Involvement of N-terminal-extended Form of Sphingosine Kinase 2 in Serum-dependent Regulation of Cell Proliferation and Apoptosis*\[S\]

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Sphingosine kinase (SPHK) 1 is implicated in the regulation of cell proliferation and anti-apoptotic processes by catalyzing the formation of an important bioactive messenger, sphingosine 1-phosphate. Unlike the proliferative action of SPHK1, another isozyme, SPHK2, has been shown to possess anti-proliferative or pro-apoptotic action. Molecular mechanisms of SPHK2 action, however, are largely unknown. The present studies were undertaken to characterize the N-terminal-extended form of SPHK2 (SPHK2-L) by comparing it with the originally reported form, SPHK2-S. Real-time quantitative PCR analysis revealed that SPHK2-L mRNA is the major form in several human cell lines and tissues. From sequence analyses it was concluded that SPHK2-L is a species-specific isoform that is expressed in human but not in mouse. At the protein level it has been demonstrated by immunoprecipitation studies that SPHK2-L is the major isoform in human hepatoma HepG2 cells. SPHK2-L, when expressed in human embryonic kidney (HEK) 293 cells, did not show any inhibition of DNA synthesis in the presence of serum, whereas it showed marked inhibition in the absence of serum. Moreover, serum deprivation resulted in the translocation of SPHK2-L into the nuclei. In addition, serum deprivation induced SPHK2-L expression in HEK 293 cells. Furthermore, suppression of SPHK2 by small interfering RNA treatment prevented serum deprivation- or drug-induced apoptosis in HEK293 cells. Taken together, these results indicate that a major form of SPHK2 splice variant, SPHK2-L, in human cells does not inhibit DNA synthesis under normal conditions and that SPHK2-L accumulation in the nucleus induced by serum deprivation may be involved in the cessation of cell proliferation or apoptosis depending on the cell type.

The lipid second messenger sphingosine 1-phosphate (S1P)2 has been implicated in the regulation of a variety of important mammalian cell processes, including proliferation, differentiation, and apoptosis (1–3). Interest in S1P has focused recently on two distinct cellular actions of this lipid, namely the function of S1P as an extracellular ligand activating specific G protein-coupled receptors and the role of S1P as an intracellular second messenger (4). Noticeably, some of the diverse signaling roles attributed to elevated cellular S1P levels include prevention of ceramide-induced apoptosis (5, 6) and calcium mobilization (7).

Sphingosine kinase (SPHK), the enzyme that catalyzes the phosphorylation of sphingosine, plays a central role in the regulation of intracellular levels of S1P. Two isoforms of mammalian SPHK (SPHK1 and SPHK2) have been cloned and characterized (8, 9). SPHK1 is a cytosolic enzyme with an apparent molecular mass of 49 kDa and contains five conserved domains, the second of which has a conserved ATP binding motif found in diacylglycerol kinases (8). Overexpression of SPHK1 induces cell proliferation by promoting the G1 to S transition of the cell cycle as well as by inhibiting the apoptotic response to serum deprivation or ceramide treatment (10). SPHK2 contains the same five conserved domains found in SPHK1 while also having divergent sequences at the N-terminal and in the middle regions, resulting in a protein 200 amino acids larger than SPHK1. In addition, heterogeneity in the N terminus was found in SPHK2 (11), whose mechanism of generation remains unknown. The role of SPHK2, however, has not been elucidated until recently. Studies from our laboratory have demonstrated that SPHK2 is a nuclear protein and inhibits DNA synthesis when overexpressed in mammalian cells (12). Similarly, Liu et al. (13) have reported that SPHK2 induces apoptosis through its putative BH3 domain. More recently, SPHK2 has been postulated to function as a potential immunomodulator either through phosphorylation of an immunosuppressant drug, FTY720 (11, 14), or association with interleukin-12 receptor in T cells (15). However, the physiological role of SPHK2 remains largely unknown.

The present studies were undertaken to determine the physiological role of N-terminal-extended SPHK2 (SPHK2-L) in comparison with the originally reported form (SPHK2-S). Here we have shown that SPHK2-L is the predominant form, at least in human cell lines and tissues tested. We have also presented data that SPHK2-L is involved in the regulation of cell proliferation and apoptosis in concert with serum.

EXPERIMENTAL PROCEDURES

cDNA Cloning and Mammalian Expression Vectors—The human SPHK2-L cDNA (DDBJ/EMBL/GenBank™ accession number NM_020126) was amplified from a human liver cDNA library by PCR using KOD-PLUS polymerase (Toyobo, Tokyo) with 5′-AC AGA TCT ACG AGA GCA GAG GAC CAG CAG-3′ and 5′-AC AGA TCT AAG CTT GTT TAT TTT CAG GGC GGC-3′ sense and antisense primers, respectively. The PCR product was subcloned into pCMV5 to make an N-terminal influenza hemagglutinin (HA) epitope-tagged construct.

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9 The on-line version of this article (available at http://www.jbc.org) contains supplemental Figs. S1–53.

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2 The abbreviations used are: S1P, sphingosine 1-phosphate; SPHK, sphingosine kinase; hSPHK2, human SPHK2; SPHK2-L, N-terminal-extended form of SPHK2; SPHK2-S, originally reported form of SPHK2; HA, influenza hemagglutinin; FCS, fetal calf serum; BrdUrd, bromodeoxyuridine; siRNA, small interfering RNA; HEK, human embryonic kidney; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.
Construction of human SPHK2-S expression vectors has been described previously (12).

**Real-time Quantitative Reverse Transcription-PCR**—Total RNA was isolated from HEK293 cells using the Nucleospin RNA II kit (Macherey-Nagel, Duven, Germany). Total RNAs from various human tissues were purchased from Ambion (Austin, TX). cDNA synthesis was performed with 1 μg of total RNA using MuLV reverse transcriptase (Applied Biosystems, Foster City, CA) priming with random hexamers. Quantitative PCR was performed by applying the real-time SYBR Green PCR technology with the use of an ABI PRISM 7000 sequence detection system (Applied Biosystems). The human SPHK2-specific primers were designed by using Primer Express software (Applied Biosystems), and their sequences were as follows: SPHK2-L mono-specific, 5′-ATG AAT GGA CAC CTT GAA GCA G-3′ and 5′-CAT GGC CTT AGC CCT GAC CAG-3′, located in exon 1 and exon 2, respectively (Fig. 2A, Pair 1); SPHK2-L and SPHK2-S duo-specific, 5′-CTG TCT GCT CCG AGG ACT GC-3′ and 5′-CAA AGG GAT TGA CCA ATA GAA GC-3′, located in exon 2 and exon 3, respectively (Fig. 2A, Pair 2); and for glyceraldehyde 3-phosphate dehydrogenase (GAPDH), 5′-GCC AAT GAC CCC TTC ATT-3′ and 5′-TCT CGC TCC TGG AAG ATG G-3′. Amplification reaction was performed with SYBR Premix Ex Taq (Takara, Otsu, Japan). Thermal cycling conditions were as follows: 10 s at 95 °C, 40 cycles of 5 s at 95 °C, and 31 s at 60 °C. The expression of human SPHK2 (hSPHK2) mRNA was normalized to GAPDH mRNA expression.

**Immunoprecipitation and SPHK Assay**—Preparation of an antibody against hSPHK2 has been described previously (12). An antibody against the N terminus of SPHK2-L (anti-SPHK2-L) was generated by immunizing rabbits with an oligopeptide (MNGHLEAEQQDQRPGELTGSWGHP GPRSTLVRKA) containing the N-terminal 36 amino acids of SPHK2-L fused with glutathione S-transferase. The antibody was affinity purified by using the immunogen-immobilized Sepharose 4B.

HEK293 cells were harvested and washed with phosphate-buffered saline. Crude supernatant fractions were prepared as described previously (16) except that detergent was excluded from the lysis buffer and cells were disrupted by sonication. SPHK2 was immunoprecipitated using anti-SPHK2 or anti-SPHK2-L antibody and assayed for SPHK activity as reported earlier (12).

**Immunocytochemistry**—Cells were grown on 4-chambered slides (Nalge/Nunc) and transfected with SPHK2-L using FuGENE 6 reagent according to the manufacturer’s instructions (Roche Applied Science). Subcellular localization studies using confocal microscopy were performed as described previously (17).

**Small Interfering RNA (siRNA)**—hSPHK2-targeted siRNA-1 (5′-UCAUCCAGACAGACAGACAGACAGACAG-3′ and 5′-UGGUUCUGUCUGUGUAU-3′), siRNA-2 (5′-GCUGGCGUGCCUUCAACC-3′ and 5′-AAGGGUAGGGACGCCCAGC-3′), and control siRNA were synthesized (iGENE Therapeutics, Inc., Tsukuba, Japan). HEK293 cells were transfected with siRNA at a final concentration of 50 nM/slide chamber using Lipofectamine 2000 reagent (Invitrogen). 24 h after transfection, cells were cultured for another 72 h either with 10 or 0.1% FCS (serum deprived) and then fixed with 4% paraformaldehyde in phosphate-buffered saline. The cells were stained with Hoechst 33258 (20 μg/ml) and analyzed for morphological studies.
Other Procedures—Bromodeoxyuridine (BrdUrd) incorporation was measured as described previously (12).

RESULTS

mRNA for SPHK2-L Is the Predominant Form in Various Human Tissues and Cells—BLAST searches of the human genome sequence data base revealed the existence of SPHK2-L. The SPHK2-L gene is ~10 kb long and consists of 6 exons separated by 5 introns (Fig. 1A). The deduced amino acid sequence of this cDNA contained 654 residues and a calculated molecular weight of 69,213. SPHK2-S, originally reported by Liu et al. (9), lacks the exon 1, and the first initiation codon for translation was within the exon 2 (Fig. 1B). It is reasonable to assume that SPHK2-S is generated by means of alternative splicing from the same gene that encodes SPHK2-L. It should be noted that prior to the initiating codon in mouse exon 2 there is a stop codon (Fig. 1B, double underlined TGA), strongly suggesting that no SPHK2-L exists in mouse.

We quantified the amount of each splice variant in various human cell lines by real-time quantitative PCR using two pairs of primer sets (Fig. 2A). In primer set 1 the forward primer was located within exon 1 and the reverse primer on exon 2 so that the specific mRNA encoding SPHK2-L could be detected, whereas in primer set 2 the forward primer was located within exon 2 and the reverse primer on exon 3, which enabled us to recognize mRNA encoding both SPHK2-L and SPHK2-S. Assuming that the molar ratio of SPHK2-L to SPHK2-S mRNA is 1:1, the ratio of the amount of PCR products obtained with these primer pairs is anticipated to be 1:2. Surprisingly, in various human tissues and

FIGURE 2. SPHK2-L and SPHK2-S mRNA expression levels determined by real-time quantitative reverse transcription-PCR in various human tissues and cell lines. A, for specific detection of SPHK2-L, primers (Pair 1) overlapping the exon1/exon2 boundary that encodes the N-terminal-extended part of SPHK2-L were used. For detection of both SPHK2-L and SPHK2-S, primers (Pair 2) overlapping the exon2/exon3 boundary were used. B and C, reverse transcription was performed on total RNA isolated from various human cell lines (B) and tissues (C). The cDNAs were used for real-time PCR with primers specific for SPHK2-L (Pair 1), SPHK2-L plus SPHK2-S (Pair 2), and for the housekeeping gene, GAPDH. Values of mRNA amounts were normalized to GAPDH expression and expressed relative to SPHK2-S expression and are the mean ± S.E. from three independent experiments done six times.
cell lines the relative amount of PCR product generated with primer Pair 1 is almost similar to that obtained with the primer Pair 2, indicating that SPHK2-L is the major form expressed in most human tissues and cell lines except brain and kidney (Fig. 2, B and C), where SPHK2-S might be expressed significantly.3 Using a different pair of primers in which the forward primer was located within exon 5 and the reverse primer on exon 6 so that the mRNA encoding both the SPHK2-L and the SPHK2-S could be detected, almost the same results as with the Pair 2 were obtained (data not shown).

Endogenous SPHK2-L Is the Predominant Form in Human Cell Lines—To detect endogenous SPHK2-L, an antibody against SPHK2-L was prepared. A rabbit polyclonal antibody raised against a glutathione S-transferase-fused oligopeptide containing the N-terminal-extended 36 amino acids of SPHK2-L specifically recognized SPHK2-L, but not SPHK2-S, on immunoblots (Fig. 3A). This antibody immunoprecipitated SPHK2 activity from HeLa cell lysates in a dose-dependent manner (Fig. 3B), confirming the existence of SPHK2-L in HeLa cells. Using this antibody, subcellular distribution of endogenous SPHK2-L was studied. SPHK2-L was mainly distributed in the nuclei and to a small extent diffusely in the cytosol of HeLa cells (Fig. 3C). This distribution pattern is similar to that of SPHK2-S and is quite reasonable because SPHK2-L contains the same nuclear localization signal as SPHK2-S (12). Similarly, in human hepatoma HepG2 cells endogenous SPHK2-L was detected in the nuclei and to a similar extent in the cytosol in a punctate pattern, suggesting that nucleocytoplasmic shuttling is regulated depending on cell types. To demonstrate further that SPHK2-L is a major form at the protein level endogenous SPHK2 was immunoprecipitated and analyzed. Anti-SPHK2-L antibody immunoprecipitated the enzyme only from human hepatoma HepG2 cells but not from mouse NIH3T3 cells, although anti-SPHK2 antibody, which recognizes both SPHK2-L and SPHK2-S, immunoprecipitated it from both cell lysates (Fig. 4A). Together with results from the sequence analyses (Fig. 1B), these results indicate that SPHK2-L is a species-specific isoform that is present in human but not in mouse, as expected from the results in Fig. 1B.

Next, the immunoreactivity of the enzyme with anti-SPHK2 and anti-SPHK2-L antibodies was tested. First, the enzyme was immunoprecipitated from HepG2 cell lysates with anti-SPHK2 antibody and dissociated from the immune complex by incubating with the immunogen peptide. The eluted enzyme was then immunoprecipitated by anti-SPHK2-L antibody. The majority of SPHK activity was immunoprecipitated by the long form-specific antibody with a concomitant loss of the activity from the supernatant in an antibody concentration-dependent manner (Fig. 4B), suggesting that SPHK2-L is a major form in HepG2 cells. Similar results were obtained when HEK293 cell lysates were used as endogenous enzyme sources (data not shown).

Inhibition of DNA Synthesis by SPHK2-L Is Overcome by Serum—We have previously shown that SPHK2 enters nucleus and inhibits DNA

3 Using a human brain total RNA purchased from a different commercial source (BD Biosciences), the observed proportion between SPHK2-L and total SPHK2 mRNAs was similar to that obtained previously and shown in Fig. 2C, suggesting the possibility of expression of SPHK2-S in these tissues.
To determine whether this was true for the SPHK2 variant as well, DNA synthesis was measured in HEK293 cells expressing HA-SPHK2-L or HA-SPHK2-S. As shown in Fig. 5, BrdUrd incorporation was markedly suppressed in cells transiently expressing HA-SPHK2-S compared with green fluorescent protein-expressing control cells, which is consistent with our previous report (12). In contrast, when HA-SPHK2-L was expressed the inhibition of BrdUrd uptake was minimal in 10% serum-containing medium. Even comparing the cells that expressed the recombinant proteins to a similar extent, SPHK2-L has little or no effect on BrdUrd uptake (not shown), indicating that SPHK2-L does not inhibit DNA synthesis significantly under normal culture conditions (in the presence of 10% serum). We have previously reported that inhibition of DNA synthesis in HeLa cells by SPHK2 is more easily observed when cells are cultured under low (0.1%) serum conditions (12). To demonstrate this tendency with SPHK2-L, DNA synthesis was measured in HEK293 cells transiently expressing SPHK2-L under low or high serum conditions. Under low serum conditions, BrdUrd incorporation was strongly suppressed in cells expressing SPHK2-L compared with green fluorescent protein-expressing control cells, which is consistent with our previous report (12). In contrast, when HA-SPHK2-L was expressed the inhibition of BrdUrd uptake was minimal in 10% serum-containing medium. Even comparing the cells that expressed the recombinant proteins to a similar extent, SPHK2-L has little or no effect on BrdUrd uptake (not shown), indicating that SPHK2-L does not inhibit DNA synthesis significantly under normal culture conditions (in the presence of 10% serum). We have previously reported that inhibition of DNA synthesis in HeLa cells by SPHK2 is more easily observed when cells are cultured under low (0.1%) serum conditions (12). To demonstrate this tendency with SPHK2-L, DNA synthesis was measured in HEK293 cells transiently expressing SPHK2-L under low or high serum conditions. Under low serum conditions, BrdUrd incorporation was strongly suppressed in cells expressing SPHK2-L compared with control (green fluorescent protein expressing) cells (Fig. 5). The serum concentration-dependent inhibition of DNA synthesis by SPHK2-L was further demonstrated by cell cycle analysis showing that the population of G2/M cells expressing SPHK2-L was remarkably low in 0.1% but not in 10% serum-containing medium as compared with respective control cells expressing green fluorescent protein (see supplemental materials).

SPHK2-L Tends to Enter the Nucleus under Low Serum Conditions—The observation that serum (10%) overcomes the inhibition of DNA synthesis by SPHK2-L prompted us to study the regulation of nucleocytoplasmic shuttling of SPHK2-L by serum. HEK293 cells were transiently transfected with HA-SPHK2-L, and its subcellular distribu-
tion was analyzed under high or low serum conditions. Under high serum conditions (10% FCS), SPHK2-L tended to be mainly localized in the cytosol with a small amount in the nucleus (Fig. 6, A and C). Under the low serum conditions (0.1% FCS), the population of cells with predominant nuclear localization was increased (Fig. 6, B and C), indicating the regulation of nucleocytoplasmic shuttling of SPHK2-L by serum. This tendency of serum deprivation-induced accumulation of SPHK2-L in the nucleus was confirmed using endogenous enzyme in non-neoplastic human skin fibroblasts (see supplemental materials).

**Serum Deprivation Results in Increased SPHK2-L Expression in HEK293 Cells**—During the course of studies on the regulation of nucleocytoplasmic shuttling of HA-SPHK2-L by serum in HEK293 cells, we noticed that the amount of endogenous SPHK2 tended to increase under conditions when cells were cultured in low serum medium. This observation prompted us to measure endogenous SPHK2 levels under different serum conditions. HEK293 cells were cultured for 2 days either in a low (0.1%) or high (10%) serum medium and assayed for SPHK2 activity or mRNA levels by real-time quantitative PCR using primer Pairs 1 and 2 to detect SPHK2-L and total SPHK2 mRNA, respectively (Fig. 2A). Experiments were done three times in hexaplicate, and the mRNA levels were normalized to GAPDH mRNA expression.

| SPHK2 activity | Total SPHK2 mRNA | SPHK2-L mRNA |
|----------------|------------------|--------------|
| pmol/min/10⁶ cells | -Fold | Relative expression | -Fold | Relative expression | -Fold |
| + Serum | 1.33 ± 0.06 | 1 | 1.0 ± 0.22 | 1 | 1.0 ± 0.06 | 1 |
| − Serum | 2.15 ± 0.08 | 1.6 | 5.4 ± 0.37 | 5.4 | 5.5 ± 0.33 | 5.5 |

**TABLE ONE**

**Serum deprivation-induced SPHK2 expression**
HEK293 cells were cultured for 2 days with 10% (+ serum) or 0.1% FCS (− serum). SPHK2 was immunoprecipitated with anti-SPHK2 antibody and assayed for enzymatic activity. Total RNA was isolated from aliquots of HEK293 cells, reverse transcribed, and analyzed by real-time quantitative PCR using primer Pairs 1 and 2 to detect SPHK2-L and total SPHK2 mRNA, respectively (Fig. 2A). Experiments were done three times in hexaplicate, and the mRNA levels were normalized to GAPDH mRNA expression.
S1P did not change significantly as compared with the cells cultured in high serum medium (see supplemental materials).

**Suppression of SPHK2 Expression by siRNA Protects HEK293 Cells from Serum Deprivation- or Drug-induced Apoptosis**—To assess the physiological role of SPHK2 we analyzed cellular responses induced by serum starvation in HEK293 cells where the level of endogenous SPHK2 was specifically suppressed by SPHK2-targeted siRNA. Compared with control siRNA, treatment with two different SPHK2-siRNAs suppressed the SPHK2 mRNA level in HEK293 cells (Fig. 7A). HEK293 cells underwent apoptosis after serum deprivation as seen in control siRNA-treated cells (Fig. 7, B and C). Importantly, serum deprivation-induced apoptosis was strongly inhibited by SPHK2-targeted siRNA treatment (Fig. 7, B and D). This protective effect by suppression of SPHK2 expression was evident within 2 days after serum deprivation. Prolonged cell culture under low serum conditions resulted in a dramatic loss of cell number due to detachment of cells, especially in control siRNA-treated cells (data not shown). SPHK2-targeted siRNA treatment also protected the cells from tumor necrosis factor α plus cycloheximide-induced apoptosis. These results strongly indicate that SPHK2 may be widely involved in the regulation of apoptosis.

**DISCUSSION**

We have demonstrated the existence of an N-terminal-extended form of SPHK2 (SPHK2-L) in human cell lines and tissues, and it may be a species-specific isotype that is the major isotype in human but that may not exist in mouse, as suggested by several observations. First, a specific antibody against the N-terminal-extended part of SPHK2-L, which interacted specifically with SPHK2-L but not with SPHK2-S, immunoprecipitated SPHK activity from HeLa (Fig. 3, A and B) and HepG2 but not from NIH3T3 cell lysates (Fig. 4A). Second, almost all of the SPHK2 protein immunoprecipitated using an anti-SPHK2 antibody was recognized by an anti-SPHK2-L antibody (Fig. 4B). Third, the amount of mRNA detected by using primer Pair 1 (between exon 1 and exon 2), which is specific to SPHK2-L, is almost the same as that detected by primer Pair 2 (between exon 2 and exon 3), which is common to both SPHK2-S and SPHK2-L (Fig. 2). Sometimes the former was larger than the latter for unknown reasons (Fig. 2B, HeLa cells). We could not resolve whether SPHK2-S is also present in the human cell lines tested due to the lack of an antibody or primer pairs that specifically recognize only SPHK2-S protein or mRNA.

Both anti-SPHK2 and anti-SPHK2-L antibodies used in the present studies immunoprecipitated the endogenous enzyme only partially, although these antibodies immunoprecipitated the recombinant enzyme almost completely (data not shown). Some of the endogenous SPHK2-L may be in a non-immunoreactive pool for reasons not fully clear yet. For instance, N-terminal HA-tagged SPHK2-L when expressed in HeLa cells and detected by using an anti-HA antibody showed a cytosolic, but not a nuclear, staining pattern. However, it could be demonstrated both in the cytosol and the nucleus when detected by using an anti-SPHK2 antibody. C-terminal FLAG-tagged SPHK2-L could be stained both in the cytosol and the nucleus when detected by either anti-FLAG or anti-SPHK2 antibody (data not shown). These results suggest that SPHK2-L may associate in a complex with other molecule(s) via its N-terminal region in the nucleus and may interfere with the recognition of the N-terminal-tagged epitope by antibody. SPHK2-L may be present in heterogeneous forms because HA-tagged SPHK2-L expressed in HeLa cells showed multiple bands in immunoblot analysis, suggesting posttranslational modification(s) (data not shown). This heterogeneity makes the identification of the specific band of interest on immunoblots difficult.

The cellular distribution of SPHK2-L varies depending on the cell type. In HeLa cells it was predominantly distributed in the nucleus, whereas it was distributed evenly in the cytosol and the nucleus in HepG2 cells (Fig. 3C). On the other hand, it was distributed mainly in the cytosol of HEK293 cells (Fig. 6A). Cellular distribution of SPHK2-L was tightly regulated depending not only on the cell type (Fig. 3C) but also on cellular milieu (Fig. 6). Identification of a nuclear export signal may be necessary for understanding the mechanism of nucleocytoplasmic shuttling of SPHK2-L.

Recently we have reported that recombinant SPHK2-S strongly inhibits DNA synthesis and causes cell cycle arrest at G1 phase when expressed in various mammalian cells (12). In the present studies we have demonstrated that SPHK2-L has a decreased ability to inhibit DNA synthesis (Fig. 5). Given that SPHK2-L is the major form in human, this observation is reasonable because HeLa cells proliferate actively even under conditions where endogenous SPHK2 is predominantly localized in the nucleus (12). It is important to note that endogenous SPHK2-S may still possess the ability to inhibit cell proliferation and help induce apoptosis under certain conditions, including low serum stress, because suppression of SPHK2 expression by siRNA prevented serum deprivation-induced apoptosis (Fig. 7). The ability of SPHK2 to inhibit cell proliferation is strikingly different from that of SPHK1, which promotes cell growth and survival (10). We have previously demonstrated that contrary to wild type SPHK1, recombinant NLS-SPHK1, which acquires the ability to enter nuclei by virtue of fusion with the nuclear localization signal from SPHK2, inhibits BrdUrd incorporation in NIH3T3 cells (12). Similarly, when SPHK1 was targeted to plasma membrane by tagging with a myristoylation sequence the fusion protein strongly inhibited cell proliferation by delaying cell cycle exit from G0/G1 (18). Moreover, Pitson et al. (19) have reported that translocation of SPHK1 to the plasma membrane, triggered by phosphorylation at Ser-225 by extracellular signal-regulated kinase 1/2, is essential for oncogenic signaling of this enzyme. Taken together, these reports strongly suggest that topology of S1P generated, i.e., extracellular milieu, plasma membrane, or nucleus, is an important determinant of cell fate along with cellular ratio of S1P versus sphingosine + ceramide level as postulated by the “rheostat model.” Based on the present results that serum withdrawal caused both SPHK2 translocation into the nucleus (Fig. 6) and increased de novo expression of SPHK2 (TABLE ONE), serum deprivation-induced apoptosis may require SPHK2-L to accumulate to a certain level in the nucleus. The excess amount of intranuclear SPHK2-L/S1P beyond the “threshold” might trigger apoptosis. Molecular mechanism of SPHK2/S1P action in the nucleus remains to be clarified.

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