Role of Human Sphingosine-1-phosphate Phosphatase 1 in the Regulation of Intra- and Extracellular Sphingosine-1-phosphate Levels and Cell Viability*

Korey R. Johnson‡, Kristy Y. Johnson§, Kevin P. Becker¶, Jacek Bielawski†, Cungui Mao‡, and Lina M. Obeid‡‡

From the Division of General Internal Medicine, Ralph H. Johnson Veterans Administration Hospital, Charleston, South Carolina 29401, the Departments of Medicine and Biochemistry and Molecular Biology, Medical University of South Carolina, Charleston, South Carolina 29425, and the Department of Biology, Citadel, Charleston, South Carolina 29409

Sphingosine-1-phosphate (S1P)1 is a highly bioactive lipid that exerts numerous biological effects both intracellularly as a second messenger and extracellularly by binding to its G-protein-coupled receptors of the endothelial differentiation gene family (S1P receptors-(1–5)). Intracellularly, at least two enzymes, sphingosine kinase and S1P phosphatase, regulate the activity of S1P by governing the phosphorylation status of S1P. To study the regulation of S1P levels, we cloned the human isoform of S1P phosphatase 1 (hSPPase1). The hSPPase1 has 78% homology to the mouse SPPase at the amino acid level with 6–8 possible transmembrane domains. Confocal microscopy revealed green fluorescent protein-tagged hSPPase1, expressed in either MCF7 or HEK293 cells, co-localized to endoplasmic reticulum with calreticulin. According to Northern blot analysis, hSPPase1 is expressed in most tissues, with the strongest levels found in the highly vascular tissues of placenta and kidney. Transient overexpression of hSPPase1 exhibited a 2-fold increase in phosphatase activity against S1P and dihydro-S1P, indicating that the expressed protein was functional. Small interfering RNA (siRNA) knockdown of endogenous hSPPase1 drastically reduced hSPPase1 mRNA levels, as confirmed by reverse transcription PCR, and resulted in an overall 25% reduction of in vitro phosphatase activity in the membrane fractions. Sphingolipid mass measurements in hSPPase1 siRNA knockdown cells revealed a 2-fold increase of S1P levels and concomitant decrease in sphingosine. In vivo labeling of hSPPase1 siRNA-treated cells showed accumulation of S1P within cells, as well as significantly increased secretion of S1P into the media, indicating that hSPPase1 regulates secreted S1P. In addition, siRNA-induced knockdown of hSPPase1 endowed resistance to tumor necrosis factor-α and the chemotherapeutic agent daunorubicin. Collectively, these data suggest that regulation of hSPPase1 with the resultant changes in cellular and secreted S1P could have important implications to cell proliferation, angiogenesis, and apoptosis.

Sphingosine-1-phosphate (S1P)1 is a highly bioactive sphingolipid implicated in a variety of intracellular signaling events that regulate such cellular functions as differentiation, proliferation, migration, cytoskeletal reorganization, senescence, and apoptosis (1–5). Intracellularly, S1P may act as a second messenger on undefined targets, or S1P may act extracellularly through binding to its G-protein-coupled receptors of the endothelial differentiation gene family (S1P receptors-(1–5)) (6). The emergence of S1P as an important signaling molecule raises the question of how S1P levels are regulated to synchronize S1P-mediated cell signaling events. S1P is generated by sphingosine kinase phosphorylating sphingosine and is dephosphorylated by S1P phosphatase 1 (SPPase1). Additionally, S1P may be degraded by S1P lyase into hexadecenal and phosphoethanolamine.

Evidence for the existence of sphingoid base-specific phosphatases was put forth by Van Veldhoven et al. (7) using cultured skin fibroblasts and rat liver (7, 8). Subsequently, utilizing Saccharomyces cerevisiae-based genetic manipulation, yeast sphingoid base-specific phosphatase enzymes (Ysr2p/Lbp1p/Lcb3p and Ysr3p/Lbp2p) were identified (9–11). Ultimately, these findings led to the cloning of both the murine and the human SPPase (12–14). The mammalian SPPases are a part of the type 2 lipid phosphate phosphatases that are magnesium-independent, N-ethylmaleimide insensitive, and contain several transmembrane domains (12–14). Additionally, these SPPases contain the three conserved motifs: 1) XXXXRR, 2) SXH, and 3) SRXXXXXXHXXD found in the superfamily of type 2 lipid phosphate phosphatases believed to constitute the site of enzymatic activity (15). These mammalian SPPases exhibit substrate specificity against sphingoid base 1-phosphates, including S1P, dihydro-S1P, and phyto-S1P (12–14).

Despite the availability of yeast and mammalian SPPases, little is known about their physiologic function. Studies in S. cerevisiae indicate a possible role for Ysr2 in the uptake and transport of long-chain bases (9). These studies also indicate that Ysr2 has a role in S. cerevisiae cell cycle regulation and thermotolerance such that overexpression of YSR2 caused cell cycle arrest and its disruption endowed thermotolerance (16). As for the role of mammalian SPPase, studies performed

---

* This work is supported by National Institutes of Health Grants GM62887 and 1P20RR17677 (to L. M. O.) and National Institutes of Health Postdoctoral Training Grant NIH-HL 07260 (to K. R. J.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

** To whom correspondence should be addressed: Dept. of Medicine, Medical University of South Carolina, 114 Doughty St., P.O. Box 250779, Charleston, SC 29425. Tel.: 843-876-5169; Fax: 843-876-5172; E-mail: obeidl@musc.edu.

---

1 The abbreviations used are: S1P, sphingosine-1-phosphate; SPPase, S1P phosphatase; hSPPase, human isoform of S1P phosphatase; mSPPase, mouse amino acid sequence for sphingosine-1-phosphate phosphatase; GFP, green fluorescent protein; siRNA, small interfering RNA; TNF-α, tumor necrosis factor; ORF, open reading frame; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; ER, endoplasmic reticulum.
with overexpression of the murine enzyme demonstrated an increase in cellular levels of ceramide and enhancement of apoptosis (17). However, because these studies relied on overexpressed enzyme it is yet to be determined what the physiologic role of the endogenous enzyme is. In addition because SPPase also functions extracellularly on its cell surface receptor it is not yet known if SPPase regulates extracellular levels of S1P. In fact we have recently demonstrated that sphingosine kinase can regulate S1P secretion (18).

In this study we identified the human SPPase1 homologue that localizes to the endoplasmic reticulum and possesses enzymatic activity toward S1P and dihydro-S1P. Northern blot analysis revealed hSPPase1 to be expressed in a variety of tissues with the highest levels occurring in the highly vascular tissues of the kidney and placenta. Enforced knockdown of endogenous hSPPase1 by siRNA resulted in a 2-fold increase of S1P within the cells with a concomitant decrease of sphingosine levels, as well as a significant increase of S1P secretion. In addition, knockdown of hSPPase1 resulted in significant resistance to growth inhibition induced by either cytotoxic agents tumor necrosis factor (TNF)-α or daunorubicin. Collectively, we demonstrate for the first time a physiologic function of endogenous hSPPase1 in the regulation of cellular and secreted S1P levels and its role in apoptosis.

**EXPERIMENTAL PROCEDURES**

**Cell Culture—**HEK293 (ATCC CRL-1573) and MCF-7 (ATCC HTB-22) cells were cultured in Eagle's minimum essential medium or RPMI 1640, respectively, containing 10% fetal bovine serum, 2 mM glutamine, 100 units/ml penicillin, and 100 μg/ml streptomycin. Cells were transfected using LipofectAMINE 2000 (Invitrogen) according to the manufacturer’s protocol.

cDNA Cloning—A tBLASTn search using the mouse amino acid sequence for sphingosine-1-phosphate phosphatase (mSPPase1), identified several human expressed sequence tags homologous to the mouse 3′ sequence: 35435 (GenBank accession number AA331563), 374988 (AW982915), 375872 (AW963799), and 376286 (AW955216). A similar tBLASTn search scanning the human genomic sequence data bank revealed several regions of chromosome 14q23 (clone R-804M7, RPCI-11 BAC library, AL161670), with high homology to the mouse SPPase1. Using PCR-based cloning strategies, we used primers P5 (5′-GCTCATCTATCATCTAGCG-3′) and P6 (5′-ATAATAGATCTT-CAGAGATACAAATGAAAAGATATGTG) to clone a 456-bp fragment from the 3′-region of the proposed open reading frame (ORF) for human SPPase using human fetal kidney Marathon-ready cDNA (Clontech). With the same cDNA library, we cloned a 679-bp fragment using PCR primers P3 (5′-CTACTGCTCTTCTTGTCCG) and P4 (5′-TGTTGTCTCTC-TGCGGTGATG) that spanned the central region of the proposed ORF and overlapped with the 3′-fragment. Combining the 456-bp and 679-bp fragments together with PCR primers P5 and P6, we obtained a 938-bp fragment that spanned two-thirds of the proposed ORF. Finally, we cloned a 452-bp fragment from the 5′-region of the proposed ORF using PCR primers P1 (5′-CTGTTATCATGCTGTGGACGG) and P2 (5′-CAGAAGGGAAGAAACAGGATG) that overlapped with the 938-bp fragment. The full-length 1322 bp cDNA clone was obtained by combining the overlapping 452-bp and 938-bp fragments together with PCR primers P1 and P6, then was subsequently cloned into pcDNA TA-Cloning Vector (Invitrogen). Digestion of the clone with KpnI and EcoRI allowed for subcloning the hSPPase ORF into pcDNA3 (Invitrogen). Digestion of pcDNA3-hSPPase1 with HindIII and EcoRI was necessary to subclone hSPPase into pEGFP-C3 (Clontech). Additionally, the 5′- and 3′-untranslated region were cloned using PCR primers (5′-CTCCGCTTTCCTCACTCCGCG and 5′-TCCACCTCTTCTCCAGCGCGCGCGG) and overlapped with the 938-bp fragment. In Vitro Phosphatase Activity Assay—Cells were transfected and allowed to continue to grow in media containing 0.5% fetal bovine serum.

**Reverse Transcription PCR—**cDNA was isolated from both HEK293 and MCF-7 cells using the RNaseasy mini kit from Qiagen. cDNA was generated using SuperScript II TaqH RNA polymerase (Invitrogen). Reverse transcription reactions were performed using retroase Gradient 96 Robocycler using Platinum TaqDNA polymerase (Invitrogen) to determine optimal conditions for the linear range of cDNA amplification. Linear amplification of a 679-bp fragment of hSPPase1 using PCR primers (5′-CTACTGCTCTTCTTGTCCGCG and 5′-TGTTGTCTCTC-TGCGGTGATG) was achieved at 30 cycles of replication at 94 °C, 60 s; 68 °C, 2 min; 72 °C, 2 min) and normalized to 28 S ribosomal RNA.

**MTT Assay—**Thirty-six hours after transfection with siRNA, cells were treated with 5 μM daunorubicin, 5 nM TNF-α, or vehicle for 18 h. The relative number of viable cells was determined using the MTT based on the toxicity assay kit as described by the manufacturer (Roche Applied Science) and lysed by sonication. Following centrifugation at 1000 × g for 10 min, the supernatant containing disruption cells were subjected to centrifugation at 100,000 × g for 1 h. Supernatant containing the cytosolic fraction was transferred to a separate tube, whereas the pellet containing the membranous fragment was resuspended in 250 μl buffer A. Protein concentration was determined by Bradford assay.

**Phosphatase activity was measured by incubating 4 μg of cytosol or membrane fractions in buffer A with 10 μM [3H]Hidihydro-S1P or [3H]S1P (50,000 cpm; American Radiolabeled Chemical, Inc.) complexed with 0.3% fatty acid-free bovine serum albumin. After 30 min at 37 °C, the lipids were extracted with 3 volumes of chloroform/methanol (2:1) followed by the addition of 0.7 volumes of water. The organic phase was dried under a nitrogen stream and resuspended in chloroform/methanol (2:1). Lipids were resolved by TLC on silica gel G60 with chloroform/methanol/4.2 N ammonium hydroxide (9:7:2). To assay for phosphatase activity of immunoprecipitated hSPPase tagged at the carboxyl terminus with e-Myc, pcDNA4-hSPPase-e-Myc, or vector alone were overexpressed in HEK293 cells for 48 h. Membrane fractions were collected as described above, except for the addition of 0.1% Tween 20 to block the nonspecific background. For detection of the membrane fractions (500 μg) with protein A/G agarose (Santa Cruz), protein was immunoprecipitated with 5 μg of mouse anti-e-c-myc antibody (Santa Cruz) for 2 h at 4 °C. Then, 30 μl of protein A/G agarose was added for 1 h. The beads were washed four times with buffer A and resuspended 60 μl for assaying phosphatase activity. Aliquots of the membrane fractions (25 μg) were resolved on 7.5% polyacrylamide gels by SDS-PAGE and electroblotted onto nitrocellulose membranes. The blots were probed with mouse anti-c-myc antibody and visualized using goat anti-mouse conjugated to horseradish peroxidase along with the ECL immunoblotting detection system (Amersham Biosciences).

**Knockdown of hSPPase1 Expression Using Small Interfering RNA—**human SPPase1 knockdown was performed using sequence-specific siRNA reagents (www.mpibpc.gwdg.de/abteilungen/100/105/sirna.html). Twenty-one nucleotide siRNA for hSPPase (GAAAUGGGC-CCGCUUUCAGGTT and CUGGAAAAACCGCCACUUCCGTT) were targeted, 54 nucleotides downstream of the start codon. Transfection of siRNA duplex was performed using OligofectAMINE reagent as described by the manufacturer with slight modifications (Invitrogen). Cells were transfected and allowed to continue to grow in media containing 0.5% fetal bovine serum.

**Northern Blot Analysis—**Poly(A)+ RNA using 1 μg RNA from 12 different human tissues (Clontech) were probed with a 1.3-kb KpnI/EcoRI fragment from pCDNA3-hSPPase1 generated by HiFiCTP cloning. Blots were hybridized for 2 h in EXEC04Hyb buffer at 68 °C and washed according to manufacturer’s protocol.
extracted by the addition of 2.5 volumes of chloroform/butanol/HCl (50:50:1), and the organic layer was dried under nitrogen. Lipids were separated using thin layer chromatography in chloroform/methanol/15 mM calcium chloride (60:35:8). Bands corresponding to S1P standards were scraped and the radioactivity measured using a scintillation counter.

**RESULTS**

Cloning of Human SPPase—To identify the human isoform for SPPase, we performed a tBLASTn search in the GenBank™ EST data bank using the mouse SPPase amino acid sequence and found several clones with homology: AA331563, AW962915, AW963799, and AW955216. A similar tBLASTn search scanning the human genomic sequence data bank revealed several regions of chromosome 14q23.1 (AL161670), with high homology to the mouse SPPase. Using PCR-based cloning strategies, the entire ORF of human SPPase1 was cloned from a human fetal kidney cDNA library (AF349315). The hSPPase1 ORF encoded a protein with 441 amino acids (approximate molecular mass = 48.9 kD) possessing 78% identity with an additional 6% similarity or 19% identity with an additional 17% similarity to the mSPPase and YSR2, respectively (Fig. 1A). Examination of the sequence revealed the hSPPase1 contained all three conserved motifs: 1) \( K XXXXX R P \), 2) \( S X H \), and 3) \( S R XXXX X H X X D \) found in the superfamily of type 2 lipid phosphate phosphatases. Of note, hSPPase1 and 2 contain a consensus PSTH sequence in the second conserved motif which differs slightly from XSGH sequence found in the other members of type 2 lipid phosphate phosphatase family. Plotting the hydrophilicity using the Kyte-Doolittle algorithm predicted hSPPase1 to contain 6–8 possible transmembrane domains with the three conserved lipid phosphatase domains oriented on the same surface (Fig. 1B).

Subcellular Localization of hSPPase1—To determine the subcellular localization of hSPPase1, the amino terminus was tagged with GFP. Transient expression of hSPPase1-GFP in both MCF-7 and HEK293 cells revealed a membranous reticular pattern consistent with the endoplasmic reticulum (ER).
Fig. 2. hSPPase1 is localized to the endoplasmic reticulum. Representative confocal images of MCF7 cells transiently transfected with pEGFP-hSPPase1 (left panel) and immunostained with rabbit anti-calreticulin antibody (center panel). Merged images generated by superimposing left and right panels indicate colocalization, as seen by yellow color (right panel).

Immunostaining these cells, transiently expressing hSPPase1-GFP, with the well known ER marker calreticulin demonstrated clear colocalization represented by the superimposable images (Fig. 2). Overlay with markers to other endomembrane compartments such as lysosomes, mitochondria, and golgi demonstrated no significant colocalization (data not shown). Similar ER localization was found in cells transiently expressing carboxyl terminus c-Myc-tagged hSPPase1 as far out as 96 h post-transfection (data not shown). Thus, the subcellular localization of hSPPase1 is consistent with the subcellular localization of its yeast (YSR2 and YSR3) and mouse (mSPPase1) homologues (13, 16).

Tissue-specific Expression of hSPPase1 mRNA—To examine tissue-specific expression of hSPPase1 mRNA, we utilized Northern blot analysis. A single 3.3-kb transcript was detected in all tissues. The expression levels varied among tissues, with the highest expression found in kidney and placenta, and the lowest expression found in peripheral blood leukocytes (Fig. 3). These results parallel recently reported results by Ogawa et al. (14).

To examine tissue-specific expression of hSPPase1 mRNA, we utilized Northern blot analysis. A single 3.3-kb transcript was detected in all tissues. The expression levels varied among tissues, with the highest expression found in kidney and placenta, and the lowest expression found in peripheral blood leukocytes (Fig. 3). These results parallel recently reported results by Ogawa et al. (14).

Dephosphorylation of S1P and Dihydro-S1P by hSPPase1—To determine whether the ER-localized hSPPase1 possesses enzymatic activity, HEK293 cells were transiently transfected with pcDNA3-hSPPase 1 or vector alone for 48 h. Cytosolic fractions from either transfectant had modest SPPase activity. In contrast, the membrane fraction of hSPPase overexpressors exhibited an ~2-fold increase in phosphatase activity over vector control membrane fractions (Fig. 4A). A similar profile of phosphatase activities was seen when dihydro-S1P was substituted as a substrate (Fig. 4B).

To confirm that the expressed protein itself accounted for the increased activity, in vitro phosphatase activity assays were conducted on immunoprecipitates of c-Myc-tagged hSPPase1. The results demonstrated significant SPPase activity in immunoprecipitates (Fig. 4C), and therefore they establish that the increase in phosphatase activity observed above was a direct result of hSPPase1 and not due to activation of a regulatory protein or by a nonspecific phosphatase. Immunoblot analysis of the membrane fractions confirmed c-Myc-tagged hSPPase1 is indeed expressed in its intact full-length form (Fig. 4C, inset).

siRNA Knockdown of hSPPase1 mRNA in S1P Accumulation—Thus far, studies have concentrated on the effects of overexpression of SPPases. To establish whether endogenous hSPPase1 is a key enzyme regulating S1P levels within the cell, RNAi technology was utilized to “knockdown” endogenous hSPPase1. Within 24–48 h of gene-specific siRNA treatment, reverse transcription PCR detection showed significantly reduced levels of hSPPase1 (Fig. 5A). In contrast, mock or scrambled siRNA treatment resulted in no change of hSPPase1 levels as compared with untreated control (Fig. 5A). Reverse transcription PCR of 28 S ribosomal RNA controlled for differences in loading in all samples and for the presence of housekeeping gene mRNA in the hSPPase1 siRNA treated samples. In vitro phosphatase activity on the membrane fraction of hSPPase1 siRNA-treated cells demonstrated a 25% overall reduction in S1P phosphatase activity as compared with the scrambled control (Fig. 5B). These data confirmed siRNA treatments resulted in significant reduction in hSPPase1 levels. Examination of MCF-7 cells for mRNA expression levels of both hSPPase1 and hSPPase2 revealed an approximate 6-fold higher level of hSPPase1 mRNA expression as compared with hSPPase2 (data not shown).

Pulsing cells with [3H]sphingosine resulted in a nearly 2-fold increase in S1P accumulation within hSPPase1 knockdown cells as compared with mock or scrambled (Fig. 6, A and B). Interestingly, a similar fold increase was also seen in the secretion of S1P into the extracellular medium of hSPPase1 knockdown cells as compared with mock or scrambled. These data suggest that hSPPase1 not only is a key enzyme responsible for the regulation of intracellular levels of S1P, but also is involved in the regulation of extracellular S1P levels.

siRNA Knockdown of hSPPase1 Increases Resistance to Cytotoxic Agents—S1P is known to exert cytoprotective effects...
within cells; therefore, we implemented siRNA knockdown of endogenous hSPPase1 to explore the biological significance of the subsequent S1P accumulation in cells exposed to cytotoxic agents. Cells transfected with scrambled siRNA and treated with 5 \( \mu \)M daunorubicin or 5 nM TNF-\( \alpha \) for 18 h resulted in a drastic reduction in viable cells to 40 and 33%, respectively (Fig. 7). Significantly, knockdown of hSPPase1 conferred a nearly 2-fold increase in cell viability, resulting in 70 and 56% cell survival, respectively (Fig. 7). Collectively, these data indicate that by regulating S1P levels, hSPPase1 mediates cellular response to challenges by cytotoxic agents.

DISCUSSION

In this manuscript we describe the cloning of the human SPPase1, which contains multiple transmembrane domains, localizes to the endoplasmic reticulum, and possesses the three conserved catalytic domains present in the superfamily of type 2 lipid phosphate phosphatases. Tissue-specific expression patterns revealed hSPPase1 levels to be greatest in highly vascularized tissues of the kidney and placenta. hSPPase1 possessed enzymatic activity against both S1P and dihydro-S1P, and has previously been shown to dephosphorylate phyto-S1P (13). Enforced knockdown of endogenous hSPPase1 expression led to not only the accumulation of intracellular S1P, but also the extracellular release of S1P. Furthermore, the reduced expression of hSPPase1 conferred significant resistance to cytotoxic challenge by daunorubicin or TNF-\( \alpha \).

S1P-mediated signaling is involved in regulating numerous biological processes; therefore, it is not surprising that S1P levels within the cell are tightly regulated. A wide variety of stimuli have been shown to increase sphingosine kinase activity and generate S1P (20). S1P-mediated signaling occurs through the binding to cell surface receptors S1P\( _{1-5} \) and activating G-protein-coupled receptors pathways (21). In addition, S1P signals intracellularly through a proposed G-protein-coupled receptor-independent pathway (21). One line of evidence
presented by Waggoner et al. (22) suggests that lipid phosphate phosphatases localizing to the plasma membrane may regulate S1P-mediated signaling of cell surface receptors through ecto-phosphatase activity. The most recent evidence for a sphingoid base phosphate-specific phosphatase regulating S1P signaling comes from recent studies by Le Stunff et al. (17) in which mSPPase1 was overexpressed and cells were given exogenous S1P. These studies found mSPPase1 dephosphorylation of S1P could result in synthesis of ceramide and ultimately lead to programmed cell death.

Although these previous studies have utilized overexpression of lipid phosphate phosphatases or mSPPase1 to examine the regulation of S1P-mediated signaling, our studies describe for the first time a role for endogenous hSPPase1 in the regulation of intracellular and extracellular S1P levels. How S1P levels are regulated in cells remains poorly understood. S1P may be metabolized by S1P lyase into phosphoethanolamine and hexadecenal; however, little is known about the role S1P lyase plays in the regulation of S1P signaling (23). S1P is also regulated by SPPases. Using siRNA knockdown of hSPPase1, we show significant accumulation of S1P intracellularly and increased secretion of S1P into the extracellular milieu. These studies indicate hSPPase1 is important for governing S1P levels, thereby ultimately regulating S1P signaling. One possibility is that S1P generated in the cytosol is somehow accessible to membrane-associated components. Cytosolic S1P could be transported and metabolized by hSPPase1 in the ER or released extracellularly. Upon siRNA knockdown of hSPPase1, S1P may be preferentially secreted (Fig. 8).

The above findings are particularly interesting in light of our recent report showing activation of sphingosine kinase by the potent mitogen, phorbol myristate acetate, led to increased secretion of S1P (18). Thus, it is reasonable to assume that hSPPase1 may act to mute mitogen-induced S1P secretion. Because S1P is known to be involved in various mitogenic pathways, the elucidation of its enzymatic regulation is of paramount importance in dissecting the pathophysiological processes of inflammation, angiogenesis, and oncogenesis.

Regulation of sphingolipid metabolites is crucial to cell survival. Tipping the balance toward S1P generation by sphingosine kinase activation precludes induction of apoptosis (24), whereas overexpression of SPPase leads to the breakdown of S1P into sphingosine and ceramide, thereby promoting induction of apoptosis (17). Our studies show that regulation of endogenous levels of hSPPase1 by siRNA leads to increased levels of S1P and results in a marked resistance to apoptosis. As shown in Table I, S1P levels indeed doubled in response to siRNA knockdown of hSPPase1 concomitant with a significant decrease in sphingosine, thus possibly shifting the cellular balance from pro-apoptotic to pro-survival. In fact previous studies by Nava et al. (25) found increases of S1P levels in...
Regulation of S1P Levels by hSPPase1

MCF-7 cells overexpressing sphingosine kinase conferred resistance to cell death induced by the cytotoxic agents doxorubicin and TNF-α (25). Therefore, hSPPase1 may be a key component in deciding whether a cell survives or enters apoptosis. Intuitively, one can envision a tumor suppressor role for hSPPase1 whereby in neoplasia hSPPase1 down-regulation would lead to uninhibited cell growth and angiogenesis. In fact, the Mitelman Database of Chromosome Aberrations in Cancer2 reveals several deletions in the region of chromosome 14q23 to which hSPPase1 maps, corresponding to diffuse B-cell lymphoma, mesothelioma, and multiple myeloma. Assuming oncogenic processes depend upon a reduction in SPPase activity, manipulation of this activity may have therapeutic implications.

In conclusion, these findings suggest intracellular regulation of hSPPase1 is necessary for the cell to modulate levels of S1P to control signaling events in an autocrine/paracrine fashion leading to biological processes, such as cell proliferation, migration, differentiation, angiogenesis, and apoptosis. Future studies dissecting regulatory domains and signaling events of hSPPase1 will enable us to characterize further the roles this enzyme plays in cell homeostasis.

Acknowledgments—We thank Dr. Yusuf A. Hannun for helpful discussion and careful review of this manuscript and the Lipidomics Core in the Department of Biochemistry and Molecular Biology at MUSC for lipid measurements.

REFERENCES
1. Hannun, Y. A. (1996) Science 274, 1855–1859
2. Hannun, Y. A., and Obeid, L. M. (1997) Biochem. Soc. Trans. 25, 1171–1175

2 Mitelman Database of Chromosome Aberrations in Cancer (2003) (Mitelman, F., Johansson, B., and Mertens, F., eds.), cgap.nci.nih.gov/Chromosomes/Mitelman.