

# Regulation of Autophagy by Sphingosine Kinase 1 and Its Role in Cell Survival during Nutrient Starvation\*

Received for publication, June 7, 2005, and in revised form, January 12, 2006 Published, JBC Papers in Press, January 16, 2006, DOI 10.1074/jbc.M506182200

Grégory Lavie<sup>‡</sup>, Francesca Scarlatti<sup>§</sup>, Giusy Sala<sup>§</sup>, Stéphane Carpentier<sup>¶</sup>, Thierry Levade<sup>¶</sup>, Riccardo Ghidoni<sup>§</sup>, Joëlle Botti<sup>‡||</sup>, and Patrice Codogno<sup>‡||1</sup>

From the <sup>‡</sup>INSERM U504, Institut André Lwoff, 94807 Villejuif Cedex, France, <sup>||</sup>INSERM U756, Faculté de Pharmacie, Université Paris-Sud, 92296 Châtenay-Malabry, France, the <sup>§</sup>Laboratory of Biochemistry and Molecular Biology, San Paolo Medical School, via A. di Rudini 8, 20142 Milan, Italy, and the <sup>¶</sup>INSERM U466, Institut Louis Bugnard, Centre Hospitalier Universitaire de Rangueil, BP 84225, 31432 Toulouse Cedex 4, France

The sphingolipid ceramide induces macroautophagy (here called autophagy) and cell death with autophagic features in cancer cells. Here we show that overexpression of sphingosine kinase 1 (SK1), an enzyme responsible for the production of sphingosine 1-phosphate (S1P), in MCF-7 cells stimulates autophagy by increasing the formation of LC3-positive autophagosomes and the rate of proteolysis sensitive to the autophagy inhibitor 3-methyladenine. Autophagy was blocked in the presence of dimethylsphingosine, an inhibitor of SK activity, and in cells expressing a catalytically inactive form of SK1. In SK1<sup>wt</sup>-overexpressing cells, however, autophagy was not sensitive to fumonisin B1, an inhibitor of ceramide synthase. In contrast to ceramide-induced autophagy, SK1(S1P)-induced autophagy is characterized by (i) the inhibition of mammalian target of rapamycin signaling independently of the Akt/protein kinase B signaling arm and (ii) the lack of robust accumulation of the autophagy protein Beclin 1. In addition, nutrient starvation induced both the stimulation of autophagy and SK activity. Knocking down the expression of the autophagy protein Atg7 or that of SK1 by siRNA abolished starvation-induced autophagy and increased cell death with apoptotic hallmarks. In conclusion, these results show that SK1(S1P)-induced autophagy protects cells from death with apoptotic features during nutrient starvation.

Macroautophagy (hereafter referred to as autophagy) is a lysosomal catabolic pathway for macromolecules and organelles (1–3). The discovery of ATG<sup>2</sup> in yeast has revealed the evolutionarily conserved mechanism for the formation of autophagosomes that sequester cytoplasmic material before they fuse with the endo/lysosomal compartment (4, 5). Stimulation of autophagy during the limitation of nutrient

supply is a cell-defense mechanism by which amino acids and other intracellular nutrients are recycled to maintain survival (6, 7) and to repress the induction of apoptosis (8). The role of starvation-induced autophagy is vital for newborn mice after the maternal nutrient supply is interrupted (9).

Several years ago, cell death was divided into three types (10–13). Type I is apoptosis, type II is autophagic cell death, and type III is cytoplasmic cell death. Type II is characterized by the accumulation of autophagic vacuoles. Recent studies have shown that autophagic machinery plays a role in cell death occurring in the absence of pro-apoptotic members of the Bcl-2 family (14) and when caspases are inhibited (15). However, in many situations apoptosis and autophagy may both contribute to cell death making difficult to clearly distinguish type I from type II cell death (16–19).

One characteristic of autophagy is its dependence on several tumor suppressor genes acting both in the formation of the autophagosome and in signaling pathways controlling autophagy (20, 21). Autophagy is thought to be a tumor suppressor mechanism, because cancer cells often display reduced autophagic capacities (20, 21). Beclin 1 is a tumor suppressor gene product that plays an important role, together with the class III phosphatidylinositol 3-kinase (PI3K), during the formation of the autophagosome (22, 23). *Beclin 1* haploinsufficiency induces a high level of tumor development in various mouse tissues (24, 25). The tumor suppressor PTEN (phosphatase and tensin homolog deleted on chromosome 10), which deactivates the class I PI3K signaling pathway, has a stimulating effect on autophagy (26). Its deletion favors the inhibition of autophagy by the class I PI3K signaling pathway by activating mTOR (target of rapamycin), a conserved nutritional sensor that represses autophagy (6, 27, 28).

However, the role of autophagy during tumor progression is probably more complex, because autophagic vacuoles have been observed in several types of human tumors (29). Whether these autophagic vacuoles are associated with cell survival or cell death remains to be clearly demonstrated.

Numerous drugs have been reported to trigger cell death with autophagic features in cancer cells (20, 21, 30). However, the events that lead to the accumulation of autophagic vacuoles by cancer treatments have only begun to be identified (30). The antiestrogen drug tamoxifen triggers autophagic cell death in human breast cancer MCF-7 cells (31). We have recently shown that the sphingolipid ceramide is a downstream target of tamoxifen during autophagy in MCF-7 cells (32). Ceramide stimulates autophagy by relieving the class I PI3K/Akt/PKB pathway and provoking the accumulation of the autophagy gene product Atg6/Beclin 1. In line with these findings, a cell-permeable analog of ceramide has recently been shown to induce autophagic cell death in glioma cells

\* This work was supported by institutional funding from the Institut National de la Santé et de la Recherche Médicale (INSERM) and from University Paris-Sud, by grants from the Association pour la Recherche sur le Cancer (n°3503 to PC) and a bilateral French-Italian exchange program (Galileo). 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.

<sup>1</sup> To whom correspondence should be addressed: INSERM U756 Faculté de Pharmacie 92296 Châtenay-Malabry, France. Tel.: 33-1-468-35528; Fax: 33-1-468-35844; E-mail: codogno@vjf.inserm.fr.

<sup>2</sup> The abbreviations used are: ATG, autophagy-related genes; C<sub>2</sub>-Cer, C<sub>2</sub>-ceramide; DGK, diacylglycerol kinase; DMEM, Dulbecco's modified Eagle's medium; 4E-BP1, eukaryotic translational initiation factor 4E-binding protein-1; FB1, fumonisin B1; GFP, green fluorescent protein; LC3, light chain 3; 3MA, 3-methyladenine; PBS, phosphate-buffered saline; Akt/PKB, protein kinase B; p70S6K, p70S6 kinase; PI3K, phosphatidylinositol 3-kinase; PLD, phospholipase D; S1P, sphingosine 1-phosphate; SK, sphingosine kinase; mTOR, mammalian target of rapamycin; z-VAD-fmk, N-benzyloxycarbonyl-Val-Ala-DL-Asp-fluoromethylketone; DMS, dimethylsphingosine; HA, hemagglutinin; siRNA, small interference RNA; PIC, Protease Inhibitor Mixture; wt, wild type; PARP, poly(ADP-ribose) polymerase.

by increasing the expression of the mitochondrial bound BH3-only BNIP3 protein (33).

Sphingolipids are ubiquitous constituents of eukaryotic membranes. Their metabolism is a highly dynamic process generating second messengers, including ceramide, sphingosine, and sphingosine 1-phosphate (S1P) (34–38). Ceramide is deacylated by ceramidases to generate sphingosine, which is then phosphorylated by sphingosine kinases to produce S1P (36). It is now generally admitted that ceramide, sphingosine, and S1P have contrasting effects on the response to stress (34–38). Ceramide and sphingosine are often associated with cell-growth arrest and the induction of cell death, whereas S1P favors cell proliferation and cell survival. Thus, the dynamic balance between ceramide, sphingosine, and S1P is important in determining whether cells survive or die (36, 39). The existence of this sphingolipid rheostat stress regulator led us to consider the role sphingosine kinase 1 (SK1) that produces S1P, in the control of autophagy.

In the present work we show that SK1 modulates two major players of autophagy: the mTOR signaling pathway and the expression of Beclin 1. Stimulation of autophagy by SK1 is dependent upon the production of S1P. In contrast to ceramide-induced autophagy, SK1(S1P)-induced autophagy did not induce cell death but has a protective effect toward apoptosis during nutrient starvation. These findings, and others in the literature (32, 33), show that both the tumor-suppressor ceramide and the tumor-promoter S1P (38) are able to trigger autophagy but with different outcomes on cancer cell survival and death.

## EXPERIMENTAL PROCEDURES

### Reagents

C<sub>2</sub>-Cer and DMS were from Calbiochem (VWR International). Fumonisin B1 (FB1) was purchased from Sigma. 3MA was from Fluka. z-VAD-fmk was from Apotech. Cell culture medium and fetal bovine serum were from Invitrogen. Nitrocellulose membranes were from Schleicher & Schüll. Ceramide from porcine brain, used as an internal standard, was from Avanti Polar Lipids Inc. The radioisotopes L-[U-<sup>14</sup>C]valine (5.47 GBq/mmol) and [<sup>32</sup>P]ATP (110 TBq/mmol) and [9,10-<sup>3</sup>H]myristic acid (1.69 TBq/mmol), the ECL<sup>TM</sup> Western blotting detection kit, and the donkey anti-rabbit antibody were purchased from BD Biosciences. The mouse monoclonal anti-Beclin1 was from Amersham Bioscience; the mouse monoclonal anti-PARP and the rabbit polyclonal anti-phospho-PKB Ser<sup>473</sup> were from Cell Signaling. Goat anti-PKB was from Santa Cruz Biotechnology. The mouse monoclonal anti-actin was from Chemicon International Inc. The rabbit polyclonal anti-p70S6K, anti-phospho-p70S6K Thr<sup>389</sup>, anti-phospho-S6 Ser<sup>235</sup>, and anti-4E-BP1 were from Ozyme, and anti-FLAG M2 was from Sigma. The rabbit polyclonal anti-Atg7 was kindly provided by W. A. Dunn (University of Florida, Gainesville). Goat anti-mouse and swine anti-goat antibodies were obtained from Bio-Rad and Caltag, respectively.

### Plasmids

The plasmid expression vectors for human sphingosine kinase 1 and catalytically inactive mutant human sphingosine kinase 1 (pcDNA3-hSK1-FLAG and pcDNA3-hSK1<sup>G82D</sup>-FLAG) were a gift from S. Pitson and B. Wattenberg (Hanson Institute, Adelaide, Australia) (40). The plasmid expression vector encoding for GFP-LC3 (pEGFP-LC3) was kindly provided by T. Yoshimori (National Institute of Genetics, Mishima, Japan). The plasmid expression vectors for PLD1 and catalytically inactive mutant PLD1 (pCGN-HA-hPLD1 and pCGN-HA-hPLD1<sup>K898R</sup>) were kindly given by M. Frohman (SUNY, New York).

### Cell Culture and Transfection

Human breast cancer MCF-7 cells were maintained at 37 °C in 10% CO<sub>2</sub> in DMEM, supplemented with 10% fetal bovine serum and 100 ng/ml each of penicillin and streptomycin as previously reported (32). Beclin 1 MCF-7/tet-off (MCF-7.*beclin 1*) cells kindly provided by B. Levine (University of Texas Southwest Medical Center, Dallas) were used during starvation-induced autophagy. Briefly, cells were maintained in DMEM with 200 μg/ml hygromycin B and 2 μg/ml tetracycline (41). Expression of Beclin 1 was analyzed by Western blotting 5 days after tetracycline withdrawal. Starvation was induced by incubating cells in nutrient-free medium (Hanks' balanced salt solution plus 0.1% bovine serum albumin). Cells were transfected by using FuGENE 6 transfection reagent (Roche Applied Science), as recommended by the manufacturer. Briefly, cells (0.5 × 10<sup>6</sup> cells/well) were plated in 6-well plates. Two days later, 1 μg of plasmid and 3 μl of FuGENE 6 were suspended in 100 μl of DMEM and added to the culture medium. When cells were co-transfected with SK1, PLD1 (or empty vector), and GFP-LC3 vectors, the transfection efficiency was 40% as determined by counting GFP-positive cells. Cells were analyzed 24 or 48 h after co-transfection as detailed below.

### siRNA

SK1 and Atg7 knockdowns were accomplished by transfecting MCF-7/GFP-LC3 cells with siRNAs. The RNAi target sequences were: sense, 5'-GGGCAAGCCUUGCAGCUC-3' for SK1 (42) and sense, 5'-CCAACACACUCGAGUCUUU-3' for Atg7. As a control, siRNA targeting the unrelated protein phosphomannomutase-2 (sense, 5'-CUGGGAAAUGAUGUGGUUG) was used. siRNA were purchased from Eurogentec (Seraing, Belgium). MCF-7 cells were seeded at 8 × 10<sup>4</sup>/cm<sup>2</sup>. After 24 h, cells were transfected for 4 h with siRNA using Oligofectamine (Invitrogen) as recommended by the manufacturer. Cells were cultured for 24 h before analysis.

### Reverse Transcription-PCR

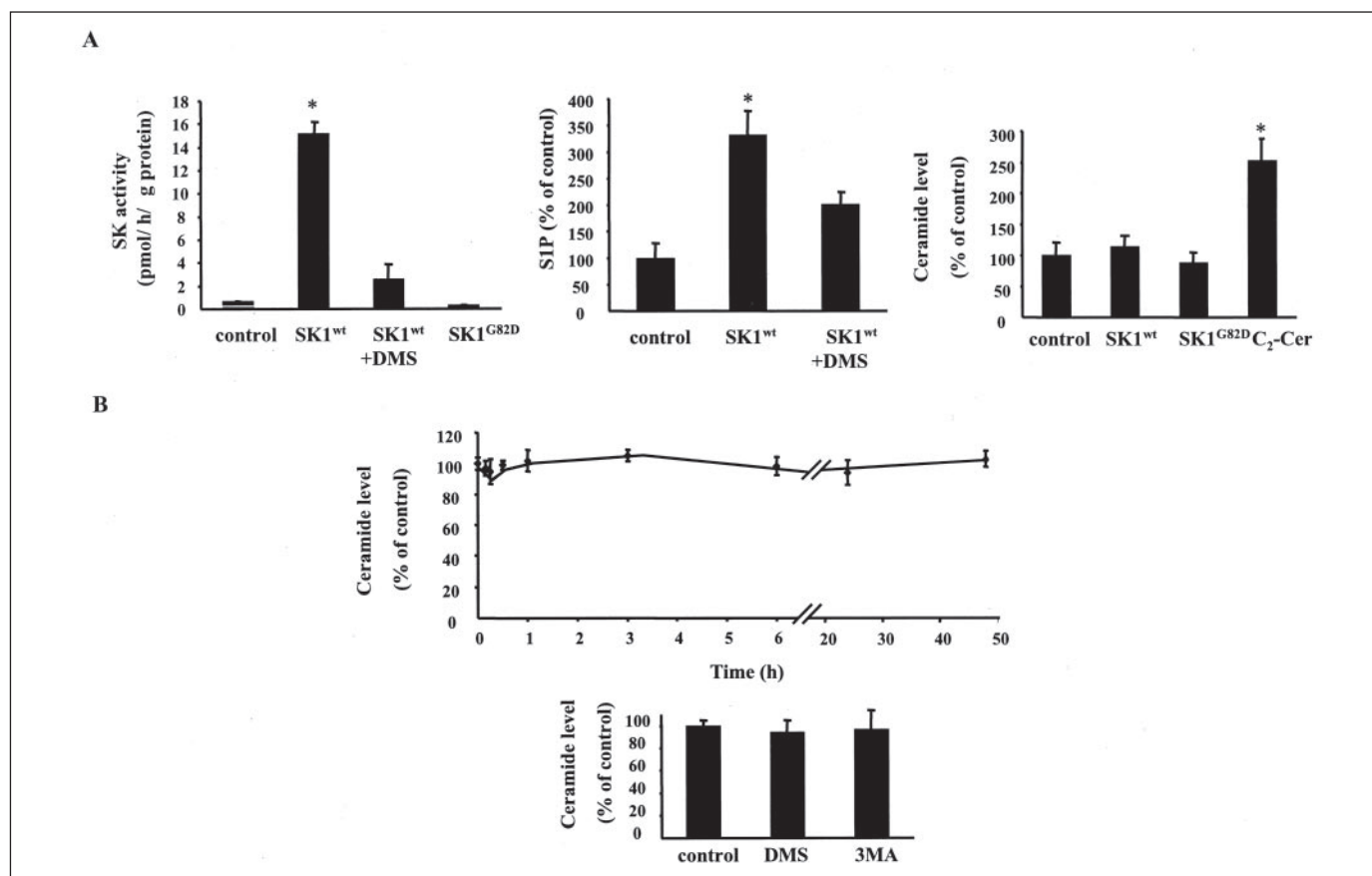
Cells were disrupted in 4 M guanidine thiocyanate, and total RNA was isolated by sedimentation in cesium chloride. RNA was transcribed with superscript II (Invitrogen). SK1 was amplified by the following specific primers (42): forward, 5'-ATCCAGAAGCCCCTGTGTAGCCTCC-3'; reverse, 5'-GCAGCAAACATCTCACTGCCAGGT-3'. Glyceraldehyde-3-phosphate dehydrogenase was amplified by following primers: forward, 5'-CGGAGTCAACGGATTTGGTCGTAT-3'; reverse, 5'-AGCCTTCTCCATGGTCGTGAAGAC-3'.

### Autophagic Parameters

**GFP-LC3 Staining**—GFP-LC3 staining was carried out essentially as described previously (43). At the indicated time after transfection GFP-LC3 staining was visualized using an Axioplan Zeiss microscope, and the number of GFP-LC3-positive cells with GFP-LC3 dots was determined.

**Proteolysis**—Proteolysis was determined as described previously (44). Briefly, cells were incubated for 24 h at 37 °C in normal culture medium containing 7400 Bq/ml of [<sup>14</sup>C]valine. Cells were rinsed three times with PBS, pH 7.4, and then the cells were incubated for 1 h in complete medium supplemented with 10 mM valine. After incubating for 1 h, during which time short-lived proteins had been degraded, the medium was replaced by fresh chase medium for another 4 h. When required, C<sub>2</sub>-Cer (75 μM), FB1 (100 μM), DMS (1.5 μM), and 3MA (10 mM) were added to the chase medium. For the PLD1- or SK1-overexpressing cells, radiolabeling with [<sup>14</sup>C]valine was started 24 h after transfection. Cells and radiolabeled proteins from the 4-h chase medium were precipitated

## Control of Macroautophagy by Sphingosine Kinase 1



**FIGURE 1. Sphingosine kinase activity and sphingolipid levels in MCF-7 cells.** *A*, MCF-7 cells were transfected with an empty vector (control), cDNAs encoding the wild-type SK1 (SK1<sup>WT</sup>) or a catalytically inactive SK1 mutant (SK1<sup>G82D</sup>). *Left*, SK activity was analyzed as described under "Experimental Procedures." When required 1.5  $\mu$ M DMS was added at the beginning of the experiment. *Middle*, S1P was determined after [<sup>3</sup>H]sphingosine incorporation in control cells or SK1<sup>WT</sup>-overexpressing cells in the presence or absence of DMS. [<sup>3</sup>H]Sphingosine recovery in control cells was set to 100%. *Right*, endogenous long-chain ceramides were quantified by the DGK assay in control cells, SK1<sup>WT</sup>-overexpressing cells, SK<sup>G82D</sup>-expressing cells, and cells treated by C<sub>2</sub>-Cer (75  $\mu$ M, 2 h). *B*, quantification of ceramide. Endogenous long-chain ceramides were quantified by the DGK assay in SK1<sup>WT</sup>-overexpressing cells cultured in complete medium at different time points. When required 3MA (10 mM) and DMS (1.5  $\mu$ M) were added, and the ceramide level was analyzed after 24 h of treatment. The amount of ceramide (5.2 pmol of Cer/nmol of P<sub>i</sub>) in MCF-7 cells cultured in complete medium was set to 100%. Values are the mean  $\pm$  S.D. of three independent experiments. \*,  $p < 0.05$  versus control cells.

in trichloroacetic acid at a final concentration of 10% (v/v) at 4 °C. Radioactivity was determined by liquid scintillation counting. Protein degradation was calculated by dividing the acid-soluble radioactivity recovered from both cells and medium by the radioactivity contained in the precipitated proteins from both cells and medium.

### Cell Death Assay

Cell viability was determined by the trypan blue exclusion test as previously described (32). When required, 40  $\mu$ M z-VAD-fmk was added prior to incubation in nutrient-free medium (Hanks' balanced salt solution plus 0.1% of bovine serum albumin).

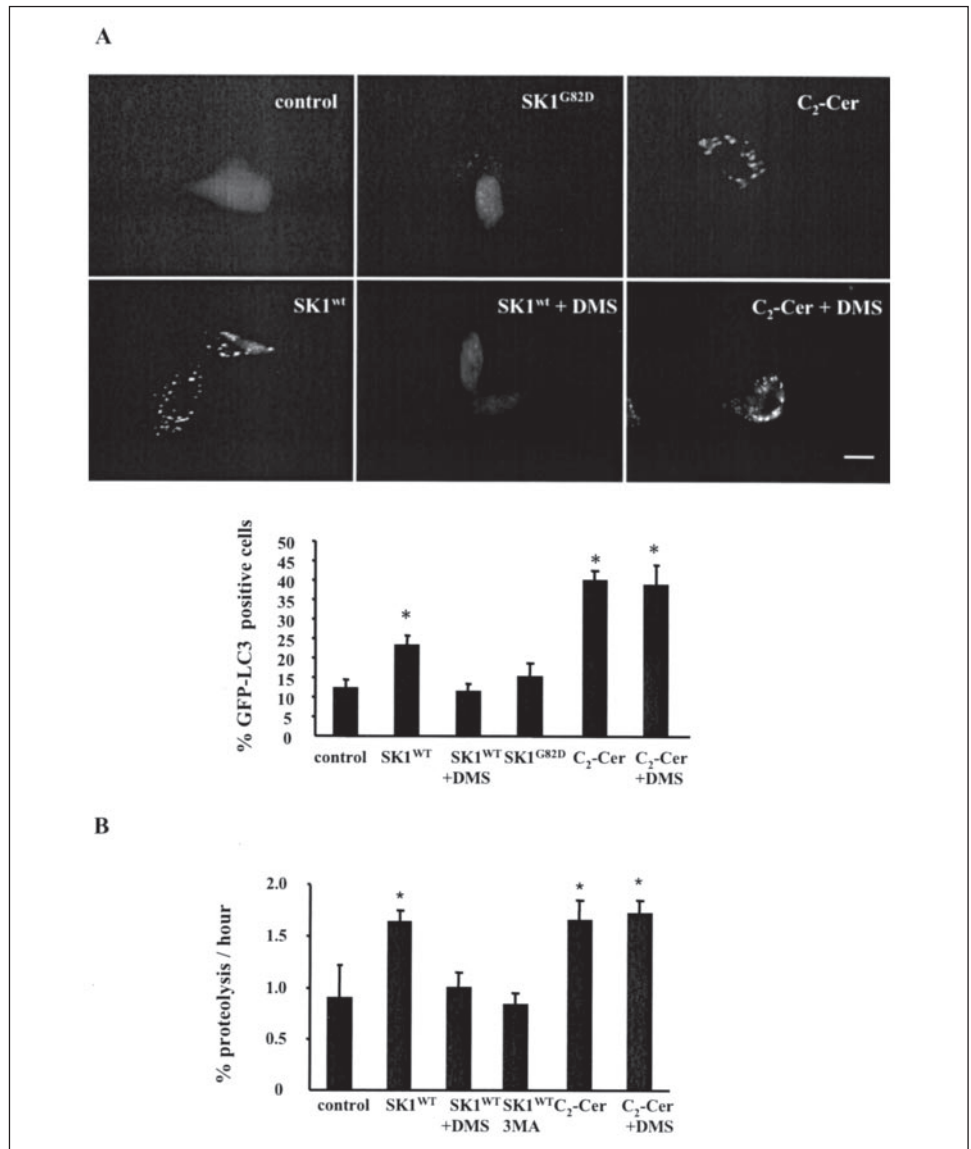
### Western Blot Analyses

Cells were collected by centrifuging (10 min, at 1200  $\times$  g), washing with PBS, and resuspending in lysis buffer (1% Triton X-100, 25 mM Tris-HCl, pH 7.4, 1 mM phenylmethylsulfonyl fluoride, 1% Protease Inhibitor Mixture (PIC), 1 mM Na<sub>3</sub>VO<sub>4</sub>, 1 mM NaF) for 1 h at 4 °C. The cell lysate was centrifuged (20,000  $\times$  g for 30 min at 4 °C), and the supernatant was recovered. Protein concentrations were determined by the bicinchoninic acid (BCA) method as recommended by the manufacturer. Extracted proteins were first separated in SDS-polyacrylamide gels and then electrotransferred onto nitrocellulose membranes. After blocking overnight with fat-free milk, the membranes were incubated with appropriate primary antibodies: anti-Beclin 1 (1/2000), anti-PARP

(1/1000), anti-p70S6K (1/1000), anti-phospho-p70S6K Thr<sup>389</sup> (1/1000), anti-phospho-S6 Ser<sup>235</sup> (1/1000), anti-4E-BP1 (1/1000), anti-FLAG (1/1000), anti-Atg7 (1/1000), and anti-PKB and anti-phospho-PKB Ser<sup>473</sup> (1/1000). Primary antibodies were detected by chemiluminescence using horseradish peroxidase-conjugated secondary antibodies against rabbit, goat, or mouse immunoglobulins. Fluorographs were quantitatively scanned using the NIH image software.

### Quantification of Ceramide

Ceramide levels were measured using the DGK assay as described previously (45). Briefly, cells were collected, and lipids were extracted according to a previous study (46). The organic phase was divided into 1/2 and 1/6 aliquots, dried, and used for ceramide and total phospholipid measurements (47), respectively. Briefly, 30 nmol of extracted lipids was incubated at room temperature for 45 min in the presence of  $\beta$ -octylglucoside/dioleoylphosphatidylglycerol micelles, 2 mM dithiothreitol, 5  $\mu$ g of proteins of DGK containing membranes (Calbiochem), and 1 mM ATP mixed with [ $\gamma$ -<sup>32</sup>P]ATP (4.8  $\times$  10<sup>5</sup> Bq/ml) in a final volume of 0.1 ml. At the end of the reaction, lipids were extracted, and [<sup>32</sup>P]ceramide 1-phosphate was determined by TLC separation in chloroform/acetone/methanol/acetic acid/water (10:4:3:3:1, v/v). The radioactivity associated with ceramide 1-phosphate spots was determined after scraping and counting in a scintillation counter. Ceramide levels were expressed in terms of the total phospholipid content.



**FIGURE 2. Sphingosine kinase 1 stimulates autophagy in MCF-7 cells.** *A*, GFP-LC3 staining and quantitative analysis in MCF-7 transfected with an empty vector (control), in SK1<sup>WT</sup>-overexpressing cells, in SK1<sup>G82D</sup>-expressing cells, and in cells treated with C<sub>2</sub>-Cer (75 μM, 2 h). When required, 1.5 μM DMS was added. The bar represents 10 μm. *B*, degradation of [<sup>14</sup>C]valine-labeled long-lived proteins was determined in control cells, in SK1<sup>WT</sup>-overexpressing cells, and in cells treated with C<sub>2</sub>-Cer (75 μM) as described under "Experimental Procedures." When required 3MA (10 mM) and DMS (1.5 μM) were added during the chase period. Values reported are the mean ± S.D. of four independent experiments. \*, *p* < 0.05 versus control cells.

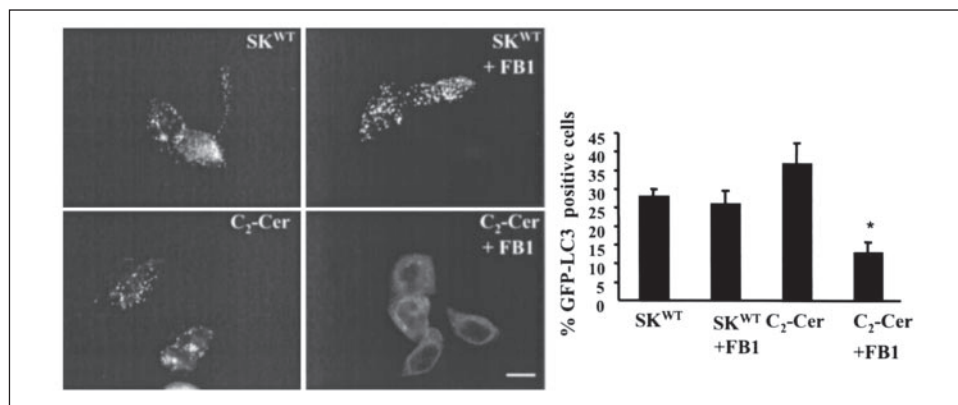
### Sphingosine Kinase Activity and Quantification of S1P

Sphingosine kinase activity was determined as described previously (48). Briefly, cells were collected and lysed by repeated freeze-thawing cycles in 200 μl of lysis buffer (20 mM Tris, pH 7.4, 20% glycerol, 1 mM EDTA, 1 mM dithiothreitol, 0.01 mM MgCl<sub>2</sub>, 1 mM Na<sub>3</sub>VO<sub>4</sub>, 15 mM NaF, 1 mM phenylmethylsulfonyl fluoride, 1% PIC, 0.1% Triton X-100, 0.5 mM 4-deoxy pyridoxine). After centrifuging at 13,000 × *g* for 30 min, the protein concentration of supernatant was determined with a Coomassie Plus Protein Assay kit (Pierce). Proteins (100 μg) were then incubated with 25 μM D-erythrosphingosine dissolved in 0.1% Triton X-100, 2 mM ATP, and [γ-<sup>32</sup>P]ATP (3.7 × 10<sup>5</sup> Bq dissolved in 20 mM MgCl<sub>2</sub>) for 30 min at 37 °C in a final volume of 200 μl. The reaction was stopped by adding 20 μl of HCl, 1 N, followed by 800 μl of chloroform/methanol/HCl (100:200:1, v/v). After vigorous vortexing, 250 μl of chloroform and 250 μl of KCl 2 M were added, and phases were separated by centrifugation. The organic layer was dried and resuspended in chloroform/methanol/HCl 37% (100:100:0.2, v/v). Lipids were resolved on silica TLC plates (Whatman) in 1-butanol/acetic acid/water (3:1:1, v/v). Labeled S1P spots were visualized by autoradiography and quantified by scraping and counting in a scintillation counter.

For quantification of S1P, MCF-7 cells were labeled for 7 h in serum-free medium with 0.3 μCi/ml D-erythro-[3-<sup>3</sup>H]sphingosine (PerkinElmer Life Sciences). After washing with PBS, cells were scraped and lysed in water. Radiolabeled S1P was extracted by phase partition as previously described (49, 50) and counted by liquid scintillation.

**Phospholipase D Activity**—PLD activity was determined as described previously (51) with some modifications. Briefly, 2 × 10<sup>6</sup> cells were plated on 25-cm<sup>2</sup> flasks for 24 h; when required, cells were transfected with PLD1 vectors (wild-type and K898R mutant). After 24 h, cells were preincubated in 0.5% fetal bovine serum containing DMEM for 1 h. After removing the preincubation medium, cells were labeled with [<sup>3</sup>H]myristic acid (Amersham Biosciences) for 1 h at 1.11 × 10<sup>5</sup> Bq/ml in 0.5% fetal bovine serum containing DMEM. Cells were rinsed with PBS and incubated for 30 min in complete medium containing 0.5% butanol. Cells were rinsed with PBS and scraped in methanol/water (98:2, v/v). Cells were rinsed with methanol, and the lipids were extracted by adding equal amounts of chloroform and water. After centrifuging, the lower phase was collected and dried under vacuum before being redissolved in chloroform. Lipids were separated by TLC on silica-coated plastic sheets (Merck) in a solvent system of chloroform:

**FIGURE 3. Effect of fumonisin B1 (FB1) on autophagy.** GFP-LC3 staining and quantitative analysis in SK1<sup>WT</sup>-overexpressing cells and in cells treated with C<sub>2</sub>-Cer (75 μM 2 h). When required, 100 μM FB1 was added. The bar represents 10 μm. Values are representative of three independent experiments. \*, *p* < 0.05 versus C<sub>2</sub>-Cer-treated cells.



methanol:acetic acid:water (75:45:3:0.4, v/v). Radioactive components were detected by autoradiography. PLD activity was determined by the [<sup>3</sup>H]phosphatidylbutanol/[<sup>3</sup>H]phosphatidylcholine ratio. Phosphatidylcholine (Avanti polar) and phosphatidylbutanol (Biomol) diluted in chloroform were loaded as standards and were visualized with iodine vapor.

### Statistical Analysis

Statistical analysis of the differences between the groups was performed using Student's *t* test. *p* < 0.05 was considered statistically significant.

## RESULTS

**Sphingosine Kinase 1 Overexpression Stimulates Autophagy**—MCF-7 cells were transfected either with the cDNA encoding the wild-type SK1 (SK1<sup>WT</sup>) or that encoding a mutant inactive form (SK1<sup>G82D</sup>). The activity of SK was significantly increased in SK1<sup>WT</sup>-overexpressing cells when compared with that observed in control cells transfected with an empty vector or in SK1<sup>G82D</sup>-expressing cells (Fig. 1A). Accordingly, SK1<sup>WT</sup>-overexpressing cells displayed a higher level of S1P compared with control cells (Fig. 1A). In the presence of the SK inhibitor DMS, both S1P production and SK activity were dramatically reduced in these cells. In contrast, no change in the level of ceramide was detected in SK1<sup>WT</sup>-overexpressing cells at any time points after transfection (Fig. 1, A and B).

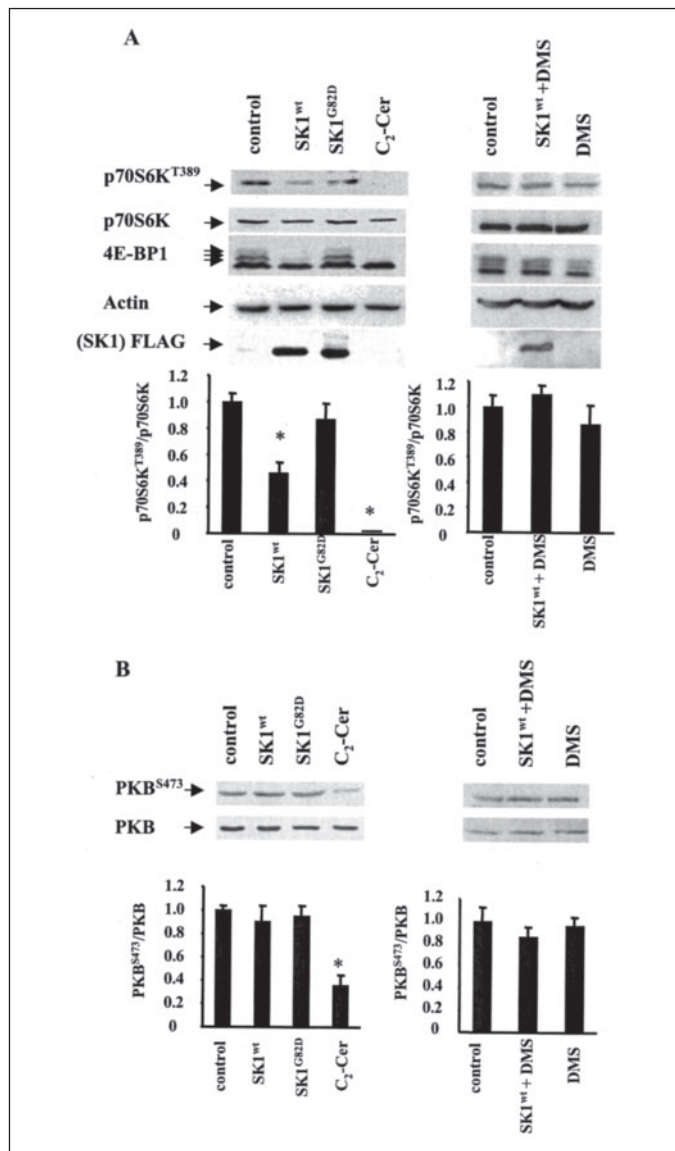
Autophagy was first analyzed after transfection of GFP-LC3 (Fig. 2A). LC3 is a reliable marker of autophagosomes in mammalian cells, and its localization changes from a diffuse cytosolic pattern to a punctuate pattern representing its recruitment to the autophagosomal membrane during the induction of autophagy (43). An accumulation of autophagic vacuoles was observed in the SK1<sup>WT</sup>-overexpressing cells but not in the SK1<sup>G82D</sup>-expressing cells (Fig. 2A). Accordingly, proteolysis sensitive to 3MA, another hallmark of autophagy (52), was increased in SK1<sup>WT</sup>-overexpressing cells (Fig. 2B). In addition, the autophagic phenotype was abolished when SK1<sup>WT</sup>-overexpressing cells were treated with 1.5 μM DMS. However, DMS did not block C<sub>2</sub>-Cer-induced autophagy in MCF-7 (Fig. 2), suggesting that this drug does not inhibit autophagy in an unspecific manner. FB1, an inhibitor of CoA-dependent dihydroceramide/ceramide synthase, which inhibits C<sub>2</sub>-Cer-induced autophagy, by impairing its conversion to long-chain ceramides (see ref. 32), did not block autophagy in SK1<sup>WT</sup>-overexpressing cells (Fig. 3). Overall, these findings led us to conclude that the production of S1P by SK1 has a stimulatory effect on autophagy, which is not merely a consequence of an increase in ceramide level.

**Role of mTOR in Controlling SK1(S1P)-induced Autophagy**—On the basis of genetics studies and the use of the immunosuppressor rapamycin,

it has been demonstrated that the inhibition of TOR plays a central role in stimulating autophagy throughout the eukaryotic kingdom (6, 27, 28). To confirm that a reduction of mTOR signaling activity was involved in the control of SK1(S1P)-induced autophagy, we investigated the phosphorylation of two known downstream effectors of mTOR, namely p70S6K and the eukaryotic translation inhibitor, 4E-BP1 (Fig. 4A). The phosphorylation of p70S6K Thr<sup>389</sup>, the major rapamycin-sensitive site (53), was greatly reduced in SK1<sup>WT</sup>-overexpressing cells. In line with the mTOR-dependent phosphorylation of 4E-BP1 (54), the hyperphosphorylated state of 4E-BP1 was dephosphorylated to a fast-migrating band in SK1<sup>WT</sup>-overexpressing cells (Fig. 4A). In contrast, mTOR signaling was neither affected in DMS-treated SK1<sup>WT</sup>-overexpressing cells nor in SK1<sup>G82D</sup>-expressing cells (Fig. 4A). However, the phosphorylation of Akt/PKB that regulates autophagy upstream of mTOR (26) was not significantly reduced in SK1<sup>WT</sup>-overexpressing cells treated or not by DMS (Fig. 4B). In contrast, C<sub>2</sub>-Cer induced both an inhibition of Akt/PKB and mTOR signaling (see Ref. 32 and Fig. 4). These data suggest that SK1 may stimulate autophagy independently of the inhibition of the class I PI3K/Akt/PKB signaling pathway.

Alternatively, we reasoned that forced stimulation of mTOR should affect SK1(S1P)-induced autophagy. Recently, phosphatidic acid produced by phospholipase D has been shown to activate mTOR in a rapamycin-dependent manner in nutrient-rich medium (55–57). First MCF-7 cells were transfected with cDNAs encoding PLD1<sup>WT</sup> and a mutant inactive form PLD1<sup>K898R</sup>. An increase in PLD activity was only observed in the PLD1<sup>WT</sup>-overexpressing cells (Fig. 5A). According to previous results (55), mTOR signaling was increased in MCF-7 cells overexpressing PLD1<sup>WT</sup> as revealed by the increase of p70S6K Thr<sup>389</sup> phosphorylation and that of the p70S6K substrate ribosomal protein S6 (Fig. 5B). This stimulatory effect was abolished in the presence of 100 nM rapamycin (Fig. 5B). Moreover, the expression of PLD1<sup>WT</sup> reversed the inhibition of mTOR signaling pathway in SK1<sup>WT</sup>-overexpressing cells (Fig. 5B). Interestingly, expression of the PLD1<sup>WT</sup> was able to abrogate both the accumulation of GFP-LC3 dots and the stimulation of [<sup>14</sup>C]valine-labeled protein degradation observed in SK1<sup>WT</sup>-overexpressing cells (Fig. 5C, lower panel). In contrast, expression of PLD1<sup>K898R</sup> did not inhibit autophagy in SK1<sup>WT</sup>-overexpressing cells, as exemplified by its lack of inhibition on proteolysis (Fig. 5C, lower panel).

**Effect of SK1 on the Accumulation of Beclin 1**—Ceramide-induced autophagy is characterized by the robust accumulation of the autophagy protein, Beclin 1 (32). A slight but reproducible accumulation of Beclin 1 was observed in SK1<sup>WT</sup>-overexpressing MCF-7 but not in cells expressing the mutant form of SK1 or in cells transfected with an empty vector (Fig. 6). However, SK1-induced accumulation of Beclin 1 was less pronounced than cells having an elevated level of ceramide after C<sub>2</sub>-Cer supplementation (Fig. 6 and see Ref. 32). However, both minute changes



**FIGURE 4. Effect of SK1 on mTOR signaling pathway and Akt/PKB activation.** *A*, analysis of mTOR signaling. Cell lysates of control MCF-7 cells (empty vector) treated or not by 1.5  $\mu$ M DMS, C<sub>2</sub>-Cer (75  $\mu$ M, 2 h)-treated cells, SK1<sup>wt</sup>-overexpressing cells treated or not by 1.5  $\mu$ M DMS, and SK1<sup>G82D</sup>-expressing cells were analyzed by Western blotting for p70S6K phosphorylation at Thr<sup>389</sup> using a phosphospecific antibody. The expression of total p70S6K was detected by using an anti-pan p70S6K antibody. Densitometry was performed on the original fluorographs, and the ratio of phospho-Thr<sup>389</sup>/total p70S6K in control cells was set to 1. The phosphorylation of 4E-BP1 was evaluated by phosphorylation-dependent band shift using anti-4E-BP1. SK1 was detected in cell lysates of SK1<sup>wt</sup>-overexpressing cells and SK1<sup>G82D</sup>-expressing cells by anti-FLAG. Actin was used as loading control. When required, 1.5  $\mu$ M DMS was added. *B*, Akt/PKB activation. Cell lysates obtained as described above were analyzed by Western blotting for Akt/PKB phosphorylation at Ser<sup>473</sup> using a phosphospecific antibody. The expression of total Akt/PKB was detected by using an anti-pan Akt/PKB antibody. Densitometry was performed on the original fluorographs, and the ratio of phospho-Ser<sup>473</sup>/total Akt/PKB in control cells was set to 1. Values are the mean  $\pm$  S.D. of three independent experiments. \*,  $p < 0.05$  versus control cells.

in Beclin 1 accumulation and inhibition of mTOR signaling pathway could be responsible for the stimulatory effect S1P on autophagy.

**SK1(S1P)-induced Autophagy Protects Cells During Starvation—**Next, we investigated the physiological consequence of SK1(S1P)-induced autophagy. According to previous results, enforced expression of SK1<sup>wt</sup> has no deleterious effect on cell viability in MCF-7 cells (58). In addition and as previously reported (58), the chronic supplementation of MCF-7 cells with 100 nM S1P during 10 days neither affected cell

growth nor viability, yet autophagic vacuoles were detected (data not shown).

To investigate whether SK1(S1P)-induced autophagy can contribute to cell survival during starvation, we used stably transfected MCF-7.*beclin1* sensitive to tetracycline, which express an increased expression of Beclin 1 after removing tetracycline from the medium (Fig. 7*A* and Ref. 41). According to Liang *et al.* (41), autophagy was stimulated in MCF-7.*beclin1* cells in the absence of nutrients (Fig. 8*A*). Inhibition of autophagy by 10 mM 3MA treatment or silencing the expression of the autophagy protein Atg7, involved in the formation of autophagosomes (59, 60), reduced cell viability after 48 h in nutrient-free medium (Fig. 8, *A* and *B*). When MCF-7.*beclin1* cells were incubated for 48 h in nutrient-free medium, a 2-fold increase in SK activity was observed (Fig. 7*B*) without any significant change in ceramide level (Fig. 7*C*). Inhibition of SK activity by DMS or SK1-specific siRNA severely impaired starvation-induced autophagy and cell viability of MCF-7.*beclin1* cells (Fig. 8, *A* and *B*) in the absence of any effect on ceramide level (Fig. 7*C*). Whatever the approach used to inhibit starvation-induced autophagy, cell death was characterized by the appearance of the cleaved form of the caspase substrate PARP (Fig. 8). Both cell death and PARP cleavage were abolished in the presence of 40  $\mu$ M of the pan caspase inhibitor z-VAD-fmk (Fig. 8*B*). These results suggest that apoptosis is involved in the loss of viability of MCF-7.*beclin1* cells during starvation when autophagy is inhibited by manipulating the regulation by SK1 or the downstream formation of autophagosomes. In contrast, FB1 did not inhibit autophagy (Fig. 9) or cell viability (data not shown). These findings suggest that ceramide produced by the activity of CoA-dependent dihydroceramide/ceramide synthase is not required to control autophagy under these conditions.

## DISCUSSION

On the basis of overexpression and siRNA approaches, the present work shows the role of SK1 in the regulation of autophagy. Moreover, the production of S1P by SK1 is necessary to stimulate autophagy, because the pharmacological inhibition of SK activity or transfection of a catalytically inactive form of SK1 blunts autophagic capacities. However, a role for a metabolite derived from S1P cannot be totally excluded in the regulation of autophagy because of the rapid turnover of S1P (36, 38). We had previously shown that an elevation of the level of ceramide triggers autophagy in MCF-7 cells and in human colon cancer cells (32). However, on the basis of measurement of ceramide level and inhibition of ceramide synthase by FB1, we conclude that SK1-induced autophagy is not merely due to an increase of ceramide level. Moreover, and in contrast to ceramide-induced autophagy, the activation of Akt/PKB was not impaired during S1P-induced autophagy. The absence of any effect of SK1 overexpression on Akt/PKB is consistent with previous data and with its role in cell survival and tumor growth (36). However, inhibition of mTOR signaling was observed during SK1(S1P)-induced autophagy. Interestingly, enforced expression of PLD1 that produces the mTOR activator phosphatidic acid (57) counteracts the SK1(S1P)-induced autophagy and relieves mTOR inhibition. These findings show that SK1 controls autophagy by inhibiting mTOR independently of the class I PI3K arm. Although, the inhibition of mTOR signaling by S1P seems odd at a first glance, in fact it is not incompatible with its stimulating effect on cell proliferation (36, 61). mTOR is present in two complexes that have different functions. The raptor-mTOR complex is sensitive to rapamycin and controls cell growth, whereas the rictor-mTOR complex controls cell proliferation and activates Akt/PKB via a feedback loop (62). This complex has a low sensitivity to rapamycin inhibition. Further

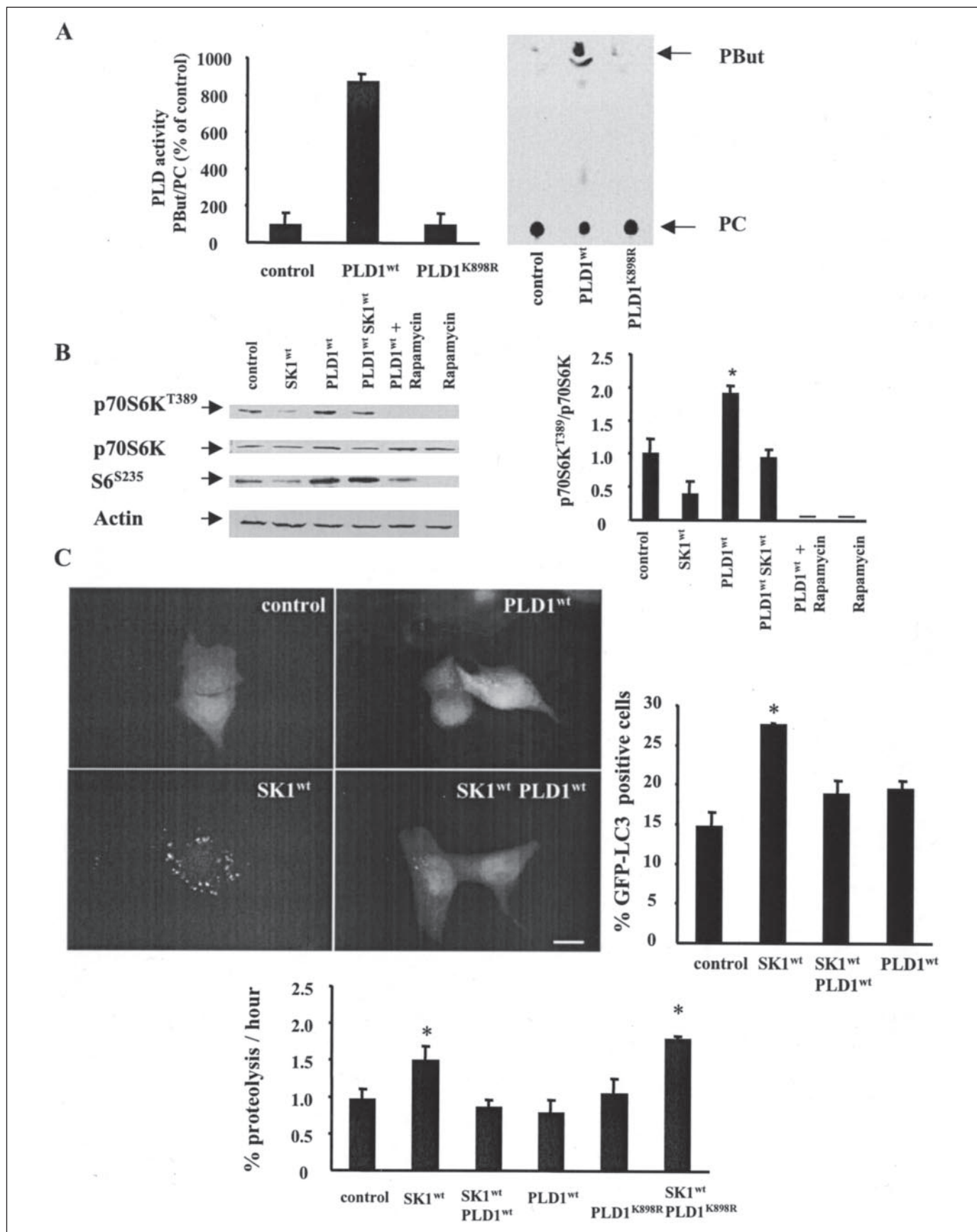


FIGURE 5. PLD1 overexpression inhibits S1P-induced autophagy and relieves the SK1 inhibition of mTOR signaling. A, PLD activity. Control MCF-7 cells (empty vectors) and cells transfected with cDNAs encoding the wild-type PLD1 (PLD1<sup>wt</sup>) or a catalytically inactive PLD1 mutant (PLD1<sup>K898R</sup>) were labeled with [<sup>3</sup>H]myristic acid and incubated in 0.5% 1-butanol-containing medium. PLD activity was determined after lipid extraction and separation by TLC (PBut, phosphatidylbutanol; PC, phosphatidylcholine) as described under

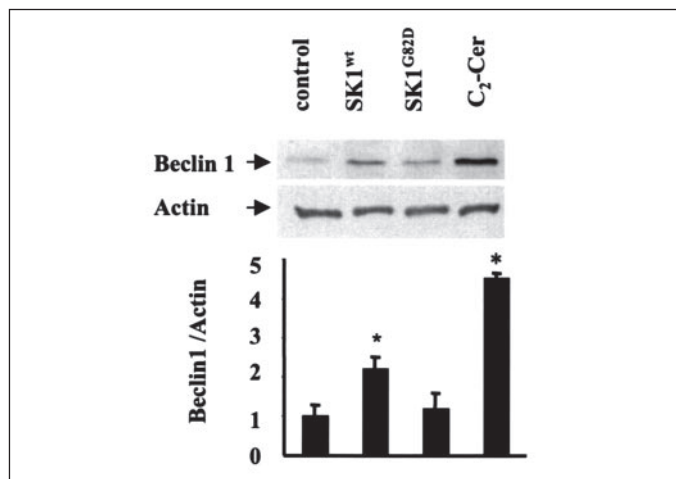


FIGURE 6. **Effect of SK1 on Beclin 1 accumulation.** Cell lysates of control MCF-7 cells (empty vector), C<sub>2</sub>-Cer (75  $\mu$ M, 2 h)-treated cells, SK1<sup>wt</sup>-overexpressing cells, and SK1<sup>G82D</sup>-expressing cells were analyzed by Western blotting for Beclin 1 and actin expression. Densitometry was performed on the original fluorographs, and the Beclin 1/actin ratio in control cells was set to 1. The Western blot is representative of three independent experiments. \*,  $p < 0.05$  versus control cells.

studies are needed to elucidate the effect of S1P on the activity of mTOR complexes.

S1P, which acts intracellularly, also binds to SP1 receptors at the cell surface (36). The role of intracellular and exogenous S1P on autophagy remains to be investigated. Preliminary data show that dihydrospingosine, which activates cell-surface S1P receptors but does not control cell survival (63), did not trigger autophagy, suggesting that autophagy and cell survival are controlled by the second-messenger activity of S1P. However, the possibility that S1P may also have a role in controlling autophagy *via* its receptors cannot be ruled out, because extracellular S1P activates signaling pathways (36, 61) such as ERK1/2, p38 mitogen-activated protein kinase, and Akt/PKB, which are known to modulate autophagy (26, 64, 65).

Starvation stimulates both autophagy and endogenous SK activity. Inhibition of SK activity by DMS or silencing the expression of SK1 by siRNA strongly impairs starvation-induced autophagy in MCF-7.*beclin 1* cells. These findings show that SK1 has to be added to the list of nutrient-sensitive proteins that regulate autophagy (66). Interestingly, the stimulation of SK activity during nutrient deprivation has also been reported in *Saccharomyces cerevisiae* (67) suggesting a general role for SK during starvation in eukaryotic cells. In line with the role of autophagy in maintaining cell survival and repressing apoptosis during starvation (7–9, 68), cell death with biochemical hallmarks of apoptosis is detectable when SK activity is inhibited or after invalidation of the expression of SK1. These findings suggest that autophagy is a novel facet of the role of SK1(S1P) in cell survival (36).

The cell death mediator ceramide is also able to stimulate autophagy in human cancer cells. However, this autophagy can contribute to the execution of a cell-death program as recently shown in glioma cells (33). The role of the mitochondrial-associated cell-death protein BNIP3 downstream of ceramide during autophagic cell death has recently been

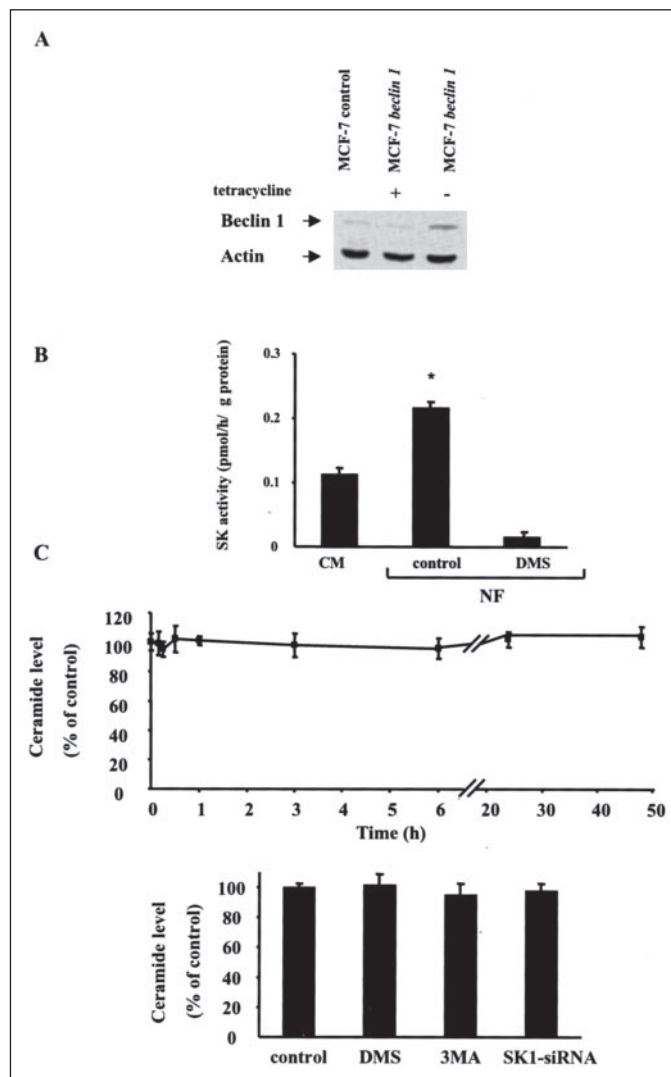


FIGURE 7. **Effect of starvation on SK activity and ceramide level in MCF7. *beclin 1* cells.** A, expression of Beclin1 was analyzed in control MCF-7 and MCF-7/tet-off clone (MCF-7.*beclin 1*) before and after tetracycline removal. Actin was used as a loading control. B, SK activity was analyzed as determined under "Experimental Procedures" in MCF-7.*beclin 1* cells cultured in tetracycline-free complete medium (CM) and in nutrient-free medium (NF) in the presence or absence of 1.5  $\mu$ M DMS for 4 h. C, quantification of ceramide. Endogenous long-chain ceramides were quantified by the DGK assay in starved MCF-7.*beclin 1* cells at different time points. When required 3MA (10 mM) and DMS (1.5  $\mu$ M) were added, and the ceramide level was analyzed after 24 h of treatment. Ceramide was quantified in cells transfected with SK1-siRNA 24 h post-transfection. The amount of ceramide (5.2 pmol of Cer/nmol of P<sub>i</sub>) in control MCF-7.*beclin 1* cells cultured in complete medium was set to 100%. Values are the mean  $\pm$  S.D. of two independent experiments. Values are representative of three independent experiments. \*,  $p < 0.05$  versus control cells.

proposed in glioma cells. The involvement of other sensors of the integrity of organelles such as death-associated protein kinase (69) is worth considering, because death-associated protein kinase, which is regulated by ceramide (70–73), also triggers autophagic cell death in cancer cells (74).

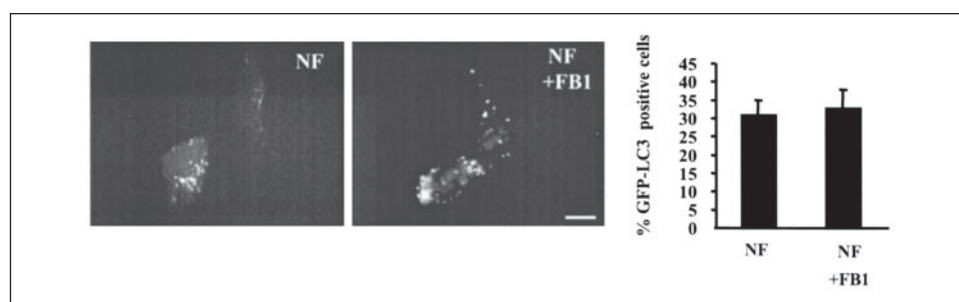
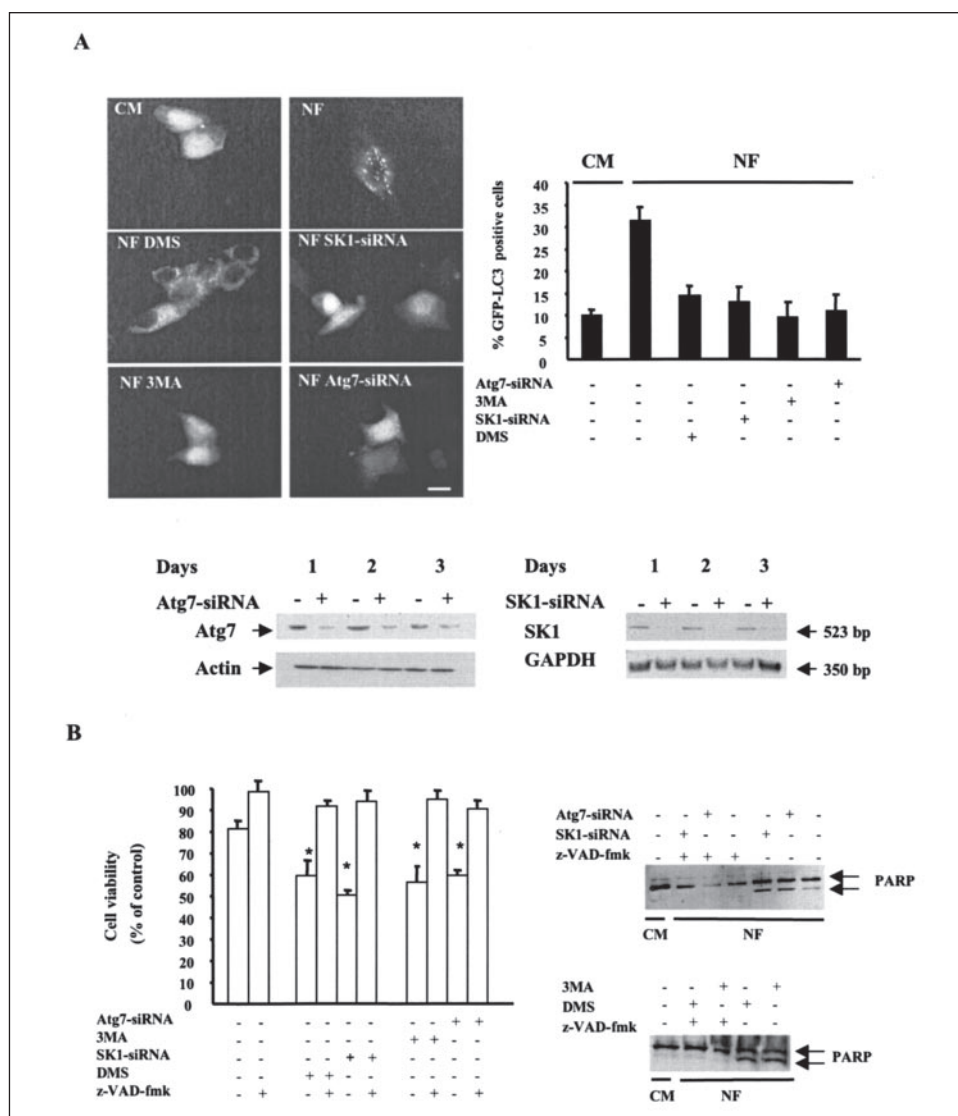
"Experimental Procedures." B, analysis of mTOR signaling. Cell lysates of control cells, SK1<sup>wt</sup>-overexpressing cells co-transfected or not with the cDNA encoding PLD1<sup>wt</sup> were analyzed by Western blotting for p70S6K phosphorylation at Thr<sup>389</sup> using a phosphospecific antibody. The expression of total p70S6K was detected by using an anti-pan p70S6K antibody. Densitometry was performed on the original fluorographs and the ratio phospho Thr<sup>389</sup>/total p70S6K in control cells was set to 1. Ribosomal S6 protein phosphorylation at Ser<sup>235</sup> was detected using a phosphospecific antibody. Actin was used as loading control. When required 100 nM rapamycin was added for 2 h. C, autophagic capacities: *Upper panels*, GFP-LC3 staining and quantitative analysis in control MCF-7 cells, SK1<sup>wt</sup>-overexpressing cells, PLD1<sup>wt</sup>-overexpressing cells, SK1<sup>wt</sup>-overexpressing cells co-transfected with the cDNA encoding PLD1<sup>wt</sup>. The bar represents 10  $\mu$ m. *Lower panel*, degradation of [<sup>14</sup>C]valine-labeled long-lived proteins was determined in control cells and in SK1<sup>wt</sup>-overexpressing cells, PLD1<sup>wt</sup>-overexpressing cells, PLD1<sup>K898R</sup>-expressing cells, SK1<sup>wt</sup>-overexpressing cells co-transfected with cDNA encoding PLD1<sup>wt</sup> or PLD1<sup>K898R</sup> as described under "Experimental Procedures." Values reported are the mean  $\pm$  S.D. of four independent experiments. \*,  $p < 0.05$  versus control cells.



## Control of Macroautophagy by Sphingosine Kinase 1

### FIGURE 8. SK1-induced autophagy protects cells from apoptosis during starvation.

In *A*: Upper panel, GFP-LC3 staining and quantitative analysis in MCF-7.*beclin 1* cells transfected with control siRNA, Atg7-siRNA, or SK1-siRNA and incubated in complete medium or in nutrient-free (NF) medium for 48 h. When required 1.5  $\mu$ M DMS or 10 mM 3MA were added to control cells (control siRNA). The bar represents 10  $\mu$ m. Lower panel, Atg7 and actin were detected by Western blot in cells transfected with control siRNA or Atg7-siRNA (left). SK1 RT-PCR in cells transfected with control siRNA, SK1-siRNA (right panel). Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) was used as a control. *B*: Left, MCF-7.*beclin 1* cells transfected with control siRNA, Atg7-siRNA, or SK1-siRNA were incubated in nutrient-free medium for 48 h. Cell viability was determined by the trypan blue exclusion test. Cell viability in complete medium was set to 100%. When required, DMS (1.5  $\mu$ M) or 3MA (10 mM) were added to control cells (control siRNA). z-VAD-fmk (40  $\mu$ M) was added 1 h before starvation and was present throughout the incubation period in nutrient-free (NF) medium. Right, analysis of PARP cleavage in MCF-7.*beclin 1* cells transfected with control siRNA, Atg7-siRNA, SK1-siRNA, and in MCF-7.*beclin 1* cells treated by 1.5  $\mu$ M DMS or 10 mM 3MA. z-VAD-fmk (40  $\mu$ M) was added 1 h before starvation and was present throughout the incubation period in nutrient-free (NF) medium. Values are representative of three independent experiments. \*,  $p < 0.05$  versus control cells in nutrient-free medium.



**FIGURE 9. Effect of fumonisin B1 (FB1) on autophagy in starved MCF-7.*beclin 1* cells.** GFP-LC3 staining and quantitative analysis in MCF-7.*beclin 1* cells incubated in nutrient-free medium (NF) for 48 h treated or not with 100  $\mu$ M FB1. The bar represents 10  $\mu$ m. Values are representative of three independent experiments.  $p < 0.05$  versus  $C_2$ -treated cells.

The role of ceramide in autophagy and cell death points to cross-talk occurring between the control of autophagy and apoptosis (14, 15, 75). Recently, it has been reported that the interaction between Bcl-2 and Beclin 1 is required to keep autophagy in a physiological range (76). Unbalancing this interaction in favor of Beclin 1 triggers unchecked autophagy and autophagy gene-dependent cell death. It is of note that ceramide-induced autophagy is characterized by an accumulation of Beclin 1, whereas only a slight change in the accumulation of Beclin 1 is observed during SK1(S1P)-induced autophagy. Whether the changes in the ratio between ceramide and S1P can alter the Beclin 1/Bcl-2 rheostat is an attractive possibility that deserves further investigations.

In conclusion, the control of autophagy by ceramide and S1P suggests a novel cell function for the sphingolipid rheostat (36, 39). In cancer cells, the survival role of SK1(S1P)-induced autophagy and the role of ceramide in cell death with autophagic features are compatible with their functions as tumor-promoting and tumor-suppressing molecules, respectively (38). The balance between ceramide (sphingosine) and S1P is under the control of sphingosine kinase and S1P-phosphatase. Overexpression of S1P phosphohydrolase induces apoptosis by increasing the intracellular level of ceramide (77). From this, it can be hypothesized that S1P phosphohydrolase could also trigger other forms of cell death, including autophagic cell death. Alternatively, S1P lyase

that hydrolyzes S1P into hexadecenal and ethanolamine phosphate has been shown to enhance stress-induced ceramide and apoptosis (78). Deletion of the gene encoding S1P lyase has been shown to protect yeast against nutrient-deprivation induced death (79). The study of enzymes that regulate the balance between S1P and ceramide is a promising route toward a better understanding of the regulation of autophagy by sphingolipids and its consequence for cell survival and cell death.

*Acknowledgments*—We are grateful to William A. Dunn Jr., Michael Frohman, Beth Levine, Stuart M. Pitson, Brian Wattenberg, and Tamotsu Yoshimori for providing us with reagents used in this study. Thanks are also due to Mojgan Djavaheri-Mergny, Gérard Pierron, and Yannick Pilatte for critical reading of the manuscript.

## REFERENCES

- Seglen, P. O., and Bohley, P. (1992) *Experientia* **48**, 158–172
- Dunn, W. A., Jr. (1994) *Trends Cell Biol.* **4**, 139–143
- Klionsky, D. J., and Emr, S. D. (2000) *Science* **290**, 1717–1721
- Klionsky, D. J., and Ohsumi, Y. (1999) *Annu. Rev. Cell Dev. Biol.* **15**, 1–32
- Klionsky, D. J., Cregg, J. M., Dunn, W. A., Jr., Emr, S. D., Sakai, Y., Sandoval, I. V., Sibirny, A., Subramani, S., Thumm, M., Veenhuis, M., and Ohsumi, Y. (2003) *Dev. Cell* **5**, 539–545
- Scott, R. C., Schuldiner, O., and Neufeld, T. P. (2004) *Dev. Cell* **7**, 167–178
- Lum, J. J., Bauer, D. E., Kong, M., Harris, M. H., Li, C., Lindsten, T., and Thompson, C. B. (2005) *Cell* **120**, 237–248
- Boya, P., Gonzalez-Polo, R. A., Casares, N., Perfettini, J. L., Dessen, P., Larochette, N., Metivier, D., Meley, D., Souquere, S., Yoshimori, T., Pierron, G., Codogno, P., and Kroemer, G. (2005) *Mol. Cell Biol.* **25**, 1025–1040
- Kuma, A., Hatano, M., Matsui, M., Yamamoto, A., Nakaya, H., Yoshimori, T., Ohsumi, Y., Tokuhisa, T., and Mizushima, N. (2004) *Nature* **432**, 1032–1036
- Schweichel, J. U., and Merker, H. J. (1973) *Teratology* **7**, 253–266
- Clarke, P. G. H. (1990) *Anat. Embryol.* **181**, 195–213
- Bursch, W. (2001) *Cell Death Differ.* **8**, 569–581
- Lockshin, R. A., and Zakeri, Z. (2004) *Int. J. Biochem. Cell Biol.* **36**, 2405–2419
- Shimizu, S., Kanaseki, T., Mizushima, N., Mizuta, T., Arakawa-Kobayashi, S., Thompson, C. B., and Tsujimoto, Y. (2004) *Nat. Cell Biol.* **6**, 1221–1228
- Yu, L., Alva, A., Su, H., Dutt, P., Freundt, E., Welsh, S., Baehrecke, E. H., and Lenardo, M. J. (2004) *Science* **304**, 1500–1502
- Edinger, A. L., and Thompson, C. B. (2004) *Curr. Opin. Cell Biol.* **16**, 663–669
- Baehrecke, E. H. (2005) *Nat. Rev. Mol. Cell Biol.* **6**, 505–510
- Golstein, P., and Kroemer, G. (2005) *Cell Death Differ.* **12**, 1490–1496
- Levine, B., and Yuan, J. (2005) *J. Clin. Invest.* **115**, 2679–2688
- Ogier-Denis, E., and Codogno, P. (2003) *Biochim. Biophys. Acta* **1603**, 113–128
- Gozuacik, D., and Kimchi, A. (2004) *Oncogene* **23**, 2891–2906
- Liang, X. H., Jackson, S., Seaman, M., Brown, K., Kempkes, B., Hibshoosh, H., and Levine, B. (1999) *Nature* **402**, 672–676
- Kihara, A., Kabeya, Y., Ohsumi, Y., and Yoshimori, T. (2001) *EMBO Rep.* **2**, 330–335
- Qu, X., Yu, J., Bhagat, G., Furuya, N., Hibshoosh, H., Troxel, A., Rosen, J., Eskelinen, E.-L., Mizushima, N., Ohsumi, Y., Cattoretti, G., and Levine, B. (2003) *J. Clin. Invest.* **112**, 1809–1820
- Yue, Z., Jin, S., Yang, C., Levine, A. J., and Heintz, N. (2003) *Proc. Natl. Acad. Sci. U. S. A.* **100**, 15077–15082
- Arico, S., Petiot, A., Bauvy, C., Dubbelhuis, P. F., Meijer, A. J., Codogno, P., and Ogier-Denis, E. (2001) *J. Biol. Chem.* **276**, 35243–35246
- Blommaert, E. F., Luiken, J. J., Blommaert, P. J., van Woerkom, G. M., and Meijer, A. J. (1995) *J. Biol. Chem.* **270**, 2320–2326
- Noda, T., and Ohsumi, Y. (1998) *J. Biol. Chem.* **273**, 3963–3966
- Alva, A. S., Gultekin, S. H., and Baehrecke, E. H. (2004) *Cell Death Differ.* **11**, 1046–1048
- Kondo, Y., Kanzawa, T., Sawaya, R., and Kondo, S. (2005) *Nat. Rev. Cancer* **5**, 726–734
- Bursch, W., Ellinger, A., Kienzl, H., Torok, L., Pandey, S., Sikorska, M., Walker, R., and Hermann, R. S. (1996) *Carcinogenesis* **17**, 1595–1607
- Scarlatti, F., Bauvy, C., Ventruti, A., Sala, G., Cluzeaud, F., Vandewalle, A., Ghidoni, R., and Codogno, P. (2004) *J. Biol. Chem.* **279**, 18384–18391
- Daido, S., Kanzawa, T., Yamamoto, A., Takeuchi, H., Kondo, Y., and Kondo, S. (2004) *Cancer Res.* **64**, 4286–4293
- Mathias, S., Pena, L. A., and Kolesnick, R. N. (1998) *Biochem. J.* **335**, 465–480
- Hannun, Y. A., Luberto, C., and Argraves, K. M. (2001) *Biochemistry* **40**, 4893–4903
- Spiegel, S., and Milstien, S. (2003) *Nat. Rev. Mol. Cell Biol.* **4**, 397–407
- Levade, T., Malagarie-Cazenave, S., Gouaze, V., Ségui, B., Tardy, C., Betito, S., Andrieu-Abadie, N., and Cuvillier, O. (2002) *Neurochem. Res.* **27**, 601–607
- Ogretmen, B., and Hannun, Y. A. (2004) *Nat. Rev. Cancer* **4**, 604–616
- Cuvillier, O., Pirianov, G., Kleuser, B., Vanek, P. G., Coso, O. A., Gutkind, S., and Spiegel, S. (1996) *Nature* **381**, 800–803
- Pitson, S. M., Moretti, P. A., Zebul, J. R., Xia, P., Gamble, J. R., Vadas, M. A., D'Andrea, R. J., and Wattenberg, B. W. (2000) *J. Biol. Chem.* **275**, 33945–33950
- Liang, X. H., Yu, J., Brown, K., and Levine, B. (2001) *Cancer Res.* **61**, 3443–3449
- Bektas, M., Jolly, P. S., Muller, C., Eberle, J., Spiegel, S., and Geilen, C. C. (2005) *Oncogene* **24**, 178–187
- Kabeya, Y., Mizushima, N., Ueno, T., Yamamoto, A., Kirisako, T., Noda, T., Komiyama, E., Ohsumi, Y., and Yoshimori, T. (2000) *EMBO J.* **19**, 5720–5728
- Pattingre, S., Petiot, A., and Codogno, P. (2004) *Methods Enzymol.* **390**, 17–31
- Perry, D. K., and Hannun, Y. A. (1999) *Trends Biochem. Sci.* **24**, 226–227
- Bligh, E. G., and Dyer, W. J. (1959) *Can. J. Biochem. Physiol.* **37**, 911–917
- Ames, B. N., and Dubin, D. T. (1960) *J. Biol. Chem.* **235**, 769–775
- Granata, R., Trovato, L., Garbarino, G., Taliano, M., Ponti, R., Sala, G., Ghidoni, R., and Ghigo, E. (2004) *FASEB J.* **18**, 1456–1458
- Gijsbers, S., Van der Hoeven, G., and Van Veldhoven, P. P. (2001) *Biochim. Biophys. Acta* **1532**, 37–50
- Vessey, D. A., Kelley, M., and Karliner, J. S. (2005) *Anal. Biochem.* **337**, 136–142
- O'Luanaigh, N., Pardo, R., Fensome, A., Allen-Baume, V., Jones, D., Holt, M. R., and Cockcroft, S. (2002) *Mol. Biol. Cell* **13**, 3730–3746
- Seglen, P. O., and Gordon, P. B. (1982) *Proc. Natl. Acad. Sci. U. S. A.* **79**, 1889–1892
- Burnett, P. E., Barrow, R. K., Cohen, N. A., Snyder, S. H., and Sabatini, D. M. (1998) *Proc. Natl. Acad. Sci. U. S. A.* **95**, 1432–1437
- Gingras, A. C., Kennedy, S. G., O'Leary, M. A., Sonenberg, N., and Hay, N. (1998) *Genes Dev.* **12**, 502–513
- Chen, Y., Rodrik, V., and Foster, D. A. (2005) *Oncogene* **24**, 672–679
- Fang, Y., Park, I. H., Wu, A. L., Du, G., Huang, P., Frohman, M. A., Walker, S. J., Brown, H. A., and Chen, J. (2003) *Curr. Biol.* **13**, 2037–2044
- Fang, Y., Vilella-Bach, M., Bachmann, R., Flanagan, A., and Chen, J. (2001) *Science* **294**, 1942–1945
- Nava, V. E., Hobson, J. P., Murthy, S., Milstien, S., and Spiegel, S. (2002) *Exp. Cell Res.* **281**, 115–127
- Tanida, I., Mizushima, N., Kiyooka, M., Ohsumi, M., Ueno, T., Ohsumi, Y., and Kominami, E. (1999) *Mol. Biol. Cell* **10**, 1367–1379
- Tanida, I., Tanida-Miyake, E., Ueno, T., and Kominami, E. (2001) *J. Biol. Chem.* **276**, 1701–1706
- Spiegel, S., and Milstien, S. (2002) *J. Biol. Chem.* **277**, 25851–25854
- Sarbasov, D. D., Guertin, D. A., Ali, S. M., and Sabatini, D. M. (2005) *Science* **307**, 1098–1101
- Van Brocklyn, J. R., Lee, M. J., Menzeleev, R., Olivera, A., Edsall, L., Cuvillier, O., Thomas, D. M., Coopman, P. J., Thangada, S., Liu, C. H., Hla, T., and Spiegel, S. (1998) *J. Cell Biol.* **142**, 229–240
- Haussinger, D., Schliess, F., Dombrowski, F., and Vom Dahl, S. (1999) *Gastroenterology* **116**, 921–935
- Pattingre, S., Bauvy, C., and Codogno, P. (2003) *J. Biol. Chem.* **278**, 16667–16674
- Meijer, A. J., and Codogno, P. (2004) *Int. J. Biochem. Cell Biol.* **36**, 2445–2462
- Lanterman, M. M., and Saba, J. D. (1998) *Biochem. J.* **332**, 525–531
- Levine, B. (2005) *Cell* **120**, 159–162
- Shang, T., Joseph, J., Hillard, C. J., and Kalyanaraman, B. (2005) *J. Biol. Chem.* **280**, 34644–34653
- Yamamoto, M., Hioki, T., Ishii, T., Nakajima-Iijima, S., and Uchino, S. (2002) *Eur. J. Biochem.* **269**, 139–147
- Shohat, G., Spivak-Kroizman, T., Eisenstein, M., and Kimchi, A. (2002) *Eur. Cytokine Netw.* **13**, 398–400
- Martinet, W., Schrijvers, D. M., De Meyer, G. R., Thielemans, J., Knaepen, M. W., Herman, A. G., and Kockx, M. M. (2002) *Arterioscler. Thromb. Vasc. Biol.* **22**, 2023–2029
- Pelled, D., Raveh, T., Riebeling, C., Fridkin, M., Berissi, H., Futerman, A. H., and Kimchi, A. (2002) *J. Biol. Chem.* **277**, 1957–1961
- Inbal, B., Bialik, S., Sabanay, I., Shani, G., and Kimchi, A. (2002) *J. Cell Biol.* **157**, 455–468
- Thorburn, J., Moore, F., Rao, A., Barclay, W. W., Thomas, L. R., Grant, K. W., Cramer, S. D., and Thorburn, A. (2005) *Mol. Biol. Cell* **16**, 1189–1199
- Pattingre, S., Tassa, A., Qu, X., Garuti, R., Liang, X. H., Mizushima, N., Packer, M., Schneider, M. D., and Levien, B. (2005) *Cell* **122**, 927–939
- Le Stunff, H., Galve-Roperh, I., Peterson, C., Milstien, S., and Spiegel, S. (2002) *J. Cell Biol.* **158**, 1039–1049
- Reiss, U., Oskouian, B., Zhou, J., Gupta, V., Sooriyakumaran, P., Kelly, S., Wang, E., Merrill, A. H., Jr., and Saba, J. D. (2004) *J. Biol. Chem.* **279**, 1281–1290
- Gottlieb, D., Heideman, W., and Saba, J. D. (1999) *Mol. Cell Biol. Res. Commun.* **1**, 66–71

## **Regulation of Autophagy by Sphingosine Kinase 1 and Its Role in Cell Survival during Nutrient Starvation**

Grégory Lavieu, Francesca Scarlatti, Giusy Sala, Stéphane Carpentier, Thierry Levade, Riccardo Ghidoni, Joëlle Botti and Patrice Codogno

*J. Biol. Chem.* 2006, 281:8518-8527.

doi: 10.1074/jbc.M506182200 originally published online January 16, 2006

---

Access the most updated version of this article at doi: [10.1074/jbc.M506182200](https://doi.org/10.1074/jbc.M506182200)

### 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 79 references, 34 of which can be accessed free at <http://www.jbc.org/content/281/13/8518.full.html#ref-list-1>