevated SRPK1 and SRSF1 expression.[62] It has yet to be determined if SRPK1 inhibition alters other tumorigenic splice events like Rac1 and MCL^{-}1, but the potential for a single drug to target multiple aspects of tumorigenesis holds promise. Of course, SRPK1-mediated regulation is not limited to pro-tumorigenic pre-mRNA splicing events, raising concern over potential "off-target" effects of SRPK1 inhibition. High throughput next generation sequencing will allow for a more comprehensive understanding of changes to the transcriptome in response to SRPK1 inhibition, and provide insight into these potential off-target effects. In fact, an understanding of transcriptome changes in response to alterations in all splicing kinases would help identify which kinase, or combination of kinases, represents the best therapeutic target, which kinases to avoid due to potential off target effects, and the degree of redundancy between kinases which could be a potential mechanism of resistance for splicing kinase inhibition.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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