Cell Adhesion & Migration 7:1, 1–10; January/February 2013; © 2013 Landes Bioscience

Ste20-like kinase SLK, at the crossroads
A matter of life and death

Khalid N. Al-Zahrani,[1,2,†] Kyla D. Baron[1,2,†] and Luc A. Sabourin[1,2,3,*]

[1]Ottawa Hospital Research Institute; Cancer Therapeutics; Ottawa, ON Canada; [2]Department of Cellular and Molecular Medicine; University of Ottawa; Ottawa, ON Canada; [3]Department of Medicine; University of Ottawa; Ottawa, ON Canada

[†]These authors contributed equally to this work.

Keywords: Ste20-like kinase, cancer and metastasis, development, cell migration, cell cycle, apoptosis, focal adhesion turnover, cytoskeletal dynamics, HER2/Neu/ErbB2 signaling, FAK/src signaling

Abbreviations: AT_2R, angiotensin II type 2 receptor; ASK1, apoptosis signal-regulating kinase-1; ATH, AT1-46 homology; CHK2, checkpoint kinase-2; DAPK3, death-associated protein kinase-3; ER, endoplasmic reticulum; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; EMT, epithelial-to-mesenchymal transition; FA, focal adhesion; FAK, focal adhesion kinase; GCKs, germinal center kinases; JNK1, c-Jun N-terminal kinase-1; Ldb, LIM domain binding protein; LOK, lymphocyte oriented kinase; MST1, mammalian sterile twenty 1; MKK, MAPK kinase; MEK1, MAPK kinase 1; MAPK, mitogen-activated protein kinase; NLS, nuclear localization signal; PAK, p21-activated kinase; PH-PAK, Pleckstrin-homology domain-containing PAK; Plk1, polo-like kinase homolog 1; RTK, receptor tyrosine kinase; SLK, Ste20-like kinase; TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling; TNFα, tumor necrosis factor α; xPlkk1, Xenopus polo-like kinase kinase-1

Reorganization of the cytoskeleton is necessary for apoptosis, proliferation, migration, development and tissue repair. However, it is well established that mutations or overexpression of key regulators contribute to the phenotype and progression of several pathologies such as cancer. For instance, c-src mutations and the overexpression of FAK have been implicated in the invasive and metastatic process, suggesting that components of the motility system may represent a new class of therapeutic targets. Over the last several years, we and others have established distinct roles for the Ste20-like kinase SLK, encompassing apoptosis, growth, motility and development. Here, we review the SLK field from its initial cloning to the most recent findings from our laboratory. We summarize the various roles of SLK and the biochemical mechanisms that regulate its activity. These various findings reveal very complex functions and pattern of regulation for SLK in development and cancer, making it a potential therapeutic target.

Introduction

There are over 1,000 different mammalian members of the protein kinase family[1,2] which can be divided into four categories: receptor tyrosine kinases (RTKs), non-receptor tyrosine kinases, receptor serine/threonine kinases, and non-receptor serine/threonine kinases.[3-5] The catalytic domain of most protein kinases is highly conserved and may reflect evolution from a common precursor.[6,7] The Ste20 family of serine/threonine protein kinases represents one important subset of protein kinases involved in cellular proliferation, migration and terminal differentiation.[8,9] The Ste20 serine/threonine protein kinase archetype is an important signaling molecule that has been well studied in yeast as a regulator of a MAPK (mitogen-activated protein kinase)-dependent pathway involved in the control of mating response.[10] Consistent with the activation of all G-protein coupled receptors, α factor binding results in the release and activation of the βγ complex. The βγ complex activates Ste20 and results in its translocation to Ste5, a scaffolding protein. This translocation results in the subsequent activation of Ste11, Ste7 and Fus3/Kss1.[11] In studying the Ste20 kinase in yeast, a group of similar Ste20-like kinases (SLKs) were identified. These SLKs can be divided into three major families based on structure: p21-activated kinases (PAKs), pleckstrin-homology domain-containing PAKs (PH-PAKs) and germinal center kinases (GCKs).[12]

*Correspondence to: Luc A. Sabourin; Email: lsabourin@ohri.ca
Submitted: 08/31/12; Revised: 09/21/12; Accepted: 10/09/12
http://dx.doi.org/10.4161/cam.22495

The Ste20-Like Kinase SLK

The mammalian Ste20-like kinase SLK was first isolated from guinea pig liver.[13] In 1999 the mouse homolog was successfully cloned, followed by human SLK.[14,15] The kinase was shown to be ubiquitously expressed in adult tissues and cell lines, as well as muscle and neuronal lineages in the developing embryo.[14,16] SLK was first identified and characterized as a caspase3-activated kinase which played a role in the induction of apoptosis.[14,17-19] SLK is classified as a GCK-related kinase that shares extensive sequence similarity with lymphocyte oriented kinase (LOK).[20] SLK is comprised of an N-terminal Ste20 catalytic kinase domain (amino acids 1–338) and predicted coil regions between amino acids 339–788 and residues 825–1,180, respectively (**Fig. 1**). The

carboxy-terminus of SLK shares a high degree of identity with AT1–46 at amino acids 788–936 (63%) and 957–1171 (71%) as well as with LOK (56%) between amino acids 867 and 1178.[14,17,21,22] This C-terminal coil motif was termed the ATH domain, for **AT**1–46 **h**omology domain.[14,23] A putative caspase 3 consensus cleavage site (DXXD) is also contained within the central domain at amino acid residue 436 of the mouse sequence and is loosely conserved in several other species.[17] Mutation of the putative cleavage site did not however affect the ability of SLK to be cleaved by caspase3 in vitro, suggesting the presence of additional sites and more complex regulation[17]

The N-terminal catalytic domain is most similar to LOK (74%) and mammalian sterile 20 1 (MST1) (26%) from amino acids 40–307 (**Fig. 1**). The characteristic Ste20 kinase signature sequence (TPYWMAPE) is located in the kinase subdomain VIII at amino acid position 193. SLK contains an activation segment characterized by a DFG motif at the beginning of the segment and an αEF/αF loop at the end.[24] The activation segment of SLK, mapped to amino acids 173–222, has been shown to contain short helices located at the C-terminal end of the DFG motif.[24] SLK has also been shown to be phosphorylated at T183 and S189, resulting in the auto-activation of kinase activity. Supporting this, T183 and S189 are located within the activation segment; subdomains VII and VIII, respectively.[24,25] Albeit not well conserved,[17,26] a putative consensus SH3 binding site (PXXPX) is found at position 735 of the murine SLK sequence, suggesting that SLK may interact with SH3 domain containing proteins that have yet to be identified.[17,26]

A number of protein kinases regulate themselves by autophosphorylation on at least one key residue within the activation segment, usually contained within the kinase lobe of the protein.[27] Autophosphorylation sites on SLK have been mapped to T183 and S189 through electron density map analysis obtained from mass spectrometry, however neither site appeared to be completely phosphorylated.[24] Phosphorylation of residues T183 and S189 are the important post-translational modifications required for the activation of SLK. In an SLK T183A mutant, kinase activity dropped by 60% compared with wild-type, while the S189A mutant and T183A/S189A double mutant showed an 80% reduction in catalytic activity.[25] For kinases like SLK that require phosphorylation in order to become activated, the phosphate group often confers a conformational change in the activation segment that promotes substrate binding, compared with the unphosphorylated activation segment which would remain unstructured.[28,29] As both T183 and S189 are never fully phosphorylated, it may be possible that both are phosphorylated in a primary and secondary manner. The role of the secondary phosphorylation site has been suggested to be involved in substrate recruitment after the first phosphorylation event has stabilized the activation segment.[30] Emphasizing the importance of T183 in SLK activation, this residue is a highly conserved phosphorylation site, also present in DAPK3 (death-associated protein kinase-3) and CHK2 (checkpoint kinase-2).[24]

The mechanism of SLK activation is under ongoing investigation, however it has been demonstrated that SLK is able to self-associate in a *trans* orientation and autophosphorylate.[24] An SLK point-mutant

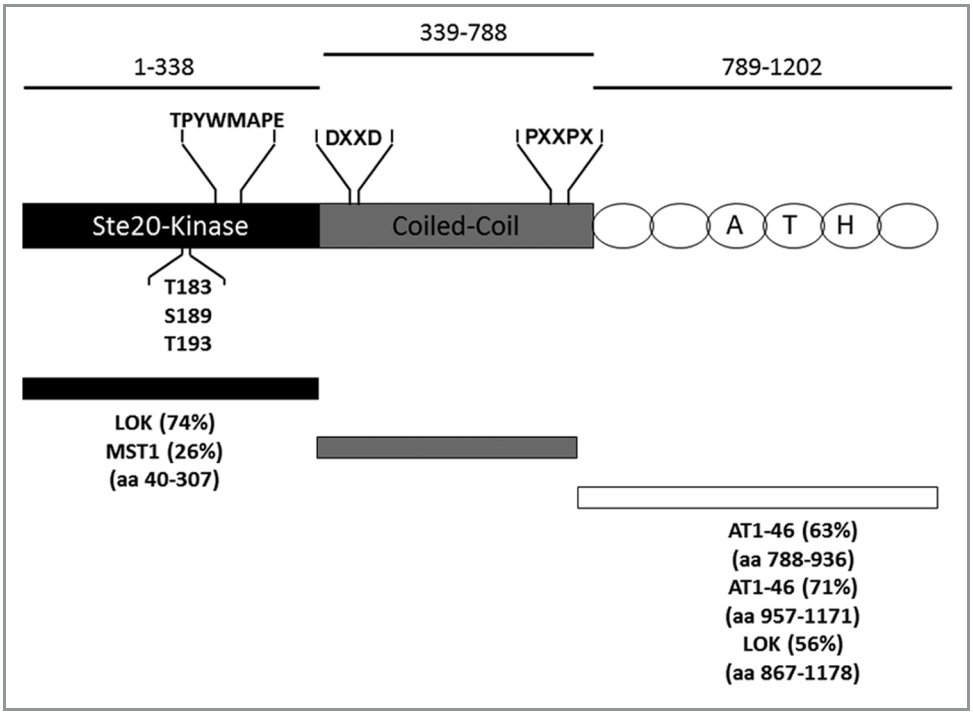

Figure 1. SLK structure and homologies. Schematic representation of murine SLK structure showing the Ste20 kinase domain at the N-terminus and the AT1–46 homology region at the C-terminal end. Amino acid numbers are shown above the structure and homologies to related kinases are shown in percentage. The Ste20 signature sequence (TPYWMAPE), the consensus caspase cleavage site (DXXD) and a putative SH3-binding domain (PXXPX) are shown.

Q185P exists as a monomer in solution and is unable to autophosphorylate, suggesting that dimerization of the kinase is critical for activity.[24] The C-terminus of SLK contains a coiled-coil region, which may facilitate dimerization allowing the catalytic site to come into proximity of the activation segment on the *trans* molecule and phosphorylate it.[22] The consensus target sequence of SLK appears to differ from its activation sequence, but the kinase is still able to recognize and phosphorylate both.[24] The GCK-related kinases have been shown to contain C-terminal auto-regulatory regions.[31,32] It was then hypothesized that the ATH domain could regulate SLK kinase activity. Indeed, a C-terminal truncation deleting the C-terminal two-thirds of the ATH domain ($SLK^{\Delta 950-1202}$) dramatically increases SLK kinase activity.[17] Further truncation of the kinase up to residue 373 (SLK^{1-373}) demonstrates a 10- to 15-fold increase in kinase activity, suggesting that kinase activity may be regulated by multiple domains.[17] Interestingly, other studies have shown that a smaller deletion of SLK (SLK^{1-592}) has no effect on its activity when compared with the full length kinase.[15] Similarly, we have shown that an SLK^{1-551} construct results in a reduction in kinase activity compared with the SLK^{1-373} truncation.[17] These data suggest that an important region required for the regulation of SLK kinase activity may lay within amino acids 373 to 592. Interestingly, different effects have been reported on the role of the ATH domain in SLK regulation. It was reported that complete deletion (SLK^{1-373}) or addition of the c-terminus has no effect of SLK kinase activity.[21,22] Another study has shown that deletion of the ATH increased SLK kinase activity.[17] One explanation is the fact that different cell lines (COS-1 and 293) and kinase assay protocols were used in the different studies. Nevertheless, these findings suggest that SLK is under complex regulation and that additional studies are required to fully understand the role of its various domains. During the investigation of the auto-inhibitory role of the ATH domain, a yeast two-hybrid screen identified the LIM domain binding transcriptional cofactor proteins (Ldb1 and Ldb2) as SLK-binding factors. The Ldbs were found to bind preferentially within the central 86 amino acids (a.a. 981–1,067) of the ATH domain ($SLK^{950-1202}$).[33] Further study demonstrated that the dimerization and nuclear localization signal (NLS) within Ldb1/2 were required for the interaction with SLK.[33] In vitro, the ATH domain of SLK inhibited the kinase activity of the SLK^{1-373} fragment and the addition of either Ldb1 or -2 further reduced activity.[33] In vivo experiments demonstrated that the kinase and ATH domain fragments were more readily co-immunoprecipitated in the presence of Ldb1/2, suggesting that Ldb1/2 aid in the stabilization of the auto-inhibitory function of the kinase domain by the ATH domain.[33] A reduction in the expression of Ldb1 caused a reduction in the amount of Ldb2 associated with the SLK complex and increased SLK activity. Conversely, the overexpression of Ldb1/2 resulted in a titration effect whereby less Ldb1/2 was bound in the SLK complex and instead, heterodimerized with each other. Again, this action increased SLK kinase activity and as such, it was suggested that the stoichiometry of the Ldb-SLK complex is highly sensitive and thus, kinase activity is very tightly controlled.[33] It is currently unknown whether there is another scaffolding-type protein facilitating the recruitment of various components to this complex (**Fig. 2**).

The Multiple Roles of SLK

Apoptosis. SLK was first characterized by Sabourin and Rudnicki in 1999 as a mediator of apoptosis. Stable overexpression of SLK in

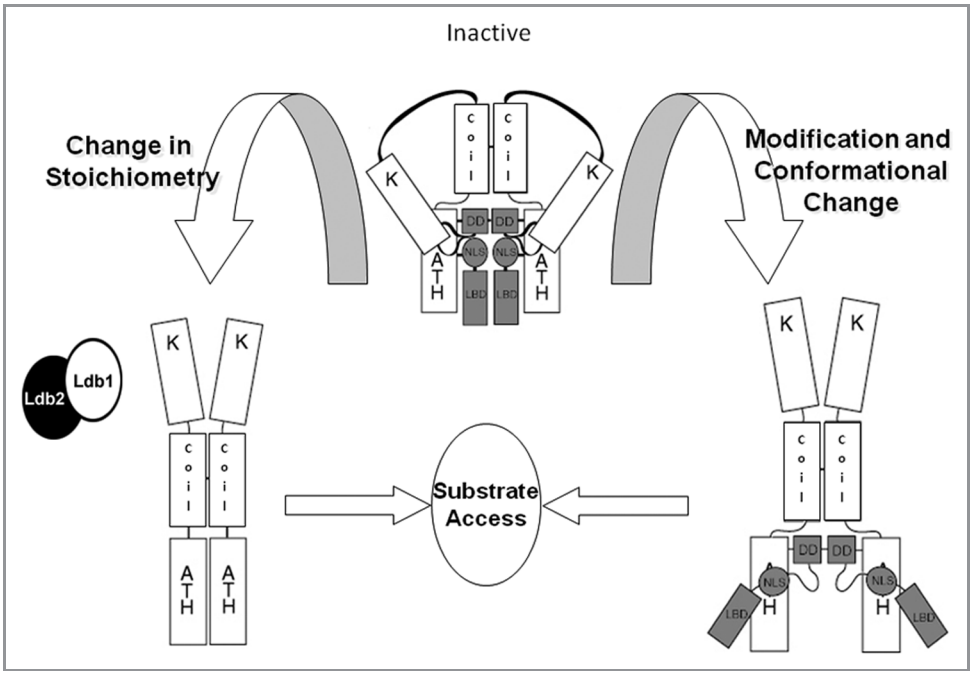

Figure 2. The SLK Ldb complex. The SLK dimer may be held in a "closed" conformation through bridging of the ATH region to the catalytic domain by Ldb dimers bound to the SLK C-terminus. Upon activation, SLK or Ldb post-translational modifications result in "opening" of the kinase and substrate access. Alternatively, changes in the Ldb stoichiometry can also result in SLK activation.

C2C12 myoblasts induced apoptosis via a c-Jun N-terminal kinase-1 (JNK1)-dependent pathway, similar to the Ste20 kinase homolog recently characterized in the helminth parasite *Schistosoma mansoni*, SmSLK.[14,34] It was found that fibroblasts expressing a kinase inactive SLK^{1-373} ($SLK^{1-373\ K63R}$) were Annexin V negative, suggesting that SLK kinase activity was required for the induction of apoptosis.[14] Interestingly, the expression of full length SLK^{K63R} also induced an apoptotic response albeit delayed, implying that perhaps SLK has the ability to induce programmed cell death independently of its kinase activity.[14] In a follow-up study, the authors found that preceding apoptosis, SLK overexpression caused the dissolution of actin stress fibers, the redistribution of actin to the cell periphery, membrane blebbing and loss of substrate adhesion.[17] The stress fiber dissolution and actin reorganization observed was similar to that seen by overexpression of activated Rac1 and PAK3.[17] It was established that expression of the kinase domain of SLK rapidly induced apoptosis due to its unregulated activity, whereas expression of the auto-regulatory ATH domain induced a delayed apoptotic response, preceded by actin stress fiber dissolution, cellular retraction and loss of adhesion.[17] Interestingly, incubation of SLK with recombinant caspase 3 or apoptotic lysates (Rat1-Myc/ER) resulted in the release of the kinase and ATH domains and the induction of apoptosis. Supporting this, exposure of cell lines to various apoptotic stimuli, including myc expression, UV radiation and tumor necrosis factor (TNF)-α resulted in caspase 3-mediated SLK cleavage.[17]

In a study of renal ischemia-reperfusion injury, the mechanism of SLK-mediated apoptosis was established. SLK activity attenuated the endoplasmic reticulum (ER) stress response and induced p38 MAPK via the apoptosis signal-regulating kinase-1 (ASK1).[18] SLK activity released cytochrome C and activated caspases-8 and -9 (**Fig. 3**). The lack of JNK activation was attributed to cell line specificity as COS-1 only weakly express MAPK kinase (MKK) 4 and MKK7.[18] SLK overexpression in glomerular epithelial cells also stimulated p53 transactivational activity via the JNK pathway.[19] SLK-induced apoptosis was attenuated with the use of the p53 inhibitor, pifithrin-α.[19] This was confirmed in vivo using a transgenic mouse line over-expressing SLK in the kidney glomerular podocytes.[35] Overexpression of the kinase in these cells resulted in injury and loss of these podocytes, accompanied by an increase in phosphorylation of p38.[35]The multi-faceted role of SLK in mediating the apoptotic response suggests that it may play a significant role in apoptosis-dependent physiological processes such as development and tumorigenesis.

The cell cycle. Initial characterization of SLK centered on the apoptotic response. However, the presence of active kinase in healthy exponentially growing cells suggested that the function of SLK was much more complex. Indeed, stable expression of kinase inactive SLK in fibroblasts cannot be achieved.[36] The lack of an apoptotic response in cells transiently expressing kinase dead SLK lacking the ATH suggests that it interferes with proliferation, supporting a role for SLK in cell cycle progression.[36] In the search for an upstream kinase to activate the polo-like kinase homolog (Plk1), SLK emerged as a candidate due to its high homology to the Xenopus polo-like kinase kinase 1 (xPLKK1).[37] Plk1 was identified as a novel substrate of SLK, which phosphorylates and activates Plk1 during progression through the G2/M transition, at which point the kinase activity of SLK is highest.[37] This phosphorylation by SLK is enhanced by the presence of the polo-box binding domain of Plk1.[38] SLK was subsequently found to be required upstream of Cdc2 and depletion of SLK caused cells to arrest in early G2 (**Fig. 3**). This arrest was accompanied by an inability to downregulate cyclin A and progress through mitosis.[39] Confirming a link between SLK and the microtubule network, the kinase co-localized with α-tubulin at the mitotic spindle and overexpression of the kinase caused ectopic spindle assembly.[39,40] Further study revealed that SLK was required for radial microtubule organization during interphase as depletion of the kinase resulted in the inability of centrosomes to anchor or cap microtubules.[21] Interestingly, overexpression of SLK induced cell cycle re-entry of Xenopus oocytes.[39] These observations offer a possible mechanism by which overexpression of SLK leads to apoptosis. Overexpression of the kinase may force cells to prematurely enter mitosis leading to their death through mitotic catastrophe.

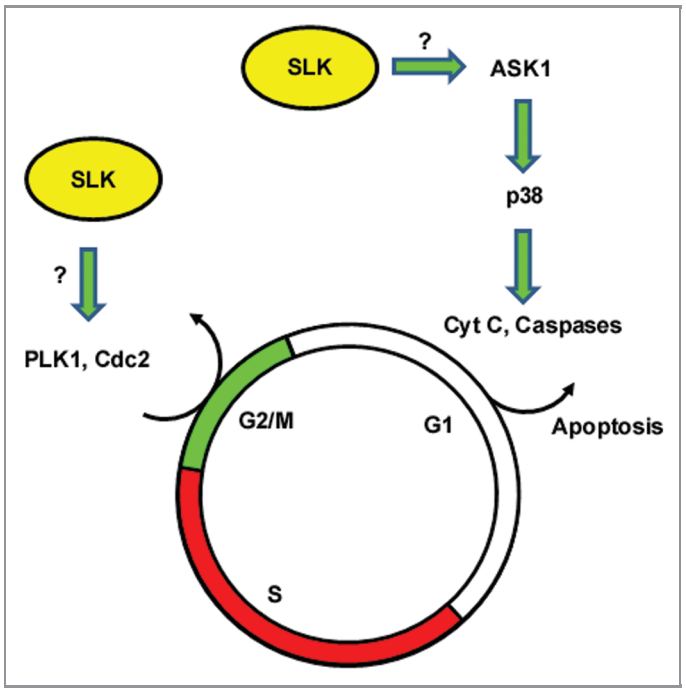

Figure 3. SLK signaling in apoptosis and cell cycle. (Left) As cells go through the cell cycle, SLK can act upstream of Plk1 and Cdc2 to allow progression through G2/M. Phosphorylation of Plk1 may be a direct event whereas the effect on Cdc2 remains to be tested. (Right) During apoptosis, active SLK can stimulate the p38 pathway through ASK1. This results in cytochrome C release and caspase 8 and 9 activation. Whether this occurs through direct phosphorylation, remains to be elucidated. For clarity, the apoptotic cascade is shown in the G1 phase but the insult could be received at any point.

Cytoskeletal Dynamics and Cell Migration

The recurrent theme in the characterization of SLK is its association with the cytoskeleton and the microtubule network.

Initial work has shown that SLK localizes to the cell periphery. Furthermore, SLK overexpression induces the disassembly of actin stress fibers, cellular retraction and the re-localization of actin to the cell periphery.[14,17] Interestingly, disassembly of actin stress fibers induced by the overexpression of SLK can be attenuated by the expression of a dominant-negative mutant of Rac1.[40] Accordingly, SLK co-localizes with Rac1 and the microtubule network in fibroblasts actively spreading on fibronectin.[40] RhoA activation is known to promote stress fiber and focal adhesion (FA) complex assembly, downstream of Rac1.[41,42] In a study of vasodilation induced by angiotensin II type 2 receptor (AT2R) activation, it was reported that AT2R signals through SLK which in turn phosphorylates Ser188 of RhoA, thus inhibiting its activity, preventing vascular smooth muscle cell contraction.[43] This interpretation supports a mechanism by which SLK mediates cytoskeletal reorganization and stress fiber breakdown through a Rho/Rac pathway.

Actin fibers are anchored at FAs through protein complexes composed of α-actinin, vinculin, talin and zyxin.[44] The FAs control adhesion dynamics and contain focal adhesion kinase (FAK), paxillin and vinculin. In response to cell adhesion or migration stimuli, cells form integrin/FAK/src complexes which recruit and activate numerous other adaptor molecules leading to FA turnover.[45,46] The formation of a functional FAK/src complex is critical for efficient cell migration by modulating FA turnover, while disruption of this complex results in reduced cell migration.[45-56] Following fibronectin stimulation, it was observed that SLK co-localized with vinculin at large podosome-like adhesions.[40] SLK ablation resulted in an increase in the size and density of vinculin-positive adhesions, indicative of stable FAs in non-migrating cells.[57] These results suggest that SLK may play a role in adhesion dynamics. Supporting this, SLK kinase assays from cells induced to migrate by scratch-wounding show an upregulation in kinase activity.[57,58] This activation was found to be dependent on c-src and the MAPK kinase pathway as pre-treatment with the PP2 and U0126 inhibitors abrogated kinase activation.[57] In addition, expression of src family kinases was found to be required for SLK recruitment at the leading edge of migrating cells.[57] Interestingly, overexpression of v-src leads to inhibition of SLK activity through hyper-phosphorylation of the kinase domain through casein kinase II, suggesting that the src family kinases can also function to negatively regulate SLK activity during cell migration, perhaps during focal contact assembly.[59] Confirming a role for SLK in cell migration, knockdown experiments and expression of dominant negative SLK show marked reductions in migration.[57]

Scratch wounding of confluent monolayers causes FAK activation as the cells migrate into the wound.[60] FA turnover during cell migration induces the formation of a functional FAK/src complex initiated by auto-phosphorylation of FAK at tyrosine residue 397 (pY397), stable focal adhesion assembly and FA turnover and migration.[41-43,57-60] Similarly, nocodazole treatment of fibroblasts results in microtubule depolymerization and FA stabilization as evidenced by high levels of phospho-FAK-Y397.[61] Nocodazole wash-out and microtubule regrowth is accompanied by cyclical changes in the levels of FAK pY397.[61] Nocodazole wash-out in MEF-3T3 fibroblasts that express kinase inactive SLK or have been siRNA treated show impaired focal adhesion turnover as demonstrated by stabilization of FAK pY397 levels.[57] SLK-deficient cells also displayed enlarged adhesions following the wash-out, further supporting impaired FA turnover.[57] Supporting a role for SLK in turnover, scratch wounding of a confluent monolayer of FAK-null fibroblasts results in almost no upregulation in SLK kinase activity, suggesting that SLK activation is FAK-dependent.[57]

Further investigation into the mechanisms of SLK activation and activity during cell migration showed that the regulatory Ldb1/2 co-factors co-localized with SLK at the leading edge of migrating cells.[33] Both the knockdown and overexpression of Ldb1/2 caused an increase in the rate of cell migration, recapitulating the changes in SLK activity seen in the SLK-Ldb complex studies in vitro.[33] Changes in the expression level of Ldb1 resulted in the loss of either Ldb factor from the SLK complex, activating the kinase and increasing cell migration.[33] Adding complexity, as SLK kinase activity increased during cell migration, the amount of Ldb1 associated with the kinase increased but SLK-associated Ldb2 remained stable, suggesting that the stoichiometry of the SLK-Ldb complex is crucial for cell migration.[33] These observations also hint that a heretofore unidentified component of the SLK-Ldb complex initiates a shift in the stoichiometry of this complex thus allowing the kinase to activate and signal downstream to the FAs (**Fig. 4**). Recent evidence from our lab shows that activated SLK regulates FAK-mediated FA turnover by phosphorylating paxillin on serine 250, an integral FA complex component (Quizi et al., in press). A paxillin serine 250 mutant (S250T) abolished phosphorylation of paxillin by SLK resulting in stable FAK pY397 levels, stabilized FAs and impaired cell migration, further supporting a role for SLK as a modulator of adhesion turnover and cell migration (Quizi et al., in press). As proposed previously, SLK might represent part of a microtubule-associated complex that targets focal adhesions for disassembly (**Fig. 4**).[62,63]

Physiological Relevance

HER2/Neu/ErbB2 signaling. Advanced tumors have acquired the ability to invade surrounding tissues and migrate throughout the vasculature to colonize distant sites within the body.[64] The process of metastasis is highly dependent on an active migration system and cross-talk between growth factor receptors and focal adhesion signaling. HER2/Neu/ErbB2, a transmembrane receptor belonging to the epidermal growth factor receptor (EGFR) family, is an oncoprotein that is overexpressed in approximately 30% of human breast cancers and mediates anchorage-independent growth.[65-68] Patients whose tumors overexpress HER2 receptors (categorized as HER2-positive cases) present with a more metastatic and invasive disease with poorer prognosis.[66] Specific docking proteins target trans-phosphorylated receptors and link activated HER2 to the Ras/MAPK-, PI3K- and src-dependent signaling cascades, initiating such cellular processes as migration, survival and apoptosis.[69-72] The phosphorylation of tyrosine residues on the cytoplasmic tail of HER2 (Y1201 or Y1226/7)

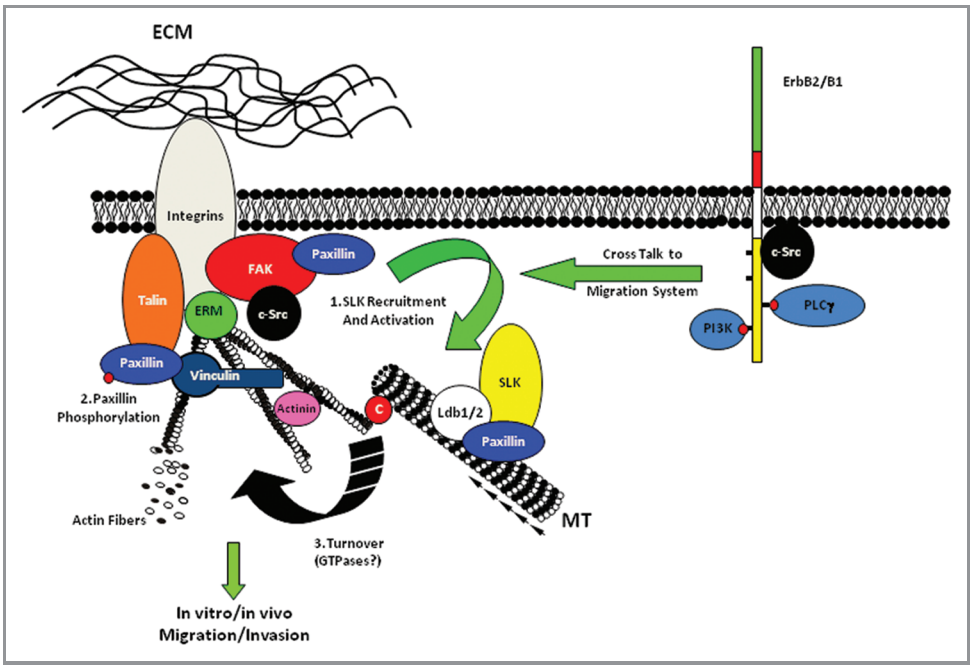

Figure 4. Upstream signals and SLK activation. Upon integrin engagement, the FAK/src complex is activated and focal contacts are assembled while SLK is held inactive. Further signaling activates and recruits SLK through the microtubule network, along the actin fibers.[57] SLK can phosphorylate paxillin in the vicinity of adhesions to induce adhesion turnover (Quizi et al., in press). Alternatively, microtubule-bound paxillin could be phosphorylated and recruited to newly formed adhesion and induce destabilization. Stimulation of growth factor receptor such as ErbB2 can also activate SLK through receptor-integrin cross talk.[76]

has been shown to mediate the epithelial-to-mesenchymal transition (EMT) induced by overexpression of HER2 in Madin-Darby canine kidney epithelial cells.[73] Memo, a mediator of ErbB2-driven cell motility, has been found to interact with pY1227 through the Shc adaptor protein, associating HER2 with the microtubule network.[74] Activation of the MAPK, PI3K and src cascades are critical for HER2-induced motility of breast carcinoma cells, but stimulation of these signals is not sufficient to induce migration in the absence of HER2 phosphorylation at Y1201 or Y1227.[74]

FAK signaling is also required for HER2-induced transformation and invasion.[75] Chemotaxis and invasion of breast cancer cells in the HER2 tumor model has been shown to signal through SLK via the FAK complex.[76] SLK has been shown to be upregulated in cell lines that express high levels of the HER2 receptor, including the T47D and 4T1 lines.[76] In cell lines where basal expression of the HER2 receptor is relatively low (HeLa and NIH3T3 cells) overexpression of an activated Neu (Neu V64E) was sufficient to upregulate SLK activity in the absence of heregulin stimulation.[76] Expression of kinase inactive SLK (SLK^{K63R}) reduced heregulin-induced cell migration by up to 75% as measured in a Boyden chamber assay.[76] Similarly, chemotaxis was inhibited in MCF-7, MDA-MB-231 and Sk-Br-3 cells when SLK^{K63R} was expressed.[76] Autophosphorylation of Neu on tyrosine 1201 and tyrosine 1226/7 has been shown to couple the Neu receptor to its downstream signaling cascades.[74] The presence of a tyrosine residue at either of the two autophosphorylation sites (Y1202 or Y1226/7), in an otherwise phosphorylation-deficient mutant (NYPD), is sufficient to activate SLK downstream of heregulin stimulation.[76] As previously noted, both MEK1 and src family kinase inhibitors are sufficient for the attenuation of SLK-mediated cell migration. Similarly, they were also shown to inhibit Neu-mediated SLK activation.[57,76] The use of inhibitors for both PI3K and PLCγ results in a partial inhibition of SLK activation in cells expressing activated Neu.[76] In cells expressing Neu with only Y1201 available for phosphorylation, inhibition of PLCγ prevented SLK activation, but inhibition of PI3K had no effect on SLK activity.[76] In cells expressing Neu with only Y1226/7 available for phosphorylation, the opposite effects are observed.[76] These results suggest that multiple signaling pathways contribute to SLK activation downstream of HER2/Neu/ErbB2. Overexpression of activated Neu in a FAK-null fibroblast cell line is not sufficient to induce SLK activity upon heregulin stimulation, suggesting that FAK is required for Neu-mediated SLK activation.[76] Although it is unclear how SLK is directly activated downstream of the Neu-FAK cross-talk, it appears to be an important downstream mediator of the Neu receptor (**Fig. 4**). Albeit much remains to be deciphered, the kinase presents itself as a potential therapeutic target in HER2-positive breast cancer patients. Specific inhibition of SLK activity may abrogate the progression of early-stage HER2-positive tumors or stabilize disease in advanced-stage cases and may achieve favorable responses in a combinatorial therapeutic approach.

Role in development. SLK is ubiquitously expressed in adult tissues and preferentially expressed in neuronal and muscle

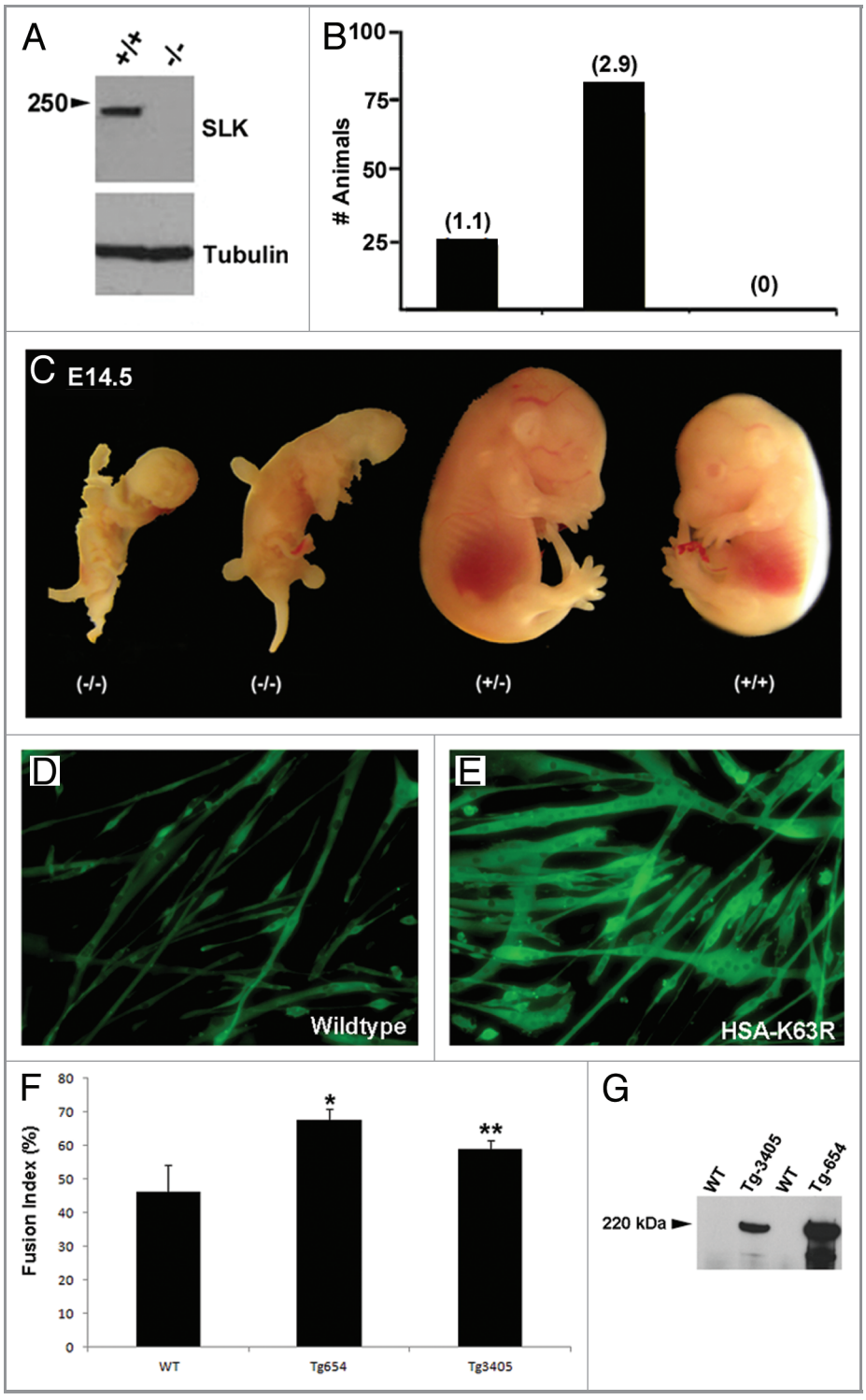

Figure 5. Effects of SLK levels on development. SLK gene trap knockouts do not express SLK protein (**A**) and do not give rise to viable SLK(–/–) pups. (**B**) Mendelian ratios are shown above the histogram boxes (n = 106). (**C**) SLK-null embryos (–/–) show marked developmental defects at embryonic day 14.5. All SLK-null embryos are reabsorbed with no viable pups at birth. (**D and E**) Primary myoblast cultures were established from 4–6 week old mice and induced to differentiate by serum withdrawal. After 3 d the cultures were stained for myosin heavy chain (MHC) and DAPI to establish the fusion index. (**F**) Quantitation of the fusion index from MHC-stained myotubes cultures. The fusion index was established from myotubes bearing 3 or more nuclei using the following calculations: (# nuclei in myotubes/total # nuclei) × 100. The fusion indices were obtained from triplicate cultures of two independent animals from two transgenic lines. At least 200 nuclei were counted. (*$p < 0.008$ Tg654 vs WT, **$p < 0.05$ Tg3405 vs WT). (**G**) Total lysates from anterior hind leg muscles were immunoprecipitated with anti-HA antibodies (12CA5). Subsequent probing for SLK shows expression of HA-tagged kinase in the 654 and 3405 transgenic lines. Kinases assays showed that total SLK activity was markedly reduced in both transgenic lines (not shown).

lineages during embryonic development.[14,16] Supporting this, SLK has been shown to be activated during myoblast differentiation and the expression of SLK^{K63R} impaired C2C12 myoblast fusion in vitro.[77] Expression of the kinase is upregulated in activated satellite cells during muscle regeneration supporting a key role for the kinase in muscle differentiation.[77] Recently, we have characterized transgenic mice expressing SLK^{K63R} from the human skeletal actin promoter. SLK^{K63R} expression negatively affects litter sizes, muscle development and muscle fatigue in the transgenic animals (Storbeck et al., unpublished). Counter-intuitively, despite decreased kinase activity in the muscle fibers of transgenic animals, muscle regeneration was enhanced in vivo and myoblast fusion was increased in vitro (**Fig. 5**; Storbeck et al., unpublished). These results demonstrate the complexity of SLK functions in vivo and will require further investigation to fully delineate the role of SLK in adult muscle function. An investigation into the rat ortholog of SLK, SK2, showed increased expression and activity within renal tissues in the developing embryo and adult renal tissues suffering from acute renal failure.[78] Ischemia-reperfusion and acute renal failure stimulated tubular epithelial cell regeneration which emulates kidney developmental processes. Tubular epithelial cells are the primary location of increased expression of SLK during both renal development and renal tissue injury.[18,19,78] Further study presented SLK as a mediator of apoptosis in response to acute renal failure and ischemia-reperfusion injury via ASK1 and p38 as well as p53, and thus as a rational therapeutic target to prevent excessive renal tissue damage.[18,19] Further highlighting the importance of this kinase in development, a gene trap-mediated knockout of SLK is embryonic lethal with affected embryos displaying severe patterning defects and impaired organogenesis (**Fig. 5**; Al-Zahrani, unpublished). These results strongly suggest that SLK represents a component of a highly complex signaling system with a prominent role in various essential physiological and pathological processes, including embryonic development, cancer and metastasis, hypertension, and tissue injury.

Perspectives and Future Directions

Over the last few years, it has become apparent that SLK plays multiple roles under normal physiological conditions. However, to our knowledge, other than sequence variants, no human mutations have been identified in the *slk* gene. Although, its regulation appears to be quite complex, it is likely to be dependent on the biological context, whether it is apoptosis, motility or proliferation (**Fig. 6**). In addition, SLK responds to oncogenic signals such as activated HER2/Neu/ErbB2 to drive chemotaxis of breast cancer cells, suggesting that SLK inhibition might suppress the invasive and metastatic process. Whether SLK is required at any other levels during cancer progression remains to be elucidated. Similarly, interfering with SLK function may also halt the cell cycle, inducing a proliferation block. However, these possibilities will have to be tested in animal models. As the global SLK knockout is embryonic lethal, the establishment of a conditional SLK allele in a cancer model will allow for the testing of its therapeutic potential. In addition, a conditional allele will also allow the dissection of its role in development and in various models of injury.

As SLK is ubiquitously expressed in adult tissues, the use of SLK inhibitors might be detrimental to normal tissue function. Therefore, the identification of additional regulatory pathways or binding partners that are tissue-specific will be beneficial, providing an opportunity to target upstream regulators in a tissue-specific manner. Similarly, the identification of additional downstream targets may allow for a more specific targeting of SLK-dependent pathways. As the SLK field is still in its infancy,

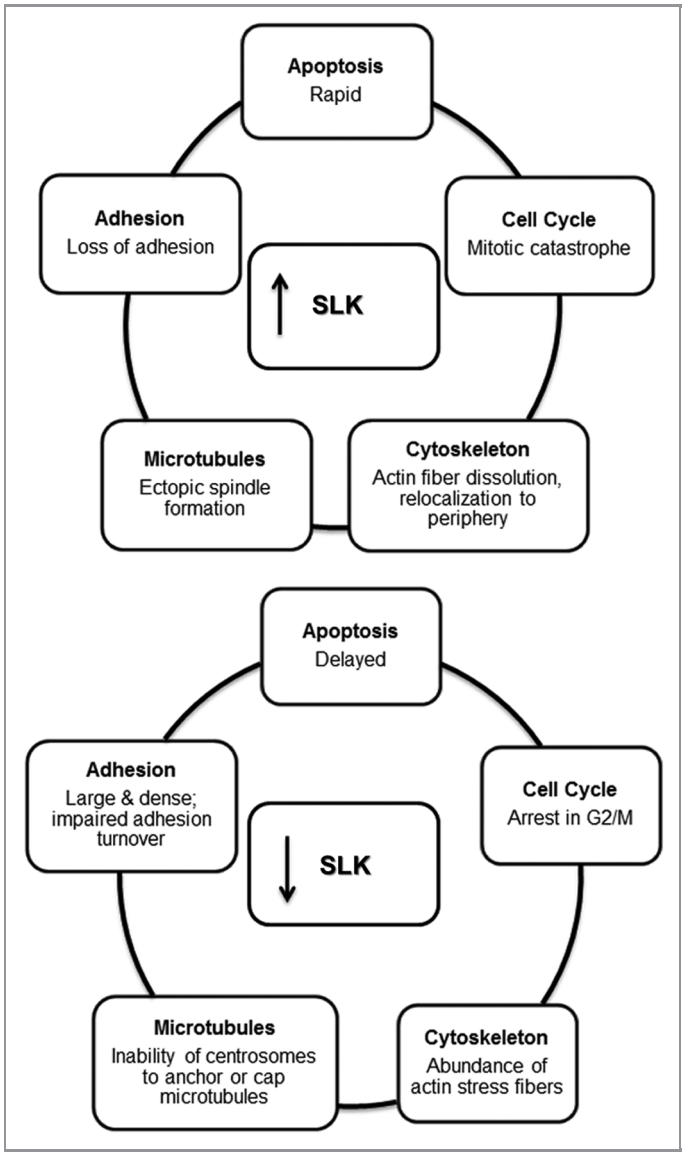

Figure 6. Summary of SLK functions. Studies have shown that high levels of SLK activity induce apoptosis, loss of actin fibers and adhesion, and ectopic spindle formation. Conversely, knock down experiments or expression of kinase inactive SLK results in delayed cell death, cell cycle arrest and inhibition of cell migration. Together, these data suggest a role for SLK in multiple processes. Whether the role of SLK is restricted to cytoskeletal remodeling in all cases remains to be verified.

little is known about its regulation under various biological contexts and how it transduces the different signals to its downstream effectors. Our ability to dissect these diverse inputs and responses awaits the identification of additional regulators, substrates and genetic models.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

The authors thank Dr. Douglas Gray for helpful comments. K.N.A. is a recipient of a Canadian Breast Cancer Foundation—Ontario Region Masters Fellowship. K.D.B. is a recipient of an Ontario Graduate Scholarship and a Canadian Breast Cancer Foundation—Ontario Region Doctoral Fellowship. This work was supported by the Canadian Institute of Health Research and the Canadian Breast Cancer Foundation. The authors declare no conflict of interest.

References

1. Hunter T. A thousand and one protein kinases. Cell 1987; 50:823-9; PMID:3113737; http://dx.doi.org/10.1016/0092-8674(87)90509-5
2. Hunter T. Protein kinases and phosphatases: the yin and yang of protein phosphorylation and signaling. Cell 1995; 80:225-36; PMID:7834742; http://dx.doi.org/10.1016/0092-8674(95)90405-0
3. Hanks SK, Quinn AM, Hunter T. The protein kinase family: conserved features and deduced phylogeny of the catalytic domains. Science 1988; 241:42-52; PMID:3291115; http://dx.doi.org/10.1126/science.3291115
4. Kingsley DM. The TGF-beta superfamily: new members, new receptors, and new genetic tests of function in different organisms. Genes Dev 1994; 8:133-46; PMID:8299934; http://dx.doi.org/10.1101/gad.8.2.133
5. Clutterbuck E, Shields JG, Gordon J, Smith SH, Boyd A, Callard RE, et al. Recombinant human interleukin 5 is an eosinophil differentiation factor but has no activity in standard human B cell growth factor assays. Eur J Immunol 1987; 17:1743-50; PMID:3500861; http://dx.doi.org/10.1002/eji.1830171210
6. Hanks SK, Quinn AM. Protein kinase catalytic domain sequence database: identification of conserved features of primary structure and classification of family members. Methods Enzymol 1991; 200:38-62; PMID:1956325; http://dx.doi.org/10.1016/0076-6879(91)00126-H
7. Hanks SK, Hunter T. Protein kinases 6. The eukaryotic protein kinase superfamily: kinase (catalytic) domain structure and classification. FASEB J 1995; 9:576-96; PMID:7768349
8. Davis RJ. The mitogen-activated protein kinase signal transduction pathway. J Biol Chem 1993; 268:14553-6; PMID:8325833
9. Fanger GR, Gerwins P, Widmann C, Jarpe MB, Johnson GL. MEKKs, GCKs, MLKs, PAKs, TAKs, and tpls: upstream regulators of the c-Jun amino-terminal kinases? Curr Opin Genet Dev 1997; 7:67-74; PMID:9024636; http://dx.doi.org/10.1016/S0959-437X(97)80111-6
10. Zhao ZS, Leung T, Manser E, Lim L. Pheromone signalling in Saccharomyces cerevisiae requires the small GTP-binding protein Cdc42p and its activator CDC24. Mol Cell Biol 1995; 15:5246-57; PMID:7565673
11. Leberer E, Thomas DY, Whiteway M. Pheromone signalling and polarized morphogenesis in yeast. Curr Opin Genet Dev 1997; 7:59-66; PMID:9024634; http://dx.doi.org/10.1016/S0959-437X(97)80110-4
12. Sells MA, Chernoff J. Emerging from the Pak: the p21-activated protein kinase family. Trends Cell Biol 1997; 7:162-7; PMID:17708935; http://dx.doi.org/10.1016/S0962-8924(97)01003-9
13. Itoh S, Kameda Y, Yamada E, Tsujikawa K, Mimura T, Kohama Y. Molecular cloning and characterization of a novel putative STE20-like kinase in guinea pigs. Arch Biochem Biophys 1997; 340:201-7; PMID:9143322; http://dx.doi.org/10.1006/abbi.1997.9893
14. Sabourin LA, Rudnicki MA. Induction of apoptosis by SLK, a Ste20-related kinase. Oncogene 1999; 18:7566-75; PMID:10602516; http://dx.doi.org/10.1038/sj.onc.1203119
15. Yamada E, Tsujikawa K, Itoh S, Kameda Y, Kohama Y, Yamamoto H. Molecular cloning and characterization of a novel human STE20-like kinase, hSLK. Biochim Biophys Acta 2000; 1495:250-62; PMID:10699464; http://dx.doi.org/10.1016/S0167-4889(99)00164-0
16. Zhang YH, Hume K, Cadonic R, Thompson C, Hakim A, Staines W, et al. Expression of the Ste20-like kinase SLK during embryonic development and in the murine adult central nervous system. Brain Res Dev Brain Res 2002; 139:205-15; PMID:12480135; http://dx.doi.org/10.1016/S0165-3806(02)00551-5
17. Sabourin LA, Tamai K, Seale P, Wagner J, Rudnicki MA. Caspase 3 cleavage of the Ste20-related kinase SLK releases and activates an apoptosis-inducing kinase domain and an actin-disassembling region. Mol Cell Biol 2000; 20:684-96; PMID:10611247; http://dx.doi.org/10.1128/MCB.20.2.684-696.2000
18. Hao W, Takano T, Guillemette J, Papillon J, Ren G, Cybulsky AV. Induction of apoptosis by the Ste20-like kinase SLK, a germinal center kinase that activates apoptosis signal-regulating kinase and p38. J Biol Chem 2006; 281:3075-84; PMID:16316999; http://dx.doi.org/10.1074/jbc.M511744200
19. Cybulsky AV, Takano T, Guillemette J, Papillon J, Volpini RA, Di Battista JA. The Ste20-like kinase SLK promotes p53 transactivation and apoptosis. Am J Physiol Renal Physiol 2009; 297:F971-80; PMID:19640899; http://dx.doi.org/10.1152/ajprenal.00294.2009
20. Kuramochi S, Moriguchi T, Kuida K, Endo J, Semba K, Nishida E, et al. LOK is a novel mouse STE20-like protein kinase that is expressed predominantly in lymphocytes. J Biol Chem 1997; 272:22679-84; PMID:9278426; http://dx.doi.org/10.1074/jbc.272.36.22679
21. Burakov AV, Zhapparova ON, Kovalenko OV, Zinovkina LA, Potekhina ES, Shanina NA, et al. Ste20-related protein kinase LOSK (SLK) controls microtubule radial array in interphase. Mol Biol Cell 2008; 19:1952-61; PMID:18287541; http://dx.doi.org/10.1091/mbc.E06-12-1156
22. Delarosa S, Guillemette J, Papillon J, Han YS, Kristof AS, Cybulsky AV. Activity of the Ste20-like kinase, SLK, is enhanced by homodimerization. Am J Physiol Renal Physiol 2011; 301:F554-64; PMID:21677149; http://dx.doi.org/10.1152/ajprenal.00062.2011
23. Schaar DG, Varia MR, Elkabes S, Ramakrishnan L, Dreyfus CF, Black IB. The identification of a novel cDNA preferentially expressed in the olfactory-limbic system of the adult rat. Brain Res 1996; 721:217-28; PMID:8793103; http://dx.doi.org/10.1016/0006-8993(96)00176-X
24. Pike AC, Rellos P, Niesen FH, Turnbull A, Oliver AW, Parker SA, et al. Activation segment dimerization: a mechanism for kinase autophosphorylation of non-consensus sites. EMBO J 2008; 27:704-14; PMID:18239682; http://dx.doi.org/10.1038/emboj.2008.8
25. Luhovy AY, Jaberi A, Papillon J, Guillemette J, Cybulsky AV. Regulation of the Ste20-like kinase, SLK: involvement of activation segment phosphorylation. J Biol Chem 2012; 287:5446-58; PMID:22203681; http://dx.doi.org/10.1074/jbc.M111.302018
26. Pawson T, Scott JD. Signaling through scaffold, anchoring, and adaptor proteins. Science 1997; 278:2075-80; PMID:9405336; http://dx.doi.org/10.1126/science.278.5346.2075
27. Nolen B, Taylor S, Ghosh G. Regulation of protein kinases; controlling activity through activation segment conformation. Mol Cell 2004; 15:661-75; PMID:15350212; http://dx.doi.org/10.1016/j.molcel.2004.08.024
28. Sicheri F, Moarefi I, Kuriyan J. Crystal structure of the Src family tyrosine kinase Hck. Nature 1997; 385:602-9; PMID:9024658; http://dx.doi.org/10.1038/385602a0
29. Meng W, Swenson LL, Fitzgibbon MJ, Hayakawa K, Ter Haar E, Behrens AE, et al. Structure of mitogen-activated protein kinase-activated protein (MAPKAP) kinase 2 suggests a bifunctional switch that couples kinase activation with nuclear export. J Biol Chem 2002; 277:37401-5; PMID:12171911; http://dx.doi.org/10.1074/jbc.C200418200
30. Hu J, Liu J, Ghirlando R, Saltiel AR, Hubbard SR. Structural basis for recruitment of the adaptor protein APS to the activated insulin receptor. Mol Cell 2003; 12:1379-89; PMID:14690593; http://dx.doi.org/10.1016/S1097-2765(03)00487-8
31. Graves JD, Gotoh Y, Draves KE, Ambrose D, Han DK, Wright M, et al. Caspase-mediated activation and induction of apoptosis by the mammalian Ste20-like kinase Mst1. EMBO J 1998; 17:2224-34; PMID:9545236; http://dx.doi.org/10.1093/emboj/17.8.2224
32. Pombo CM, Bonventre JV, Molnar A, Kyriakis J, Force T. Activation of a human Ste20-like kinase by oxidant stress defines a novel stress response pathway. EMBO J 1996; 15:4537-46; PMID:8887545
33. Storbeck CJ, Wagner S, O'Reilly P, McKay M, Parks RJ, Westphal H, et al. The Ldb1 and Ldb2 transcriptional cofactors interact with the Ste20-like kinase SLK and regulate cell migration. Mol Biol Cell 2009; 20:4174-82; PMID:19675209; http://dx.doi.org/10.1091/mbc.E08-07-0707
34. Yan Y, Tulasne D, Browaeys E, Cailliau K, Khayath N, Pierce RJ, et al. Molecular cloning and characterisation of SmSLK, a novel Ste20-like kinase in Schistosoma mansoni. Int J Parasitol 2007; 37:1539-50; PMID:17651740; http://dx.doi.org/10.1016/j.ijpara.2007.06.001
35. Cybulsky AV, Takano T, Papillon J, Guillemette J, Herzenberg AM, Kennedy CR. Podocyte injury and albuminuria in mice with podocyte-specific overexpression of the Ste20-like kinase, SLK. Am J Pathol 2010; 177:2290-9; PMID:20889563; http://dx.doi.org/10.2353/ajpath.2010.100263

36. O'Reilly PG, Wagner S, Franks DJ, Cailliau K, Browaeys E, Dissous C, et al. The Ste20-like kinase SLK is required for cell cycle progression through G2. J Biol Chem 2005; 280:42383-90; PMID:16236704; http://dx.doi.org/10.1074/jbc.M510763200
37. Ellinger-Ziegelbauer H, Karasuyama H, Yamada E, Tsujikawa K, Todokoro K, Nishida E. Ste20-like kinase (SLK), a regulatory kinase for polo-like kinase (Plk) during the G2/M transition in somatic cells. Genes Cells 2000; 5:491-8; PMID:10886374; http://dx.doi.org/10.1046/j.1365-2443.2000.00337.x
38. Johnson TM, Antrobus R, Johnson LN. Plk1 activation by Ste20-like kinase (Slk) phosphorylation and polo-box phosphopeptide binding assayed with the substrate translationally controlled tumor protein (TCTP). Biochemistry 2008; 47:3688-96; PMID:18298087; http://dx.doi.org/10.1021/bi702134c
39. O'Reilly PG, Wagner S, Franks DJ, Cailliau K, Browaeys E, Dissous C, et al. The Ste20-like kinase SLK is required for cell cycle progression through G2. J Biol Chem 2005; 280:42383-90; PMID:16236704; http://dx.doi.org/10.1074/jbc.M510763200
40. Wagner S, Flood TA, O'Reilly P, Hume K, Sabourin LA. Association of the Ste20-like kinase (SLK) with the microtubule. Role in Rac1-mediated regulation of actin dynamics during cell adhesion and spreading. J Biol Chem 2002; 277:37685-92; PMID:12151406; http://dx.doi.org/10.1074/jbc.M205899200
41. Van Aelst L, D'Souza-Schorey C. Rho GTPases and signaling networks. Genes Dev 1997; 11:2295-322; PMID:9308960; http://dx.doi.org/10.1101/gad.11.18.2295
42. Nobes CD, Hall A. Rho, rac, and cdc42 GTPases regulate the assembly of multimolecular focal complexes associated with actin stress fibers, lamellipodia, and filopodia. Cell 1995; 81:53-62; PMID:7536630; http://dx.doi.org/10.1016/0092-8674(95)90370-4
43. Guilluy C, Rolli-Derkinderen M, Loufrani L, Bourgé A, Henrion D, Sabourin L, et al. Ste20-related kinase SLK phosphorylates Ser188 of RhoA to induce vasodilation in response to angiotensin II Type 2 receptor activation. Circ Res 2008; 102:1265-74; PMID:18420945; http://dx.doi.org/10.1161/CIRCRESAHA.107.164764
44. Craig SW, Johnson RP. Assembly of focal adhesions: progress, paradigms, and portents. Curr Opin Cell Biol 1996; 8:74-85; PMID:8791409; http://dx.doi.org/10.1016/S0955-0674(96)80051-2
45. Mitra SK, Schlaepfer DD. Integrin-regulated FAK-Src signaling in normal and cancer cells. Curr Opin Cell Biol 2006; 18:516-23; PMID:16919435; http://dx.doi.org/10.1016/j.ceb.2006.08.011
46. Brown MC, Turner CE. Paxillin: adapting to change. Physiol Rev 2004; 84:1315-39; PMID:15383653; http://dx.doi.org/10.1152/physrev.00002.2004
47. Webb DJ, Parsons JT, Horwitz AF. Adhesion assembly, disassembly and turnover in migrating cells – over and over and over again. Nat Cell Biol 2002; 4:E97-100; PMID:11944043; http://dx.doi.org/10.1038/ncb0402-e97
48. Sieg DJ, Hauck CR, Schlaepfer DD. Required role of focal adhesion kinase (FAK) for integrin-stimulated cell migration. J Cell Sci 1999; 112:2677-91; PMID:10413676
49. Ren XD, Kiosses WB, Sieg DJ, Otey CA, Schlaepfer DD, Schwartz MA. Focal adhesion kinase suppresses Rho activity to promote focal adhesion turnover. J Cell Sci 2000; 113:3673-8; PMID:11017882
50. Webb DJ, Donais K, Whitmore LA, Thomas SM, Turner CE, Parsons JT, et al. FAK-Src signalling through paxillin, ERK and MLCK regulates adhesion disassembly. Nat Cell Biol 2004; 6:154-61; PMID:14743221; http://dx.doi.org/10.1038/ncb1094
51. Kaplan KB, Bibbins KB, Swedlow JR, Arnaud M, Morgan DO, Varmus HE. Association of the amino-terminal half of c-Src with focal adhesions alters their properties and is regulated by phosphorylation of tyrosine 527. EMBO J 1994; 13:4745-56; PMID:7525268
52. Fincham VJ, Frame MC. The catalytic activity of Src is dispensable for translocation to focal adhesions but controls the turnover of these structures during cell motility. EMBO J 1998; 17:81-92; PMID:9427743; http://dx.doi.org/10.1093/emboj/17.1.81
53. Kaverina I, Krylyshkina O, Small JV. Microtubule targeting of substrate contacts promotes their relaxation and dissociation. J Cell Biol 1999; 146:1033-44; PMID:10477757; http://dx.doi.org/10.1083/jcb.146.5.1033
54. Cary LA, Chang JF, Guan JL. Stimulation of cell migration by overexpression of focal adhesion kinase and its association with Src and Fyn. J Cell Sci 1996; 109:1787-94; PMID:8832401
55. Owen JD, Ruest PJ, Fry DW, Hanks SK. Induced focal adhesion kinase (FAK) expression in FAK-null cells enhances cell spreading and migration requiring both auto- and activation loop phosphorylation sites and inhibits adhesion-dependent tyrosine phosphorylation of Pyk2. Mol Cell Biol 1999; 19:4806-18; PMID:10373530
56. Cary LA, Han DC, Polte TR, Hanks SK, Guan JL. Identification of p130Cas as a mediator of focal adhesion kinase-promoted cell migration. J Cell Biol 1998; 140:211-21; PMID:9425168; http://dx.doi.org/10.1083/jcb.140.1.211
57. Wagner S, Storbeck CJ, Roovers K, Chaar ZY, Kolodziej P, McKay M, et al. FAK/src-family dependent activation of the Ste20-like kinase SLK is required for microtubule-dependent focal adhesion turnover and cell migration. PLoS One 2008; 3:e1868; PMID:18382658; http://dx.doi.org/10.1371/journal.pone.0001868
58. Etienne-Manneville S, Hall A. Integrin-mediated activation of Cdc42 controls cell polarity in migrating astrocytes through PKCzeta. Cell 2001; 106:489-98; PMID:11525734; http://dx.doi.org/10.1016/S0092-8674(01)00471-8
59. Chaar Z, O'reilly P, Gelman I, Sabourin LA. v-Src-dependent down-regulation of the Ste20-like kinase SLK by casein kinase II. J Biol Chem 2006; 281:28193-9; PMID:16837460; http://dx.doi.org/10.1074/jbc.M605665200
60. Gates RE, King LE, Jr., Hanks SK, Nanney LB. Potential role for focal adhesion kinase in migrating and proliferating keratinocytes near epidermal wounds and in culture. Cell Growth Differ 1994; 5:891-9; PMID:7986754
61. Bershadsky A, Chausovsky A, Becker E, Lyubimova A, Geiger B. Involvement of microtubules in the control of adhesion-dependent signal transduction. Curr Biol 1996; 6:1279-89; PMID:8939572; http://dx.doi.org/10.1016/S0960-9822(02)70714-8
62. Palazzo AF, Gundersen GG. Microtubule-actin cross-talk at focal adhesions. Sci STKE 2002; 2002:pe31; PMID:12096217; http://dx.doi.org/10.1126/stke.2002.139.pe31
63. Kaverina I, Krylyshkina O, Small JV. Regulation of substrate adhesion dynamics during cell motility. Int J Biochem Cell Biol 2002; 34:746-61; PMID:11950592; http://dx.doi.org/10.1016/S1357-2725(01)00171-6
64. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell 2000; 100:57-70; PMID:10647931; http://dx.doi.org/10.1016/S0092-8674(00)81683-9
65. Dankort DL, Muller WJ. Signal transduction in mammary tumorigenesis: a transgenic perspective. Oncogene 2000; 19:1038-44; PMID:10713687; http://dx.doi.org/10.1038/sj.onc.1203272
66. Mansour EG, Ravdin PM, Dressler L. Prognostic factors in early breast carcinoma. Cancer 1994; 74 (Suppl):381-400; PMID:8004612; http://dx.doi.org/10.1002/cncr.2820741326
67. Nanni P, Pupa SM, Nicoletti G, De Giovanni C, Landuzzi L, Rossi I, et al. p185(neu) protein is required for tumor and anchorage-independent growth, not for cell proliferation of transgenic mammary carcinoma. Int J Cancer 2000; 87:186-94; PMID:10861472; http://dx.doi.org/10.1002/1097-0215(20000715)87:2<186::AID-IJC5>3.0.CO;2-1
68. Schechter AL, Stern DF, Vaidyanathan L, Decker SJ, Drebin JA, Greene MI, et al. The neu oncogene: an erb-B-related gene encoding a 185,000-Mr tumour antigen. Nature 1984; 312:513-6; PMID:6095109; http://dx.doi.org/10.1038/312513a0
69. Dankort D, Jeyabalan N, Jones N, Dumont DJ, Muller WJ. Multiple ErbB-2/Neu Phosphorylation Sites Mediate Transformation through Distinct Effector Proteins. J Biol Chem 2001; 276:38921-8; PMID:11500516; http://dx.doi.org/10.1074/jbc.M106239200
70. Dankort D, Maslikowski B, Warner N, Kanno N, Kim H, Wang Z, et al. Grb2 and Shc adapter proteins play distinct roles in Neu (ErbB-2)-induced mammary tumorigenesis: implications for human breast cancer. Mol Cell Biol 2001; 21:1540-51; PMID:11238891; http://dx.doi.org/10.1128/MCB.21.5.1540-1551.2001
71. Harari D, Yarden Y. Molecular mechanisms underlying ErbB2/HER2 action in breast cancer. Oncogene 2000; 19:6102-14; PMID:11156523; http://dx.doi.org/10.1038/sj.onc.1203973
72. Ménard S, Tagliabue E, Campiglio M, Pupa SM. Role of HER2 gene overexpression in breast carcinoma. J Cell Physiol 2000; 182:150-62; PMID:10623878; http://dx.doi.org/10.1002/(SICI)1097-4652(200002)182:2<150::AID-JCP3>3.0.CO;2-E
73. Khoury H, Dankort DL, Sadekova S, Naujokas MA, Muller WJ, Park M. Distinct tyrosine autophosphorylation sites mediate induction of epithelial mesenchymal like transition by an activated ErbB-2/Neu receptor. Oncogene 2001; 20:788-99; PMID:11314013; http://dx.doi.org/10.1038/sj.onc.1204166
74. Marone R, Hess D, Dankort D, Muller WJ, Hynes NE, Badache A. Memo mediates ErbB2-driven cell motility. Nat Cell Biol 2004; 6:515-22; PMID:15156151; http://dx.doi.org/10.1038/ncb1134
75. Benlimame N, He Q, Jie S, Xiao D, Xu YJ, Loignon M, et al. FAK signaling is critical for ErbB-2/ErbB-3 receptor cooperation for oncogenic transformation and invasion. J Cell Biol 2005; 171:505-16; PMID:16275754; http://dx.doi.org/10.1083/jcb.200504124
76. Roovers K, Wagner S, Storbeck CJ, O'Reilly P, Lo V, Northey JJ, et al. The Ste20-like kinase SLK is required for ErbB2-driven breast cancer cell motility. Oncogene 2009; 28:2839-48; PMID:19525980; http://dx.doi.org/10.1038/onc.2009.146
77. Storbeck CJ, Daniel K, Zhang YH, Lunde J, Scime A, Asakura A, et al. Ste20-like kinase SLK displays myofiber type specificity and is involved in C2C12 myoblast differentiation. Muscle Nerve 2004; 29:553-64; PMID:15052621; http://dx.doi.org/10.1002/mus.20000
78. Cybulsky AV, Takano T, Papillon J, Khadir A, Bijian K, Chien CC, et al. Renal expression and activity of the germinal center kinase SK2. Am J Physiol Renal Physiol 2004; 286:F16-25; PMID:12965890; http://dx.doi.org/10.1152/ajprenal.00144.2003