Available online at www.scholarsresearchlibrary.com

Extended Abstract

Journal of Computational Methods in Molecular Design, 2021 **ISSN 2231-3176**
https://www.scholarsresearchlibrary.com/journals/journal-of-computational-methods-in-molecular-design/

SRMS, an unheralded Src-related kinase- Biochemistry and phosphoproteomics

K. Lukong, University of Saskatchewan
E-mail: kiven.lukong@usask.ca

SRMS (Src-related kinase lacking C-terminal regulatory tyrosine and N-terminal myristoylation sites) is a non-receptor tyrosine kinase that belongs to the BRK family kinases (BFKs) whose other two members include BRK (Breast tumor kinase or protein tyrosine kinase 6) and FRK (Fynrelated kinase). Like BRK, FRK and Src-family kinases, SRMS possesses the prototypical SH3 (Src-homology 3) and SH2 (Src-homology 2) domains involved in intra/inter-molecular interactions as well as a catalytic kinase domain. However, unlike BRK and Src family kinases, SRMS lacks a conserved C-terminal auto-regulatory tyrosine residue implicated in the regulation of enzymatic activity. Furthermore, unlike the BRK and Src family kinases whose roles have been well established in signal transduction, the cellular roles of SRMS are unknown. We performed the first biochemical characterization studies on SRMS and discovered that the 50 amino acid-long N-terminal sequence in the kinase plays a critical role in regulating the enzymatic activity and consequent substrate phosphorylation. We further applied global and shot-gun phosphoproteomic approaches to identify the candidate cellular substrates and signaling intermediates of SRMS which provided key insights into the cellular roles played by the kinase. Highlights of these studies will be discussed. SRMS (Src-related tyrosine kinase lacking C-terminal regulatory tyrosine and N-terminal myristoylation sites) belongs to a family of nonreceptor tyrosine kinases, which also includes breast tumour kinase and Fyn-related kinase. SRMS, similar to breast tumour kinase and Fyn-related kinase, harbours a Src homology 3 and Src homology 2, as well as a protein kinase domain. However, unlike breast tumour kinase and Fyn-related kinase, SRMS lacks a C-terminal regulatory tail but distinctively possesses an extended N-terminal region. Both breast tumour kinase and Fyn-related kinase play opposing roles in cell proliferation and signalling. SRMS, however, is an understudied member of this family. Although cloned in 1994, information on the biochemical, cellular and physiological roles of SRMS remains unreported. The present study is the first to explore the expression pattern of SRMS in breast cancers, its enzymatic activity and autoregulatory elements, and the characterization of docking protein 1 as its first bonafide substrate. We found that, similar to breast tumour kinase, SRMS is highly expressed in most breast cancers compared to normal mammary cell lines and tissues. We generated a series of SRMS point and deletion mutants and assessed enzymatic activity, subcellular localization and substrate recognition. We report for the first time that ectopically-expressed SRMS is constitutively active and that its N-terminal region regulates the enzymatic activity of the protein. Finally, we present evidence indicating that docking protein 1 is a direct substrate of SRMS. Our data demonstrate that, unlike members of the Src family, the enzymatic activity of SRMS is regulated by the intramolecular interactions involving the N-terminus of the enzyme and that docking protein 1 is a bona fide substrate of SRMS. SRMS (Src-related kinase lacking C-terminal regulatory tyrosine and N-terminal myristoylation sites), also known as PTK 70 (Protein tyrosine kinase 70), is a non-receptor tyrosine kinase that belongs to the BRK family of kinases (BFKs). To date less is known about the cellular role of SRMS primarily because of the unidentified substrates or signaling intermediates regulated by the kinase. In this study, we used phosphotyrosine antibody-based immunoaffinity purification in large-scale label-free quantitative phosphoproteomics to identify novel candidate substrates of SRMS. Our analyses led to the identification of 1258 tyrosine-phosphorylated peptides which mapped to 663 phosphoproteins, exclusively from SRMS-expressing cells. DOK1, a previously characterized SRMS substrate, was also identified in our analyses. Functional enrichment analyses revealed that the candidate SRMS substrates were enriched in various biological processes including protein ubiquitination, mitotic cell cycle, energy metabolism and RNA processing, as well as Wnt and TNF signaling. Analyses of the sequence surrounding the phospho-sites in these proteins revealed novel candidate SRMS consensus substrate motifs. We utilized customized high-throughput peptide arrays to validate a subset of the candidate SRMS substrates identified in our MS-based analyses. Finally, we independently validated Vimentin and Sam68, as bona fide SRMS substrates through in vitro and in vivo assays. Overall, our study identified a number of novel and biologically relevant SRMS candidate substrates, which suggests the involvement of the kinase in a vast array of unexplored cellular functions. The cellular proto-oncogene c-Src is a nonreceptor tyrosine kinase involved in cell growth and cytoskeletal regulation. Despite being dysregulated in a variety of human cancers, its precise functions are not fully understood. Identification of the substrates of c-Src remains a major challenge, because there is no simple way to directly stimulate its activity. Here we combine the chemical rescue of mutant c-Src and global quantitative phosphoproteomics to obtain the first high resolution snapshot of the range of tyrosine phosphorylation events that occur in the cell immediately after specific c-Src stimulation. After enrichment by anti-phosphotyrosine antibodies, we identified 29 potential novel c-Src substrate proteins. Tyrosine phosphopeptide mapping allowed the identification of 382 nonredundant tyrosine phosphopeptides on 213 phosphoproteins. Stable isotope labeling of amino acids in cell culture-based quantitation allowed the detection of 97 nonredundant tyrosine phosphopeptides whose level of phosphorylation is increased by c-Src. A large number of previously uncharacterized c-Src putative protein targets and phosphorylation sites are presented here, a majority of which play key roles in signaling and cytoskeletal networks, particularly in cell adhesion. Integrin signaling and focal adhesion kinase signaling pathway are two of the most altered pathways upon c-Src activation through chemical rescue. In this context, our study revealed the temporal connection between c-Src activation and the GTPase Rap1, known to stimulate integrin-dependent adhesion. Chemical rescue of c-Src provided a tool to dissect the spatiotemporal mechanism of activation of the Rap1 guanine exchange factor, C3G, one of the identified potential c-Src substrates that plays a role in focal adhesion signaling.

Bottom Note: *This work is partly presented at EuroScicon congress on Biochemistry, Molecular Biology & Allergy* October 11 - 12, 2018 Amsterdam, Netherlands