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Review

# Sphingosine arrests the cell cycle and induces apoptosis by targeting sphingosine-dependent protein kinase and protein kinase Cδ in vitro

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ABSTRACT

*Purpose:* Emerging evidence has indicated that the sphingolipid sphingosine is involved in cellular differentiation, cell growth, and apoptosis. Here, we elucidated the sphingosine signaling pathways underlying apoptosis and cell growth inhibition.
*Study section and results:* Sphingosine induced mitochondria-mediated apoptosis in rat hippocampal neurons and astrocytes by activating caspase-3/-9 via the sphingosine-dependent protein kinase (SDK)/14-3-3 protein/Bax/cytochrome c pathway. This may account for the implication of sphingosine/SDK in the pathogenesis of Alzheimer's disease. Additionally, sphingosine induced apoptosis in MKN-28 human gastric cancer cells in an SDK-dependent manner. In the human malignant pleural mesothelioma cell lines, sphingosine suppressed cell growth by arresting the cell cycle at the $G_0/G_1$ phase, along with inhibiting protein kinase Cδ (PKCδ). This suggests that sphingosine/SDK/PKCδ signaling can be potentially used in treating many types of cancers.
*Conclusion:* The present review shows that sphingosine induces apoptosis and inhibits the cell cycle by targeting SDK and PKCδ. Our findings represent a fresh insight into sphingosine signaling pathways, and may provide a blueprint for developing drugs for treating Alzheimer's disease and many types of cancers.


## 1. Introduction

Sphingolipids such as ceramide, ceramide 1-phosphate, sphingosine, and sphingosine 1-phosphate (S1P) mediate diverse cellular processes, including differentiation, growth, and apoptosis. Ceramide, which is produced from sphingomyelin by sphingomyelinase, induces apoptosis in neurons and cancer cells via diverse signal transduction pathways [1,2]. Sphingosine, which is produced from ceramide by ceramidase, also plays a role in apoptosis [3,4]. S1P, which is produced from sphingosine by sphingosine kinases (SphKs) such as SphK1 and SphK2, plays an important role in neuronal cell apoptosis, tumorigenesis, and neovascularization as a lipid mediator [5,6]. Sphingosine induces apoptosis in rhabdomyosarcoma cells by promoting Bax release from the mitochondria, thereby activating caspase-9 and its effector, caspase-3 [7]. Sphingosine also induces apoptosis in mouse BALB/c 3T3 clone A31 cells in a sphingosine-dependent protein kinase (SDK)-dependent manner [8]. SDK is produced through proteolytic processing of protein kinase Cδ (PKCδ) and is activated by its binding to sphingosine [8]. SDK phosphorylates the 14-3-3 proteins as substrates [9,10] to dissociate Bax, a member of the Bcl-2 family, from the 14-3-3 protein/Bax complex, thereby creating a Bax/Bax complex and causing mitochondrial damage, followed by apoptosis. Sphingosine also inhibits PKC activity in vitro and in human platelets [11].

In the present review, we showed that SDK and PKCδ are critical targets in sphingosine-induced apoptosis and cell cycle arrest.

## 2. Sphingosine induces apoptosis in rat hippocampal neurons and astrocytes

Sphingolipids may participate in the pathogenesis of neurodegenerative diseases such as Alzheimer's disease [12,13]. Intriguingly, ceramides are upregulated and glycosphingolipids and S1P are downregulated during progression of dementia and Alzheimer's disease [14]. Amyloid-β oligomers activate acid sphingomyelinase, causing an increase in ceramide and sphingosine levels, and a decrease in S1P levels [15]. Therefore, we also hypothesized that sphingosine might be involved in the progression of Alzheimer's disease.

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To address this point, we examined whether sphingosine induces apoptosis in hippocampal neurons. Sphingosine reduced cell viability in a concentration (in the range of 1–100 μM)-dependent manner, with the number of viable cells reaching nearly 0% at 100 μM (Fig. 1A). Most of the cells treated with sphingosine were positive for terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) (Fig. 1B). Following sphingosine treatment, nuclear fragmentation was observed in cultured hippocampal neurons, as labeled by an anti-microtubule-associated protein 2 (MAP2) antibody, and in astrocytes, as tagged by an anti-glial fibrillary acidic protein (GFAP) antibody (Fig. 1C). These results confirmed that sphingosine induces apoptosis in hippocampal neurons and astrocytes. Similar to sphingosine, SphK inhibitors such as dimethylsphingosine (DMS) and 2-(p-hydroxyanilino)-4-(p-chlorophenyl) thiazole (HACPT) also reduced cell viability in a concentration (1–100 μM)-dependent manner and increased TUNEL-positive cells (Fig. 1A and B). SDK, produced through the proteolytic processing of PKCδ and activated by sphingosine binding, is known to phosphorylate the 14-3-3 proteins as their effector substrate. Sphingosine increased SDK levels in a time-dependent manner, in parallel with a time-dependent decrease in PKCδ levels (Fig. 2A). Sphingosine phosphorylated the 14-3-3 proteins and converted their dimer forms to monomers in cultured rat hippocampal cells (Fig. 2B). In response to apoptotic stimuli, 14-3-3 proteins capturing Bax, disassociates from Bax, which is in turn translocated to the mitochondria where it perturbs the mitochondrial membrane potential, allowing the release of cytochrome c into the cytosol. Then, cytochrome c associates with Apaf-1/dATP to activate caspase-9 and the effector caspase-3, thereby inducing apoptosis. Sphingosine decreased Bax levels in the cellular cytosol, but increased them in the mitochondrial components (Fig. 2C). To evaluate mitochondrial damage, we monitored mitochondrial membrane potentials by staining the cells with the DePsipher dye, which produces orange-red fluorescence at a wavelength of 590 nm for normal-functioning mitochondrial membrane, but green fluorescence at 530 nm under disrupted mitochondrial membrane potentials. Sphingosine produced green fluorescent signals, and no orange-red fluorescence, in the cultured rat hippocampal cells (Fig. 2D). Moreover, sphingosine markedly enhanced the activities of caspase-9 and caspase-3 in hippocampal cells (Fig. 2E). Taken together, these results indicate that sphingosine increases the levels of and activates SDK, phosphorylates the

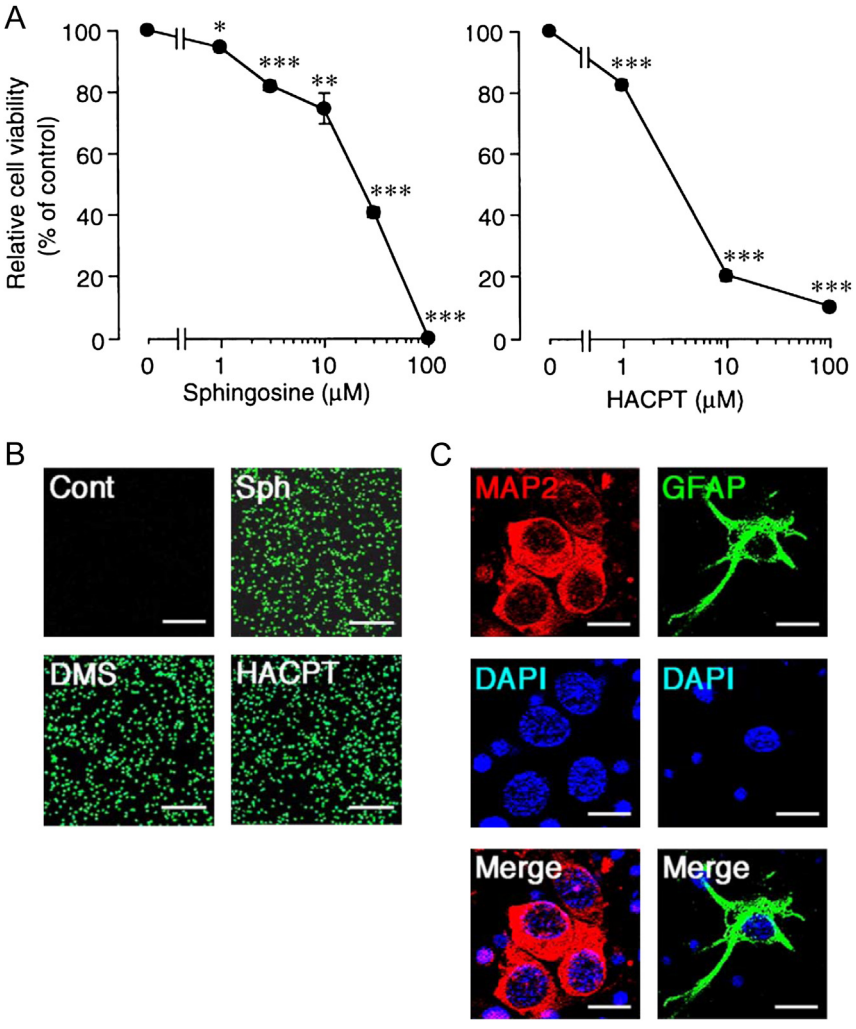


**Fig. 1.** Sphingosine-induced apoptosis in cultured rat hippocampal cells (A) Cultured rat hippocampal cells were separately treated with sphingosine and HACPT at the indicated concentrations for 24 h, and their cell viability was quantified with an MTT assay. In the graphs, each point represents the mean (±SEM) percentage of control (MTT intensities for cells untreated with any drug) ($n = 4$ independent experiments). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ as compared with basal levels, unpaired $t$-test. (B) TUNEL staining of untreated (Cont) cultured rat hippocampal cells and those treated with sphingosine (Sph; 100 μM), DMS (10 μM), and HACPT (3 μM) for 6 h. Scale bars, 100 μm. (C) Cultured rat hippocampal neurons and astrocytes were identified by immunostaining for MAP-2 and GFAP, respectively, and were also stained with DAPI after a 6-h treatment with sphingosine (100 μM). Scale bars, 10 μm.