Cellular and Molecular Life Sciences

REVIEW

Role of SRC-like adaptor protein (SLAP) in immune and malignant cell signaling

Julhash U. Kazi[1,2,3] · **Nuzhat N. Kabir**[3] · **Lars Rönnstrand**[1,2]

Abstract SRC-like adaptor protein (SLAP) is an adaptor protein structurally similar to the SRC family protein kinases. Like SRC, SLAP contains an SH3 domain followed by an SH2 domain but the kinase domain has been replaced by a unique C-terminal region. SLAP is expressed in a variety of cell types. Current studies suggest that it regulates signaling of various cell surface receptors including the B cell receptor, the T cell receptor, cytokine receptors and receptor tyrosine kinases which are important regulator of immune and cancer cell signaling. SLAP targets receptors, or its associated components, by recruiting the ubiquitin machinery and thereby destabilizing signaling. SLAP directs receptors to ubiquitination-mediated degradation and controls receptors turnover as well as signaling. Thus, SLAP appears to be an important component in regulating signal transduction required for immune and malignant cells.

Keywords SLAP-2 · FLT3 · c-KIT · PDGFR · EPHA2 · EPOR · CSF1R · SYK

✉ Lars Rönnstrand
lars.ronnstrand@med.lu.se

1 Division of Translational Cancer Research, Lund University, Medicon Village 404:C3, 223 63 Lund, Sweden

2 Lund Stem Cell Center, Lund University, Lund, Sweden

3 Laboratory of Computational Biochemistry, KN Biomedical Research Institute, Barisal, Bangladesh

Introduction

A majority of cellular processes are controlled by extracellular factors, which bind to cell surface receptors and transduce signals to intracellular signaling molecules. Adaptor proteins play, by linking signaling molecules to the receptors, an important role in signal transduction. This class of proteins contains multiple functional domains and thus can create multi-protein complexes that facilitate signal transduction. Adaptor proteins can regulate cell surface receptor signaling by amplifying the signal or diminishing the signal. In the first case, it recruits signaling molecules that further transduce the signal through the downstream signaling cascades. In the other cases, it recruits negative regulators such as ubiquitin ligases or phosphatases that shut down the signal either by destabilizing the receptor and/or downstream signaling molecule or by de-phosphorylating the receptor, which leads to inactivation of the receptors. SRC-like adaptor protein (SLAP) is an adaptor protein that displays considerable structural homology with SRC [1]. *SLAP* is expressed in a variety of tissues and known to regulate the signaling of multiple receptors in different systems.

SLAP-deficient mice have been generated. Mice lacking *SLAP* appear to be healthy, and do not display any obvious physical abnormalities suggesting that SLAP is not essential for embryonic development [2]. However, in adult mice, SLAP negatively regulates differentiation of osteoclasts and proliferation of their precursors [3]. Furthermore, *SLAP*-deficient mice have higher TCR signaling in comparison to wild-type mice [4]. *SLAP2* knockout and *SLAP/SLAP2* double-knockout mice have also been described [5]. *SLAP2* knockout mice did not show any considerable effect on ZAP70 or TCR signaling. Furthermore, double-knockout mice displayed only the phenotype that *SLAP* knockout

mice show in TCR signaling suggesting that SLAP, but not SLAP2, is required for maintaining TCR signaling. Therefore, it is more likely that SLAP is involved in controlling normal cellular signaling and that loss of function results in aberrant activation of survival pathways.

The SLAP gene and protein

SLAP was initially identified in mice using a yeast two-hybrid screen as a 34 kDa protein [6]. In the same year, *SLAP* gene was mapped in humans to chromosome 8 (8q22.3) and in mice to chromosome 15 (15 D2) [7]. Both human and mouse *SLAP* genes contain seven exons and six introns [7–9]. SLAP is also known as SLA and SLA1. The closest homolog of SLAP, SLAP2 was described after identification of SLAP [10, 11]. The *SLAP2* gene is located on human chromosome 20q11.23 consisting of seven exons [12]. According to the Gene database (http://www.ncbi.nlm.nih.gov/gene/) the human *SLAP* gene encodes 5 different isoforms due to the alternative splicing (Fig. 1). The alternative splicing human *SLAP* gene displays differences in tissue abundance [9]. While the human *SLAP2* gene encodes a 261 amino acid protein, an alternative spliced isoform of 210 amino acids (SLAP-2-v) has also been described [12]. SLAP a, b and c isoforms have a short N-terminal uncharacterized region followed by an SH3 and an SH2 domain. All isoforms have a similar C-terminal uncharacterized region. Isoform d lacks a functional SH2 domain and isoform e lacks both SH3 and SH2 domains. The N-terminus of the b, c and d isoforms contains a PAAP motif, indicating the possibility of interaction with SH3 domain-containing proteins. Although the tissue specific roles of different splice variants have not yet been defined, it is likely that due to the difference in the arrangements of functional domains, different splice variants of SLAP play different roles in different tissues.

The expression of SLAP

SLAP is expressed in a variety of human and murine tissues (Table 1). Although data from Northern blotting suggest that spleen, lung, thymus and lymph nodes express relatively higher levels of *SLAP* mRNA [6, 9, 13], expression data from RNAseq and microarray analysis display discrepancies in lungs and other non-hematopoietic organs (Table 1). B cells and T cells from spleen, and thymocytes express moderate levels of *SLAP* mRNA [13]. Therefore, it has been suggested that SLAP may play an important role in the B cell and T cell lineages. *SLAP* expression has also been described in Jurkat T cells [14]. Differences in *SLAP* expression for the same tissue in the Table 1, for example, mouse heart, liver, skeletal muscle and kidney, are not surprising as the experimental settings were different in different studies. Therefore, mRNA levels depicted in Table 1 reflect the relative expression of *SLAP* within each experimental setting. Besides abundant

Fig. 1 Structure of SLAP. The SLAP gene is expressed as 5 transcript variants. Variants a, b and c contain intact SH2 and SH3 domains, while the d variant lacks functional SH2 domain and the e variant lacks both domains. The functional domains of SLAP are highly conserved between species including human, mouse, chicken and frog. The uncharacterized C-terminal region displays comparatively poor conservation. SLAP shares considerable sequence similarity with its homolog SLAP2. SLAP has a similar domain structure as SRC except for the presence of a functional kinase domain in SRC
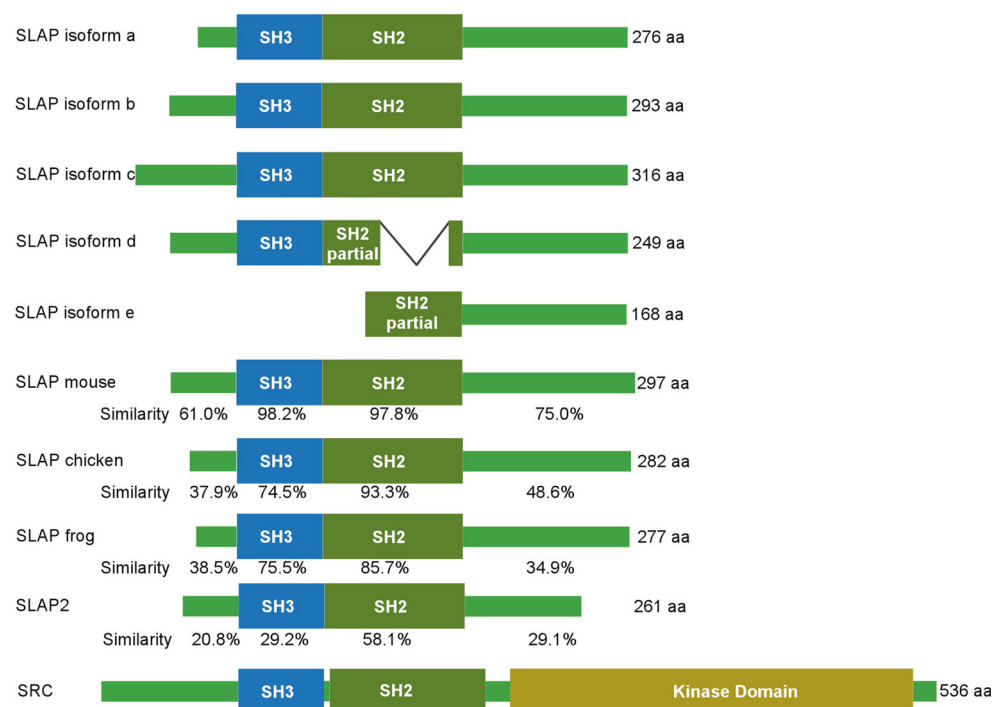
Table 1 *SLAP* mRNA expression in human and murine tissues using Northern blotting (NB) comparing with RNAseq and microarray data

Tissue/cell line	Species	NB	References	FPKM[a]	RPKM[b]	Expression[c]
Heart	Human	+	[9]	2	2.1	13
	Mouse	+	[6]			
		−	[13]			
Brain	Human	+	[9]	NF	3.7	NF
	Mouse	++	[6]			
		+	[13]			
Spleen	Mouse	++++	[6]	50	38.6	NF
		+++	[13]			
Lung	Human	+++	[9]	16	13.3	35
	Mouse	+++	[6]			
		+	[13]			
Liver	Human	+	[9]	5	1.3	13
	Mouse	+	[6]			
		−	[13]			
Skeletal muscle	Human	+	[9]	0	0.4	17
	Mouse	+	[6]			
		−	[13]			
Kidney	Mouse	+	[6]	3	2	8
		−	[13]			
	Human	+	[9]			
Testis	Mouse	−	[6]	2	0.5	10
		−	[13]			
Placenta	Human	+	[9]	8	NF	12
Pancreas	Human	+	[9]	3	0.9	9
Lymph nodes	Mouse	++	[13]	72	NF	91
Thymus	Mouse	++++	[13]	NF	NF	460

− absent, + lower, ++ moderate, +++ higher, ++++ very high, *NF* not found

[a] Data from the Human Protein Atlas

[b] Data from GTExPortal

[c] Data from BioGPS

expression of *SLAP* in B cells and T cells, several factors induce *SLAP* expression in hematopoietic cell lines. For example, all-trans retinoic acid (ATRA) induces *SLAP* expression in U937, HL60 and NB-4 cells [15]. Since, ATRA induces differentiation in these cell lines, it is possible that *SLAP* expression is required for the cell maturation process [15]. However, the role of SLAP in cell differentiation is still largely unknown. Like ATRA, dexamethasone exposure also markedly increases expression of *SLAP* in RBL-2H3 mast cells [16]. This regulation is mediated through the transcriptional activation of the glucocorticoid response element (GRE) [17] and thus it has been suggested that SLAP is a regulator of glucocorticoid-induced cell death [18].

SLAP2 expression overlaps in many cases with *SLAP* expression. For example *SLAP2* expression is maintained in thymocytes development in a similar fashion to *SLAP*

expression [5]. *SLAP2* is highly expressed in bone marrow macrophages, hematopoietic cells such as platelets, monocytes, T cells, B cells, spleen, leukocytes, lung and thymus [3, 10–12, 19, 20]. Therefore, it is more likely that SLAP and SLAP2 share similar functions in many cases.

SLAP in intracellular signaling

So far 18 different proteins have been described to associate with SLAP including cell surface receptors, membrane-bound non-receptor proteins and cytosolic proteins, although in several cases it remains unknown whether the interaction was direct between SLAP and the partner protein (Table 2). Therefore, it has been suggested that SLAP regulates multiple signaling pathways by interacting with various proteins. Since SLAP remains

Table 2 SLAP-interacting proteins

Name	Method and domain involved	Functions	References
CBL	In vitro GST pull-down, co-transfection in COS7 cells, phosphorylation-independent interaction, associates through C-terminal hydrophobic region	positive regulation of NFAT-AP1 activity	[14]
	Co-immunoprecipitation in transfected Jurkat T cells	Negative regulation	[33]
	Co-immunoprecipitation, independent of PDGF stimulation	Negative regulation	[45]
CD247/CD3ζ/TCRζ	Co-immunoprecipitation, TCR activation, indirect interaction, associates through SH2 domain	Positive regulation of NFAT-AP1 activity	[14]
	Co-immunoprecipitation in Jurkat T, associates through SH2 domain	Down-regulates TCRζ	[32]
CD79A/Igα	Co-immunoprecipitation, TCR activation, associates through SH2 domain	Negative regulation	[29]
EPHA2/ECK	In vitro GST pull-down, ligand stimulation-dependent associates through SH2 domain	Unknown	[6]
		Tumor suppressor functions	[44]
FLT3	Co-immunoprecipitation in COS1 cells, ligand stimulation	Positive regulation of AKT, ERK, p38 signaling	[22]
KIT	Co-immunoprecipitation in transfected COS1 cells, endogenous in P815 cells, ligand stimulation, associates through SH2 domain	Negative regulation	[23]
PDGFRB	Overexpression in COS7 cells, endogenous in NIH3T3 cell line, ligand stimulation-dependent, associates through SH2 domain	SLAP tyrosine phosphorylation, negatively regulates mitogenesis	[1]
	Binding was not been determined	Strongly inhibits PDGF response	[21]
	Co-immunoprecipitation, ligand dependent	Negative regulation	[45]
SYK	Co-immunoprecipitation in transfected COS7 cells, associates through SH2 domain	SLAP dimerization is independent of SYK or LCK activity, required for positive regulation of NFAT-AP1 activity	[14]
ZAP70			
LAT/pp36			
SLAP	Forms homodimers through C-terminal region		
VAV1	GST pull-down and immunoprecipitation in SLAP-transfected Jurkat TAg cells	Inhibited TCR-induced NFAT activation	[13]
LCK			
SLP76			
EPOR	Co-immunoprecipitation in overexpressed 293T cells and EI-11 erythroblasts	Negative regulation	[24]
UB4EA	Through the SH3 domain	Tumor suppressor functions	[44]
GM-CSFRα	GST pull-down, both SH3 and SH2 domains are involved	Regulates monocytic dendritic cell maturation	[41]
CSF1R	GST pull-down, CSF1-dependent	Unknown	[47]

myristoylated in G2 and localizes to the cell membrane [21], it is expected that SLAP associates with membrane-bound proteins. Several receptor tyrosine kinases including EPHA2 [6], FLT3 [22], KIT [23], PDGFR [1] and the cytokine receptor EPOR [24] interact with SLAP in a tyrosine phosphorylation-dependent manner. Receptor tyrosine kinases transduce signal partially through adaptor proteins, which recruits downstream signaling components or negative regulators [25–28]. Thus, association of SLAP with receptor tyrosine kinases may have a role in receptor downstream signaling. SLAP also interacts with multiple intracellular tyrosine kinases, such as SYK [14], LCK [21] and ZAP70 [14] which are involved in T cell receptor (TCR) or B cell receptor (BCR) signaling. SLAP interacts with the TCR and BCR component CD3ζ [14], Igα [29] and LAT [14]. In contrast, SLAP2 interacts with ZAP70 and CD3ζ but not with LAT [11, 19]. There is also evidence that both SLAP and SLAP2 associate with the ubiquitin E3 ligase CBL [11, 14, 19]. Furthermore, SLAP associates with the guanine nucleotide exchange factor VAV1 [13] and SH2 domain-containing leukocyte protein SLP76 [13]. Most of the SLAP-binding proteins are

important regulators of T cell and B cell signaling suggesting that SLAP is involved in the regulation of normal immunity.

SLAP-mediated regulation of TCR signaling

The T cell receptor (TCR) activation is a critical event for T cell development in the thymus. TCR activation is also required for initiating immune responses in mature T cells. Activation of the TCR is initiated by SRC family kinases (such as LCK) resulting in activation of multiple protein tyrosine kinases which in turn phosphorylate various substrates and thereby initiating transcriptional activation of pro-survival genes [30]. Our current understanding suggests that SLAP plays important roles in the control of TCR signaling by regulating at least three different signaling pathways (Fig. 2). SLAP associates with multiple TCR signaling components including CBL, ZAP70, SYK, LAT, CD3ζ, SLP76 and VAV1 upon TCR activation in Jurkat cells [13, 14]. The SH2 domain of SLAP is involved in interaction with those tyrosine-phosphorylated proteins, except CBL that associates through the C-terminal hydrophobic region (Table 2). This probably allows SLAP to recruit the ubiquitin machinery to the signaling proteins
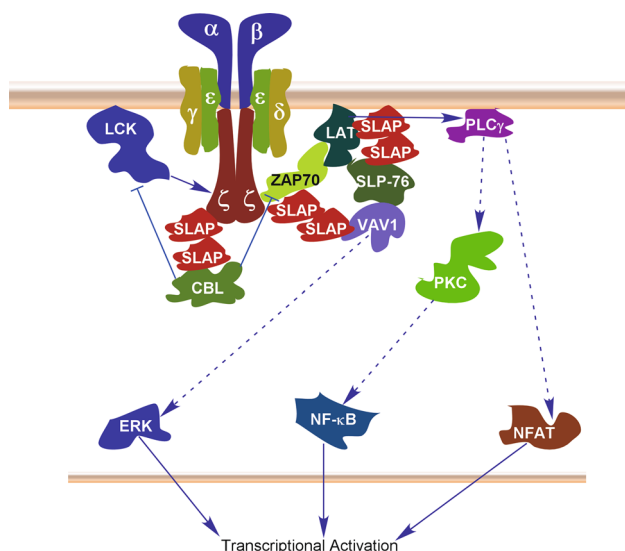

Fig. 2 SLAP in regulation of TCR signaling. Activation of the TCR results in activation of ERK1/2, NF-κB and NFAT signaling through different mechanisms. Activation of TCR signaling initiates tyrosine phosphorylation mediated by tyrosine kinases such as LCK. Tyrosine phosphorylation creates docking site for SLAP that in turn recruits the ubiquitin ligase CBL. SLAP associates with TCRζ, ZAP70 and LAT through its SH2 domain, while CBL associates with SLAP through its C-terminal hydrophobic region. The C-terminal region is also involved in the dimerization of SLAP. Recruitment of CBL directs TCR components to proteasomal degradation resulting in negative regulation of all three pathways

which subsequently leads to their degradation. Such regulation has been reported where SLAP binds with CD3ζ-chain of TCR and directs it for proteasomal degradation [31]. In addition, cells lacking SLAP express higher levels of CD3ζ which further suggests that SLAP is required to maintain basal CD3ζ levels [32]. This regulation is mediated through the recruitment of the ubiquitin ligase CBL to SLAP through LCK-dependent phosphorylation [33]. SLAP dimerizes through its C-terminal region which is also required for SLAP-mediated down-regulation of AP1-activity in T cells [14]. This dimerization property allows SLAP to create multi-protein complexes.

LAT is an upstream activator of PLCγ which in turn activates NFAT and AP-1 in a calcium- and protein kinase C (PKC)-dependent manner. Overexpression of SLAP inhibits the activity of NFAT and AP-1 which can be restored by treatment with ionomycin and phorbol myristate acetate (PMA) [13]. Ionomycin boosts the cellular calcium ion concentration and PMA is a well-known activator of the PKC family proteins [34, 35]. Thus, SLAP controls LAT signaling by deregulating its activity through association. It is also evident that SLAP deficiency leads to up-regulation of TCRβ, CD3, CD4, CD5, CD8 and CD69 levels [2] suggesting that SLAP destabilizes multiple proteins in T cells. Furthermore, SLAP deficiency rescues T cell development in ZAP70 null mice [2]. Therefore, it is suggested that SLAP is an important adaptor protein which is required for the control of normal TCR signaling.

SLAP-mediated regulations of BCR signaling

B cell receptor (BCR) signaling is essential for the development and function of B cell. BCR signaling contributes to the antigen-triggered differentiation of memory B cells and antibody-secreting plasma cells [36]. Activation of BCR induces tyrosine phosphorylation on Ig chains, thereby creating docking sites for the SH2 domain-containing tyrosine kinase SYK. These events in turn activate AKT, NF-κB and NFAT signaling leading to transcriptional activation of effector genes. SLAP expression controls the levels of B cell receptor expression and it has been demonstrated that SLAP expression is required to maintain normal levels of BCR expression [37]. SLAP also controls signaling through the BCR complex and B cell development. SLAP has been shown to be directly associated with tyrosine-phosphorylated components of the BCR complex and down-regulates the surface levels and total BCR levels in a CBL-dependent manner [29]. The C-terminal region of SLAP is required for CBL-mediated down-regulation of BCR, suggesting that SLAP recruits the ubiquitin machinery to the signaling proteins through its multiple functional units. SLAP deficiency leads to a

decreased number of autoreactive B cells, which is maintained by an inefficient receptor regulation [38]. Since SLAP is capable of binding to SYK as well [14], it is likely that SLAP binds to multiple components of the BCR complex and negatively regulates signaling by directing proteins for CBL-mediated degradation (Fig. 3). SLAP associates with tyrosine-phosphorylated SYK and BCR Igα through its SH2 domain, and destabilizes these two intermediate components of BCR signaling by linking to CBL. Therefore, the SLAP activity is important for maintaining normal BCR signaling.

SLAP-mediated regulations of cytokine signaling

EPOR

The cytokine erythropoietin (EPO) and its receptor (EPOR) are important regulators of erythroid progenitor cells. Activation of EPOR blocks apoptosis, and promotes terminal differentiation and proliferation of erythroid progenitor cells [39]. These processes are mediated through activation of several signaling cascades including the PI3-K/AKT, JAK2/ERK1/2 and STAT5 pathways [40]. SLAP counteracts EPO-induced anti-apoptotic properties by inhibiting EPO-induced STAT5 activation in erythroblasts [24]. SLAP directly associates with EPO receptor, EPOR and

thus controls EPO signaling probably through CBL-dependent degradation of the receptor, as CBL [33] has been shown to be associated with SLAP (Fig. 4). SLAP probably inhibits EPO-induced AKT, STAT5 and ERK1/2 activation by CBL-mediated degradation of EPOR; however, it yet has to be determined whether SLAP downregulates EPOR signaling through CBL-dependent degradation.

GM-CSFR

The cytokine receptor GM-CSFR plays an important role in maturation of dendritic cells. Bone marrow-derived dendritic cells derived from mice deficient of SLAP and its close homolog SLAP2 display increased GM-CSFRβ stability resulting in elevated activation of JAK2, AKT and ERK1/2 signaling in response to GM-CSF stimulation [41]. Although SLAP and SLAP2 regulated stability of GM-CSFRβ subunit, neither SLAP nor SLAP2 associated with GM-CSFRβ. However, only SLAP but not SLAP2 interacted with GM-CSFRα through SH2 and SH3 domains. These dendritic cells display poor response to lipopolysaccharide in IL-12 and TNFα production and therefore fail to stimulate T cells. SLAP function is required in maintaining baseline GM-CSFR signaling which is important for the generation of functionally mature monocytic dendritic cells [41].
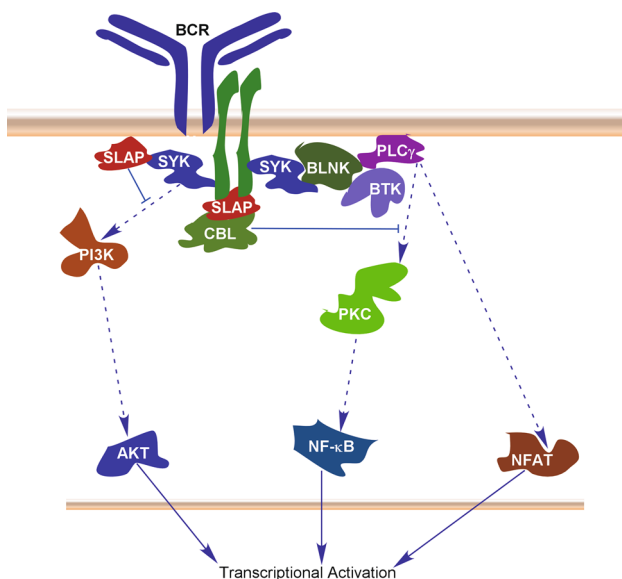

Fig. 3 SLAP in regulation of BCR signaling. Activation of BCR results in the activation of AKT, NF-κB and NFAT signaling through different mechanisms. SLAP binds to the BCR components Igα and SYK through its SH2 domain and then recruits CBL resulting in negative regulation of all three pathways. SLAP destabilizes SYK and BCR Igα which are intermediate signaling components of BCR signaling
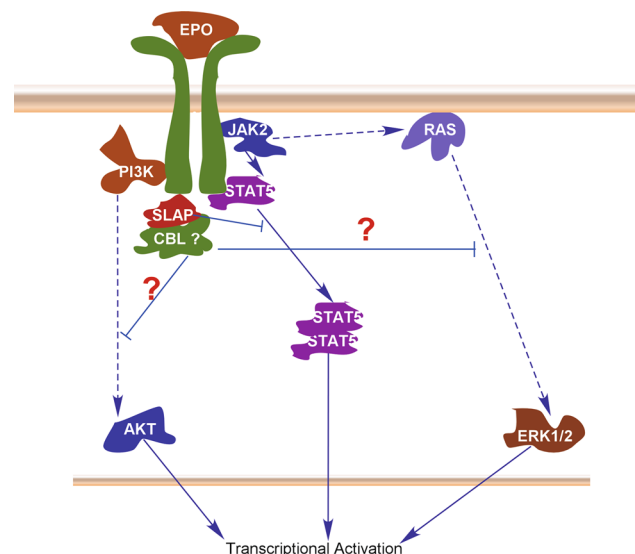

Fig. 4 SLAP in regulation of EPOR signaling. Activation of the EPOR results in the activation of AKT, STAT5 and ERK1/2 signaling. SLAP counteracts EPOR signaling probably by recruiting CBL to the EPOR. CBL-dependent degradation of EPOR results in weaker activation of AKT, STAT5 and ERK1/2. Therefore, SLAP negatively regulates all three pathways

SLAP-mediated regulation of RTK signaling

Receptor tyrosine kinases (RTKs) are cell surface receptors that activate diverse signaling cascades upon ligand stimulation. RTK signaling is tightly controlled by regulatory proteins [42]. In general, activation of RTKs initiates various signaling cascades through activation of AKT, p38, ERK1/2 and in some cases STAT5. Like other proteins SLAP associates with RTKs and induces ubiquitination-dependent degradation. Association of SLAP with RTKs is mediated by the SLAP SH2 domain and phosphotyrosine residue on the RTK. Therefore, SLAP predominantly acts as a negative regulator of RTK signaling. However, it is currently evident that SLAP also cooperates with RTK to potentiate RTK signaling (Fig. 5).

EPHA2

The adaptor protein SLAP was initially identified as an EPHA2-(also known as ECK) binding protein [6]. EPHA2 is a member of EPH receptors family of the RTKs that binds to membrane-bound ligands denoted ephrins. SLAP binding to EPHA2 leads to recruitment of the N-methyl-D-aspartate (NMDA) receptor as well, and down-regulates this receptor as well as downstream signaling [43]. Furthermore, a SLAP-mediated tumor suppressor functions in

Fig. 5 SLAP in regulation of RTK signaling. Activation of RTK signaling results in the activation of AKT, p38, STAT5 and ERK1/2 signaling through different mechanisms. SLAP binds to RTKs and display different functions. SLAP associates with phospho-tyrosine residues of the activated receptor through its SH2 domain. While SLAP association increases receptor ubiquitination and accelerates the degradation, it both negatively and positively regulates receptor signaling depending on the receptor type

colorectal cancer have been found to be mediated through destabilization of the EPHA2 receptor [44]. EPHA2 is a well-known substrate of SRC and by destabilizing EPHA2, SLAP controls SRC-EPHA2-AKT signaling. SLAP recruits the ubiquitin ligase UBE4A to the EPHA2 through the SLAP-SH3 domain and the SLAP-SH2 domain interacts with EPHA2 through pY594 which is a SRC phosphorylation site. Therefore, SLAP function is required for controlling EPHA2 receptor activity which is partially mediated through destabilization of the receptor by ubiquitination.

PDGFR

SLAP negatively regulates PDGF signaling by associating with PDGFRB in response to PDGF stimulation in NIH3T3 cells [1]. The binding sites of SLAP were mapped in PDGFRB. The SLAP-binding sites are completely overlapping with the SRC-binding sites (Y579 and Y581) in PDGFRB, which are located in the juxtamembrane domain. Overexpression of SLAP in NIH3T3 cells inhibits PDGF-induced DNA synthesis and the mitogenic response to both serum and PDGF. An intact SH2 domain was essential for this effect but the SH3 domain was dispensable, suggesting that inhibition is partially due to the competition for binding of SRC to tyrosine-phosphorylated residues in PDGFRB [1, 21]. Later studies also demonstrated that SLAP controls PDGFR signaling by competing with SRC, but it recruits CBL to the receptor as well [45]. Thus, SLAP controls PDGF signaling by two different mechanisms: by limiting access of SRC and by destabilizing the receptor through CBL-mediated ubiquitination.

CSF1R

Colony-stimulating factor 1 receptor (CSF1R) regulates proliferation, differentiation and function of macrophages when stimulated with its ligand colony-stimulating factor 1 (CSF1). Activation of CSF1R results in activation of multiple downstream signaling cascades including PI3 K/AKT and RAS/ERK pathways through SH2 domain-containing adaptors or kinases. Since *SLAP* and *SLAP2* genes are constitutively expressed in bone marrow macrophages [3, 46], it is expected that these genes will contribute to the regulation of CSF1R signaling. SLAP2 remains constitutively associated with the CSF1R independent of ligand stimulation [46, 47]. However, how SLAP2 associates with the CSF1R remains debated. While overexpression studies in HEK293 cells suggest that SLAP2 associates with the CSF1R independent of both the N-terminal region, the SH2 domain, the SH3 domain and the C-terminal region [46], another study suggests that association was dependent on the SLAP2-SH2 domain but not on the C-terminal region,
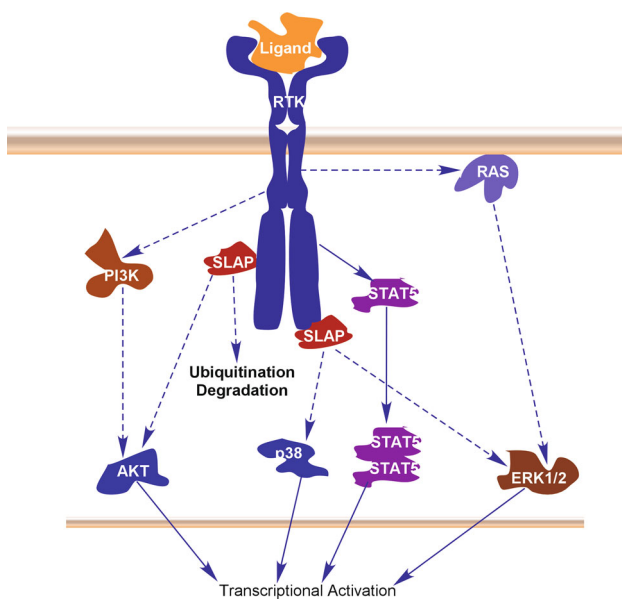
as a point mutation in the phosphotyrosine-binding pocket of the SH2 domain completely abolished association in GST pull-down assay [47]. Since SLAP2 constitutively associates with CSF1R, it would be more likely that SLAP2 uses other binding sites than the SH2 domain. However, association with SLAP was completely dependent on CSF1 stimulation [47]. Therefore, it is suggested that the mechanism of interaction between CSF1R and SLAP or between CSF1R and SLAP2 is different. Interaction between SLAP2 and CSF1R results in down-regulation of the CSF1R in response to ligand stimulation of the CSF1R. Furthermore, SLAP2 has been shown to be associated with CBL in primary bone marrow-derived macrophages and expression of a SLAP2 SH2 domain mutant stabilizes CSF1R [10, 47] suggesting that SLAP2 down-regulates CSF1R through CBL-dependent degradation.

FLT3

FLT3 and its ligand (FL) play vital roles in the immune system and also in certain types of cancers. SLAP associates with FLT3 in response to FL stimulation [22]. Although association with SLAP induces accelerated ubiquitination and degradation of FLT3, it also enhances receptor signaling by an unknown mechanism [22]. Probably, the SLAP SH3 domain is involved in the amplification of signals from FLT3. Since SLAP associates with FLT3 through its SH2 domain, the SH3 domain is available to interact with SH3 domain-binding proteins resulting in activation of downstream signaling cascades.

KIT

Another receptor tyrosine kinase KIT and its oncogenic mutants are involved in several cancers including mastocytoma, gastrointestinal stromal tumor (GIST), melanoma and certain type of acute myeloid leukemia. Both wild-type and oncogenic mutants of KIT associate with SLAP. While the interaction between wild-type KIT and SLAP is dependent of stem cell factor (SCF) stimulation, oncogenic KIT-D816V mutant displays constitutive binding [23]. SLAP can block KIT signaling by inducing ubiquitination followed by degradation, but it was unable to block oncogenic KIT-D816V-mediated mitogenic signaling [23]. KIT-D816V can selectively phosphorylate SLAP on three tyrosine residues of which two are located in the C-terminal and involved in dimerization. Probably, KIT-D816V-mediated tyrosine phosphorylation blocks dimerization of SLAP and subsequently makes SLAP inactive, which can be restored by a triple (Y-F) SLAP mutant [23].

SLAP regulates SYK signaling

Besides cell surface receptor tyrosine kinases, SLAP associates with the intracellular tyrosine kinase SYK and partially blocks SYK tyrosine phosphorylation as well as phosphorylation of PLCγ2 and ERK suggesting that SLAP acts as a negative regulator of SYK signaling [16, 48]. Therefore, the role of SLAP in receptor tyrosine kinase signaling is fairly complicated. SLAP can both inhibit and activate signaling downstream of receptors.

Conclusions

Current studies suggest that SLAP mainly acts as an adaptor to recruit the ubiquitination machinery resulting in negative regulation of receptor signaling. Although a majority of studies support this idea, there is sufficient evidence that SLAP can contribute to positive regulation of receptor signaling. The presence of multiple domains, proline-rich motif and the ability to dimerize leads to a multitude of levels of regulation of receptor signaling. Our current knowledge is still mainly limited to the regulation of mitogenesis by SLAP and future studies should define the role of SLAP in the regulation of other cellular processes such as metabolism, cell cycle regulation, intracellular trafficking, etc. The role of SLAP has been defined in the regulation of limited number of receptors, and its expression in a variety of tissues suggests that SLAP might be important in the regulation of diverse signaling pathways. Therefore, identification of novel SLAP-binding proteins will enhance our knowledge about the mechanisms of cellular regulatory mechanisms. Although SLAP is abundantly expressed in the healthy intestinal epithelium, it is strongly down-regulated in 50 % of colorectal cancer patients. Silencing of SLAP enhances tumorigenicity and invasiveness in colorectal cancer cell lines, while overexpression of SLAP suppresses these transforming properties. Regulation of receptor tyrosine kinases frequently mutated or involved in cancers also provide evidence that SLAP is an important regulator for maintaining normal cellular functions.

References

1. Roche S, Alonso G, Kazlauskas A, Dixit VM, Courtneidge SA, Pandey A (1998) Src-like adaptor protein (Slap) is a negative regulator of mitogenesis. Curr Biol 8:975–978. doi:10.1016/S0960-9822(98)70400-2

2. Sosinowski T, Killeen N, Weiss A (2001) The Src-like adaptor protein downregulates the T cell receptor on $CD4^+$ $CD8^+$ thymocytes and regulates positive selection. Immunity 15:457–466. doi:10.1016/S1074-7613(01)00195-9

3. Kim HJ, Zou W, Ito Y et al (2010) Src-like adaptor protein regulates osteoclast generation and survival. J Cell Biochem 110:201–209. doi:10.1002/jcb.22527

4. Friend SF, Peterson LK, Kedl RM, Dragone LL (2013) SLAP deficiency increases TCR avidity leading to altered repertoire and negative selection of cognate antigen-specific $CD8^+$ T cells. Immunol Res 55:116–124. doi:10.1007/s12026-012-8354-y

5. Dragone LL, Shaw LA, Myers MD, Weiss A (2009) SLAP, a regulator of immunoreceptor ubiquitination, signaling, and trafficking. Immunol Rev 232:218–228. doi:10.1111/j.1600-065X.2009.00827.x

6. Pandey A, Duan H, Dixit VM (1995) Characterization of a novel Src-like adapter protein that associates with the Eck receptor tyrosine kinase. J Biol Chem 270:19201–19204

7. Angrist M, Wells DE, Chakravarti A, Pandey A (1995) Chromosomal localization of the mouse Src-like adapter protein (Slap) gene and its putative human homolog SLA. Genomics 30:623–625. doi:10.1006/geno.1995.1289

8. Kratchmarova I, Sosinowski T, Weiss A, Witter K, Vincenz C, Pandey A (2001) Characterization of promoter region and genomic structure of the murine and human genes encoding Src like adapter protein. Gene 262:267–273. doi:10.1016/S0378-1119(00)00516-3

9. Meijerink PH, Yanakiev P, Zorn I et al (1998) The gene for the human Src-like adaptor protein (hSLAP) is located within the 64-kb intron of the thyroglobulin gene. Eur J Biochem 254:297–303. doi:10.1046/j.1432-1327.1998.2540297.x

10. Holland SJ, Liao XC, Mendenhall MK et al (2001) Functional cloning of Src-like adapter protein-2 (SLAP-2), a novel inhibitor of antigen receptor signaling. J Exp Med 194:1263–1276

11. Pandey A, Ibarrola N, Kratchmarova I et al (2002) A novel Src homology 2 domain-containing molecule, Src-like adapter protein-2 (SLAP-2), which negatively regulates T cell receptor signaling. J Biol Chem 277:19131–19138. doi:10.1074/jbc.M110318200

12. Loreto MP, McGlade CJ (2003) Cloning and characterization of human Src-like adaptor protein 2 and a novel splice isoform, SLAP-2-v. Oncogene 22:266–273. doi:10.1038/sj.onc.1206114

13. Sosinowski T, Pandey A, Dixit VM, Weiss A (2000) Src-like adaptor protein (SLAP) is a negative regulator of T cell receptor signaling. J Exp Med 191:463–474

14. Tang J, Sawasdikosol S, Chang JH, Burakoff SJ (1999) SLAP, a dimeric adapter protein, plays a functional role in T cell receptor signaling. Proc Natl Acad Sci USA 96:9775–9780. doi:10.1073/pnas.96.17.9775

15. Ohtsuki T, Hatake K, Ikeda M et al (1997) Expression of Src-like adapter protein mRNA is induced by all-trans retinoic acid. Biochem Biophys Res Commun 230:81–84. doi:10.1006/bbrc.1996.5887

16. Hiragun T, Peng Z, Beaven MA (2006) Cutting edge: dexamethasone negatively regulates Syk in mast cells by up-regulating SRC-like adaptor protein. J Immunol 177:2047–2050. doi:10.4049/jimmunol.177.4.2047

17. Park SK, Beaven MA (2009) Mechanism of upregulation of the inhibitory regulator, src-like adaptor protein (SLAP), by glucocorticoids in mast cells. Mol Immunol 46:492–497. doi:10.1016/j.molimm.2008.10.011

18. Mansha M, Carlet M, Ploner C et al (2010) Functional analyses of Src-like adaptor (SLA), a glucocorticoid-regulated gene in acute lymphoblastic leukemia. Leukoc Res 34:529–534. doi:10.1016/j.leukres.2009.06.029

19. Loreto MP, Berry DM, McGlade CJ (2002) Functional cooperation between c-Cbl and Src-like adaptor protein 2 in the negative regulation of T-cell receptor signaling. Mol Cell Biol 22:4241–4255

20. Sugihara S, Katsutani S, Deckmyn H, Fujimura K, Kimura A (2010) Roles of Src-like adaptor protein 2 (SLAP-2) in GPVI-mediated platelet activation SLAP-2 and GPVI signaling. Thromb Res 126:e276–e285. doi:10.1016/j.thromres.2010.07.010

21. Manes G, Bello P, Roche S (2000) Slap negatively regulates Src mitogenic function but does not revert Src-induced cell morphology changes. Mol Cell Biol 20:3396–3406. doi:10.1128/MCB.20.10.3396-3406.2000

22. Kazi JU, Rönnstrand L (2012) Src-Like adaptor protein (SLAP) binds to the receptor tyrosine kinase Flt3 and modulates receptor stability and downstream signaling. PLoS ONE 7:e53509. doi:10.1371/journal.pone.0053509

23. Kazi JU, Agarwal S, Sun J, Bracco E, Rönnstrand L (2014) Src-like-adaptor protein (SLAP) differentially regulates normal and oncogenic c-Kit signaling. J Cell Sci 127:653–662. doi:10.1242/jcs.140590

24. Lebigot I, Gardellin P, Lefebvre L, Beug H, Ghysdael J, Quang CT (2003) Up-regulation of SLAP in FLI-1-transformed erythroblasts interferes with EpoR signaling. Blood 102:4555–4562. doi:10.1182/blood-2003-06-2077

25. Kazi JU, Kabir NN, Flores-Morales A, Rönnstrand L (2014) SOCS proteins in regulation of receptor tyrosine kinase signaling. Cell Mol Life Sci 71:3297–3310. doi:10.1007/s00018-014-1619-y

26. Kabir NN, Sun J, Rönnstrand L, Kazi JU (2014) SOCS6 is a selective suppressor of receptor tyrosine kinase signaling. Tumour Biol. doi:10.1007/s13277-014-2542-4

27. Kabir NN, Kazi JU (2014) Grb10 is a dual regulator of receptor tyrosine kinase signaling. Mol Biol Rep 41:1985–1992. doi:10.1007/s11033-014-3046-4

28. Lennartsson J, Rönnstrand L (2012) Stem cell factor receptor/c-Kit: from basic science to clinical implications. Physiol Rev 92:1619–1649. doi:10.1152/physrev.00046.2011

29. Dragone LL, Myers MD, White C et al (2006) Src-like adaptor protein (SLAP) regulates B cell receptor levels in a c-Cbl-dependent manner. Proc Natl Acad Sci USA 103:18202–18207. doi:10.1073/pnas.0608965103

30. Fu G, Rybakin V, Brzostek J, Paster W, Acuto O, Gascoigne NR (2014) Fine-tuning T cell receptor signaling to control T cell development. Trends Immunol 35:311–318. doi:10.1016/j.it.2014.05.003

31. Ersek B, Molnar V, Balogh A et al (2012) CD3zeta-chain expression of human T lymphocytes is regulated by TNF via Src-like adaptor protein-dependent proteasomal degradation. J Immunol 189:1602–1610. doi:10.4049/jimmunol.1102365

32. Myers MD, Dragone LL, Weiss A (2005) Src-like adaptor protein down-regulates T cell receptor (TCR)-CD3 expression by targeting TCRzeta for degradation. J Cell Biol 170:285–294. doi:10.1083/jcb.200501164

33. Myers MD, Sosinowski T, Dragone LL et al (2006) Src-like adaptor protein regulates TCR expression on thymocytes by linking the ubiquitin ligase c-Cbl to the TCR complex. Nat Immunol 7:57–66. doi:10.1038/ni1291

34. Kazi JU, Kabir NN, Rönnstrand L (2013) Protein kinase C (PKC) as a drug target in chronic lymphocytic leukemia. Med Oncol 30:757. doi:10.1007/s12032-013-0757-7

35. Kazi JU (2011) The mechanism of protein kinase C regulation. Front Biol 6:328–336. doi:10.1007/s11515-011-1070-5

36. Kurosaki T (2011) Regulation of BCR signaling. Mol Immunol 48:1287–1291. doi:10.1016/j.molimm.2010.12.007

37. Dragone LL, Myers MD, White C, Sosinowski T, Weiss A (2006) SRC-like adaptor protein regulates B cell development and function. J Immunol 176:335–345. doi:10.4049/jimmunol.176.1.335

38. Peterson LK, Pennington LF, Shaw LA et al (2014) SLAP deficiency decreases dsDNA autoantibody production. Clin Immunol 150:201–209. doi:10.1016/j.clim.2013.12.007

39. Kagaya Y, Asaumi Y, Wang W et al (2012) Current perspectives on protective roles of erythropoietin in cardiovascular system: erythropoietin receptor as a novel therapeutic target. Tohoku J Exp Med 227:83–91. doi:10.1620/tjem.227.83

40. Munugalavadla V, Kapur R (2005) Role of c-Kit and erythropoietin receptor in erythropoiesis. Crit Rev Oncol Hematol 54:63–75. doi:10.1016/j.critrevonc.2004.11.005

41. Liontos LM, Dissanayake D, Ohashi PS, Weiss A, Dragone LL, McGlade CJ (2011) The Src-like adaptor protein regulates GM-CSFR signaling and monocytic dendritic cell maturation. J Immunol 186:1923–1933. doi:10.4049/jimmunol.0903292

42. Marshall CJ (1995) Specificity of receptor tyrosine kinase signaling: transient versus sustained extracellular signal-regulated kinase activation. Cell 80:179–185. doi:10.1016/0092-8674(95)90401-8

43. Semerdjieva S, Abdul-Razak HH, Salim SS et al (2013) Activation of EphA receptors mediates the recruitment of the adaptor protein Slap, contributing to the downregulation of N-methyl-D-aspartate receptors. Mol Cell Biol 33:1442–1455. doi:10.1128/MCB.01618-12

44. Naudin C, Sirvent A, Leroy C et al (2014) SLAP displays tumour suppressor functions in colorectal cancer via destabilization of the SRC substrate EPHA2. Nat Commun 5:3159. doi:10.1038/ncomms4159

45. Sirvent A, Leroy C, Boureux A, Simon V, Roche S (2008) The Src-like adaptor protein regulates PDGF-induced actin dorsal ruffles in a c-Cbl-dependent manner. Oncogene 27:3494–3500. doi:10.1038/sj.onc.1211011

46. Manes GA, Masendycz P, Nguyen T et al (2006) A potential role for the Src-like adapter protein SLAP-2 in signaling by the colony stimulating factor-1 receptor. FEBS J 273:1791–1804. doi:10.1111/j.1742-4658.2006.05199.x

47. Pakuts B, Debonneville C, Liontos LM, Loreto MP, McGlade CJ (2007) The Src-like adaptor protein 2 regulates colony-stimulating factor-1 receptor signaling and down-regulation. J Biol Chem 282:17953–17963. doi:10.1074/jbc.M701182200

48. Park SK, Qiao H, Beaven MA (2009) Src-like adaptor protein (SLAP) is upregulated in antigen-stimulated mast cells and acts as a negative regulator. Mol Immunol 46:2133–2139. doi:10.1016/j.molimm.2009.03.013