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Identification of functional nuclear export sequences in human sphingosine kinase 1☆

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Abstract

Sphingosine kinase (SPHK) is an enzyme that phosphorylates sphingosine to form sphingosine 1-phosphate (S1P). Human SPHK1 (hSPHK1) was localized predominantly in the cytoplasm when transiently expressed in Cos7 cells. In this study, we have found two functional nuclear export signal (NES) sequences in the middle region of hSPHK1. Deletion and mutagenesis studies revealed that the cytoplasmic localization of SPHK1 depends on its nuclear export, directed by the NES. Furthermore, upon treatment with leptomycin B, a specific inhibitor of the nuclear export receptor CRM1, a marked nuclear accumulation of hSPHK1 was observed, indicating that hSPHK1 shuttles between the cytoplasm and the nucleus. Our results provide the first evidence of the active nuclear export of SPHK1 and suggest it is mediated by a CRM1-dependent pathway.

Keywords: Sphingosine 1-phosphate; Sphingosine; Sphingosine kinase; Nuclear export signal

The sphingolipid metabolites ceramide, sphingosine, and sphingosine 1-phosphate (S1P) play important roles in the regulation of cell proliferation, survival, and apoptosis [1–3]. S1P is a bioactive lipid mediator that regulates diverse biological effects and signaling pathways. S1P has been shown to act both as an intracellular messenger and an extracellular ligand for the S1P/Edg family of G protein-coupled receptors (GPCR) [4–6]. Recent studies have shown that intracellular S1P regulates apoptosis [7], calcium mobilization [8,9], inflammatory responses [10], and cell differentiation [11]. However, intracellular targets of S1P have not yet been identified.

Sphingosine kinase (SPHK) is an enzyme that phosphorylates sphingosine to form S1P. The distribution of SPHK1 activity varies among cell types and tissues [12,13]. Many growth and survival factors, including platelet-derived growth factor (PDGF) [14], nerve growth factor (NGF) [15], epidermal growth factor (EGF) [16], and vascular endothelial growth factor (VEGF) [17], reportedly increase SPHK activity. To date, two isoforms of mammalian SPHK (SPHK1 and SPHK2) have been identified [18,19], and the existence of other isoforms has been suggested [13]. SPHK1 is predominantly localized in the cytoplasm [7]. In contrast, SPHK2 is localized in the nucleus, due to its nuclear localization signal (NLS) sequence [20].

Interestingly, although SPHK1 is predominantly localized in the cytoplasm, PDGF induces the translocation of SPHK1 to the nucleus after long-term stimulation [21]. This observation suggests that mechanisms exist for shuttling SPHK1 between the cytoplasm and the nucleus, and that growth and survival factors might regulate this action. So far, however, little is known about the mechanism that regulates the localization of SPHK1. In this study, we have found two functional nuclear export signal (NES) sequences in the middle region of hSPHK1. NES sequences are short

☆ *Abbreviations:* SPHK, sphingosine kinase; S1P, sphingosine 1-phosphate; NES, nuclear export signal; NLS, nuclear localization signal; CRM1, chromosomal region maintenance 1; LMB, leptomycin B; PCR, polymerase chain reaction; PBS, phosphate-buffered saline; GFP, green fluorescent protein.

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sequence motifs which mediate the nuclear export of large carrier proteins. The hydrophobic NES sequence is specifically recognized by the protein chromosomal region maintenance 1 (CRM1), which enables a carrier protein to exit through the nuclear pore [22,23]. Our results suggest that the predominantly cytoplasmic localization of SPHK1 is dependent on its nuclear export, directed by NES.

Materials and methods

Materials. Leptomycin B was a kind gift from Dr. M. Yoshida (RIKEN, Discovery Research Institute, Japan). Anti-HA Y-11 polyclonal antibody was purchased from Santa Cruz Biotechnology and the anti-HA HA-7 monoclonal antibody was from Sigma. Alexa 488-conjugated anti-rabbit IgG and Alexa 488-conjugated anti-mouse IgG were from Molecular Probes. DAPI was from Boehringer–Mannheim. Bovine serum albumin (BSA, fraction V) was from Sigma (St. Louis, MO). All other reagents were of the highest purity available.

Cell culture and DNA transfection. Cos7 and HeLa cells were grown in Dulbecco's modified Eagle's medium containing 1000 mg/L glucose (Sigma), 10% (v/v) fetal bovine serum (Asahi techno glass, Funabashi, Japan), 2 mM glutamine, 100 U/ml penicillin, and 10 μg/ml streptomycin, at 37 °C, in a humidified atmosphere of 5% carbon dioxide. Cells were transfected using 6 μl LIPOFECTAMINE-PLUS and 4 μl LIPOFECTAMINE (Invitrogen) according to the manufacturer's instructions. Plasmids used in the transfection were prepared with Quantum Prep Plasmid Miniprep Kit (BIO-RAD) according to the manufacturer's instructions.

DNA construction. pcDNA3-HA, a derivative of pcDNA3 (Invitrogen), was constructed to create an N-terminal hemagglutinin (HA)-tagged gene [24]. The following primers were used to amplify deletion mutants of HA-hSPHK1: for pcDNA3HA-hSPHK1-ΔN141, 5′-CGGGATCCCCGGCTGCTGTCACCCATGAAC-3′ and 5′-GGGAATTCATAAGGGCTCTTCTGGCGGTG-3′; for pcDNA3HA-hSPHK1-ΔN154, oligonucleotides 5′-TGGGATCCGGCTTCGGGGCTGCGCCTCTTC-3′ and 5′-GGGAATTCATAAGGGCTCTTCTGGCGGTG-3′; for pcDNA3HA-hSPHK1-ΔN164, oligonucleotides 5′-CGGGATCCCAGCCTGGCCTGGGGCTTCATT-3′ and 5′-GGGAATTCATAAGGGCTCTTCTGGCGGTG-3′; for pcDNA3HA-hSPHK1-ΔN176, oligonucleotides 5′-CGGGATCCAGAGAGTGAGAAGTATCGGCGT-3′ and 5′-GGGAATTCATAAGGGCTCTTCTGGCGGTG-3′; and for pcDNA3HA-hSPHK1-ΔN205, oligonucleotides 5′-CGGGATCCCCGACTGGCCTACCTCCCTGTA-3′ and 5′-GGGAATTCATAAGGGCTCTTCTGGCGGTG-3′. All oligonucleotides were synthesized with *Bam*HI and *Eco*RI ends. The antisense primers were treated with T4 polynucleotide kinase (Takara, Ohtsu, Japan). The PCR was carried out with Pfu turbo (Stratagene) in the presence of 0.2 mM dNTPs, 1 mM $MgCl_2$, and 0.3 μM polynucleotide kinase-treated primers for 25 cycles. The PCR products were ligated by Ligation High (Toyobo, Osaka, Japan). After *Bam*HI–*Eco*RI digestion, the fragment was ligated into a *Bam*HI–*Eco*RI-digested pcDNA3-HA vector. pcDNA3HA-hSPHK1-disNES1 and pcDNA3-HA-hSPHK1-disNES2 were constructed by introducing two point mutations. For the first point mutation, oligonucleotides 5′-CACACGGCTTCGGGGCTGCGC-3′ and 5′-CGCAGACAGCAGGTTCATGGGTGACAG-3′ (disNES1) and oligonucleotides 5′-GTGCTCAGCGCGGCCTGGGGCTTC-3′ and 5′-AGAGAAGAGGCGCAGCCCCGAAGC-3′ (disNES2) were used. The PCR product was used as a template for a second PCR. For the second point mutation, oligonucleotides 5′-CTGCTGTCACCCATGAACCTGGCGTCT-3′ and 5′-CCGGCGGCACAGCAATAGCGTGCAG-3′ (disNES1) and oligonucleotides 5′-GTGGCCAGCGCGGCCTGGGGCTTC-3′ and 5′-AGAGAAGAGGCGCAGCCCCGAAGC-3′ (disNES2) were used. For pcDNA3HA-hSPHK1-ΔNES, oligonucleotides 5′-GCCTGGGGCTTCATTGCTGATGTGGACC-3′ and 5′-CCGGCGGCACAGCAATAGCGTG CAG-3′ were used. The PCR and ligation were performed as described above. For pcDNA3HA-hSphK1-NESmut, oligonucleotides (5′-GCGGCGTCACCCATGAACGCGGCGTCTGCGCACACGGCTTCGGGGGCGCGCGCCTTCTCTGTGGCCAGCGCG-3′ and 5′-CGCGCTGGCCACAGAGAAGGCGCGCGCCCCCGAAGCCGTGTGCGCAGACGCCGCGTTCATGGGTGACGCCGC-3′) were annealed. Annealed oligonucleotides were treated with T4 polynucleotide kinase and ligated with the PCR product for pcDNA3HA-hSPHK1-ΔNES. For the expression of GFP fusion proteins (shown in Fig. 2), we employed the pEGFP-C1 vector (Clontech). Oligonucleotides 5′-GATCCCGGCTGCTGTCACCCATGAACCTGCTGTCTCTGCACG-3′ and 5′-AATTCGTGCAGAGACAGCAGGTTCATGGGTGACAGCAGCCGG-3′, encoding NES1; 5′-GATCCCGGCTGCTGT CACCCATGAACCTGGCGTCTGCGCACG-3′ and 5′-AATTCGTGCGCAGACGCCAGGTTCATGGGTGACAGCAGCCGG-3′, encoding disNES1 (L153A/L155A); 5′-GATCCTCGGGGCTGCGCCTCTTCTCTGTGCTCAGCCTGGCCG-3′ and 5′-AATTCGGCCAGGCTGAGCACAGAGAAGAGGCGCAGCCCCGAG-3′, encoding NES2; and 5′-GATCCTCGGGGCTGCGCCTCTTCTCTGGGCCAGCGCGGCCG-3′ and 5′-AATTCGGCCGCGCTGGCCACAGAGAAGAGGCGCAGCCCCGAG-3′, encoding disNES2 (L167A/L169A) were synthesized with *Bam*HI and *Eco*RI ends. Annealed oligonucleotides were ligated into *Bam*HI–*Eco*RI-digested pEGFP-C1 vectors.

Immunofluorescence microscopy. Immunohistochemistry was performed essentially as described previously [24]. The cells were washed twice with PBS, fixed for 15 min in 3.7% formaldehyde in PBS, washed three times with PBS, and permeabilized in 0.2% Triton X-100 in PBS for 5 min. Blocking was performed with PBS containing 10% BSA for 30 min at room temperature. Primary antibodies were anti-HA Y-11 polyclonal antibody (Santa Cruz Biotechnology) and anti-HA HA-7 monoclonal antibody (Sigma) that were used at 1:100 dilutions. After a 1 h incubation with the primary antibody, the cells were washed three times with PBS and incubated for 30 min with Alexa 488-conjugated anti-rabbit IgG (Molecular Probes) or Alexa 488-conjugated anti-mouse IgG (Molecular Probes), each diluted 1:300 in BSA/PBS. The cells were washed three times with PBS for 5 min each and then the coverslips were rinsed in water and mounted onto glass slides using Mowiol 4-88 (Calbiochem, San Diego, CA). Images of the stained cells were analyzed by fluorescence microscopy (Zeiss AxioSkop2) or confocal microscopy (Zeiss LSM510).

Results

A specific region of hSPHK1 is required for its cytoplasmic localization in Cos7 cells

We analyzed the mechanisms controlling the cytoplasmic localization of hSPHK1 in Cos7 cells. To determine which region of the protein is required for the cytoplasmic localization of hSPHK1, N-terminal deletion mutants of hSPHK1 were used (Fig. 1A). Expression vectors encoding N-terminally HA-tagged forms of hSPHK1 (HA-hSPHK1) or the deletion mutants were transfected into Cos7 cells, and their subcellular localization was analyzed by fluorescence microscopy (Fig. 1B). HA-hSPHK1, HA-hSPHK1 ΔN141, and HA-hSPHK1 ΔN154 were found to be predominantly localized in the cytoplasm, whereas HA-hSPHK1 ΔN164, HA-hSPHK1 ΔN176, and HA-hSPHK1 ΔN205

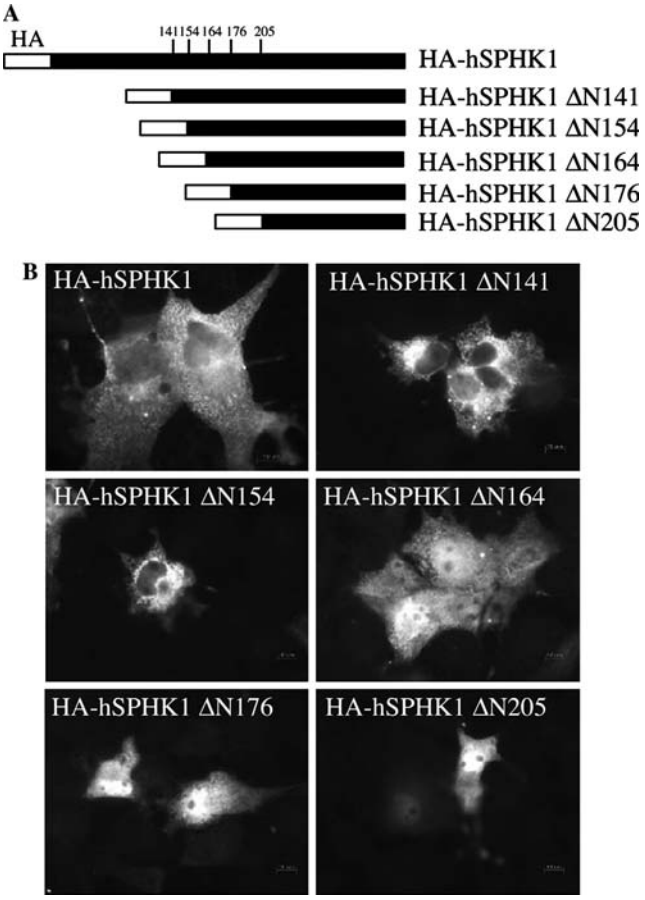

Fig. 1. Domains of SPHK1 involved in its cytoplasmic localization in Cos7 cells. (A) SPHK1 deletion mutants. Vectors expressing the indicated HA-tagged SPHK1 deletion mutants were constructed as described in the Materials and methods. (B) Subcellular localization of deletion mutants in Cos7 cells. Vectors expressing HA-tagged SPHK1 deletion mutants were transiently transfected in Cos7 cells. Twenty-four hours after transfection, cells were fixed and stained with anti-HA antibody (Y-11) and Alexa 488 goat anti-rabbit IgG and then visualized by immunofluoresence microscopy.

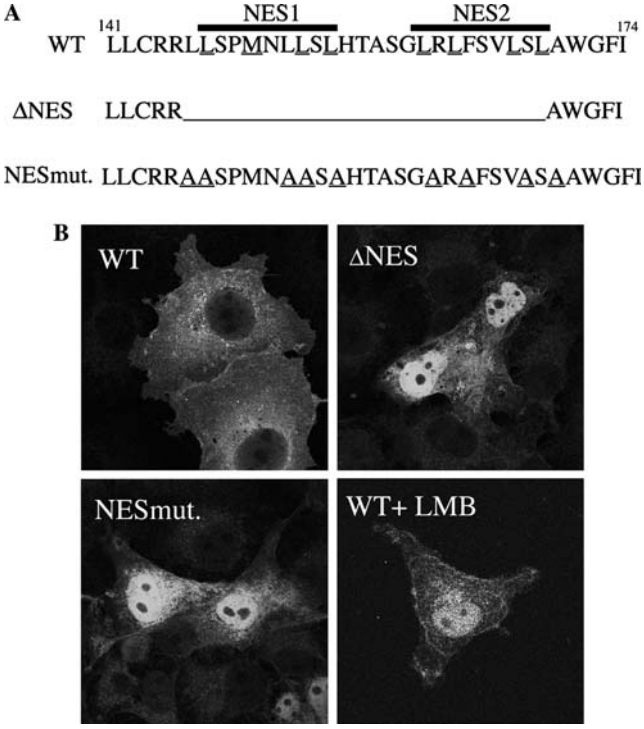

Fig. 2. The cytoplasmic localization of hSPHK1 depends on its CRM1/NES-mediated nuclear export. (A) Putative NES sequences in SPHK1. (B) Effect of deletions or mutations in the putative NES on the subcellular localization of HA-hSPHK1. Wild type (WT), deleted (ΔNES) or mutated forms (NESmut.) of HA-hSPHK1 were transfected in Cos7 cells. The effect of LMB was also analyzed. Cos7 cells were transfected with HA-hSPHK1 and treated with 20 ng/ml leptomycin B for 16 h. Twenty-four hours after transfection, the cells were fixed and stained with anti-HA antibody (HA-7). HA-hSPHK1 was visualized by confocal microscopy.

were observed in the nucleus. These results suggest that amino acid residues 155–164 are required for the cytoplasmic localization of hSPHK1.

Two NES sequences in hSPHK1

The results described above led us to speculate on the presence of a leucine-rich NES sequence in hSPHK between amino acid residues 155–164. Upon examination of the sequence at this region, we found two putative NES sequences (Fig. 2A) that fit the consensus Z-X_{1-4}-J-X_{2-3}-Z-X-Z, where Z is Leu, Ile, or Val; J is Z, Met, or Phe; and X is any amino acid [25,26]. To examine whether hSPHK1 contains one or more functional NES, the putative NES sequences of HA-hSPHK1 were deleted and all leucine residues in the sequence were mutated to alanines (Fig. 2A). Expression vectors encoding the HA-tagged forms of NES-deleted hSPHK1 (ΔNES) or mutated hSPHK1 (NESmut.) were transfected into Cos7 cells and their subcellular localization was analyzed by confocal microscopy (Fig. 2B). Consistent with previous reports [7,12,20,21], HA-hSPHK1 (WT) was predominantly localized in the cytoplasm. In contrast to the wild type, the deleted (ΔNES) and mutated forms (NESmut.) were predominantly localized in the nucleus (Fig. 2B). These results indicate that the cytoplasmic localization of hSPHK1 is dependent on the NES sequences.

CRM1-dependent nuclear export of hSPHK1

To examine whether CRM1 is responsible for the nuclear export of hSPHK1, we used leptomycin B (LMB), a specific inhibitor of CRM1. Binding of NES to the exportin CRM1 is inhibited by LMB and, consequently, the nuclear export of proteins that use the CRM1 pathway is blocked [27,28]. Treatment of HA-hSPHK1-transfected Cos7 cells with LMB caused a translocation of hSPHK1 from the cytoplasm to the nucleus (Fig. 2B). This result indicates that binding of

NES sequences to CRM1 mediates nuclear export of hSPHK1.

Both NES1 and NES2 mediate the cytoplasmic localization of hSPHK1

To confirm that NES1 and NES2 behave as nuclear export signal, we first studied their ability to export hSPHK1. Previous studies have determined that mutations in both the third and fourth hydrophobic residues in NES abolish the nuclear export activity [22,23,25,29]. Thus, we constructed NES1-disrupted hSPHK1 (disNES1-hSPHK1) (L153A/L155A) and NES2-disrupted hSPHK1 (disNES2-hSPHK1) (L167A/L169A) (Fig. 3A). HA-tagged forms of these mutants or of wild type hSPHK1 were transfected into Cos7 cells and their subcellular localization was analyzed by immunohistochemistry. Wild type hSPHK1 (WT) was predominantly localized in the cytoplasm (Fig. 3B). The deleted and mutated forms of hSPHK1, both of which lack NES1 and NES2, were found predominantly in the nucleus (Fig. 2B). In contrast, disNES1-hSPHK1 and disNES2-hSPHK1 were only partially localized in the nucleus (Fig. 3B). Taken together these results imply that together, NES1 and NES2 cooperatively mediate the nuclear export of hSPHK1.

We next tested and compared the export activities of NES1 and NES2. GFP fusion proteins containing the NES or NES-disrupted sequence (shown in Fig. 3C) were transfected into HeLa cells and analyzed by immunohistochemistry. We counted more than 100 cells in each transfection. Although wild type GFP localized throughout the cell (data not shown), GFP-NES1 and GFP-NES2 preferentially localized to the cytoplasm (Fig. 3D). As predicted, localization of GFP-disNES1 and GFP-disNES2 was similar to that of wild type GFP (Fig. 3D). Although NES1 seems to be more active than NES2 (Fig. 3D), these results demonstrate that both NES1 and NES2 are functional nuclear export sequences.

Discussion

In this study, we identified two functional NES sequences in hSPHK1. Deletion and mutagenesis studies revealed that the cytoplasmic localization of SPHK1 depends on its nuclear export, directed by the NES. LMB treatment induced SPHK1 accumulation in the nuclei. Thus, a CRM1-dependent pathway might mediate the nuclear export of SPHK1. Although we could not predict a distinct nuclear localization signal (NLS) in hSPHK1 using database searches (http://ca.expasy.org/prosite/, http://cubic.bioc.columbia.edu/predictNLS/ [30,31]), these results also suggest that there are mechanisms for importing hSPHK1 into the nuclei, besides a simple diffusion.

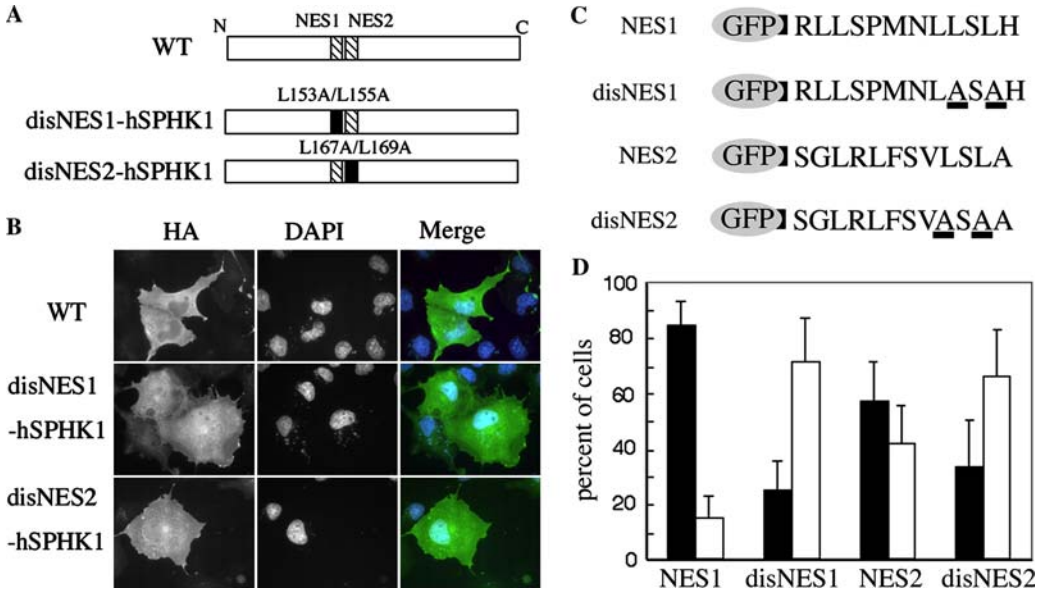

Fig. 3. Two NES sequences of SPHK1 are functional. (A) Vectors expressing NES-disrupted HA-hSPHK1 were constructed. The dark boxes indicate the disrupted positions of NES. (B) Expression of NES-disrupted hSPHK1. NES1-disrupted HA-hSPHK1 (disNES1-hSPHK1) and NES2-disrupted HA-hSPHK1 (disNES2-hSPHK1) were transfected into Cos7 cells and analyzed by immunohistochemistry. Nuclear DNA was stained with DAPI. (C) Vectors expressing the indicated NES sequence-fused GFP were constructed. (D) Quantification of the subcellular localization profiles of the NES sequence-fused GFP. NES sequence-fused GFP were transfected into HeLa cells and 24 h after the transfection, GFP fusion proteins were visualized directly. Cells displaying cytoplasmic localization of GFP were scored as nuclear export-positive (closed bar). An approximate equilibration of signal between the cytoplasm and the nucleus, and any obvious nuclear accumulation were both scored as nuclear export-negative (open bar). More than 100 cells were counted from each transfection. The mean and standard error from three independent experiments are shown.

It has been shown that PDGF can induce the nuclear localization of SPHK1 [21]. Furthermore, we observed cell-specific nuclear localization of SPHK1 (Kihara, A., et al., unpublished observation). In Cos7, CHO (Chinese hamster ovary), and HeLa cells, SPHK1 was predominantly localized in the cytoplasm, whereas in mouse embryonal carcinoma F9 cells, SPHK1 was observed throughout the cell. These observations suggest that nucleocytoplasmic translocation of SPHK1 is a physiological phenomenon.

Although physiological functions for the nucleocytoplasmic translocation of SPHK1 are not well known, a recent study provides some insight [20]. In that study, NLS-fused SPHK1 was used as a model for nuclear-localized SPHK1. NLS-SPHK1 was found to be localized in the nucleus and to inhibit DNA synthesis. Although it is also unknown whether the SPHK1 substrate, sphingosine, is present in the nuclear membrane, it is of interest to investigate the physiological functions of the nuclear translocation of SPHK1.

Our identification of NES sequences in hSPHK1 provides critical insight into the nucleocytoplasmic shuttling of this kinase. The identification of mechanisms responsible for inducing nuclear localization of SPHK1 and of the physiological functions of the nuclear-localized kinase remains to be investigated.

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References

[1] Y. Igarashi, Functional roles of sphingosine, sphingosine 1-phosphate, and methylsphingosines: in regard to membrane sphingolipid signaling pathways, J. Biochem. 122 (1997) 1080–1087.

[2] Y.A. Hannun, L.M. Obeid, Ceramide and the eukaryotic stress response, Biochem. Soc. Trans. 25 (1997) 1171–1175.

[3] M. Maceyka, S.G. Payne, S. Milstien, S. Spiegel, Sphingosine kinase, sphingosine-1-phosphate, and apoptosis, Biochim. Biophys. Acta 1585 (2002) 193–201.

[4] T. Hla, M.J. Lee, N. Ancellin, J.H. Paik, M.J. Kluk, Lysophospholipids-receptor revelations, Science 294 (2001) 1875–1878.

[5] Y. Yatomi, Y. Ozaki, T. Ohmori, Y. Igarashi, Sphingosine 1-phosphate: synthesis and release, Prostaglandins Other Lipid Mediat. 64 (2001) 107–122.

[6] Y. Takuwa, Subtype-specific differential regulation of Rho family G proteins and cell migration by the Edg family sphingosine-1-phosphate receptors, Biochim. Biophys. Acta 23 (2002) 112–120.

[7] A. Olivera, T. Kohama, L. Edsall, V. Nava, O. Cuvillier, S. Poulton, S. Spiegel, Sphingosine kinase expression increases intracellular sphingosine-1-phosphate and promotes cell growth and survival, J. Cell Biol. 147 (1999) 545–558.

[8] O.H. Choi, J.H. Kim, J.P. Kinet, Calcium mobilization via sphingosine kinase in signalling by the FcεRI antigen receptor, Nature 380 (1996) 634–636.

[9] D. Meyer zu Heringdorf, H. Lass, R. Alemany, K.T. Laser, E. Neumann, C. Zhang, M. Schmidt, U. Rauen, K.H. Jakobs, C.J. van Koppen, Sphingosine kinase-mediated Ca^{2+} signalling by G-protein-coupled receptors, EMBO. J. 17 (1998) 2830–2837.

[10] A.J. Melendez, A.K. Khaw, Dichotomy of Ca^{2+} signals triggered by different phospholipid pathways in antigen stimulation of human mast cells, J. Biol. Chem. 277 (2002) 17255–17262.

[11] A. Kihara, M. Ikeda, Y. Kariya, E.Y. Lee, Y.M. Lee, Y. Igarashi, Sphingosine-1-phosphate lyase is involved in the differentiation of F9 embryonal carcinoma cells to primitive endoderm, J. Biol. Chem. 278 (2003) 14578–14585.

[12] T. Murate, Y. Banno, K.-T. Koizumi, K. Watanabe, N. Mori, A. Wada, Y. Igarashi, A. Takagi, T. Kojima, H. Asano, Y. Akao, S. Yoshida, H. Saito, Y. Nozawa, Cell type-specific localization of sphingosine kinase1a in human tissues, J. Histochem. Cytochem. 49 (2001) 845–855.

[13] Y. Fukuda, A. Kihara, Y. Igarashi, Distribution of sphingosine kinase activity in mouse tissues: contribution of SPHK1, Biochem. Biophys. Res. Commun. 309 (2003) 155–160.

[14] A. Olivera, S. Spiegel, Sphingosine-1-phosphate as second messenger in cell proliferation induced by PDGF and FCS mitogens, Nature 365 (1993) 557–560.

[15] L.C. Edsall, G.G. Pirianov, S. Spiegel, Involvement of sphingosine 1-phosphate in nerve growth factor-mediated neuronal survival and differentiation, J. Neurosci. 17 (1997) 6952–6960.

[16] D. Meyer zu Heringdorf, H. Lass, I. Kuchar, R. Alemany, Y. Guo, M. Schmidt, K.H. Jakobs, Role of sphingosine kinase in Ca^{2+} signalling by epidermal growth factor receptor, FEBS Lett. 461 (1999) 217–222.

[17] X. Shu, W. Wu, R.D. Mosteller, D. Broek, Sphingosine kinase mediates vascular endothelial growth factor-induced activation of ras and mitogen-activated protein kinases, Mol. Cell. Biol. 22 (2002) 7758–7768.

[18] T. Kohama, A. Olivera, L. Edsall, M.M. Nagiec, R. Dickson, S. Spiegel, Molecular cloning and functional characterization of murine sphingosine kinase, J. Biol. Chem. 273 (1998) 23722–23728.

[19] H. Liu, M. Sugiura, V.E. Nava, L.C. Edsall, K. Kono, S. Poulton, S. Milstien, T. Kohama, S. Spiegel, Molecular cloning and functional characterization of a novel mammalian sphingosine kinase type 2 isoform, J. Biol. Chem. 275 (2000) 19513–19520.

[20] N. Igarashi, T. Okada, S. Hayashi, T. Fujita, S. Jahangeer, S. Nakamura, Sphingosine kinase 2 is a nuclear protein and inhibits DNA synthesis, J. Biol. Chem. (2003), in press.

[21] B. Kleuser, M. Maceyka, S. Milstien, S. Spiegel, Stimulation of nuclear sphingosine kinase activity by platelet-derived growth factor, FEBS Lett. 503 (2001) 85–90.

[22] D. Gorlich, I.W. Mattaj, Nucleocytoplasmic transport, Science 271 (1996) 1513–1518.

[23] E.A. Nigg, Nucleocytoplasmic transport: signals, mechanisms and regulation, Nature 386 (1997) 779–787.

[24] T. Kohno, A. Wada, Y. Igarashi, N-glycans of sphingosine 1-phosphate receptor Edg-1 regulate ligand-induced receptor internalization, FASEB J. 16 (2002) 983–992.

[25] B.R. Henderson, A. Eleftheriou, A comparison of the activity, sequence specificity, and CRM1-dependence of different nuclear export signals, Exp. Cell Res. 256 (2000) 213–224.

[26] I.W. Mattaj, L. Englmeier, Nucleocytoplasmic transport: the soluble phase, Annu. Rev. Biochem. 67 (1998) 265–306.

[27] N. Kudo, B. Wolff, T. Sekimoto, E.P. Schreiner, Y. Yoneda, M. Yanagida, S. Horinouchi, M. Yoshida, Leptomycin B inhibition of signal-mediated nuclear export by direct binding to CRM1, Exp. Cell Res. 242 (1998) 540–547.

[28] K. Nishi, M. Yoshida, D. Fujiwara, M. Nishikawa, S. Horinouchi, T. Beppu, Leptomycin B targets a regulatory cascade of CRM1, a fission yeast nuclear protein, involved in control of higher order chromosome structure and gene expression, J. Biol. Chem. 269 (1994) 6320–6324.

[29] A. Wada, M. Fukuda, M. Mishima, E. Nishida, Nuclear export of actin: a novel mechanism regulating the subcellular localization of a major cytoskeletal protein, EMBO J. 17 (1998) 1635–1641.

[30] C.J. Sigrist, L. Cerutti, N. Hulo, A. Gattiker, L. Falquet, M. Pagni, A. Bairoch, P. Bucher, PROSITE: a documented database using patterns and profiles as motif descriptors, Brief Bioinform. 3 (2002) 265–274.

[31] M. Cokol, R. Nair, B. Rost, Finding nuclear localization signals, EMBO Rep. 1 (2000) 411–415.