Int J Clin Exp Med 2015;8(8):11913-11921
www.ijcem.com /ISSN:1940-5901/IJCEM0007645

*Review Article*

# Sphingosylphosphorylcholine in cancer progress

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Received March 6, 2015; Accepted May 28, 2015; Epub August 15, 2015; Published August 30, 2015

**Abstract:** Sphingosylphosphorylcholine (SPC) is a naturally occurring bioactive sphingolipid in blood plasma, metabolizing from the hydrolysis of the membrane sphingolipid. It has been shown to exert multifunctional role in cell physiological regulation either as an intracellular second messenger or as an extracellular agent through G protein coupled receptors (GPCRs). Because of elevated levels of SPC in malicious ascites of patients with cancer, the role of SPC in tumor progression has prompted wide interest. The factor was reported to affect the proliferation and/or migration of many cancer cells, including pancreatic cancer cells, epithelial ovarian carcinoma cells, rat C6 glioma cells, neuroblastoma cells, melanoma cells, and human leukemia cells. This review covers current knowledge of the role of SPC in tumor.

**Keywords:** Sphingosylphosphorylcholine, second messenger, GPCRs, cancer

## Introduction

Sphingosylphosphorylcholine (SPC), sharing similar structure with sphingosine-1-phosphate (S1P) and lysophosphatidic acid (LPA), is an amphiphilic lysophospholipid composed of a sphingosine backbone and a hydrophilic phosphorylcholine. With the modulating function of the sphingolipids in cell physiology to be unveiled, SPC is now emerging as an increasingly important lipid mediator possessing the potential of regulating cell proliferation, migration, differentiation, metabolism and cell death. SPC has been shown to be a multifunctional molecule in cardiovascular system, immune system, central nervous system and skin [1, 2]. Besides, elevated level of SPC was found in some pathological conditions as atopic dermatitis, Niemann-Pick disease (NPD) and cancer [3-7]. With regard to tumor progression, SPC was reported to affect the proliferation and/or migration of pancreatic cancer cells, prostate cancer cells, epithelial ovarian carcinoma cells, rat C6 glioma cells, neuroblastoma cells, melanoma cells, and human leukemia cells. In this review, we will discuss the the current understanding of the role of SPC in tumor.

## Origination and metabolism of SPC

Sphingolipids synthesis and metabolism is a series of enzymatic process precisely regulated by a multitude of enzymes. Even with limited information available about the origination pathway of SPC, two pivotal enzymes do involve in this process: sphingomyelin deacylase and autotoxin.

Sphingomyelin deacylase was first identified in bacteria *Pseudomonassp TK4* as a 52KD protein capable of breaking down the N-Acyl linkage of both glycosphingolipids and sphingomyelin [8]. The abnormally higher expression of sphingomyelin deacylase corresponds to the upregulated SPC level in the stratum corneum of AD patients [9]. Sphingomyelin hydrolysis can be catalyzed by either sphingomyelinase or sphingomyelin deacylase to produce ceramide and SPC, respectively [9, 10]. Thus the activity balance between the two enzymes may be a critical determination of these two lipid species level. For example, the excessive expression of the deacylase leads to the ceramide deficiency which partially accounts for the pathogenesis of atopic dermatitis [10]. Besides, as is the

case with other lipids, SPC production and release should be a precisely regulated process. Incubation with endothelin-1 (ET-1) increased the generation of SPC in cardiac myocytes [11]. Despite of no direct evidence, the activation of platelets is widely believed to promote the release of SPC into the blood [12]. Pharmalogical manipulation of the sphingomyelin deacylase may provide a useful tool to understand the bioactive function of SPC [13]. Hence, it will be of grant value to determine the specific physiochemical or structural properties of this enzyme and its expression pattern in species and tissues.

Autotaxin, an ecto-nucleotide pyrophosphatase/phosphodiesterase (ENPP2), was found to show lysophospholipase D activity and extended its substrate specificity to glycerophospholipids and phosphosphingolipid [14, 15]. Thus this enzyme is involved in the production of phospholipids species such as LPA, S1P. Autotaxin catalyzed release of choline from SPC to produce S1P. This seems to be the only subsequent metabolism mechanism of SPC uncovered until now. In addition, autotoxin is an exoenzyme exsisting in blood which could explain the rapid decay of the coronary perfused SPC [16]. But the metabolism pathway of this lipid specie inside the cell remains unknown. Unlike the sphingomyelin deacylase, the architecture of rodent autotoxin and the molecular mechanism involved in the LPA production has been well analyzed [17]. This provides a foundation for the design and discovery of human ATX inhibitors. Besides, several specific inhibitors have been available now [18, 19].

## Effect of SPC on diverse cancer cells

### *SPC in endocrine tumors*

*SPC inhibits the proliferation of epithelial ovarian carcinoma (EOC):* SPC and another two bioactive lysophospholipids, LPA and S1P, were present in ascitic fluids from patients with ovarian cancer [20]. As well, SPC could inhibit the proliferation of ovarian cancer cells, which was accompanied by transient increases in cytosolic free $Ca^{2+}$ and rapid increases in tyrosine phosphorylation of specific cellular proteins, including the focal adhesion kinase p125FAK in HEY and OCC1 ovarian cancer cell lines [20]. Interleukin 8 (IL-8) is a proinflammatory and proangiogenic factor potentially involved in EOC development. LPA, S1P and SPC dose- and time-dependently upregulated IL-8 mRNA and protein levels in EOC (HEY, OCC1, and SKOV3) implicating the potential role of SPC in tumor inflammation [21]. The OGR1 receptor was first identified as a receptor in response to SPC eliciting DNA synthesis and cell proliferation through MAPK signaling in HEY ovarian cancer cell [22]. Following this report, several other structural related GPCRs for SPC were uncovered. However, those receptor clusters were found to be PH sensitive and no more powerful evidence have been provided to confirm their role as SPC's receptors [23-25]. G-protein-coupled receptor 4 (GPR4) is one of those receptors. Microvascular density in cancer is associated with lymph node metastasis and clinical stage. Analysis of the relationship between GPR4 expression and clinical and pathological characteristics of EOC indicated that SPC might also affect EOC progression by targeting GPR4 to promote microvascular density [26].

*SPC inhibits the proliferation and migration of anaplastic thyroid carcinoma cell:* SPC at 1 to 10 μM could inhibit the proliferation and migration of thyroid cancer FRO cells in a GPCR-dependent manner [27, 28]. The extracellular addition of SPC induced the rounding of FRO cells within 10 min. Accompanied by this morphologic change was inhibited proliferation caused by retarded G1/S cell cycle and impaired migration. The effect of SPC on FRO cells was modulated via a PI3K-Akt and MAP kinase signaling pathway, and phospholipase C, protein kinase C, p38 kinase, or JUN were not involved with this process.

### *SPC in central nervous system malignancies*

*SPC increases membrane potentials of glioma cells:* While information is lacking about its role in glioma cell proliferation and migration, SPC was reported to increase membrane potentials, modulate cellular phospholipid homeostasis and induce c-fos activation in rat C6 glioma cells [29]. SPC could significantly increase [14C] phosphatidylserine synthesis and decrease level of 14C-labeled phosphatidylethanolamine for a role in cellular phospholipid homeostasis. Pretreatment with pertussis toxin (PTX) could not reduce SPC-induced c-fos activation, which suggests a receptor-independent func-

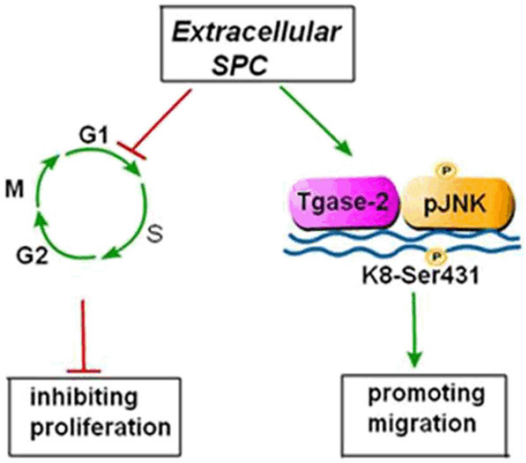


**Figure 1.** Effect of sphingosylphosphorylcholine (SPC) on pancreatic-cancer PANC-1 cells. SPC inhibits proliferation of PANC-1 cells by G1/S arresting and promotes PANC-1 migration dependent of Tgase or JNK-regulated phosphoralation of Keratin8 (K8).

tion of SPC in C6 cells [30]. Exogenous SPC treatment did not stimulate phospholipase D in C6 glioma cells [31]. As well, sphingosine stimulated phosphatidylserine synthesis independent of protein kinase C but was suppressed by thapsigargin and cholesterol 3-sulfate (an amphiphilic anion) in glioma C6 and rat liver microsomes, so SPC may function as an amphiphilic compound. Thus, SPC may be involved in the serine base exchange reaction [32, 33].

*SPC inhibits the growth of neuroblastoma cells:* SPC at < 150 μM inhibited the growth of three mouse neuroblastoma cell lines, NS-20Y, Neuro2a, and N1E-115, with less effectiveness than the other two lysosphingolipids, lysosulfatide and psychosine. Among the three kinds of lysosphingolipids, only SPC induced reversible neurite outgrowth and changed the lipid composition, modifying the amounts of cholesterol, sphingomyelin and ganglioside GM3 in all cell lines [34], which might be associated with the metabolism of SPC via sphingomyelin synthesis. The exogenous [3-3H] SPC may be first degraded into phosphocholine and sphingosine, the precursors of ceramide, which can be further synthesized to sphingomyelin [35].

*SPC in digestive cancers*

*SPC inhibits the growth but promotes the migration of pancreatic cancer cells:* The roles and mechanisms of SPC in pancreatic cancer cells are the clearest. SPC at 3 or 10 μM can inhibit the growth of the human pancreatic cancer cells MLA PaCa-2, PANC-1, PK-1 and PK-9 cells, possibly by regulating the cell cycle from the G1 to the S phase [36]. Furthermore, SPC affected cellular elasticity and migration of PANC-1 cancer cells by reorganization of keratin [37-39]. Phosphorylation of Keratin 8 Ser431 regulated by MEK-ERK and Tgase-2-JNK signaling pathways was found required for SPC-induced keratin reorganization and consequently enhanced migration of human epithelial tumor cells [40, 41] (**Figure 1**).

*SPC did not affect the growth of bladder carcinoma cells:* Research into the role of sphingolipids in bladder carcinoma cells is limited. In 2000, Jakobs et al. found that similar to S1P, SPC did not affect the growth of human bladder carcinoma (J82) cells and induced only a small, PTX-sensitive motile response in J82 cells. In addition, SPC could inhibit LPA (PTX-sensitive) but promote thrombin (PTX-insensitive)-stimulated cell motility without altering Rho-GTPase activation and the resulting actin stress fiber formation. Thus, the modulation of SPC on this process may due to GPCR expression on J82 cells, which integrates various intra- and extracellular signals [42].

*SPC in Skin cancer*

*SPC in melanoma cells:* SPC was reported to inhibit B16 murine melanoma cell migration and invasion, which was completely abolished by the S1P2-selective antagonist JTE013, suggesting the role of S1P2 receptor in SPC mediated process [43]. Notably, the involvement of SPC in melanogenesis process obtained extensively attention besides its role in melanoma.

In cultured human melanocytes, SPC was first proposed to be a melanogenic stimulator since SPC at > 5 μM could elicit the activation of melanogenic related MITF-M/tyrosinase/c-kit signal pathway and MAPK signaling cascades [44]. However, a more elaborate research was performed in human epidermal melanocytes isolated from adolescent foreskins later. In this study, SPC at 1 to 10 μM concentration-dependently inhibited melanin synthesis. In parallel, MITF-M and tyrosinase was suppressed in both mRNA level and protein level [45]. To clarifying the confliction, mechanism of

**Table 1.** Effect of sphingosylphosphorylcholine on cancer cells

| Concentration | Effect | Cell type | Pathway | Ref. |
|---|---|---|---|---|
| 3, 10 μM | Proliferation↓; migration↑ | Pancreatic cancer cells: MLA PaCa-2, PANC-1, PK-1 and PK-9 | G1/S cell cycle arrest<br>MEK/ERK Tgase-2/JNK<br>Keratin 8 Ser431 phosphorylation | [36] |
| 100 μM | Apoptosis↑ | Prostate cancer cells: DU 145, PC3 | Receptor-independent; $Ca^{2+}$ | [41] |
| 3 μM | Proliferation↓ | Epithelial ovarian carcinoma cells: HEY | $Ca^{2+}$; tyrosine phosphorylation of p125FAK | [61] |
| < 150 μM | Proliferation↓; neurite outgrowth↑ | Mouse neuroblastoma cells: NS-20Y, Neuro2a, N1E-115 | Metabolism of SPC via sphingomyelin synthesis | [34] |
| 10 μM | Migration and invasion↓ | Mouse B16 melanoma cells | S1P2 receptor | [43] |
| 5 μM/15 μM | Proliferation↓; differentiation↑ | Human leukemia cells: HL-60 | MEK/ERK | [48] |
| 10 μM | Motile response↑ | Human bladder carcinoma cells: J82 | GPCRs | [42] |
| 1-10 μM | Proliferation and migration↓ | Anaplastic thyroid carcinoma cells: FRO | PI3K-Akt; MAPK | [28] |

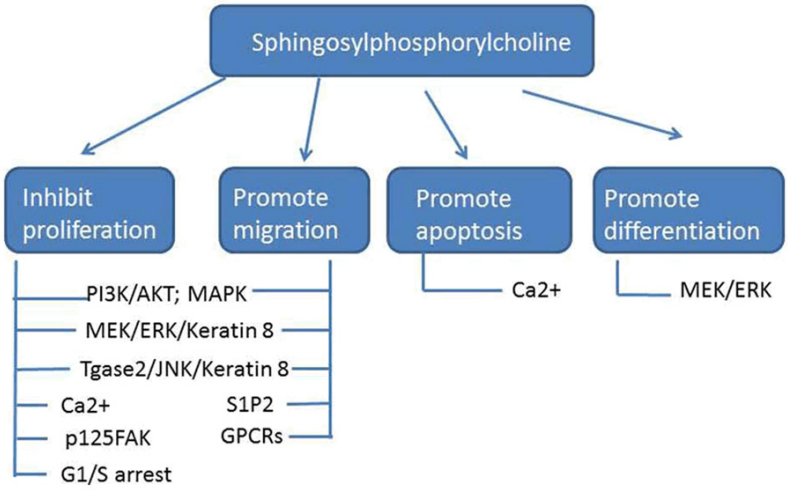


**Figure 2.** SPC affects proliferation, migration, apoptosis and differentiation of cancer cells. SPC inhibits proliferation and migration of cancer cells by PI3K/AKT, MAPK, MEK/ERK/Keratin8, Tgase2/JNK/Keratin8 pathway. The $Ca^{2+}$, p125FAK, G1/S arrest was responsible for its inhibiting proliferation, and S1P2 or other GPCRs was involved in its promoting migration. The increase of $Ca^{2+}$ was associated with its promoting apoptosis function, and MEK/ERK was also involved in its promoting differentiation role.

SPC in melanogenesis was detailed investigated [46, 47]. SPC induced hypopigmentation in Mel-Ab cell, a mouse-derived spontaneously immortalized melanocyte cell line. The activation of Akt/mTOR and ERK signaling correlated with this hypopigmentation effect. Further, two phosphatases PP2A and DUSP6 responsible for the dephosphorylation of Akt and ERK respectively were both inhibited by SPC. Due to the different origination of those melanoma cells, it seems difficult to determine the actual role of SPC in melanogenesis. Further research should be performed.

*SPC in blood cancers*

*SPC induces differentiation of human leukemia cells:* SPC is a potentially novel differentiation-inducing agent both for mouse embryo stem cells and certain human tumor cells depending on MEK-ERK signaling [2, 48]. As early as 1991, SPC (5 μM) was found to be involved in adherence during macrophage differentiation of HL-60 cells (human pro-myelocytic leukemia cells) and as effective as sphingomyelin [49]. SPC at 12.5 μM could also induce the differentiation of human NB4 promyelocytic leukemia cells via the MEK-ERK signaling pathway [48]. Besides, single-channel recording from microsomes incorporated in planar lipid bilayers revealed that GPCRs and phospholipase C were involved in the modulation of SPC-stimulated $Ca^{2+}$ in HL-60 cells [50, 51].

Overall, the reports about the function of SPC in cancers suggest a popular negative control of cancer cell proliferation or migration. Hence, targeting SPC may provide a novel strategy for tumor therapy (**Table 1**; **Figure 2**).

*SPC detection and quantification*

Since multiple studies have revealed the critical role of SPC in the pathogenesis of diverse diseases, it can be considered as a potential novel biomarker for clinical diagnosis. However, efforts addressing blood sphingolipids as biomarkers of disease are still in their infancy [52]. Thus high sensitive and precise analyze or quantification methods for this bioactive lipid molecule in various physiological and pathological conditions are in need. With the robust development of the mass spectrometry technology and its usage in lipids analyzation, the minor sphingolipid molecules including LPA, S1P and SPC are within the detectable range [53, 54]. By combining the lipids separation method as the HPLC or TLC with MS detecting and quantification system, the level of SPC in samples from both healthy people and patients with diverse diseases were evaluated [3, 16]. Under normal condition, SPC concentration in plasma and serum was estimated at 50 nM in plasma and 130 nM in serum. Under pathological condition such as the cerebrospinal fluid (CSF) of patients with SAH, the level of SPC was dramatically higher. The recently introduced electrospray ionization mass spectrometry (ESI-MS/MS) and hydrophilic interaction chromatography tandem mass spectrometry (HILIC-MS/MS) enabled the precise evaluation of the minor sphingolipid species from samples as various as blood, lipoproteins, tissues and cell cultures [55, 56] and could be possibly applied to the large clinical studies. The sphingolipids profile in ascites fluid

from patients with ovarian cancer was determined by use of the ESI-MS/MS, many of the lysophospholipid species including SPC are shown to be upregulated [57]. Even much advance has been made in those MS instruments dependent analyzation methods, there exist some disadvantages which hampered its massive application to the clinical diagnosis. To be the first, the typical sphingolipids species separation methods such as gas chromatography or high-performance liquid chromatography (HPLC) are generally time-consuming and may sometimes render the risk of incomplete separation of closely related lipid species. Besides, MS instruments are usually expensive and are not widely affordable. Thus, a much cost effective analyzation method for SPC monitoring is needed. Aptamers, oligonucleotides that can bind with specific targets including proteins and lipid species, may help to solve these problems [58, 59]. Katsunori Horii et al. screened RNA aptamers from the random RNA pool which could specifically recognize and bind with SPC [60]. Based on this, they further developed quick and high sensitive enzyme-linked aptamer assay system for SPC monitoring. This was the first trial employing aptamers in sphingolipids detection and contributed to the practical monitoring of SPC in clinical.

## Conclusion

SPC can be not only a promising biomarker for tumor diagnosis but also provides pharmalogical target for disease therapy. Thus it will be of remarkable significance to specifying its properties and bioactive functions. Much more research should be done in the following aspects: (a) The analysis of the enzymes involved in SPC production; (b) The elaboration of the action mechanism of SPC as the intracellular and extracellular signal factor: the role of GPCRs and lipid raft; (c) The elucidation of further clinical evidences and mechanisms of SPC in cancer; (d) The development of high sensitive and convenient detection technique for minor lipid species.

## Acknowledgements

We thank Laura Heraty for critical reading and English correction of the manuscript. This work was supported by the Natural Science Foundation of China (no. 31070999) and Doctoral Foundation of Shandong Province (BS2012SW021).

## Disclosure of conflict of interest

None.

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