Molecular cloning and characterization of a novel human STE20-like kinase, hSLK

Eitaro Yamada, Kazutake Tsujikawa *, Susumu Itoh, Yo-ichiro Kameda, Yasuhiro Kohama, Hiroshi Yamamoto

Department of Immunology, Graduate School of Pharmaceutical Sciences, Osaka University, 1-6 Yamadaoka, Suita, Osaka 565-0871, Japan

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Abstract

We have cloned a human counterpart to a guinea pig STE20-like kinase cDNA, designated human SLK (hSLK), from a human lung carcinomatous cell line A549 cDNA library. hSLK cDNA encodes a novel 1204 amino acid serine/threonine kinase for which the kinase domain located at the N-terminus shares considerable homology to that of the STE20-like kinase family. The C-terminal domain of hSLK includes both the coiled-coil structure and four Pro/Glu/Ser/Thr-rich (PEST) sequences, but not the GTPase-binding domain (GBD) that is characteristic of the p21-activated kinase (PAK) family, polyproline consensus binding sites, or the Leu-rich domain seen in the group I germinal center kinases (GCKs). Northern blot analysis indicated that hSLK was ubiquitously expressed. hSLK overexpressed in COS-7 cells phosphorylates itself as well as myelin basic protein used as a substrate. On the other hand, hSLK cannot activate any of the three well-characterized mitogen-activated protein kinase MAPK (ERK, JNK/SAPK and p38) pathways. Moreover, hSLK kinase activity is not upregulated by constitutive active forms of GTPases (RasV12, RacV12 and Cdc42V12). These structural and functional properties indicate that hSLK should be considered to be a new member of group II GCKs. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Serine/threonine kinase; Signal transduction; Mitogen-activated protein kinase cascade; STE20; Group II germinal center kinase

1. Introduction

Mitogen-activated protein kinase (MAPK) cascades play a critical role in transducing information from various extracellular stimuli including growth factors and environmental stresses to the nucleus...
The MAPK cascades are composed of a specific subset of MAPKK kinases (MAPKKKs), MAPK kinases (MAPKKs) and MAPks. A MAPKKK phosphorylates conserved threonine and serine residues in a MAPKK, inducing the activation of a MAPKK. An activated MAPKK leads to the activation of a MAPK through the phosphorylation of its conserved threonine and tyrosine residues. In mammals, there are three well-characterized MAPK cascades, extracellular signal-regulated kinase (ERK) [3], c-jun N-terminal kinase/stress-activated protein kinase (JNK/SAPK) [4-6], and p38 (also known as cytokine-suppressive anti-inflammatory drug-binding protein, CSBP or reactivating kinase, RK) cascades [7,8]. The ERK cascade, a classical MAPK cascade, is activated by growth factors and tumor-promoting phorbol esters. By contrast, JNK/SAPK and p38 cascades are strongly activated in response to stressful stimuli such as heat and osmotic shock, UV light, and inhibitors of protein synthesis, but not by growth factors and phorbol esters. These kinase subsets of MAPK cascades are highly conserved from yeast to mammals.

In budding yeast Saccharomyces cerevisiae, five MAPK cascades have been identified. One of these cascades regulates spore formation [9,10]. The other four cascades operate in active cells [11]: two cascades operate in response to solute concentration (Hog and MPK pathways respond to high and low osmolarities, respectively); the remaining two cascades function in relation to mating (Fus3 pathway) and filamentation (Kss1 pathway). The Fus3 pathway is activated by the binding of peptide pheromone to cell-surface receptors in haploid a or α cells. This binding activates a trimeric G protein and in turn activates STE20 (MAPKKK), STE11 (MAPKK), STE7 (MAPKK) and Fus3 (MAPK). In the Kss1 pathway, the initiation signal is not identified; however, the same set of MAPKKKK (STE20)-MAPKK (STE11)-MAPKK (STE7)-MAPK (Kss1) pathway is required to transmit signals for filamentation. Thus, STE20 functions as an important initiation kinase in MAPK pathways of S. cerevisiae.

Recently, much attention has been directed to MAPKKKKs in mammals since many novel serine/threonine kinases related to STE20 (STE20-like kinases (SLKs)) have been identified. SLKs are divided as follows into three families based on their structure: p21-activated kinase (PAKs), pleckstrin-homology domain-containing PAKs (PH-PAKs) and germlinal center kinase (GCKs) families [12]. PAKs and PH-PAKs have a common feature to their C-terminal (CT) catalytic and N-terminal regulatory domains. The CT regulatory domain includes GTPase-binding domain (GBD) [13], also known as cell division cycle (Cdc) 42/Rac-interactive binding domain (CRIB) [14], that is necessary for direct interaction with Cdc42 or Rac. The interaction induces the activation of PAK family kinases, leading to the activation of JNK/SAPK and p38 [15-17]. At present, four isoforms of PAKs have been identified in the mammalian PAK family. PH-PAKs possess the PH domain N-terminal to the GBD. No PH-PAKs have yet been found in higher eukaryotes, although PAK-like fragments that might encode pieces of a mammalian PH-PAK seem to be registered in EST data bases [12].

The GCK family includes GCK [18], GCK-related (GCKR) [19], GCK-like kinase (GLK) [20], hematopoietic progenitor kinase-1 (HPK1) [21,22], kinase homologous to SPS1/STE20 (KHS) [23], mammalian sterile 20-like (MST) 1–3 [24–26], STE20-like oxidant stress-activated kinase-1 (SOK1) [27], Nck-interacting kinase (NIK) [28] and lymphocyte-oriented kinase (LOK) [29]. All members of this family share a similar structure including an N-terminal kinase domain and a CT long regulatory domain, but the lack of a recognizable GBD motif. The GCK family can be further divided into two subfamilies based on their structural and functional properties [30]. Group I GCKs, GCK, GCKR, GLK, HPK1, NIK and KHS, interact with MAPK/ERK kinase kinase (MEKK) 1, a MAPKKK; consequently they activate the JNK/SAPK pathway, but not ERK or p38 pathways. Structurally, the kinases of this group contain at least two Pro/Glu/Ser/Thr-rich (PEST) sequences and at least two proline-rich consensus binding sites for the Src-homology (SH)-3 domain. In addition, their CT regulatory regions possess a leucine-rich domain and a 140–150 amino acid stretch, the CT region.

Group II GCKs include MST1–3, SOK1 and LOK. These kinases have no ability to activate ERK, JNK/SAPK or p38 pathways except for MST1, which has been reported to activate JNK/
SAPK and p38 MAPK pathways [31]. Although group II GCKs share a catalytic domain homology with group I GCKs, no conserved motifs on the CT domains of the group II kinases have been found.

We have previously carried out PCR-based cDNA cloning of protein kinases expressing in eosinophils, and cloned a novel putative kinase cDNA fragment. Full-length cDNA of this protein kinase was cloned from a guinea pig liver cDNA library using the cDNA fragment as a probe. The encoded polypeptide contains a conserved serine/threonine kinase domain on its N-terminus, and the kinase domain is similar to that of STE20 [32]. In this study, we cloned human SLK (hSLK) cDNA and characterized the kinase. Structurally, hSLK contained a conserved serine/threonine kinase domain on its N-terminus as well as a CT long regulatory domain that is characteristic of GCKs. Functionally, hSLK could not activate ERK, JNK/SAPK or p38 MAPK pathways. Furthermore, hSLK was not activated by small GTPases Ras, Rac or Cdc42. Considering these properties, we concluded that hSLK is a new member of group II GCKs.

2. Materials and methods

2.1. Cell lines

The cell lines U937 (human histiocytic lymphoma), A549 (human lung carcinoma), HepG2 (human hepatocellular carcinoma) and COS-7 cells were obtained from Human Science Research Resources Bank (Osaka, Japan), and EoL1 (human eosinophilic leukemia) from Riken Cell Bank (Saitama, Japan).

2.2. Molecular cloning of hSLK cDNA

Total RNA was prepared from A549 cells by the guanidinium/cesium chloride method, and the poly(A)+-rich RNA was purified by chromatography on oligo (dT)-cellulose (New England Biolabs, Beverly, MA, USA) [33,34]. cDNA synthesis was carried out by the method of Arufo and Seed [35]. Briefly, cDNAs were synthesized using random primers (Pharmacia, Uppsala, Sweden), and a BstXI adopter (Takara Shuzo, Otsu, Japan) was ligated. The synthesized cDNAs were fractionated by potassium acetate gradient (5–20%) centrifugation. The fractions in which the length of the cDNAs was over 3 kbp were pooled and ligated into a BstXI-cleave CDMD8 vector (Invitrogen, Groningen, Netherlands). The ligated plasmid DNAs were introduced into Escherichia coli MC1061P3 cells, plated and transferred to a nitrocellulose membrane. The colony hybridization assay was performed in a solution containing 50 mM Tris-HCl (pH 8.0), 1 M NaCl, 10 mM EDTA, 0.1% sodium dodecyl sulfate (SDS), 1×Denhart’s solution, and 100 μg/ml salmon sperm DNA for 16 h at 60°C. The membrane was washed with a solution containing 450 mM NaCl, 45 mM sodium citrate, and 0.1% SDS for 30 min at 60°C. By screening approximately 4.7×10^5 colonies using the guinea pig SLK cDNA fragment as a probe, two positive clones (CDM8-51 and CDM2-40) were isolated. The inserted cDNAs were subcloned into pBluescript vector (Stratagene, La Jolla, CA, USA) and sequenced. Gap nucleotides between the two clones (nucleotides 3712–3733) were amplified by reverse transcription-polymerase chain reaction (RT-PCR) using A549 cDNA library as a template. One set of oligonucleotide primers corresponding to the 3′ sequence of clone CDM8-51 (5′-ATGCCACATTGGTTGGTCAGC-3′) and the 5′ sequence of clone CDM2-40 (5′-ATGATCCGGTGGAATGCAAG-3′) were used for the RT-PCR. The amplified cDNA was ligated into pT7Blue(R) T vector (Novagen, Madison, WI, USA) and sequenced.

2.3. Northern blot analysis

RNA tissue blots were purchased from Clontech (Palo Alto, CA, USA). Each blot contained 2 μg of poly(A)+ RNA isolated from different human tissues. Prehybridization was carried out at 42°C for 6 h in hybridization buffer containing 750 mM NaCl, 50 mM NaH₂PO₄, 5 mM EDTA, 10×Denhart’s solution, 2% SDS and 100 μg/ml denatured salmon sperm DNA. The hSLK cDNA fragment to be used for a probe (nucleotides 2874–3585) was labeled with [α-³²P]dCTP (ICN Biomedicals, Costa Mesa, CA, USA) by random priming. The blot was hybridized with the labeled probe at 42°C for 12 h in hybridization buffer, washed once in washing buffer containing 300 mM NaCl, 30 mM sodium citrate, 0.05% SDS, and washed again in another washing
buffer containing 150 mM NaCl, 15 mM sodium citrate and 0.1% SDS for 1 h at 50°C. The blot was exposed on an X-ray film for 4 days at −80°C.

2.4. Plasmids

The hemagglutinin antigen (HA) epitope-tagged hSLK expression vector (pEF HA-hSLK) was constructed by adding a sequence that encodes two copies of 10 amino acid HA epitope (MYPYDVPDYA) [36] to the N-terminus of hSLK and ligating the cDNA into pEF [37]. The kinase-negative mutant of hSLK (hSLK K63R), in which the Lys-63 required for ATP-binding is replaced with Arg, was constructed by the oligonucleotide-directed mutagenesis method [38]. To construct the expression vectors for HA-hSLKΔ592–971, HA-tagged full-length hSLK cDNA was digested with NcoI/XhoI. A 5′ NeoI fragment and an XhoI 3′ fragment were ligated using suitable oligonucleotides (5′-CATGGTTGGGTGC-3′ and 5′-TCGAGCACCCAC-3′), and subcloned into pEF. To construct the expression vectors for HA-hSLKΔ582–1204 mutants, HA-tagged full-length hSLK cDNA was digested with NcoI or XhoI, respectively, and then 5′ fragments were subcloned into pEF. The expression vectors for HA-tagged MAPKs (pSRα3 HA-ERK2, pSRα3 HA-JNK1 and pSRα3 HA-p38) and for constitutive active forms of GTPases (pSRα3 RasV12, pSRα3 RacV12 and pCMV5 Cdc42V12) were donated by Dr. Michael Karin (University of California, San Diego, CA, USA) [39,40].

2.5. In vitro kinase assay

In the assay for hSLK kinase activity, the expression vectors for either HA-hSLK WT, HA-hSLK K63R, HA-hSLKΔ591–972, HA-hSLKΔ975–1204 or HA-hSLKΔ591–1204 mutants, HA-tagged full-length hSLK cDNA was digested with NcoI or XhoI, respectively, and then 5′ fragments were subcloned into pEF. The expression vectors for HA-tagged MAPKs (pSRα3 HA-ERK2, pSRα3 HA-JNK1 and pSRα3 HA-p38) and for constitutive active forms of GTPases (pSRα3 RasV12, pSRα3 RacV12 and pCMV5 Cdc42V12) were donated by Dr. Michael Karin (University of California, San Diego, CA, USA) [39,40].

2.6. Western blot

To examine the MAPK activation in response to the overexpression of hSLK, COS-7 cells were cotransfected with pEF HA-hSLK and pSRα HA-MAPKs (ERK, JNK or p38). The cell lysates were separated by SDS–polyacrylamide gel electrophoresis (PAGE). After drying, the gel was subjected to autoradiography.
3. Results

3.1. Cloning and sequencing of the hSLK

Before the construction of the cDNA library, we assessed the expression levels of hSLK mRNA in EoL1, U937, A549 and HepG2 cell lines by Northern blot analysis using a guinea pig SLK cDNA as a probe. Since hSLK mRNA was abundantly expressed in a human lung carcinomatous cell line A549 (data not shown), we prepared and screened the A549 cDNA library using the guinea pig SLK cDNA fragment as a probe. Two positive clones (3.7 kbp and 2.1 kbp) were identified. A homology

Fig. 1. Nucleotide and predicted amino acid sequences of hSLK. A: The conserved serine/threonine kinase domain is boxed, and the ATP-binding site is circled. Potential PEST sequences are double-underlined, and the region of the coiled-coil structure is shaded. The stop codon (TGA) 171 bp 5' of the putative initiation codon is underlined, and the polyadenylation signal in the 3' untranslated region is dotted. Numbering of the nucleotide and amino acid positions is indicated on the left. B: The schematic diagram illustrating the domain structure of hSLK. The serine/threonine kinase domain is drawn as a hatched box. Potential PEST sequences are drawn as solid boxes, and the region of the coiled-coil structure is drawn as a shaded box.

Fig. 2. Alignment of the catalytic domain of hSLK with that of other STE20 family members. The predicted amino acid sequence of the kinase domain of hSLK was aligned with the corresponding domains of hLOK, hMST1, hSOK1, hGCK, hHPK1, hKHS, mNIK, hPAK1 and STE20. The 11 subdomains conserved in protein kinases [41] are indicated in Roman numbers above the sequences. The identical sequences among at least five proteins are highlighted with solid boxes.

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search against guinea pig SLK showed that the 3.7 kbp and 2.1 kbp fragments included initiation and termination codons, respectively. Since these two fragments did not overlap each other, we determined the gap nucleotide sequence between these two clones (nucleotides 3713–3734) by the RT-PCR method. As shown in Fig. 1, the complete nucleotide sequence of hSLK cDNA predicted an open reading frame of 1204 amino acids with a calculated molecular mass of 138,993 Da. The deduced amino acid sequence contained the conserved serine/threonine kinase domain [41] on its N-terminus. In the kinase domain, hSLK displays a similarity to other SLKs with about 40% identity (46.1% identical to hGCK, 41.0% to hHPK1, 45.3% to hKHS, 45.1% to hMST1, 44.3% to hSOK1, 41.9% to hPAK1, 42.9% to mNIK and 39.4% to yeast STE20) (Fig. 2). hSLK is most similar to hLOK, which belongs to the SLK family, and the kinase domain of hSLK is 77.2% identical to that of hLOK. Furthermore, it is notable that the CT domain of hSLK is 43.9% identical to that of hLOK. Since the CT domain of hLOK includes the coiled-coil structure, we attempted to determine whether hSLK also contains the structure using ‘COILS’, a program that predicts for the coiled-coil region [42]. This examination showed that hSLK possesses the region of the coiled-coil structure on its C-terminus (amino acids 827–1168) (Fig. 1A,B). Since some members of the GCK family have the PEST sequence, we confirmed the existence of the sequence using a program called PESTfind [43,44]. Interestingly, hSLK was found to have four PEST sequences between its kinase domain and the region of the coiled-coil structure.

3.2. Tissue distribution of hSLK mRNA

Northern blot analysis of human tissue mRNA using the CT regulatory domain of hSLK (nucleotides 2874–3585) as a probe indicated the existence of three distinct transcripts (8.5, 7.0 and 5.0 kbp) and the most abundant expression of one 7.0 kbp transcript in all tissues. The highest levels of expression for these transcripts were detected in the heart and the skeletal muscle, with intermediate levels being detected in the placenta, pancreas, prostate, testis, uterus and small intestine (Fig. 3).

3.3. Kinase activity of hSLK

To investigate the kinase activity of hSLK, we constructed five expression vectors for HA epitope-tagged wild-type and mutated hSLKs, as shown in Fig. 4A. hSLK K63R, a kinase-dead mutant, was constructed by changing the 63rd lysine of the ATP-binding site to arginine. hSLK can be divided into three regions based on the similarities to guinea pig SLK: an N-terminal highly homologous region including the kinase domain (amino acids 1–548, 84.5% identical to guinea pig SLK), a CT highly homologous region (amino acids 733–1204, 91.7%) and an intermediate region with relatively low homology (amino acids 549–732, 43.7% identical). We hypothesized that the CT highly homologous region...
may play an important role in regulating the kinase activity of hSLK. To investigate which part of this region would regulate this kinase activity, we constructed expression vectors for three distinct hSLK deletion mutants (Δ592–971, Δ976–1204 and Δ592–1204; Fig. 4A) by dividing the CT highly homologous region into two parts at an XhoI site. To con-
firm the expression of hSLK mutants, COS-7 cells were transfected with each expression vector. Cell lysates were separated on SDS–PAGE and immunoblotted with anti-HA mAb (12CA5). As shown in Fig. 4B, clear single bands derived from transfected cDNA were detected in each lane. In the transfect-
tants of full-length hSLK cDNA (hSLK WT and K63R), the expressed proteins were detected at approximately 200 kDa. Since the calculated molecular mass of hSLK protein is approximately 140 kDa, the difference in molecular mass might be due to post-translational modification.

The expressed HA-tagged hSLK proteins were immunoprecipitated with anti-HA mAb, and an in vitro kinase assay was then performed by incubating these immunocomplexes using MBP as a substrate. hSLK WT markedly phosphorylated MBP, but hSLK K63R did not. Moreover, an approximately 200 kDa phosphorylated band was detected in hSLK WT immunoprecipitates. These results indicate that hSLK had not only the kinase activity for the sub-
strate but also autophosphorylation activity. Compared with hSLK WT, no remarkable changes in kinase activity for MBP and autophosphorylation levels were observed in the three deletion mutants (Fig. 4C).

3.4. Overexpression of hSLK could not activate MAPKs

In mammals, three MAPK cascades have been well-characterized. One is the ERK pathway, a classical MAPK cascade, that responds to growth fac-

Fig. 4. Kinase activity of hSLK expressed in COS-7 cells. A: a schematic diagram of hSLK and its mutants. Deletions in the C-terminus of hSLK were performed by using unique restriction enzyme sites as described in Section 2. hSLK K63R is a kinase inactive mutant, in which the critical lysine (amino acid 63) re-
quired for ATP-binding has been changed to an arginine. The kinase domains are drawn as hatched boxes. COS-7 cells were transfected with pEF HA (vector), wild-type HA-tagged hSLK (WT) or hSLK mutants ligated into a pEF HA vector (K63R, Δ592–971, Δ976–1204 and Δ592–1204). B: Cell lysates were sub-
jected to SDS–PAGE and immunoblotted with anti-HA mAb. C: After immunoprecipita-
tions, an in vitro kinase assay was performed using [γ-32P]ATP and MBP as a substrate. The reaction products were separated by SDS–PAGE and subjected to autoradiography.
The other two MAPKs, JNK/SAPK and p38, are activated by stressful stimuli such as anisomycin and NaCl. Many of the SLKs such as PAKs, GCK, HPK1, NIK, KHS and MST1 have been shown to activate JNK/SAPK and/or p38 MAPK cascades. We attempted to determine which of the three MAPKs would be activated by hSLK. HA-tagged hSLK or its mutants were co-transfected to COS-7 cells with HA-tagged ERK, JNK/SAPK or p38. The activation of these MAPKs was analyzed by immunoblot with anti-active ERK (Fig. 5A), anti-active JNK/SAPK (Fig. 5B) or anti-active p38 (Fig. 5C) antibodies. hSLK WT and its deletion mutants were not able to activate any of these MAPKs. In contrast, when COS-7 cells were transfected with wild-type hSLK (WT) or its mutants as described in Fig. 4A in the presence of pSRα HA-ERK (A), pSRα HA-JNK (B) and pSRα HA-p38 (C). The cell lysates were separated by SDS-PAGE and immunoblotted with anti-active MAPK, anti-active JNK or anti-active p38 pAbs (upper panel). The expression levels of ERK were checked by reprobing the same blot with anti-HA mAb (lower panel). As a positive control, the cell lysates of pSRα HA-ERK-, pSRα HA-JNK- and pSRα HA-p38-transfected COS-7 cells stimulated with EGF (A), anisomycin (B) and NaCl (C), respectively, were immunoblotted with anti-active MAPK antibodies.

3.5. Active forms of Ras, Rac1 and Cdc42 are not the up-stream regulators of hSLK

In yeast, STE20 is activated by an active form of Cdc42p. Similarly, PAK family kinases (PAKs 1–3)
have been reported to interact with and be activated by active forms of Rac1 and Cdc42 [45-47]. Therefore, we examined whether Ras, Rac1 or Cdc42 could increase the kinase activity of hSLK. Expression vectors for constitutive active forms of small G proteins (RasV12, Rac1V12 or Cdc42V12) were co-transfected with HA-tagged hSLK into COS-7 cells. Total cell lysates were immunoprecipitated with anti-HA mAb, and subjected to an in vitro kinase assay using MBP as a substrate. No changes in the phosphorylation levels of hSLK and MBP were observed, even though constitutive active forms of GTPases were co-transfected (Fig. 6A,B). In hSLK K63R transfectants, phosphorylated bands were not detected at all. In order to confirm the GTPase activity of the small G proteins, RasV12 or Rac1V12/Cdc42V12 were co-transfected with HA-tagged ERK or JNK, respectively, and the cell lysates were immunoblotted with anti-active MAPK or anti-active JNK pAbs (upper panels). The expression levels of ERK and JNK were checked by reprobing the same blot with anti-HA mAb (lower panels).

Fig. 6. No activation of hSLK by constitutive active forms of GTPases. A and B: Constitutive active forms of GTPases (RasV12, Rac1V12 or Cdc42V12) were co-transfected with HA-tagged hSLK WT (WT) or the hSLK K63R (K63R) described in Fig. 1A into COS-7 cells. The cell lysates were immunoprecipitated with anti-HA mAb, and subjected to an in vitro kinase assay using MBP as a substrate, as described in Fig. 4C. HA-tagged ERK was co-transfected with RasV12, a constitutive active form of Ras (left panels). HA-tagged JNK was co-transfected with Rac1V12 or Cdc42V12, constitutive active forms of Rac1 or Cdc42, respectively (right panels). The cell lysates were immunoblotted with anti-active MAPK or anti-active JNK pAbs (upper panels). The expression levels of ERK and JNK were checked by reprobing the same blot with anti-HA mAb (lower panels).

4. Discussion

In this study, we were able to clone a novel human serine/threonine protein kinase, hSLK. Because the kinase domain of hSLK located on its N-terminus is similar to that of STE20 (a MAPKKKK of
we characterized hSLK both structurally and functionally.

In recent years, many SLKs have been cloned. Based on their characteristics, they can be classified into three families: PAK, PH-PAK and GCK families [12]. In these families, only PH-PAK family members have not yet been found in mammals. All PAK and PH-PAK homologues possess conserved serine/threonine kinase domains on the C-terminus and GBDs on the N-terminus. Until now, four human PAKs (PAKs 1–4) have been identified [45–47,50]. These PAKs were found to interact with Cdc42 and Rac except for PAK4, which interacts with Cdc42 but not with Rac. In addition, PAKs 1–3 activate JNK/SAPK and p38 pathways, and PAK4 activates the JNK/SAPK pathway. hSLK does not belong to PAK nor PH-PAK families for the following reasons: (1) hSLK has a kinase domain on its N-terminus and no GBD domain; (2) co-transfection of hSLK with constitutive active forms of GTPases (RasV12, RacV12 and Cdc42V12) does not activate the hSLK kinase activity; and (3) hSLK does not activate ERK, JNK/SAPK or p38 pathways.

Members of the GCK family have a common feature in the localization of the kinase domain on their N-terminus. Thus, based on its domain structure, hSLK belongs to the GCK family. The GCK family can be subdivided into two groups based on the structural and functional properties of its members [30]. Group I GCKs are closely related to GCK itself and include GCK, GCKR, GLK, HPK1 and NIK. All members of this group interact with MEKK1, a MAPKKK, and consequently activate the JNK/SAPK pathway [19–21,27,51]. Structurally, group I GCKs contain at least two PEST sequences: GCK, three; GCKR, GLK, HPK1 and NIK, two; and MST1, LOK and SOK, only one. In hSLK, four potential PEST sequences were found between the N-terminal kinase domain and the CT coiled-coil region. Since hSLK is a constitutive active kinase in transfected COS-7 cells, the kinase activity may be regulated in part by a short half-life due to the PEST domain.

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To determine which stimulations activate hSLK, the COS-7 cells transfected with wild-type hSLK were stimulated with growth factors, hyperosmolarity, H₂O₂, anisomycin or UV, and the kinase activity of hSLK was examined by an in vitro kinase assay.
(data not shown). However, autophosphorylation levels were increased in none of the cells, and nor was the kinase activity of hSLK. We have previously reported that the autophosphorylation of guinea pig SLK expressed in COS-7 cells is increased by the stimulation of anisomycin in vivo [32]. The discrepancy between in vivo and in vitro examinations might be due to the high basal kinase activity of hSLK. Since wild-type hSLK and its deletion mutants already express quite high levels of kinase activity in transfected COS-7 cells, this expression may have obscured the difference in the kinase activity. These results seem to be a common feature of GCKs.

The biological function of group II GCKs including hSLK remains to be identified. Progress towards clarifying the physiological function might be made possible by identifying a molecule that binds to the PEST motif and/or the coiled-coil structure. An examination of the importance of these motifs in hSLK is now in progress.

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