Identification and Characterization of a Novel Human Sphingosine-1-phosphate Phosphohydrolase, hSPP2*

Chie Ogawa, Akio Kihara, Maiko Gokoh, and Yasuyuki Igarashi‡
From the Department of Biomembrane and Biofunctional Chemistry, Graduate School of Pharmaceutical Sciences, Hokkaido University, Kita 12-jo, Nishi 6-choume, Kita-ku, Sapporo 060-0812, Japan

Sphingosine 1-phosphate (S1P) is a bioactive lipid molecule that acts as both an extracellular signaling mediator and an intracellular second messenger. S1P is synthesized from sphingosine by sphingosine kinase and is degraded either by S1P lyase or by S1P phosphohydrolase. Recently, mammalian S1P phosphohydrolase (SPP1) was identified and shown to constitute a novel lipid phosphohydrolase family, the SPP family. In this study we have identified a second human S1P phosphohydrolase, SPP2, based on sequence homology to human SPP1. SPP2 exhibited high phosphohydrolase activity against S1P and dihydro sphingosine 1-phosphate. The dihydro sphingosine 1-phosphate phosphohydrolase activity was efficiently inhibited by excess S1P but not by lysophosphatidic acid, phosphatidic acid, or glycerol 3-phosphate, indicating that SPP2 is highly specific to sphingoid base 1-phosphates. Immunofluorescence microscopic analysis demonstrated that SPP2 is localized to the endoplasmic reticulum. Although the enzymatic properties and localization of SPP2 were similar to those of SPP1, the tissue-specific expression pattern of SPP2 was different from that of SPP1. Thus, SPP2 is another member of the SPP family that may play a role in attenuating intracellular S1P signaling.

Sphingosine 1-phosphate (S1P), a sphingolipid metabolite, regulates diverse biological processes including mitogenesis, differentiation, migration, and apoptosis both as an extracellular mediator and as an intracellular second messenger (1–3). Extracellular effects of S1P are known to be mediated via endothelial differentiation gene (Edg) family of plasma membrane G-protein-coupled receptors, whereas its intracellular targets have yet to be determined (2, 3). S1P is synthesized by the phosphorylation of sphingosine and catalyzed by sphingosine kinase. Once formed, S1P is rapidly degraded by S1P lyase to hexadecenal and phosphoethanolamine or dephosphorylated by S1P phosphohydrolase.

The existence of a S1P-specific phosphohydrolase had been suggested by biochemical analyses using cultured skin fibroblasts and rat liver (4, 5). In 2000, murine S1P phosphohydrolase (mSPP1) was cloned as a S1P phosphohydrolase based on sequence homology to the yeast sphingoid base 1-phosphate phosphatase, Lcb3p/Lbp1p/Ysr2p (6). Recently, a human homolog of mSPP1, hSPP1, which exhibits 76% identity and 81% similarity to mSPP1, was identified (7). These mammalian SPP1s and their two yeast homologs, Lcb3p and Ysr3p, constitute the SPP family, which is distinct from another lipid phosphohydrolase family, the type 2 lipid phosphohydrolases (LPP), both in sequence and in biochemical properties. Accordingly, the SPP family members are highly specific to sphingoid base 1-phosphates, including S1P, dihydro sphingosine 1-phosphate (dihydro-S1P), and phytosphingosine 1-phosphate (6–9); yet the LPP family members have broad substrate specificities including S1P, phosphatidate (PA), lysophosphatidate (LPA), ceramide 1-phosphate, and diacylglycerol pyrophosphate (10–14). Proteins from both the SPP and LPP families contain the following three conserved motifs: motif 1, KXXXXXXXRP; motif 2, PSGH; and motif 3, SRXXXHXXXD. These motifs are found in a superfamily of phosphatases that include several lipid phosphatases, glucose-6-phosphatases, bacterial nonspecific acid phosphatases, and chloroperoxidase (15). The crystal structure of chloroperoxidase disclosed that these conserved motifs were in close proximity and that together they constituted a binding pocket for the cofactor vanadate, a compound structurally similar to phosphate (16). Thus, these motifs are believed to constitute the active site in members of this phosphatase superfamily, a conclusion supported by mutational analyses of yeast Dpp1p and mammalian LPP-1 (17, 18).

We report here that we have identified a second human S1P phosphohydrolase, termed hSPP2, based on sequence homology to hSPP1. hSPP2 exhibited high phosphohydrolase activity specifically against sphingoid base 1-phosphates. Additionally, in contrast to the ubiquitous expression of hSPP1, the expression of hSPP2 was restricted to specific tissues. Using immunofluorescence microscopy we also demonstrated that hSPP2 is localized to the endoplasmic reticulum (ER), suggesting that it functions in attenuating intracellular S1P signaling.

EXPERIMENTAL PROCEDURES

DNA Construction—pEGFP-C1 was purchased from Clontech. pcDNA3 HA4, a derivative of pcDNA3 (Invitrogen), was constructed to create a C-terminal hemagglutinin (HA)-tagged gene. BLAST searches using the hSPP1 sequence identified an EST clone (GenBank™ accession number BG696302) that contains an entire hSPP2 open reading frame (ORF) but lacks 45 bp of the 5′ sequence. To obtain the entire hSPP2 cDNA, the primers 5′-GCCCACCATGGCGGACTGCTGGCG-
Identification of a Novel S1P Phosphohydrolase

Identification of hSPP2—To identify a new S1P phosphohydrolase, the GenBank™ human EST data base was searched using the BLAST program for sequences similar to the hSPP1 sequence. We found several ESTs including BG696302, BM127455, BMS80654, and AW779536 with high sequence homology to hSPP1. These ESTs contained different fragments but were derived from an identical cDNA, subsequently named human SPP2 (hSPP2). The ORF of hSPP2 encodes 399 amino acids with a molecular mass of 44.7 kDa (Fig. 1A). The hSPP2 sequence indicated 39.3% identity and 69.7% similarity to hSPP1. hSPP2 also shows significant homology to other SPP family members. Sequences from the SPP and LPP families of human and yeast were aligned using ClustalW. GenBank™ accession numbers are AJ293294 (hSPP1) and AF542512 (hSPP2). The alignment was generated using ClustalW (22) and BOXSHADE (Institute for Animal Health, Surrey, United Kingdom) programs. Black boxes indicate identical residues, and gray boxes show amino acid similarity. Underlined sequences indicate the three motifs found in a superfAMILY of phosphohydrolases, including several lipid phosphohydrolases, bacterial nonspecific acid phosphohydrolases, and chloroperoxidase (15). B, dendrogram of the SPP and LPP families. Sequences from the SPP and LPP family members of human and yeast were aligned using ClustalW. GenBank™ accession numbers are AJ293294 (hSPP1), AF542512 (hSPP2), CAAS9430 (Lpp1p), CAAS9231 (Lpp2p), CAAS9231 (Lpp3p), AP011766 (hLPP1), AF056083 (hLPP2), AP017786 (hLPP3), AAB64945 (Lpp1p), and AAB64475 (Dpp1p). C, hydropathy profiles of hSPP1 and hSPP2. The parameters used were from Kyte and Doolittle (23). Each point on the curve represents the hydropathy average of a 15-amino acid window.

RESULTS AND DISCUSSION

Identification of hSPP2—To identify a new S1P phosphohydrolase, the GenBank™ human EST data base was searched using the BLAST program for sequences similar to the hSPP1 sequence. We found several ESTs including BG696302, BM127455, BMS80654, and AW779536 with high sequence homology to hSPP1. These ESTs contained different fragments but were derived from an identical cDNA, subsequently named human SPP2 (hSPP2). The ORF of hSPP2 encodes 399 amino acids with a molecular mass of 44.7 kDa (Fig. 1A). The hSPP2 sequence indicated 39.3% identity and 69.7% similarity to hSPP1. hSPP2 also shows significant homology to other SPP family members, whereas it is divergent from the LPP family (Fig. 1B). Although the N-terminal hydrophilic region of hSPP2 was shorter than that of hSPP1, the overall hydropathy profile of hSPP2 was very similar to that of hSPP1 (Fig. 1C), suggesting that the transmembrane structure of hSPP2 is the same as that of hSPP1.

In Vitro Phosphohydrolase Assay—Cells suspended in buffer A (50 mM HEPES-NaOH (pH 7.5), 150 mM NaCl, 20% glycerol, 1 mM EDTA, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, 1% Triton X-100, 0.1% deoxycholate, 1.0 mM PMSF, 50 mM 32P-dCTP, 1,000 units/ml of L-amino acid dehydrogenase, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, 1% Triton X-100) were subjected to ultracentrifugation at 100,000 g for 2 hours. Supernatants were subjected to ultracentrifugation at 100,000 g for 2 hours. Supernatants were then separated by centrifugation, and the organic phase was recovered, dried, and dissolved in chloroform/methanol (2:1, v/v). The labeled lipids were resolved by thin layer chromatography (TLC) on Silica Gel 60 high performance TLC plates (Merek) with 1-butanol/acetate acid/water (3:1:1, v/v).

Northern Blot Analysis—Poly(A)* RNA blots containing 1 mg of RNA from human tissues (Clontech) were used. For detection of hSPP2 mRNA, blots were probed with a 0.48-kb PstI-NcoI fragment of pcDNA3 hSPP2-HA that was labeled with [32P]dCTP by random priming. For detection of hSPP1 mRNA, a hSPP1 probe was prepared as follows. A 0.5-kb DNA fragment corresponding to the 5’ region of hSPP1 ORF was amplified using the primers 5’-GGATCCATGGCCGAGCTGCTGC-3’ and 5’-TTTCAAGGTTAACCCAGCAGACCC-3’. The amplified DNA fragment was digested with HindIII and cloned into the HindIII-BamHI site of pcDNA3 HA4 to generate pcDNA3 mSPP1-HA plasmid. The pcDNA3 mSPP1-HA plasmid, which encoded C-terminal HA-tagged mSPP1, was constructed as follows. The mSPP1 cDNA was amplified from the EST clone (GenBank™ accession number BG696302), which contains an entire mSPP1 ORF, using the primers 5’-AAAACTCCGCCGATCCATCCATTGCTGTCGCCCGCTTCCAGC-3’ and 5’-GGATCCCTTATATTGATACCTTCTTG-3’. The amplified DNA fragment was digested with HindIII and BamHI and cloned into the HindIII-BamHI site of pcDNA3 HA4 to generate pcDNA3 mSPP1-HA.

Cell Culture and Transfection—HEK 293 cells were grown on coverslips and transfected with the indicated cDNA using LipofectAMINE Plus (Invitrogen).

Immunofluorescence Microscopy—HEK 293 cells grown on coverslips and transfected with the indicated cDNAs were fixed with 3.7% formaldehyde in phosphate-buffered saline (PBS) at 37°C for 10 min, permeabilized with 0.1% Triton X-100 in PBS, blocked with blocking solution (10 mg/ml bovine serum albumin in PBS), and incubated at 37°C for 30 min with primary antibodies, anti-HA Y11 (Santa Cruz Biotechnology) at 1 µg/ml, or anti-KDEL antibody (StressGen Biotechnologies) at 4.8 µg/ml in blocking solution. After washing three times with PBS, the cells were incubated for 30 min with secondary antibody, Alexa 488-conjugated anti-rabbit or Alexa 594-conjugated anti-mouse antibody (Molecular Probes) diluted in blocking solution to 5 µg/ml. Coverslips were washed in PBS three times and in deionized water three times. Coverslips were then mounted with Mowiol 4-88 (Calbiochem) and observed under a fluorescence microscope.

Electrophoresis of Proteins and Lipids—Lipids were extracted from cells using a modified Bligh and Dyer method (16) and applied to a Silica Gel 60 high performance TLC plates (Merek) with 1-butanol/acetate acid/water (3:1:1, v/v). Phases were then separated by centrifugation, and the organic phase was recovered, dried, and dissolved in chloroform/methanol (2:1, v/v). The labeled lipids were resolved by thin layer chromatography (TLC) on Silica Gel 60 high performance TLC plates (Merek) with 1-butanol/acetate acid/water (3:1:1, v/v).
Identification of a Novel S1P Phosphohydrolase

Phosphohydrolase assay was performed using [3-3H]S1P. Similar to that of mSPP1, the specific activity of hSPP2 against dihydro-S1P-HA (Fig. 2), the specific activity of hSPP2 against dihydro-S1P-HA was expressed 1.6 times more abundantly than that of hSPP1. The TopPredII 1.1 program (19) predicts hSPP2 to be an integral membrane protein with as many as nine membrane-spanning segments. The three conserved motifs found in the superfamily of phosphatases, including several lipid phosphatases, were also present in hSPP2 (Fig. 1A).

Characterization of Phosphohydrolase Activity of hSPP2—To investigate whether hSPP2 had S1P phosphohydrolase activity, HEK 293 cells were transiently transfected with a pcDNA3 hSPP2-HA encoding the C-terminal HA-tagged hSPP2 (hSPP2-HA). For comparison we also constructed a pcDNA3 mSPP1-HA that encodes the C-terminal HA-tagged mSPP1 (mSPP1-HA). Total cell lysates were prepared from HEK 293 cells, transfected with the respective plasmids, and separated by SDS-PAGE. This was followed by immunoblotting using anti-HA antibodies. A band with an apparent molecular mass of 35 kDa was specifically detected in hSPP2-HA-transfected cell lysates and was absent in vector-transfected cell lysates (Fig. 2, lanes 1 and 2). mSPP1-HA was detected as a band at 40 kDa (Fig. 2, lane 3). These mobilities were faster than the predicted molecular masses of hSPP2-HA (46.1 kDa) and mSPP1-HA (49.2 kDa).

Using membrane fractions and [4,5-3H]dihydro-S1P we also performed an in vitro phosphohydrolase assay. In contrast to vector-transfected HEK 293 cells, which had low levels of activity, cells overexpressing hSPP2-HA and mSPP1-HA exhibited a 6.3- and 11.5-fold increase in phosphohydrolase activity, respectively (Fig. 3, A and B, lanes 1 and 2). However, because mSPP1-HA was expressed 1.6 times more abundantly than hSPP2-HA (Fig. 2), the specific activity of hSPP2 against dihydro-S1P was similar to that of mSPP1.

To investigate the substrate specificity of hSPP2, the in vitro phosphohydrolase assay was performed using [3-3H]S1P. Similar to the results obtained using [4,5-3H]dihydro-S1P (Fig. 3B, lanes 1 and 2), cells overexpressing hSPP2-HA exhibited an ~6-fold higher phosphohydrolase activity against [3-3H]S1P than did vector-transfected cells (Fig. 3B, lanes 3 and 4). Next, we performed a competition assay using [4,5-3H]dihydro-S1P and various excess cold lipids, including dihydro-S1P, S1P, LPA, PA, and glycerol 3-phosphate. S1P inhibited the dihydro-S1P phosphohydrolase activity as efficiently as dihydro-S1P (Fig. 3C). However, LPA, PA, and glycerol 3-phosphate had little inhibitory effect (Fig. 3C). These results indicated that hSPP2 is a phosphohydrolase specific to sphingoid base 1-phosphates.

A previous study demonstrated that SPP1s are Mg2+-independent phosphohydrolases (7). To examine the requirement of Mg2+ for the SPP2 activity, the S1P phosphohydrolase assay was performed in the presence of the metal chelator EDTA. The activity of SPP was not affected by 10 mM EDTA (Fig. 4). Furthermore, addition of 10 mM Mg2+ did not enhance the phosphohydrolase activity (data not shown). These results indicated that SPP2 is also a Mg2+-independent phosphohydrolase.

**Fig. 2. Detection of hSPP2 by immunoblotting.** HEK 293 cells were transfected with pcDNA3 HA4 (vector, lane 1), pcDNA3 hSPP2-HA (lane 2), and pcDNA3 mSPP1-HA (lane 3). 24 h after transfection total lysates were prepared from each cell line, and fixed amounts of protein (17 μg) were subjected to immunoblotting using anti-HA antibody. The asterisk indicates nonspecific background.

**Fig. 3. Phosphohydrolase activity and substrate specificity of hSPP2.** A, samples from the same total lysates (60 μg of protein) used in Fig. 2 were ultracentrifuged, and the resulting membrane fractions were incubated with 10 μM [4,5-3H]dihydro-S1P at 37 °C for 10 min. Lipids were extracted and separated by TLC. Open bar, pcDNA3 HA4 (vector); closed bar, pcDNA3 hSPP2-HA; gray bar, pcDNA3 mSPP1-HA. Values relative to those exhibited by vector-transfected cells are shown. B, membrane fractions prepared from HEK 293 cells transfected with pcDNA3 HA4 (lanes 1 and 3) and pcDNA3 hSPP2-HA (lanes 2 and 4) were incubated with either 10 μM [4,5-3H]dihydro-S1P (lanes 1 and 2) or 10 μM [3-3H]S1P (lanes 3 and 4) at 37 °C for 10 min. Lipids were extracted and separated by TLC. Sph, sphingosine. C, membrane fractions prepared from HEK 293 cells transfected with pcDNA3 hSPP2-HA were incubated with 10 μM [4,5-3H]dihydro-S1P in the presence of 100 μM cold dihydro-S1P, S1P, LPA, PA, or glycerol 3-phosphate at 37 °C for 10 min. Lipids were extracted and separated by TLC. G3P, glycerol 3-phosphate.
exhibited by vector-transfected cells with no treatment are shown. Lipids were extracted and separated by TLC. Values relative to those presence of 10 mM EDTA, 10 mM NaF, 10 mM phosphatase inhibitors, and Triton X-100 have strong inhibitory effects on SPP1 activity, whereas β-glycerophosphate is not effective (6, 7). Similar to SPP1, SPP2 was significantly inhibited by 10 mM sodium orthovanadate and 0.2% Triton X-100 but not by 10 mM β-glycerophosphate (Fig. 4). In contrast, 10 mM NaF, which efficiently inhibited the SPP1 activity, had no effect on the SPP2 activity (Fig. 4). Moreover, even 100 mM NaF did not inhibit the SPP2 activity (data not shown).

Immunofluorescent Localization of SPP2—S1P can act both extracellularly, via cell surface Edg family receptors, and intracellularly. Therefore, it is important to distinguish whether SPP2 plays a role in attenuating intracellular or extracellular S1P signaling. For this purpose, we performed an indirect immunofluorescence microscopic analysis to determine the localization of hSPP2. HEK 293 cells transiently transfected with pCDNA3 hSPP2-HA were subjected to immunostaining with anti-HA antibody. Fig. 5A shows a reticular immunofluorescence staining pattern apparent in the perinuclear and cytosolic regions. This staining pattern was attributable to localization of hSPP2-HA in the ER, because it was superimposable upon the pattern of ER stained by an antibody against the ER retention signal, KDEL (Fig. 5A). This staining pattern of hSPP2 was similar to that of mSPP1 (Fig. 5B), which also co-localized with anti-KDEL antibody (data not shown). This result about the localization of mSPP1 is consistent with the recent report by Spiegel and co-workers (20). To examine the possibility that some fraction of hSPP2 is localized to the plasma membrane, we performed an immunofluorescence microscopic analysis using non-permeabilized cells. HEK 293 cells co-transfected with pEGFP-C1 and pCDNA3 hSPP2-HA were fixed and treated with or without Triton X-100, and hSPP2-HA was immunostained with anti-HA antibody. Permeabilized cells exhibiting green fluorescence protein fluorescence were stained with HA antibody (Fig. 5C), indicating that pEGFP-C1 and pCDNA3 hSPP2-HA were efficiently co-introduced. However, when the permeabilization step was omitted, cells exhibited only green fluorescence protein fluorescence and were not stained with HA antibody (Fig. 5D). This result indicated that permeabilization was essential for staining of hSPP2-HA.

We next examined the effects of various phosphatase inhibitors such as NaF, sodium orthovanadate, and β-glycerophosphate, as well as Triton X-100 on the SPP2 activity. Previous studies demonstrated that NaF, sodium orthovanadate, and Triton X-100 have strong inhibitory effects on SPP1 activity, whereas β-glycerophosphate is not effective (6, 7). Similar to SPP1, SPP2 was significantly inhibited by 10 mM sodium orthovanadate and 0.2% Triton X-100 but not by 10 mM β-glycerophosphate (Fig. 4). In contrast, 10 mM NaF, which efficiently inhibited the SPP1 activity, had no effect on the SPP2 activity (Fig. 4). Moreover, even 100 mM NaF did not inhibit the SPP2 activity (data not shown).

Immunofluorescent Localization of SPP2—S1P can act both extracellularly, via cell surface Edg family receptors, and intracellularly. Therefore, it is important to distinguish whether SPP2 plays a role in attenuating intracellular or extracellular S1P signaling. For this purpose, we performed an indirect immunofluorescence microscopic analysis to determine the localization of hSPP2. HEK 293 cells transiently transfected with pCDNA3 hSPP2-HA were subjected to immunostaining with anti-HA antibody. Fig. 5A shows a reticular immunofluorescence staining pattern apparent in the perinuclear and cytosolic regions. This staining pattern was attributable to localization of hSPP2-HA in the ER, because it was superimposable upon the pattern of ER stained by an antibody against the ER retention signal, KDEL (Fig. 5A). This staining pattern of hSPP2 was similar to that of mSPP1 (Fig. 5B), which also co-localized with anti-KDEL antibody (data not shown). This result about the localization of mSPP1 is consistent with the recent report by Spiegel and co-workers (20). To examine the possibility that some fraction of hSPP2 is localized to the plasma membrane, we performed an immunofluorescence microscopic analysis using non-permeabilized cells. HEK 293 cells co-transfected with pEGFP-C1 and pCDNA3 hSPP2-HA were fixed and treated with or without Triton X-100, and hSPP2-HA was immunostained with anti-HA antibody. Permeabilized cells exhibiting green fluorescence protein fluorescence were stained with HA antibody (Fig. 5C), indicating that pEGFP-C1 and pCDNA3 hSPP2-HA were efficiently co-introduced. However, when the permeabilization step was omitted, cells exhibited only green fluorescence protein fluorescence and were not stained with HA antibody (Fig. 5D). This result indicated that permeabilization was essential for staining of hSPP2-HA.

We next examined the effects of various phosphatase inhibitors such as NaF, sodium orthovanadate, and β-glycerophosphate, as well as Triton X-100 on the SPP2 activity. Previous studies demonstrated that NaF, sodium orthovanadate, and Triton X-100 have strong inhibitory effects on SPP1 activity,
Thus, SPP2 may function in the ER to dephosphorylate intracellular S1P.

**Tissue Distribution of SPP2 mRNA Expression**—To compare the tissue distribution patterns of SPP1 and SPP2 mRNAs, we first examined the expression of hSPP1 mRNA in human tissues by Northern blotting. Similar to previously reported data for mSPP1 mRNA (6), hSPP1 mRNA was found to be ubiquitously expressed (Fig. 6A). A predominant 3.4-kb hSPP1 mRNA species was detected in all tissues, but the species varied in levels. The levels of the species were highest in placenta and kidney and lowest in peripheral blood and small intestine. A 1.0-kb transcript, which may be a spliced isoform, was detected only in placenta (Fig. 6A, lane 10). In contrast, the expression of hSPP2 mRNA was rather tissue-specific (Fig. 6B). We observed two mRNAs of 5.1 and 1.7 kb in size, and the expression levels of the 5.1-kb mRNA were highest in kidney and heart. The expression pattern of the 1.7-kb mRNA was similar but weaker than that of the 5.1-kb mRNA (Fig. 6B). Thus, the expression pattern of SPP2 was quite different from that for SPP1.

In summary, we identified a second S1P phosphohydrolase that shows similar activity, substrate specificity, and localization to previously identified SPP1s. Sequence and substrate specificity of hSPP2 confirm that SPP2 belongs to the SPP family. Although the expression patterns of SPP1 and SPP2 were quite different, both were expressed abundantly in kidney. It is unclear why two such S1P phosphohydrolases with similar enzymatic properties exist in the same tissue. However, yeast *Saccharomyces cerevisiae* also contains two sphingoid base 1-phosphate phosphatases, Lcb3p and Ysr3p, which also share similar enzyme activity and substrate specificity (8) and which are both localized to the ER (21). In synthetic complete medium the Δlcb3 mutant, but not the Δysr3 mutant, was significantly protected from loss of viability after severe heat shock (9). However, the Δysr3 mutant showed a dramatic enhancement in survival when cultured in YPD medium (9). Moreover, transcription of *YSR3* was up-regulated by heat shock (9). These results suggest that Lcb3p and Ysr3p are differentially regulated by growth and stress conditions. Therefore, it is possible that SPP1 and SPP2 are differentially regulated by unidentified conditions and have different roles in such conditions. Additional work will be needed to determine their specific function and regulation.

**Acknowledgment**—We thank A. Wada for the pcDNA3 HA-4 plasmid.
Identification and Characterization of a Novel Human Sphingosine-1-phosphate Phosphohydrolase, hSPP2
Chie Ogawa, Akio Kihara, Maiko Gokoh and Yasuyuki Igarashi

J. Biol. Chem. 2003, 278:1268-1272.
doi: 10.1074/jbc.M209514200 originally published online October 30, 2002

Access the most updated version of this article at doi: 10.1074/jbc.M209514200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 23 references, 15 of which can be accessed free at
http://www.jbc.org/content/278/2/1268.full.html#ref-list-1