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Review article

Seeking a better understanding of the non-receptor tyrosine kinase, SRMS

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ABSTRACT

SRMS (Src-Related kinase lacking C-terminal regulatory tyrosine and N-terminal Myristoylation Sites) is a non-receptor tyrosine kinase first reported in a 1994 screen for genes regulating murine neural precursor cells. SRMS, pronounced "Shrims", lacks the C-terminal regulatory tyrosine critical for the regulation of the enzymatic activity of Src-family kinases (SFKs). Another remarkable characteristic of SRMS is its localization into distinct SRMS cytoplasmic punctae (SCPs) or GREL (Goel Raghuveera-Erique Lukong) bodies, a pattern not observed in the SFKs. This unique subcellular localization of SRMS could dictate its cellular targets, proteome, and potentially, substrates. However, the function of SRMS is still relatively unknown. Further, how is its activity regulated and by what cellular targets? Studies have emerged highlighting the potential role of SRMS in autophagy and in regulating the activation of BRK/PTK6. Potential novel cellular substrates have also been identified, including DOK1, vimentin, Sam68, FBKP51, and OTUB1. Recent studies have also demonstrated the potential role of the kinase in various cancers, including gastric and colorectal cancers and platinum resistance in ovarian cancer. This review discusses the advancements made in SRMS-related biology to date and the path to understanding the cellular and physiological significance of the kinase.

1. Introduction

SRMS (Src-Related kinase lacking C-terminal regulatory tyrosine and N-terminal Myristoylation Sites) is a member of the family of non-receptor type tyrosine kinases known as the BRK family kinases (BFKs). The other two members of the family include Breast Tumour Kinase (BRK)/PTK6 (protein tyrosine kinase 6) and Fyn-Related Kinase (FRK)/PTK5 (Reviewed in Refs. [1,2]). Like BRK and FRK, SRMS possesses three relatively conserved globular domains. These domains are the Src-homology 3 (SH3), Src-homology 2 (SH2) and kinase domains (Fig. 1). While BRK and FRK possess a regulatory C-terminal tyrosine residue essential for their enzymatic regulation, SRMS lacks this residue and has a truncated C-terminal tail. SRMS' autoregulation mode seems to rely on its unique N-terminal region [3].

The gene encoding SRMS was first discovered by Kohmura et al. in 1994 during a screening aimed at identifying novel genes that may be involved in neural precursor cell differentiation. The gene was named SRMS as an abbreviation for its biochemically

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descriptive nomenclature of the sequence-specific differences observed between SRMS and the Src Family Kinases (SFKs). Thus, the acronym is informative in that SRMS is a "Src-Related kinase", "lacking a C-terminal regulatory residue and N-terminal Myristoylation Sites" [4], both of which are hallmark biochemical features of the SFKs. The study also reported that the murine SRMS gene mapped to chromosome 2 [4]. However, the human *SRMS* orthologue is positioned adjacent to the BRK gene on the chromosomal locus 20q13.33. Kawachi et al. in 1996 reported cloning the same gene from murine neonatal skin tissue. The gene was named Protein Tyrosine Kinase 70 (PTK70). Given the two unique differences between the amino acid sequences of PTK70 and the SFKs, Kawachi et al. proposed that SRMS is likely a member of another non-receptor tyrosine kinase family distinct from the SFKs. Later reports noted that the SFKs possess 12 exons, whereas SRMS, BRK, and FRK display a unique intron-exon splice pattern resulting in 8 exons. Therefore, SRMS, BRK and FRK were considered a separate family of non-receptor tyrosine kinases and, after that named the BRK family kinases [1,2].

Since the discovery of SRMS in 1994, a few reports have demonstrated the properties and potential functions of SRMS (Fig. 2). The present review outlines these findings and discusses the significance and potential cellular implications of SRMS.

2. Protein organization and enzyme activity

The SRMS cDNA encodes a 54 kDa protein spanning 488 amino acids in length. It harbors an SH3, SH2 and a kinase domain: a structural framework common to BRK, FRK and the SFKs (Fig. 1). The SH3 and SH2 domains are typically involved in intra/inter-molecular interactions with distinct amino acid motifs. The SH3 domain characteristically binds to poly-proline residues, whereas the SH2 domain interacts with phosphotyrosine residue-containing motifs [5]. The enzymatic activity of BRK (Reviewed in Ref. [1]), FRK (Reviewed in Ref. [2]) and the SFKs (Reviewed in Refs. [6,7]) are regulated by the phosphorylation of a conserved C-terminal tyrosine residue. This residue corresponds to Y447 in BRK, Y497 in FRK, and Y530 in human c-Src (Y527 in chicken c-Src) [8]. Extensive analyses of the c-Src crystal structures have revealed that the enzyme natively exists in an autoinhibited state, where the phosphorylated C-terminal tyrosine residue is bound to the SH2 domain. The phosphorylation of Y530 in c-Src is catalyzed by the c-Src kinase (Csk) [7]. However, de-phosphorylation of the C-terminal tyrosine residue and the autophosphorylation of the activation loop tyrosine residue promotes conformational changes that result in the full activation of the enzyme [6]. Accordingly, the mutation of Y530 in c-Src [6] or the conserved residues in BRK [9] and human FRK [10] have shown the constitutive activity of the enzymes. However, unlike BRK, FRK and the SFKs, SRMS lacks a C-terminal regulatory region and has an extended N-terminal amino acid region. Intriguingly, SRMS exhibits two structural characteristics common to Csk. SRMS and Csk lack an N-terminal myristoylation signal and C-terminal regulatory tyrosine residue [3]. However, the mechanism of enzymatic autoregulation is distinct in both enzymes. While the kinase activity of Csk is primarily regulated through interactions involving its SH2 domain [11], we reported that unlike BRK, FRK and the SFKs, the enzymatic activity of SRMS is regulated by its extended N-terminal region [1–3]. Deletion of this 50 amino-acid long, N-terminal region has been found to abrogate SRMS enzymatic activity [3]. Analyses of the crystal structure of SRMS may better reveal the underlying structural features that support an undefined autoregulation mechanism.

3. Protein and RNA expression

The expression pattern of SRMS in normal or cancer tissues is largely unknown. We reported the expression of SRMS in breast tissue microarrays representing breast carcinoma samples and the adjacent normal mammary tissue [3]. Although the array contained only a small cohort of 24 cores derived from six patients with invasive ductal carcinoma, our results indicated that SRMS expression is

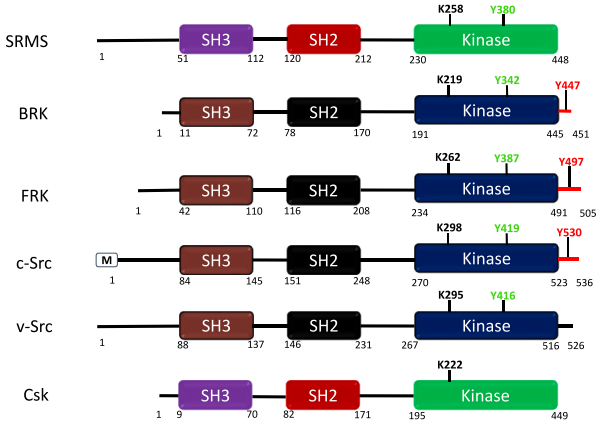

Fig. 1. Schematic representation of human SRMS, BRK, FRK, c-Src, Csk and chicken v-Src functional domains: SRMS, BRK, FRK, c-Src, v-Src and Csk possess three structured domains, namely, the SH3, SH2 and the kinase domain. Key residues implicated in the regulation of enzymatic activity of these kinases, are indicated. These are the activation loop autophosphorylation tyrosine residues (SRMS Y380, BRK Y342, FRK Y387, c-Src Y419 and v-Src Y416), the C-terminal regulatory tyrosine residues (BRK Y447, FRK Y497 and c-Src Y530) and the ATP-contacting lysine residues (SRMS K258, BRK K219, FRK K262, c-Src K298, v-Src K295 and Csk K222). All numbering is for human proteins and v-src is from retroviral source with its own numbering.

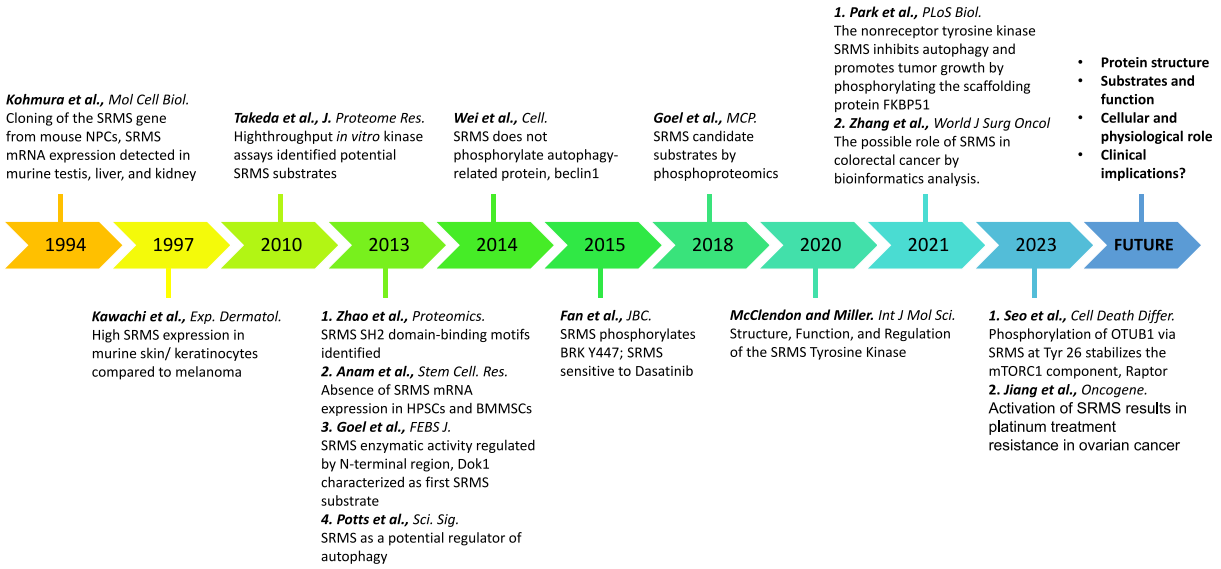

Fig. 2. SRMS-related discoveries/reports in the literature (1994–2023): A series of significant SRMS-related investigations and highlights of the findings, are listed chronologically.

potentially higher in breast carcinomas compared to the adjacent normal control. A similar observation was made through expression studies in a panel of immortalized breast cancer cell lines. From the immunohistochemistry (IHC) analyses, it also appeared that the expression levels corresponded with increasing tumour grade [3]. Breast cancer is a heterogeneous disease with different clinical, histopathological, and molecular characteristics [12,13]. Therefore, an evaluation of SRMS mRNA and protein expression in a broader panel covering the various breast cancer subgroups would be necessary to understand if SRMS expression can be statistically correlated between normal/tumour pairs. Further, SRMS expression levels in other cancer types are largely undetermined and may warrant relevant studies. The Human Protein Atlas provides an informative overview of the expression of SRMS in various cell lines and cancer types [14] (Fig. 3). SRMS expression is higher in breast and gastric cancers than in other cancer subtypes [14]. Further studies may be necessary to confirm and characterize the deregulated expression of SRMS in specific malignancies.

4. Subcellular localization

Unlike the SFKs, SRMS lacks a myristoylation/palmitoylation signal that dictates plasma-membrane anchoring of the SFKs [15] and a nuclear localization signal such as that embedded within the SH2 domain of FRK [2]. However, we determined that both the endogenous and ectopically expressed SRMS localize predominantly to punctate cytoplasmic structures in HEK 293, HeLa, and certain breast cancer cell lines [3]. We have named these unique structures SRMS cytoplasmic punctae (SCPs) or GREL (Goel Raghuveer-a-Erique Lukong) bodies. This specific expression pattern, not previously reported for the SFKs, is also regulated by the activation

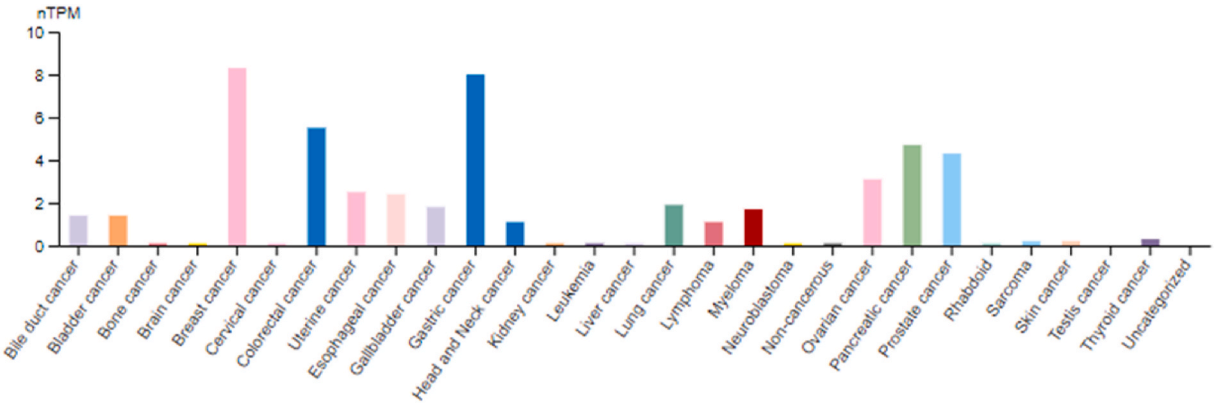

Fig. 3. Pan-cancer specific SRMS RNA expression profile: SRMS RNA expression levels are summarized based on cell line data derived from DepMap Portal (https://depmap.org/portal/download/all/). The cell lines were generated within the Human Protein Atlas project and are grouped according to cancer type. Relative RNA expression of SRMS per cancer type are shown as normalized transcript per million (nTPM) values [14].

status of the kinase. For instance, wild-type c-Src was found to localize in the perinuclear region of murine fibroblast cells, where it directly associates with the microtubule-organizing center and co-localizes with endosomal markers [16]. Interestingly, in the same cells, the constitutively active c-Src Y527F variant was found to localize to focal adhesions. Accordingly, c-Src localizes to focal adhesions in cells lacking Csk expression, implying that the phosphorylation of c-Src Y527 by Csk regulates c-Src subcellular localization [17]. Contrarily, v-Src was detected in both the plasma membrane [18] and the perinuclear region of transformed cells [19]. Ectopically expressed BRK displays a diffuse nucleo-cytoplasmic localization in HeLa and HEK293 cells [20,21]. Endogenous BRK tends to localize with its substrate Sam68 in what are known as Sam68 nuclear bodies (SNBs), while activated BRK has been widely reported to localize to the cell periphery [22,23]. Interestingly, the co-expression of BRK and Sam68 in HeLa cells regulates the distribution of SNBs in the nucleus to form multiple SNBs [20]. Whether wild-type SRMS localizes to any specific organelle or co-localizes with certain cytoplasmic markers is unknown. However, deletion of the N-terminus of SRMS or the inactivation of the kinase activity via introducing a K258 M substitution resulted in a partially diffused cytoplasmic localization as opposed to the predominant punctate pattern displayed by wild-type SRMS [3]. Therefore, like Src, the enzymatic activity of SRMS may be partly linked to its subcellular localization. It also appears that the localization of wild-type SRMS to GREL bodies is partially regulated by the unique extended N-terminus of SRMS. Identifying SRMS interacting partners may provide vital clues on the identity of the proteins that sequester with SRMS to these cytoplasmic punctae. The punctate subcellular localization of SRMS, as a potential consequence of its specific intermolecular interactions, may further provide insights into the cellular functions of the kinase.

5. Potential substrates and signaling pathways

While a multitude of binding partners/substrates has been identified and characterized for BRK and FRK [1,2], little is known about the targets of SRMS. A proteomics study by Takeda et al. revealed, for the first time, the potential substrates of SRMS and other BFK and SFK members [24]. The study primarily explored the substrate-specificity of various Src family members and other related kinases against 519 unique substrates in high-throughput *in vitro* kinase assays. The targets were ranked based on their relative substrate-specificity towards CRK (p38) as a common phosphorylated target. A fair number of SRMS potential substrates were identified (Table 1). We later validated docking protein 1 (DOK1) as the first SRMS substrate [3]. DOK1 is a key adapter protein known to function as a tumour suppressor in various cancer types [25]. It has been characterized as a substrate of several other tyrosine kinases, including BRK [26], Src [27] and Abl [28]. However, the effect of DOK1 phosphorylation by SRMS on the cellular functions of the adapter protein has yet to be discovered and may be investigated in the future. Global phosphoproteomics analyses by our group revealed that serine/threonine phosphorylation dynamics play important secondary events in the SRMS-regulated phosphoproteome [29].

More recently, Park et al. discovered that under nutrient-replete conditions, SRMS phosphorylates the stress-response, scaffolding protein FK506-binding protein 51 (FKBP51) on its tyrosine 54 residue [30]. They found that the wild-type SRMS could phosphorylate FKBP51 on tyrosine residue(s) *in vitro*, which could also be detected at endogenous levels in MDA-MB-231 cells [30]. Further, the inhibition of SRMS via gene editing or use of the tyrosine kinase inhibitor, Ibrutinib, was found to abrogate FKBP51 tyrosine phosphorylation without inducing significant changes in the global tyrosine phosphorylation [30]. These results suggest that FKBP51 is a new, bona fide, endogenous substrate of SRMS.

Further, Seo et al. have recently shown that the knockdown of SRMS decreases the phosphorylation of OTU domain-containing ubiquitin aldehyde-binding proteins Otubain1 (OTUB1), resulting in the down-regulation of Regulatory-associated protein of TOR (Raptor) [31]. The authors also reported that SRMS induced the phosphorylation of OTUB1 at Y26, which resulted in Raptor stabilization. Intriguingly, the phosphorylation of OTUB1 at Y26 did not affect the stability of other OTUB1-targeted substrates. In addition, another study conducted by Jiang et al. found that SRMS inhibited MKK4 kinase activity via the direct phosphorylation of MKK4 at the Y269 and Y307 residues, resulting in the consequent attenuation of MKK4-JNK activation [32]. Together, these findings, therefore, suggest that OTUB1 and MKK4 may be substrates of SRMS [31,32].

A study by Zhao et al. identified the SH2-binding specificities of 25 non-receptor tyrosine kinases [33]. From a library of phosphotyrosine peptides screened against the SH2 domains of several tyrosine kinases, the SRMS SH2-specific motifs were identified as preferably possessing hydrophobic residues at the P+3 position (where P is the phosphotyrosine) (Fig. 4). Specifically, it was noted

Table 1
List of the potential substrates of SRMS identified by Takeda et al., 2010 [24].

Protein name	Accession no.	Function
Tom1 like 1	AK315002	Src-activating
Dok1	AK055944	Adapter protein
Lck	BC013200	Tyrosine Kinase
CRK associated substrate	AK124815	Adapter protein
CRK associated substrate	BC053532	Adapter protein
CRK	BC001718	Adapter protein
ARG	BC065912	Tyrosine Kinase
SYK	AK075020	Tyrosine Kinase
Disabled 1	AK095513	Adapter protein
HEF-like protein	AK295613	HisKa Domain
NEK11	AK027148	S/T kinase

that the SRMS SH2 domain favours motifs possessing a phenylalanine, methionine or cysteine residue at the P+3 position. Furthermore, at the P+1 position, the SRMS SH2 domain exhibited specificity towards a histidine or glutamic acid residue, whereas, at the P-1 position, it favoured either a histidine or a tyrosine residue [33]. Characterization of the SRMS SH3/SH2 ligands may provide vital cues on SRMS substrate specificity and the regulation of associated signaling pathways. Additionally, a study by Liu et al. examined the *in vitro* binding specificities of 78 different SH2 domain-containing proteins towards tyrosine-phosphorylated motifs derived from various receptor proteins implicated in immune response signaling [34]. Specifically, compared to Src, BRK or FRK, the SRMS SH2 domain was found to exhibit generally minimal binding specificity towards either the LIRB4 pY360, DSCAM pY1708, PGRFB pY797, PGRFB pY904, CD3E pY199, SLAF pY304, STAM1 pY371, LAY1 pY71, LY9 pY626 or CD79A pY199 synthetic peptides [34]. This may imply that SRMS SH2-mediated intermolecular interaction and its role as a signaling intermediate in the immune receptor network may be limited. Further, the results of the study also indicate that, unlike other BFK and SFK members, the SRMS SH2 domain potentially interacts with a highly limited and specific cohort of tyrosine phosphorylated proteins [34]. This may further reflect on the degree and specificity of SRMS cellular functions.

We have shown that SRMS can phosphorylate and/or induce the phosphorylation of several cellular targets [3] and could therefore modulate various cellular events. We were the first to report that SRMS was an active kinase whose catalytic activity was regulated by the intramolecular interactions involving the N-terminus of the enzyme [3]. Further, via global phosphoproteomics analysis, we identified 60 hyperphosphorylated (phosphoserine/phosphothreonine) proteins mapped from 140 hyperphosphorylated peptides enriched in biological and cellular processes, including DNA repair apoptosis pathways [29]. Cellular processes related to viral infection and DNA repair, for instance, were predicted with a significantly increased activation state. In contrast, apoptosis and necrosis were predicted to display an overall decreased activation state. Furthermore, we identified Casein kinase 2 alpha (CK2α) as one of the significant potential kinases regulated by SRMS [35]. A bioinformatic tool developed to connect signaling proteins to their targets also linked casein kinase 2 to the SRMS-signaling network [36].

Additionally, we performed tyrosine phospho-enrichment mass spectrometry analysis to identify potential substrates of SRMS in cell lines ectopically expressing SRMS. We identified 1258 tyrosine-phosphorylated peptides, which mapped to 663 phosphoproteins

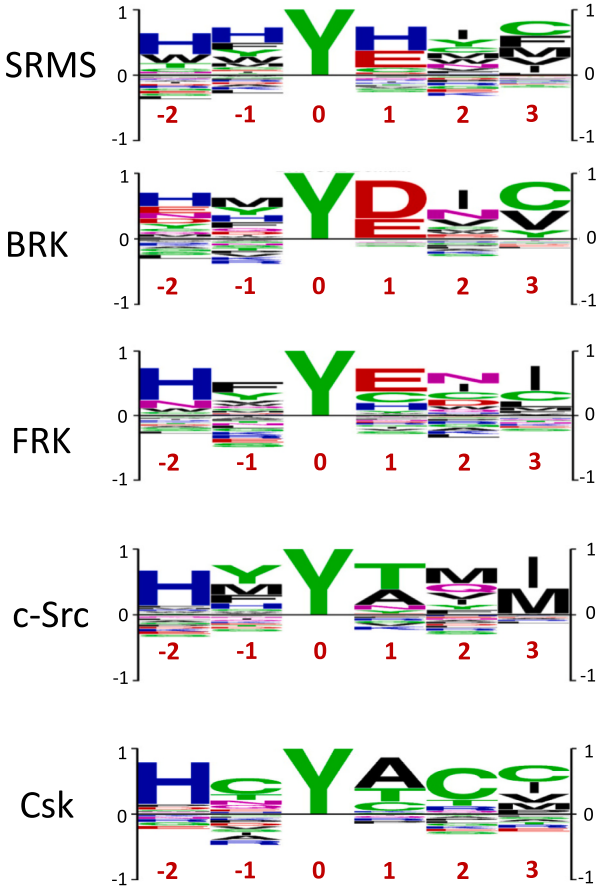

Fig. 4. SH2 domain-binding motifs of SRMS, BRK, FRK, SRMS and CSK: Zhao et al. performed high throughput *in vitro* binding assays using a synthetic phosphopeptide library, with permutatively arranged amino acid residues surrounding the phosphotyrosine residue, against the SH2 domains of various tyrosine kinases. Shown here are the resulting binding specificities of the SH2 domains of SRMS, BRK, FRK, c-Src and Csk [33]. The positions of the phosphotyrosine residue (0) and the residues surrounding the phosphotyrosine are indicated (−1,-2/+1,+2,+3).

that included DOK1 [35], which we had previously characterized as a SRMS substrate [3]. In general, candidate SRMS substrates were enriched in biological processes that included protein ubiquitination, mitotic cell cycle, energy metabolism and RNA processing. We also validated Vimentin and Sam68 as bona fide SRMS substrates [35].

6. Function and cellular roles

Information on the functional and cellular role of SRMS is relatively sparse. The lack of characterized bona fide endogenous targets/substrates of SRMS has limited our understanding of the function and role of the enzyme. However, SRMS was reported to phosphorylate the C-terminal Y447 residue in BRK, implying that SRMS may potentially regulate BRK activity, hence, BRK kinase-dependent functions [37]. In a cellular context, SRMS has also been shown to play a potential role in the regulation of autophagy [30,38] and is speculated to be involved in cell differentiation [4,39] and oncogenesis [30,40].

SRMS as a negative regulator of BRK: The SFKs and the BFK members BRK and FRK display a similar structural architecture given the presence of an SH3, SH2, and kinase domain in addition to a C-terminal region containing a conserved tyrosine residue extending beyond the kinase domain. Hence, it is probable that the mechanism associated with the regulation of enzymatic activity is likely conserved in these kinases and dependent on the phosphorylation of the conserved C-terminal tyrosine alongside other characteristic autoregulatory features. Extensive biochemical and structural characterization studies of c-Src have demonstrated that the intramolecular interaction between the C-terminal phosphorylated Y530 and the SH2 domain result in the inhibition of the c-Src enzymatic activity [7]. In 1989, Okada et al. reported the identification of a protein tyrosine kinase called C-terminal Src kinase (Csk), capable of inhibiting c-Src by specifically phosphorylating its C-terminal tyrosine [6]. Csk was later shown to phosphorylate and inactivate other SFK members as well [41–43]. Csk has been characterized as a physiological inhibitor of SFKs [44]. Studies have shown that mutating the conserved C-terminal tyrosine in BRK and FRK results in constitutive enzymatic activity, implying that the phosphorylation of this tyrosine is involved in the enzymatic regulation of these kinases. Interestingly, while Csk did not phosphorylate BRK at the conserved C-terminal residue, Y447 [45], it was found to phosphorylate FRK Y497, highlighting the potential regulatory role of Csk towards FRK enzymatic activity [46]. However, the regulation of FRK enzymatic activity by Csk has not been characterized and warrants further investigation.

In addition, a study by Feng G. et al. showed that the exogenous expression of wild-type SRMS and a kinase-dead mutant of BRK (K219 M) resulted in enhanced phosphorylation of BRK at Y447 [37]. Consistent with previous findings, ectopically expressed Csk did not phosphorylate BRK at Y447. Interestingly, while SRMS phosphorylated BRK Y447, the kinase did not phosphorylate specific synthetic peptides targeted by Src, nor Protein kinase A and Insulin receptor [37]. However, the effect of SRMS-catalyzed phosphorylation of BRK Y447 on the enzymatic activity of BRK was not reported. Given the importance of BRK Y447 in regulating enzymatic activity, it would be necessary to investigate whether SRMS is an inactivator of BRK, both *in vitro* and *in vivo*, and a regulator of the kinase-dependent cellular and physiological functions of BRK. In the case of Csk, although Csk knockout mice died at the early stages of embryogenesis, the homozygous knockout embryos, as well as Csk-deficient cell lines established from the knockout embryos, exhibited constitutive activation of Src family kinases that resulted in the accumulation of tyrosine-phosphorylated targets [47,48]. In both Drosophila and C. elegans, ablation of Csk also resulted in constitutive activation of the SFKs associated with hyperproliferation and defective cytoskeletal function, respectively [49,50]. Therefore, these studies strongly indicate that Csk is required to repress the catalytic activity of the SFKs and regulate normal physiological development. On the other hand, SRMS knockout mice were viable and fertile and displayed no apparent phenotype [4]. While this may indicate functional redundancy with perhaps other Src-related kinases, it is important to note that BRK knockout mice also displayed a normal phenotype (Review in Ref. [1]). Yet, BRK has been widely reported to be implicated in growth regulation in different cells. It is also important to note that the activation status of BRK in SRMS knockout mice has not been reported. Altogether, further studies may reveal a unique cellular and physiological function of SRMS.

Additionally, the arrangement of the functional domains of SRMS is similar to that of Csk. Both proteins lack the N-terminal fatty acylation sites and the C-terminal negative regulatory sites conserved among the SFKs, with the latter also found in BRK and FRK. However, Csk lacks the auto-phosphorylation site in the activation loop in SRMS and other BFKs and SFKs [11]. The crystal structure of full-length Csk revealed that the orientation of the binding pockets of the SH3 and SH2 domains of Csk and the SFKs are significantly different, ultimately influencing their intermolecular interactions and substrate specificity [51]. Structural analyses of wild-type SRMS may reveal distinguishing features of this kinase that may aid in understanding its substrate specificity.

SRMS as a potential regulator of cellular differentiation: Two subsequent studies in the early 1990s highlighted a possible link between SRMS expression and murine cellular differentiation biology. Kohmura et al., credited with discovering the SRMS cDNA from mouse neural precursor cells (NPCs), identified two variants of the SRMS mRNA via Northern blotting analyses possessing molecular sizes of 2 kb and 2.6 kb [4]. Though it is unclear whether these variants represent products of alternative splicing events, the expression of these variants was found to differ temporally and spatially in certain murine tissues examined. For instance, the 2.6 kb variant was abundantly expressed in tissues derived from the murine lung, liver, spleen, ovary, kidney, and intestines, while the expression of the 2 kb variant was limited to fewer organs, including the testis and cerebral tissues. Lower expression levels of the 2.6 kb variant were seen in murine heart, cerebrum, cerebellum, and thymus tissues [4]. This indicates that SRMS expression is likely spatially altered across different murine organs where the cellular roles of the kinase are yet to be examined. Additionally, while the expression of the 2.6 kb mRNA variant was detected in the embryonic day 15 (E_{15}) brain tissues, it was found to be relatively lower in the postnatal brain. Contrarily, the 2 kb mRNA variant was more abundant in the postnatal brain [4]. Interestingly, both variants were in low abundance in the E_{10} brain-derived NPCs, E_{11} brain, and adult mouse brain tissues. Therefore, given the apparent temporal regulation of SRMS expression in the murine brain, SRMS may play an essential function during specific developmental and differentiation stages of the murine brain cells [4].

Further, a study by Kawachi et al. also identified two mRNA variants in mouse tissues which were speculated to result from alternative splicing events [39]. As with the observations from Kohmura et al., Kawachi and colleagues noted organ-specific differences in SRMS mRNA expression, though these observations were not entirely identical to those indicated by Kohmura et *al.* Specifically, Kawachi et al. determined that SRMS mRNA expression was more abundant in murine liver, lung, thymus, and skin compared to the brain, kidney, heart, and spleen. Furthermore, quantitative analyses of SRMS mRNA in selected skin-derived cell lines revealed a relatively higher level of SRMS mRNA expression in the Sq-1974 cell line than in other cell lines tested such as the B16 melanoma 4A5 and NFSaY83. Additionally, SRMS mRNA levels were higher in the normal epidermal cells than in the dermal fibroblast cells. Given the abundant expression of SRMS mRNA in the keratinocyte-derived Sq-1974 cell line and the epidermal cells, it was speculated that SRMS may play a role in keratinocyte differentiation [39].

In a later study, Anam et al. quantitatively assessed the mRNA expression levels of various receptor tyrosine kinases, non-receptor tyrosine kinases, and other key signaling proteins in murine bone marrow cell (BMC)-derived hematopoietic stem/progenitor cells (HPSCs) as well as bone marrow mesenchymal stromal cells (BMMSCs) [52]. Interestingly, among other genes, a few members of the SFKs, such as Lck, Lyn, Hck, and Fyn were found to be differentially expressed in the HPSCs and BMMCs. However, the expression of SRMS and another tyrosine kinase, Tnk1, could not be detected in either cell type. The study did not report the underlying reasons supporting the absence of SRMS expression. However, it may indicate that SRMS may be functionally redundant in the differentiation process involving bone marrow-derived cells.

SRMS as an inhibitor of autophagy: Autophagy, defined as a protein and organelle degradation pathway in which cytoplasmic material is engulfed in autophagosomes and delivered to lysosomes for degradation, is involved in various diseases, including cancer [53]. Autophagy is enhanced in response to various stresses and nutrient deprivation and provides the cell with nutrients and energy released from degraded products [53]. Autophagy has paradoxical effects in cancer where it can have both tumour-suppressive and tumour-promoting roles in a context-dependent manner, such as tumour stage [54,55]. The tumour suppressive functions usually occur during tumour initiation. At the same time, the pro-oncogenic properties tend to be associated with the later stages of cancer development when tumour cells are most exposed to stress stimuli [54,55].

Potts et al. used a high throughput approach utilizing libraries of siRNA, miRNA, and marine-derived bioactive molecules to identify SRMS as a potential regulator of autophagy [38]. They showed that knockdown of SRMS resulted in increased light chain 3 (LC3)-positive puncta per cell and decreased abundance of GFP-LC3 in an autophagy-related 5 (ATG5)–dependent manner. On the other hand, overexpression of SRMS reduced the number of LC3-positive puncta per cell, suggesting that SRMS potentially inhibits autophagy upstream of autophagosome formation. Additionally, the knockdown of SRMS did not affect p70S6K phosphorylation and mTOR pathway activity, indicating that the role of SRMS in the potential inhibition of autophagy is nutrient-independent.

Park et al. have recently validated SRMS as a negative regulator of autophagy [30]. They found that in the assessment of autophagosome–lysosome fusion in U2OS RFP-GFP-LC3 cells, the depletion of SRMS increased the average number of both pre-fusion autophagosomes (RFP-positive; GFP-positive) and post-fusion autolysosomes (RFP-positive; GFP-negative) per cell [30]. Park et al. also demonstrated that under nutrient-replete conditions, SRMS phosphorylates FKBP51 on its Y54 residue [30]. FKBP51 has been previously reported to inhibit the PI3K-AKT signaling pathway. This pathway plays an important role in autophagy, promoting cell growth and proliferation and inhibiting apoptosis [56,57]. This phosphorylation disrupted the FKBP51-PHLPP phosphatase complex, promoting the degradation of FKBP51 through the ubiquitin-proteasome pathway [30]. Subsequently, this prevents the PHLPP-mediated dephosphorylation of AKT and results in the sustained activation of AKT which promotes cell growth and inhibits autophagy, as FKBP51 is a necessary scaffolding protein for the inactivation of AKT via the PHLPP phosphatase family through the dephosphorylation of p-AKT at S473 [30,58].

Additionally, there is evidence supporting a tumour-suppressive function of autophagy. For instance: (1) monoallelic loss of beclin 1, an essential autophagy-associated gene, occurs with high frequency in several cancers, including human breast, ovarian and prostate tumours [59,60]; (2) beclin $1^{+/-}$ mutant mice display an increase in tumour incidence [61]. A recent study demonstrated that Beclin 1 is regulated by tyrosine phosphorylation [62]. The epidermal growth factor receptor (EGFR) was shown to associate with and phosphorylate Beclin 1 on several tyrosine residues, resulting in the inhibition of autophagy and the promotion of tumorigenesis in non-small cell lung carcinoma (NSCLC) cells [62]. Unlike active EGFR, Wei et al. found that control tyrosine kinases, including SRMS and PDGFR, did not phosphorylate the Beclin 1 peptide *in vitro* [62]. It therefore appears that the mechanism of action of SRMS as a potential negative regulator of autophagy is not via Beclin 1 phosphorylation. However, a recent study by Park et al. found that SRMS inhibited Beclin1-dependent autophagy through the phosphorylation FKBP51 [30]. The inhibition of SRMS and the subsequent sustained activity of AKT resulted in the increased formation of pro-autophagy Beclin 1 protein complexes as demonstrated by an increased association of Beclin 1 with pro-autophagy proteins UVRAG, VPS34, and ATG14 [30]. Additionally, introducing constitutively active AKT into SRMS knockout cells rescued Beclin 1 phosphorylation at Ser295 and attenuated the lipidation of LC3. This confirmed the findings of Park et al. that AKT acts downstream of SRMS in the regulation of Beclin1-dependent autophagy [30].

7. Potential role of SRMS in cancer

In a study of copy number changes in the tyrosine kinase gene loci in gastric cancer cell lines, *PTK6*, SRMS, *PTK7* and *MET* genes were found among 24 high-level amplifications [63]. The functional effects of SRMS amplification were not reported. However, Jha et al. used population differentiation statistics and found that the *PTK6-SRMS* locus on chr20:q13.33 loci had high differentiation between different human populations worldwide [64]. Specifically, they showed a significant correlation between the *PTK6-SRMS* variant with gastric cancer incidences in East Asian populations where the cancer is most prevalent [64]. Gastric or stomach cancer is a common cancer of the gastrointestinal tract [65]. In 2020, gastric cancer was the third most common cause of cancer-related deaths

worldwide, third only to lung and liver cancer, with nearly 770,000 deaths [66]. Chronic gastric inflammation from *Helicobacter pylori* (H. pylori) infection has been identified as the strongest risk factor for gastric cancer. The *Helicobacter pylori* cagA gene-encoded *CagA* protein is phosphorylated at the Glu-Pro-Ile-Tyr-Ala (EPIYA) motifs and mediates SH2-domain interactions with multiple host signaling molecules [67]. *CagA* has been shown to interact with E-cadherin and activate β-catenin by impairing the complex formation between E-cadherin and β-catenin, promoting the nuclear accumulation of β-catenin. Activating β-catenin in a *CagA*-dependent manner has resulted in the targeted transcriptional regulation of genes related to carcinogenesis in gastric cancer, including *STAT3.* Although the role of SRMS in CagA-mediated gastric cancer has not been established, other Src-family kinases, along with signaling molecules such as STAT3, EGFR, and β-catenin, have been implicated in H. pylori infection and the carcinogenesis of gastric cancer [68–70]. The strong selection pressure of the *PTK6-SRMS* locus may indicate the possible role of both gene products in gastric cancer. Another evidence for a potential role of SRMS in gastric cancer came from a quantitative proteomic analysis in which SRMS, vitronectin, clusterin isoform 1, and thrombospondin 1 displayed significant differential expression between the gastric cancer groups and the normal controls [71]. This study, therefore, identified SRMS as a diagnostic marker for gastric cancer.

In addition to gastric cancer, SRMS may play a role in breast cancer and tamoxifen treatment resistance. Approximately 70% of breast cancers are Estrogen Receptorα-positive (ER+) and dependent on estrogen for growth and progression [72,73]. Tamoxifen is a front-line treatment for ER + breast cancer patients. However, although tamoxifen helps significantly improve patients' survival rates, acquired tamoxifen resistance remains a major clinical drawback and results in frequent cancer recurrence and mortality [72,73]. The underlying mechanisms for tamoxifen resistance are multifactorial [74]. For instance, the activation of insulin-like growth factor 1 receptor (IGF1R) signaling has been identified as one of the mechanisms that promote the proliferation of tamoxifen-resistant cells [74]. To understand the signaling cascades underlying IGF1R-mediated tamoxifen resistance in breast cancer, Zhang et al. performed a kinome siRNA screen on MCF7 estrogen receptor-positive breast cancer cell line under these conditions. They identified SRMS as a potential inducer in the proliferation of these tamoxifen-resistant cells [75]. Further, additional studies have shown that Src's increased expression and activity promote tamoxifen insensitivity in breast cancers [76]. SRMS, a Src-related kinase, is overexpressed in breast cancer [3]. Mined data from cBioportal revealed that SRMS mRNA expression was potentially increased in 10% of luminal breast cancers and that SRMS expression correlated with poor prognosis [34]. The study also demonstrated that the knockdown of SRMS inhibited the growth of the luminal breast cancer cell lines MCF7 and T47D.

In addition to breast and gastric cancers, SRMS was recently found to have potential colorectal cancer (CRC) involvement. Globocan 2020 statistics revealed nearly 2 million new cases of colorectal cancer that year, with over 900,000 resulting in mortality [66]. Further, CRC is the third most common cancer worldwide, third only to lung cancer and female breast cancer, respectively [66]. With the high incidence and mortality of CRC, a prognostic biomarker and target for therapy would be beneficial for early detection and treatment to reduce the number of annual deaths. Yoo et al. discovered SRMS to be one of four proteins and the only kinase differentially expressed in their proteomics study of new, specific biomarkers for gastric cancer [71]. Subsequently, a bioinformatics study by Zhang et al. found SRMS to be a promising prognostic biomarker and target for patients with CRC [40]. They discovered that SRMS was overexpressed in CRC tissues and correlated with a poor prognosis. Further, the expression levels of SRMS in colon adenocarcinoma (COAD) significantly correlated with nodal metastasis status and the pathological stages of CRC [40]. Mainly, SRMS was found to regulate the progression of CRC via the modulation of cytokine-cytokine receptor interaction, IL-17, chemokines, and the intestinal immune networks for signaling pathways of IgA production [40]. However, more research is needed to validate these findings in the future.

Further, SRMS was also found to play a role in tumour growth in a kinase-dependent manner. Park et al. found that the knockout of SRMS in MDA-MB-231 triple-negative breast cancer cells resulted in the profound reduction of anchorage-independent growth [30]. Furthermore, the impaired growth of SRMS knockout in MDA-MB-231 cells and U2OS human bone osteosarcoma epithelial cells were rescued by reintroducing the wild-type SRMS but not the catalytically inactive mutant, SRMS (K258A) [30]. The team also found that SRMS knockout MDA-MB-231 cells failed to grow tumours when subcutaneously injected into NSG recipient mice, while the parental MDA-MB-231 cells readily formed tumours. Additionally, the reintroduction of wild-type SRMS rescued xenograft tumour growth in the SRMS knockout cells, while the reintroduction of SRMS (K258A) had no effect [30]. These findings suggest that SRMS may play a critical role in tumour growth, both *in vitro* and *in vivo*, most likely via the PI3K-AKT signally pathway often involved in human diseases such as cancer [77].

Recently, Jiang et al. found SRMS to be involved in platinum-based chemotherapy resistance in ovarian cancer [32]. Ovarian cancer is among the leading cause of mortality in gynecological malignancies, with over 300,000 new diagnoses and over 200,000 deaths in 2020 [66]. Alongside surgery, platinum-based chemotherapy remains the standard of treatment for ovarian cancer [78,79]. Unfortunately, approximately 70% of patients will relapse following treatment. The development of platinum resistance is often observed in relapsed patients and is a significant contributing factor to ovarian cancer mortality [80,81]. Jiang et al. conducted a kinome-wide synthetic lethal RNAi screening in combination with unbiased data mining in Genomics of Drug Sensitivity in Cancer (GDSC) and Cancer Cell Line Encyclopedia (CCLE) databases of cell line platinum treatment response. They found SRMS to be a novel negative regulator of MKK4-JNK signaling under the platinum therapy [32]. Additionally, Jiang et al. observed that SRMS plays an important role in dictating the efficacy of platinum treatment in ovarian cancer, and the suppression of SRMS was found to sensitize p53-deficient ovarian cancer cells to platinum both *in vitro* and *in vivo* [32]. Furthermore, SRMS suppression led to enhanced MKK4-JNK-mediated apoptosis via the inhibition of MCL1 transcription, increasing platinum efficacy [32]. The team also discovered a small molecule selective inhibitor of B-RafV600E known as PLX4720 to be a novel inhibitor of SRMS. PLX4720 was found to potently increase the efficacy of platinum therapy in ovarian cancer both *in vitro* and *in vivo* [32]. Therefore, targeting SRMS with PLX4720 may hold the key to overcoming chemoresistance in ovarian cancer and the improvement of platinum-based chemotherapy efficacy.

Interestingly, ampelopsin, a plant product with pharmacological activity, has been shown to have anti-proliferative properties and

to induce apoptosis of cancer cells [82]. Ampelopsin C was shown to inhibit metastasis of the MDA-MB-231 breast cancer cell line in a mechanism that was reported to involve the decreased activation of a few tyrosine kinases, including SRMS, Fyn and Hck [83]. These culminated studies reveal that SRMS may be an integral kinase in various cancers and warrants further research to uncover the potential role of SRMS in oncogenesis.

8. Physiological role

SRMS-deficient mice were determined to be viable and fertile with normal offspring and no apparent histological abnormalities in the body organs [4]. A similar observation was made for BRK and FRK-deficient mice [84,85]. Yet, extensive characterization of BRK and FRK functions evidenced by ectopic expression and RNA knockdown experiments have produced notable implications in cell biology. Thus, an invariable phenotype yielded by gene knockout experiments may not necessarily be inferred as a functional redundancy of the gene product. While specific reasons may not be attributed to this cause, a rather complex network of functioning/signaling, leading to the activation of alternate protein kinases, may serve to recompense the targeted disruption of a gene product. This was especially seen in FRK knockout mice, where c-Src and other SFK expression levels were elevated to various degrees in the murine kidney and intestine [85]. This may partly explain, for instance, why SRMS knockout mice displayed no apparent changes in the phenotype [4]. Further characterization of SRMS knockout mice may reveal specific cellular aberrancies that may help determine the physiological significance of the kinase.

9. Summary

The non-receptor tyrosine kinase SRMS is a relatively understudied protein whose cellular role and function are currently understudied. Recent studies reveal SRMS' possible involvement in autophagy, cellular differentiation, BRK regulation, oncogenesis, and drug resistance. In addition, recent studies suggest that targeting SRMS or its substrates may be a selective therapeutic strategy for treating some cancers. Moreover, the unique subcellular localization of SRMS may play an essential role in dictating its cellular targets, proteome, and potential substrates, as well as its physiological functions in the cell. Although its nomenclature has much description, there is still little known about SRMS. Future studies to investigate the puncta pattern of localization and the characterization of its binding partners may hold the key to uncovering the enigma of this kinase.

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