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The sphingosine kinase-1/sphingosine-1-phosphate axis in cancer: Potential target for anticancer therapy

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ABSTRACT

Sphingolipid metabolites, such as ceramide, sphingosine and sphingosine-1-phosphate (S1P), play many important roles in cellular activities. Ceramide and sphingosine inhibit cell proliferation and induce cell apoptosis while S1P has the opposite effect. Maintaining a metabolic balance of sphingolipids is essential for growth and development of cells. Sphingosine kinase (SPHK) is an important regulator for keeping this balance. It controls the level of S1P and plays important roles in proliferation, migration, and invasion of cancer cells and tumor angiogenesis. There are two isoenzymes of sphingosine kinase, SPHK1 and SPHK2. SPHK1 is ubiquitously expressed in most cancers where it promotes survival and proliferation, while SPHK2 is restricted to only certain tissues and its functions are not well characterized. SPHK1 is currently considered as a novel target for the treatment of cancers. Targeting SPHK1 would provide new strategies for cancer treatment and improve the prognosis of cancer patients. Here we review and summarize the current research findings on the SPHK1-S1P axis in cancer from many aspects including structure, expression, regulation, mechanism, and potential inhibitors.

Contents

Abbreviations: SPHK1, Sphingosine kinase 1; SPHK2, Sphingosine kinase 2; Sph, Sphingosine; Cer, Ceramide; S1P, Sphingosine-1-phosphate; S1PR, Sphingosine-1-phosphate receptor; PKC, Protein kinase C; ERK1/2, Extracellular signal regulated kinase1/2; SPP, Sphingosine-1-phosphate phosphatase; SPL, Sphingosine-1-phosphate lyase; ER, Estrogen receptor; PI3K, Phosphatidylinositol 3-kinase; AKT, Protein kinase B, PKB; NTD, N-Terminal domain; CTD, C-Terminal domain; NSCLC, Non-small cell lung cancer; HCC, Hepatocellular carcinoma; OSCC, Oral squamous cell carcinoma; VEGF, Vascular endothelial growth factor; CRC, Colorectal cancer; ccRCC, Clear cell renal cell carcinoma; CSC, Cancer stem cell; MAPK, Mitogen-activated protein kinase; PDGF, Platelet-derived growth factor; Sp1, Specificity protein-1; AP2, Activator protein-2; HBx, Hepatitis B virus X protein; miRNA, microRNA; LncRNA, Long non-coding RNA; DMS, N,N-Dimethylsphingosine; DHS, Dihydroxysphingosine; EGCG, Epigallocatechin gallate.

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1. Introduction

Phospholipids are a class of lipid that is a major component of all cell membranes and can form lipid bilayers, which maintain the function and fluidity of membranes. The structure of the phospholipid molecule generally consists of two hydrophobic fatty acid 'tails' and a hydrophilic 'head' containing a phosphate group. Sphingolipids and glycerophospholipids are the two major kinds of phospholipids. The *de novo* synthesis pathway of sphingolipids is regulated by two key enzymes, serine palmitoyltransferase (SPT), and ketoreductase (Kds) (Ding et al., 2018). Sphingolipids are involved in various normal biological processes, but are especially active in cancer cells where they can regulate various signal pathways to promote proliferation, migration, invasion and metastasis. In the last few decades, many of the enzymes involved in sphingolipid metabolism such as ceramide synthase, ceramidase, SPP-1 and SPHK1/2 have been identified and their genes cloned. These enzymes,especially SPHK1, also play key roles in the development of tumors and may be used as potential drug targets for cancer therapy. This review discusses the research progress on the SPHK1-S1P axis in cancers and summarizes current knowledge on structure, expression, regulation, mechanisms of action, and inhibitors.

2. Sphingosine kinases and sphingosine-1-phosphate

Many metabolites of phospholipids are important mediators of cellular signal transduction. Phospholipids such as glycerophosphatides and sphingomyelin are the main components of the lipid bilayers of cell membranes. Ceramide (Cer), sphingosine (Sph) and sphingosine-1-phosphate (S1P) are metabolites of sphingomyelin, which play important roles in the development of cancer(Saddoughi, Song, & Ogretmen, 2008). Cer and Sph mediate cell cycle arrest and induce cell apoptosis, whereas S1P promotes cell growth, migration, invasion and cell survival(Haddadi, Lin, Simpson, Nassif, & McGowan, 2017). Under normal physiological conditions, the levels of Cer, Sph and S1P are maintained in dynamic balance via enzymatic reactions which can be described as forming a 'sphingolipid-rheostat' that is necessary for cell survival(Newton, Lima, Maceyka, & Spiegel, 2015) (Fig. 1).

Sphingosine kinases (SPHKs) are lipid kinases that phosphorylate Sph to S1P. There are two isoenzymes of sphingosine kinase, SPHK1 and SPHK2. SPHKs are key mediators of the 'sphingolipid-rheostat'(Newton et al., 2015) (Fig. 1). SPHK1, the key enzyme in the synthesis of S1P, can promote the transition from Cer/Sph to S1P and thus accelerate the development of cancer(Yester, Tizazu, Harikumar, & Kordula, 2011). Inhibiting the activity of SPHK1 or reducing the level of S1P may be an effective anticancer therapy. SPHK1 is mainly located in the cytoplasm (Fig. 2). SPHK1 is activated by phosphorylation in the cytoplasm and is then transferred to the plasma membrane where it catalyzes the phosphorylation of Sph to S1P, which can be secreted. Once outside the cell, S1P binds to the S1P receptor (S1PR) (Alshaker et al., 2013). Activation of the S1P-S1PR pathway promotes

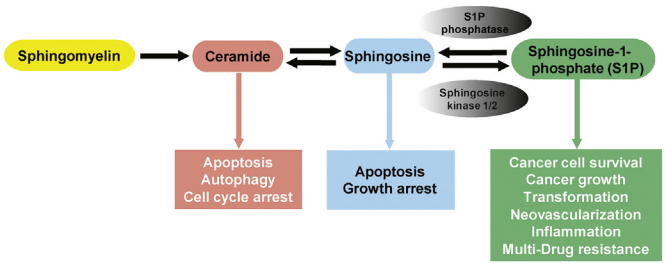


Fig. 1. Sphingolipid-rheostat. Intracellular Cer, Sph and S1P levels are kept in dynamic balance via enzymatic reactions. Cer or Sph arrest cell cycle and induce apoptosis, while S1P promotes cell proliferation, survival, angiogenesis and inflammation. SPHKs regulate generation of S1P and maintain a balance between Sph and S1P under physiological conditions.

proliferation, migration and invasion of cancer cells(Maceyka & Spiegel, 2014). SPHK1 is overexpressed in various malignant cancers and highly associated with poor prognosis. SPHK1 also participates in mediating the sensitivity of many kinds of cancer cells to certain chemotherapeutic drugs(Vadas, Xia, McCaughan, & Gamble, 2008).

SPHK2 is activated by protein kinase C (PKC) in the nucleus (Fig. 2) and converts Sph to S1P. Nuclear S1P inhibits the activity of histone deacetylase, HDAC1/2, thus preventing the deacetylation of histone H3 and facilitating the transcription of related genes(Hait et al., 2009). On the surface of mitochondria, S1P produced by SPHK2 can act on the effector protein BAX/BAK to increase the permeability of the mitochondrial membrane, promote the release of cytochrome *c* and induce cell apoptosis(Chipuk et al., 2012). S1P is degraded by S1P lyase (SPL) and S1P phosphatases (SPP)(Alvarez, Milstien, & Spiegel, 2007) to maintain a very low concentration in the cytoplasm. Therefore, under physiological conditions S1P mainly exerts its functions through binding to S1PR on the extracellular surface (Fig. 2) which promotes proliferation, migration and invasion of cancer cells. Studies have shown that S1P also stimulates angiogenesis(Camaré et al., 2015; Mukhopadhyay, Ramanathan, & Takabe, 2015; Yang et al., 2013).

SPHK1 has inherent kinase activity, and its activation can be independent of the post-translational modifications of eukaryotic cells. The production of secreted S1P needs two steps: the activation of SPHK1 and its translocation from the cytoplasm to the plasma membrane. When cells are stimulated by growth factors, cytokines, hormones, or immunoglobulins, SPHK1 can be rapidly activated. Activation may also occur by direct action of various factors on SPHK1 such as ERK1/2 phosphorylation of Ser225 (Pitson et al., 2003). This single phosphorylation site not only regulates the catalytic activity of SPHK1, but is also necessary for translocation. The conformation of SPHK1 is changed when it is activated by Ser225 phosphorylation. After activation and translocation to the plasma membrane, SPHK1 interacts with the calcium-myristoyl switch protein 1 (CIB1), which mediates the phosphorylation of Sph on the cell membrane to S1P. S1P binds to SIPR and promotes cell proliferation, survival and migration (Fig. 3).

The S1PRs are a group of G-protein-coupled receptors that are currently divided into five subtypes; S1P1, S1P2, S1P3, S1P4 and S1P5. Binding of S1P to S1PR1 promoted development of tumors and was identified as a potential drug target in activated B cell-like diffuse large B cell lymphoma(Liu et al., 2012). S1PR2 was found to induce proliferation of AML(Powell et al., 2017) and activate ezrin-radixin-moesin (ERM) proteins to induce migration and invasion of HeLa cells(Adada et al., 2015). Aldehyde dehydrogenase 1 (ALDH1) is a biomarker of breast cancer stem cells (BCSCs), and it was demonstrated that binding of S1P to S1PR3 increased ALDH1-positive BCSCs without ligand-dependent Notch activation(Hirata et al., 2014). Over-expression of S1PR4 was correlated with shorter disease-free survival in a cohort of 140 patients with estrogen receptor (ER)-negative BC in a clinical study(Ohotski et al., 2012). A recent report stated that the S1P–S1PR5 signal pathway increased mitotic progression in HeLa cells and led to chromosome segregation defects, which were caused by binding of S1P secreted through SPNS2 and S1PR5-dependent activation of the PI3K/AKT pathway(Andrieu et al., 2017). The S1PRs regulate many basic biological processes including cell proliferation, mitogenesis, cytoskeletal organization, cell migration, angiogenesis, endothelial cell chemotaxis and immune cell trafficking.

3. Structure of SPHK1

SPHK1 consists of an N-terminal domain (NTD) and a C-terminal domain (CTD). The catalytic site is in the gap between the two domains and the binding site of SPHK1 with substrate Sph is in the C-terminal domain (Fig. 4). Although SPHK1 belongs to the lipid kinase family that is highly conserved during evolution, the structure of SPHK1 is not similar to that of other lipid kinases, such as phosphatidylinositol kinase (PI3K). However, SPHK1's structure is similar to diacylglycerol

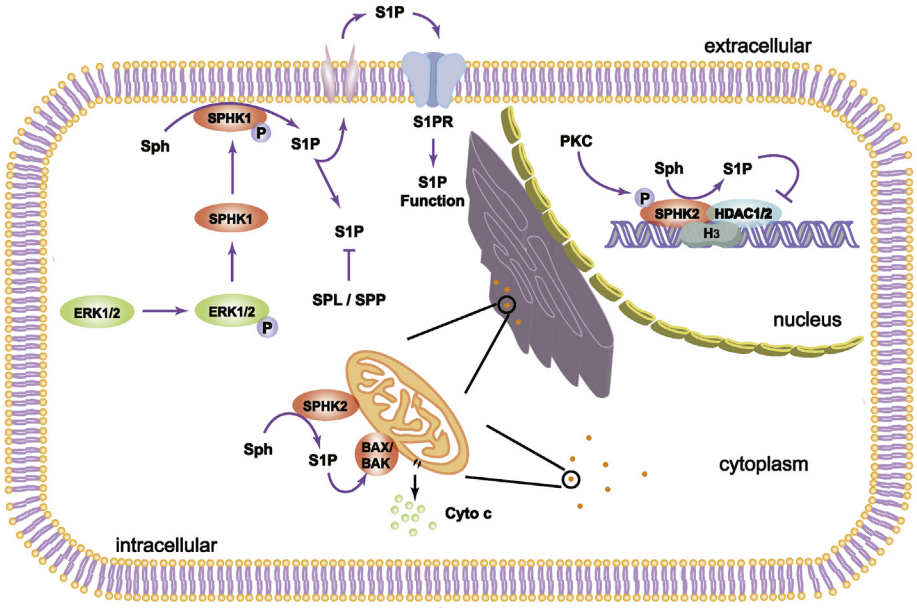


Fig. 2. Roles of SPHK1, SPHK2 and S1P in cells. SPHK1 is located in the cytoplasm, but when it is recruited to the plasma membrane, it phosphorylates Sph to S1P which is secreted. Outside the cell, S1P binds to S1PR, a family of five receptor subtypes anchored in the membrane. The S1P produced by SPHK2 is mainly located in the nucleus and regulates gene expression, but S1P can also influence mitochondrial function. S1P in the cytoplasm is degraded by S1P lyase (SPL) or dephosphorylated by S1P phosphatases (SPP).

kinases (DGKs) and ceramide kinase, although their sequence homology is low (10–20% identical)(Z. Wang et al., 2013).

4. Expression and functional role of SPHK1 in cancer

4.1. SPHK1 expression in various types of cancer

SPHK1 is overexpressed in many cancers, including lung, gastric, esophageal, liver, breast, and colon, as well as glioma, non-small cell lung cancer and chronic myeloid leukemia (Fig. 5). A high expression level of SPHK1 is associated with a great degree of tumor malignancy, occurrence, and development and with poor prognosis.

4.1.1. The expression of SPHK1 in lung cancer

Lung cancer has one of the highest incidence rates in the world and is one of the most malignant tumors. Expression of SPHK1 is significantly increased in non-small cell lung cancer (NSCLC) and the survival rate of patients with NSCLC is associated with expression of SPHK1. Over-expression of SPHK1 promotes proliferation and migration of NSCLC cells,while inhibition of SPHK1 can induce apoptosis and increase the chemosensitivity of NSCLC to cytotoxic drugs(Song et al., 2011).

4.1.2. The expression of SPHK1 in gastric cancer

Gastric cancer is one of the commonest gastrointestinal malignancies in China. Increased expression of SPHK1 results in poor survival of gastric cancer patients. ERK1 interacting with lysophosphatidic acid

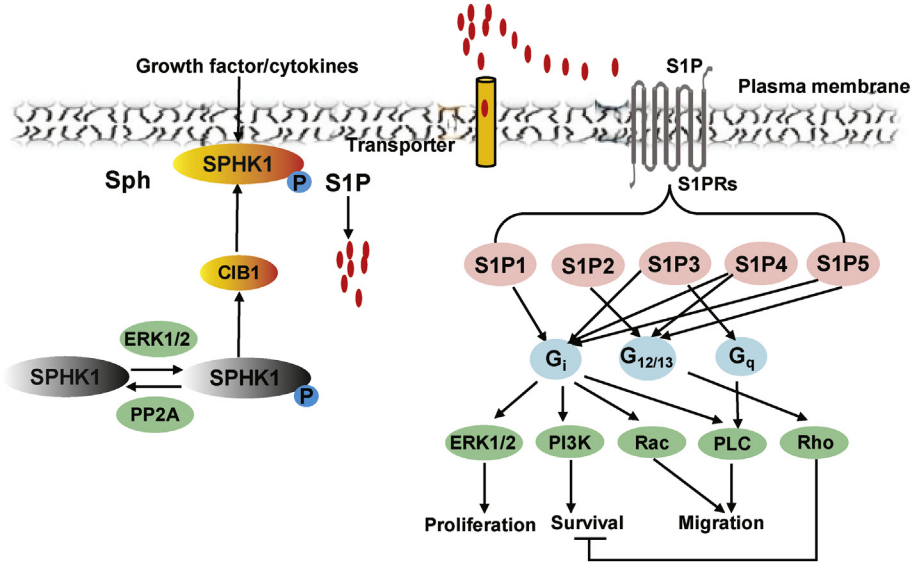


Fig. 3. Mechanism and functional roles of the SPHK1-SIP axis in cancer. SPHK1 phosphorylates Sph to S1P. Secreted S1P binds to S1PRs and activates these G protein-coupled receptors to promote cell proliferation, survival and migration by regulating different signaling pathways including ERK1/2, PI3K/Akt, Rac, PLC, and Rho.

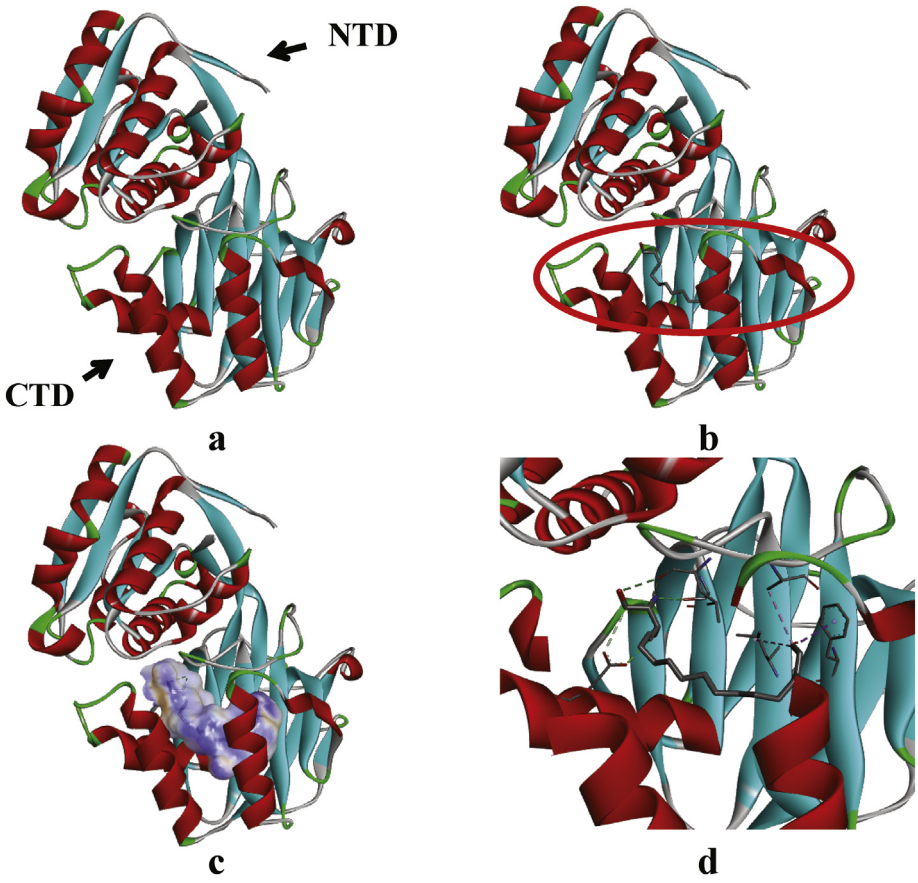


Fig. 4. Crystal structure of SPHK1. (a) The structure of SPHK1; (b) SPHK1 substrate Sph in the binding site; (c) The J-shaped binding pocket of Sph; (d) The interaction between Sph and SPHK1. (PDB ID: 3VZB). SPHK1 contains an N-terminal and a C-terminal domain. The active site is near the Sph binding pocket in the CTD. Many SPHK1 inhibitors were designed to block SPHK activity1 by binding to the substrate site and most early stage drugs were analogs of Sph.

(LPA) can increase the expression of SPHK1 mRNA and protein which promotes the proliferation, migration and invasion of gastric cancer cells. These negative effects can be reversed by inhibiting SPHK1 (Shida et al., 2008). *In vitro*, the proliferation of gastric cancer cells was inhibited and chemosensitivity to doxorubicin was increased when SPHK1 expression had been significantly inhibited by delivering locked nucleic acid-antisense oligonucleotide (LNA-ASO)(Fuereder et al., 2011). In addition, high expression of SPHK1 caused resistance of gastric cancer cells to a number of anti-tumor drugs(Matula et al., 2015).

4.1.3. The expression of SPHK1 in hepatocellular carcinoma

Hepatocellular carcinoma (HCC) is the fifth most common malignant tumor in the world. SPHK1 is overexpressed in HCC tissues compared with adjacent non-HCC tissues and this promotes the proliferation, migration and invasion of HCC cells(Uranbileg et al., 2016). SKI-II, an SPHK inhibitor, increased the cytotoxic effect of 5-fluorouracil on HepG2 cells(Grbčić et al., 2017). The microRNA, MIR-506, reduced tumor angiogenesis by inhibiting SPHK1 mRNA expression in hepatocellular carcinoma cells(Lu et al., 2015).

4.1.4. The expression of SPHK1 in breast cancer

Breast cancer is one of the commonest malignant tumors in women with high morbidity and mortality. French KJ et al. analyzed the expression of SPHK1 mRNA in a variety of solid tumors and discovered that the expression of SPHK1 in breast cancer cells was 4-fold greater than in normal tissues(K. French et al., 2003). Ruckhäberle E et al. analyzed gene expression by using microarray data of 1269 tumor samples in different subtypes of breast cancer and found that SPHK1 was overexpressed in estrogen receptor (ER)-negative breast cancer. Furthermore, survival analysis showed that breast cancer patients with high expression of SPHK1 had lower survival rates(Ruckhäberle et al., 2008). Down-regulation of SPHK1 expression can induce arrest of cell cycle and apoptosis of the human breast cancer cell line, MCF-7. In addition, ceramide can also induce apoptosis of breast cancer cells (Sarkar et al., 2005). Increased levels of estrogen (E2), an important female hormone mainly produced by the ovaries and the placenta can promote the development of estrogen receptor (ER)-positive breast cancers. SPHK1 is mostly expressed in ER-negative tumors rather than ER-positive breast cancers. However, studies showed that E2 binding to ERα enhanced the activation of SPHK1 with increased production of S1P (Sukocheva, Wadham, Gamble, & Xia, 2015). Prolactin (PRL) is also an important regulator in breast cancers. Compared with E2α, over-expression or knockdown of SPHK1 in MCF-7 breast cancer cells has similar effects on PRL-induced cell proliferation and migration (Döll, Pfeilschifter, & Huwiler, 2007).

4.1.5. The expression of SPHK1 in colorectal cancer

Colon cancers are often associated with local bleeding. Therefore, platelets may be activated at the tumor sites and that combined with S1P secretion may affect colorectal cancer progression(Shida, Takabe, Kapitonov, Milstien, & Spiegel, 2008). It was reported that there was higher expression of SPHK1 in colon cancer tissues induced by azoxymethane (AOM), a reagent which can cause colon cancer, than in polyps(Kawamori et al., 2006). Studies showed that both SPHK1 and S1PR1 were highly expressed in colon cancer. Knockout of SPHK1 in $Apc^{MIN/+}$ mice markedly reduced tumor size without changing the incidence of adenomas(Kohno et al., 2006). The expression of S1P lyase, the enzyme responsible for degrading S1P, was reduced in colon cancer tissues compared with adjacent tissues, which suggested that

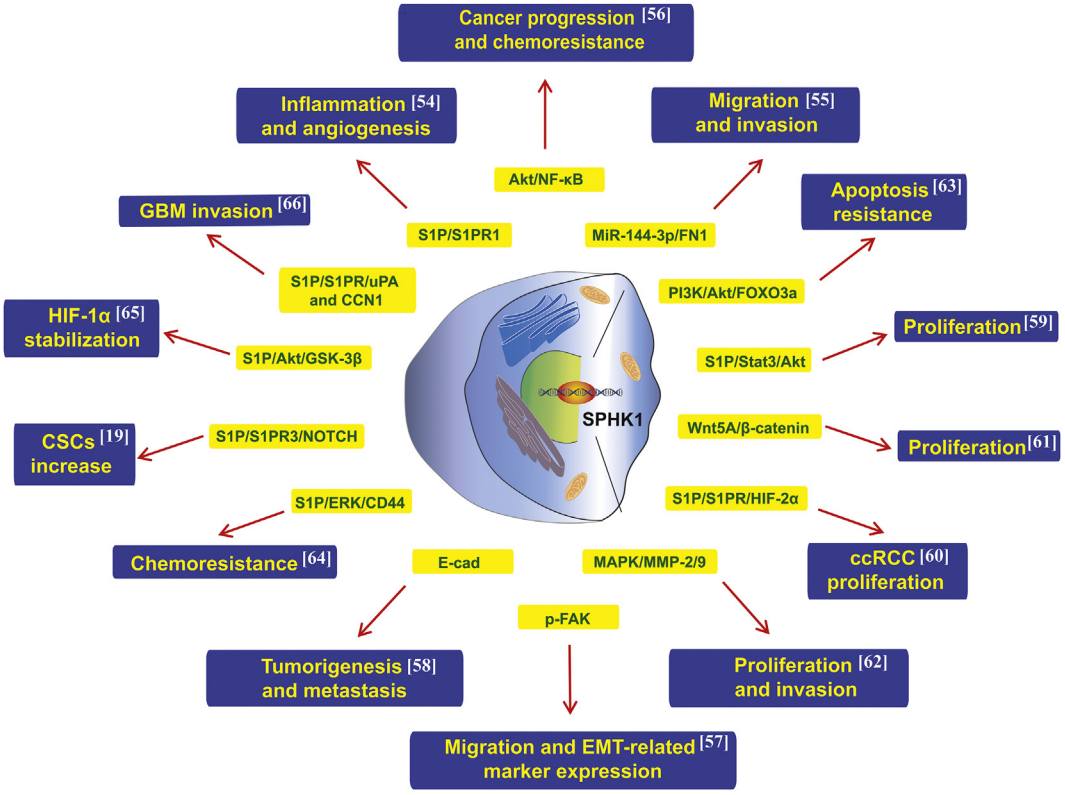


Fig. 5. Mechanisms of SPHK1 function in cancers. SPHK1 plays many functional roles in the development of cancers by several potential mechanisms. SPHK1 increased proliferation of cancer cells via the MAPK/MMP-2/9 pathway, S1P/S1PR/HIF-2a, Wnt5A/β-catenin and S1P/Stat3/AKT pathways. Over-expression of SPHK1 induced drug resistance and blocked apoptosis by the S1P/ERK/CD44 pathway, the Akt/NF-KB pathway and the PI3K/Akt/Foxo3a pathway in cancers. SPHK1 also promoted the EMT, migration, invasion and metastasis of cancer cells by regulating the E-cadherin pathway, p-FAK pathway, MiR-144-3p/FN1 pathway, S1P/S1PR/uPA and CCN1 pathways. CSCs were increased by the SPHK1/S1P/S1P3R/NOTCH pathway. SPHK1 promoted angiogenesis by regulating the S1P/S1PR1 pathway and the S1P/Akt/GSK-3β pathway.

the formation and metabolism of S1P in the cell was closely associated with the development of colon cancer (Oskouian et al., 2006). The expression of COX-2 and the production of PGE2 may be regulated by the SPHK1/S1P signal pathway, which can influence the development of colon cancer(Kawamori et al., 2006).

4.1.6. The expression of SPHK1 in glioma

SPHK1 is overexpressed in glioblastomas and is associated with poor prognosis in patients. SK1-I, an SPHK1 inhibitor, can block cell growth, induce cell death, and inhibit invasion and metastasis of the glioblastoma cell lines LN229 and U373. In addition, SK1-I also significantly reduced the growth rate of xenografted LN229 tumors and tumor angiogenesis, and increased survival in mice(Kapitonov et al., 2009). SPHK1 is also a prerequisite for the survival of glioblastoma-derived neurospheres(Van Brocklyn et al., 2005). The mean survival time of glioma patients with low SPHK1 expression was significantly longer than patients with high expression. The expression of SPHK1 mRNA and protein in astrocytomas was significantly higher than that in paracancerous tissues. In addition, expression of SPHK1 was associated with the pathological grade of astrocytoma(Li et al., 2008).

4.1.7. The expression of SPHK1 in leukemia

SPHK1 was found to be highly expressed in acute leukemia(Sobue et al., 2006).There was a positive correlation between the IC_{50} of daunorubicin and SPHK1 expression in leukemia cells. Cells with low SPHK1 expression were sensitive to daunorubicin whereas cells with high SPHK1 expression were resistant (Sobue et al., 2008). SPHK1 may be both a good marker of daunorubicin sensitivity in leukemia cells and a potential target for leukemia therapy.

4.1.8. The expression of SPHK1 in melanoma

SPHK1 and S1P are increased as melanoma develops. Anchorage-dependent and -independent growth of melanoma cells were reduced when expression of SPHK1 was inhibited by SPHK1 siRNA. The SPHK1 inhibitor, SKI-I, reduced the level of S1P and increased ceramide, which halted the cell cycle of melanomas cells in G2-M and induced apoptosis(Madhunapantula et al., 2012). Another study showed that SPHK1/S1P regulated the communication between dermal fibroblasts and melanoma cells, and that SPHK1 was a potential target in stopping the progression of melanoma(Albinet et al., 2014).

4.1.9. The expression of SPHK1 in ovarian cancer

The expression of SPHK1 was closely correlated with the microvascular density (MVD) of ovarian cancer tissue. *In vitro*, inhibition of SPHK1 expression reduced the angiogenic potential and angiogenic factor secretion of ovarian cancer cells. Angiogenesis was restored by the addition of S1P and S1P induced the secretion of angiogenic factors via S1PR1 and S1PR3, but not S1PR2(Dai et al., 2017).

4.1.10. The expression of SPHK1 in thyroid cancer

The expression of SPHK1 is generally up-regulated in thyroid cancer and is associated with the degree of thyroid malignancy. Proliferation of thyroid cancer cells was inhibited when SPHK1 expression was silenced by SPHK1 siRNA. Furthermore, SPHK1 expression was strongly associated with the expression of PCNA (proliferating cell nuclear antigen) in thyroid cancer tissues. Silencing of SPHK1 resulted in dephosphorylation of protein kinase B and GSK-3β(Guan et al., 2011).

4.1.11. The expression of SPHK1 in oral squamous cell carcinoma (OSCC)

SPHK1 was over-expressed in most OSCCs. OSCCs patients with high SPHK1 expression showed higher invasive grades and unfavorable survival rates and prognosis(Kato, Shimasaki, Kato, Segami, & Ueda, 2018). SPHK1 expression was associated with acquisition of vimentin expression and loss of E-cadherin expression.

4.1.12. The expression of SPHK1 in esophageal cancer

SPHK1 over-expression was associated with high invasive capacity and metastasis of esophageal cancer. SPHK1 expression was higher in esophageal carcinomas than in the adjacent normal tissues and its expression was significantly associated with tumor invasiveness and lymph node metastasis as determined by esophageal carcinoma tissue microarray analysis. SPHK1 was over-expressed in most esophageal cancers and was significantly associated with lymph node metastasis, poor prognosis and lower 5-year survival rates. In addition, expression levels of SPHK1 mRNA and protein and serum S1P levels in esophageal cancer patients were much higher in the metastasis-positive group than in the metastasis-negative group(Kawakita et al., 2017).

4.2. Functional role of SPHK1 in cancer

4.2.1. Role of SPHK1 in cancer

Over-expression of SPHK1 can induce neoplastic transformation of NIH3T3 fibroblasts. In Xia et al., 2000 were the first to demonstrate the oncogenic characteristics of SPHK1 by using colony formation and tumorigenicity assays in NOD/SCID mice(P. Xia et al., 2000). They found that SPHK1-activated fibroblasts had the ability to form colonies in soft agar and tumors in nude mice, demonstrating that over-expression of SPHK1 can promote cell proliferation and migration. SPHK1 is involved in H-Ras-mediated tumorigenesis(P. Xia et al., 2000) and shows oncogenic properties in leukemia. In the multi-stage leukemia spi-1 transgenic mouse model, the occurrence of tumors was correlated with the upregulation of SPHK1(Le Scolan et al., 2005). These studies have linked elevated SPHK1 activity with tumorigenesis and development of cancer.

4.2.2. Role of SPHK1 in angiogenesis in cancer

Angiogenesis is an important part of the development and metastasis of cancers, as the formation of a capillary blood supply is critical for cancer growth and survival. SPHK1 is involved in the regulation of cancer angiogenesis through S1P which is one of the most potent angiogenic factors. The ginsenoside, compound K, was found to block S1P-induced cell migration by regulating SPHK1 activity and expression in human umbilical vein endothelial cells (HUVECs) (Shin et al., 2014). S1P induces vascular mimicry in cancer cells which stimulates the growth of xenografts in nude mice by up-regulating VEGF and increasing the density of microvessels in tumors(Visentin et al., 2006). In triple-negative breast cancer cells (MDA-MB-231), S1P increased the release of angiogenic factors while S1P antibodies inhibited VEGF- and bVEGF-induced angiogenesis and prevented tumor-associated angiogenesis(Visentin et al., 2006).

5. Mechanisms of SPHK1 action and regulation of SPHK1 expression in cancer

5.1. Mechanisms of SPHK1 action in cancer

Elevated expression of SPHK1 has been found in many types of cancer and leads to increased cell proliferation, migration capability, invasiveness and angiogenesis by several mechanisms (Fig. 5).

5.1.1. SPHK1/S1P/S1PR1 pathway is associated with chronic inflammation and angiogenesis in tumors

Expression of SPHK1 was up-regulated by a high fat diet and more S1P was produced. Activation of the SPHK1-S1P-S1PR axis promoted development of breast cancer. Targeting the SPHK1/S1P/S1PR1 pathway with FTY720/fingolimod (SPHK1 inhibitor) attenuated the key pro-inflammatory cytokine IL-6, macrophage infiltration and tumor progression induced by obesity(Nagahashi et al., 2018).

5.1.2. SPHK1/miR-144-3p/FN1 promotes migration and invasion

Over-expression of SPHK1 can reduce the expression of the microRNA, miR-144-3p. MiR-144-3p can directly bind the FN1 mRNA 3′-UTR to inhibit expression of FN1 which mediated migration and invasion of papillary thyroid cancer (PTC) TPC1 cells. Silencing of SPHK1 significantly increased the expression of miR-144-3p which reduced the invasiveness of PTC cells by targeting FN1 (Liang et al., 2017).

5.1.3. SPHK1/Akt/NF-κB is associated with pancreatic cancer progression and chemoresistance

The miR-506 promoter is highly methylated in pancreatic cancer cells compared to normal tissues. SPHK1 was identified as a target of miR-506, and reduced expression of miR-506 activated the SPHK1/Akt/NF-κB signaling pathway, which is common in pancreatic cancer (Li et al., 2016).

5.1.4. SPHK1/p-FAK mediated cell migration and EMT-related marker expression in colorectal cancer (CRC) cells

SPHK1 induced the expression of epithelial-mesenchymal transition (EMT) - related markers and cell migration by regulating the expression of p-FAK in CRC cells. Silencing SPHK1 reduced CRC cell migratory ability by inhibiting the expression of EMT-related markers and p-FAK(C. Xu et al., 2017).

5.1.5. The TNFα/SPHK1/E-cadherin pathway is associated with tumorigenesis and metastasis

Elevated SPHK1 expression was associated with malignant transformation and metastasis of breast epithelial cells. Over-expression of SPHK1 reduced expression of E-cadherin and stimulated the proliferation and invasion of normal human mammary epithelial cells line, MCF-10A, while knockdown of SPHK1 inhibited cell proliferation and invasion of the human breast cancer cell line, MCF-7. TNFα up-regulated SPHK1 increased its ability to reduce expression of E-cadherin, which plays an important role in tumorigenesis and metastasis. Activation of the TNFα/SPHK1/E-cadherin pathway could be blocked by an inhibitor of SPHK1 (DMS) (Zheng et al., 2014).

5.1.6. SPHK1/S1P/Stat3/Akt signaling in colorectal cancer (CRC)

The PRSS8 gene encodes a membrane-anchored serine protease called prostasin that is associated with CRC. Over-expression of PRSS8 inhibited proliferation of CRC cells while knockdown of PRSS8 promoted proliferation of CRC cells. Over-expressing PRSS8 also inhibited growth of CRC cells in nude mice. Mechanistic studies revealed that PRSS8 exerted its function by inhibiting the SPHK1/S1P/Stat3/Akt signaling pathway(Bao et al., 2016).

5.1.7. SPHK1/S1P/HIF-2α in clear cell renal cell carcinoma (ccRCC)

SPHK1 silencing inhibited expression of the transcription factor hypoxia-inducible factor (HIF)-2α and reduced cell proliferation in ccRCC. Down-regulation of SPHK1 expression was also associated with impaired Akt and mTOR signaling in ccRCC. Inhibition of S1P extracellular signaling by S1P antibody blocked HIF-2α accumulation in ccRCC cell lines. The SPHK1/S1P signaling pathway regulated expression of HIF-2α in ccRCC(Bouquerel et al., 2016).

5.1.8. The SPHK1/Wnt5A/β-catenin signaling pathway in human hepatoma HepG2 cells

Blocking SPHK1 activity with the specific inhibitor, SKI-II, reduced the expression of β-catenin and promoted its degradation, while also affecting the downstream target signaling pathway molecules, cyclin D1 and c-Myc. SKI-II increased the expression of Wnt5A, but did not reduce

the expression of β-catenin when Wnt5A was silenced. SKI-II inhibited the proliferation of human hepatoma HepG2 cells via the Wnt5A/β-catenin signaling pathway(Liu et al., 2016).

5.1.9. SPHK1/MAPK/MMP-2/9 is associated with increased proliferation and invasion of cells

Over-expression of SPHK1 induced the constitutive expression of extracellular signal regulated kinase1/2 (ERK1/2) while reducing the constitutive expression of p38 mitogen-activated protein kinase (MAPK). Inhibiting the ERK1/2 pathway abrogated the biological effects induced by over-expression of SPHK1. Blocking the p38 MAPK pathway reversed the effects of DMS and the silencing of SPHK1 with small hairpin RNA (shRNA). SPHK1 was found to be required for activation of MMP-2/9 and urokinase plasminogen activator (uPA) in tumor cells. This effect was suppressed by the ERK1/2 inhibitor, U0126, but enhanced by the p38 MAPK inhibitor, SB203580. SPHK1 enhances proliferation and invasion of colon cancer cells by upregulating the expression of MMP-2/9 and uPA via the MAPK pathways(Liu et al., 2012).

5.1.10. The SPHK1/PI3K/Akt/FOXO3a signaling pathway is associated with apoptosis resistance

Overexpression of SPHK1 significantly elevated Akt activity and inactivated FOXO3a by phosphorylation, which led to down-regulation of Bcl-2-interacting mediator (Bim) expression in glioma cells. These effects of SPHK1 were dependent on phosphatidylinositol 3-kinase (PI3K). In addition, the effects of SPHK1 on the PI3K/Akt/FOXO3a/Bim pathway could be reversed by SPHK1 inhibitor or siRNA (Guan et al., 2011).

5.1.11. SPHK1/S1P/ERK/CD44 is associated with human colon cancer cells

CD44, a marker of cancer stem cells, increases cellular resistance to anticancer drugs. Over-expression of SPHK1 increased the expression of both CD44 and phospho-ERK. The increase in CD44 protein was abolished by the inhibition of ERK phosphorylation. SPHK1/S1P regulated expression of CD44 through the ERK signaling pathway in colon cancer cells(Kawahara et al., 2013).

5.1.12. The SPHK1/S1P/S1PR3/NOTCH pathway is active in cancer stem cells (CSC)

S1P increases aldehyde dehydrogenase ($ALDH1^+$)-positive CSC by binding S1P receptor 3(S1PR3) and subsequently activating the Notch pathway. CSCs with high SPHK1 expression had greater capacity to produce tumors in nude mice than parental cells. The tumorigenicity of CSCs with overexpressing SPHK1 was abrogated by S1PR3 knockdown or incubation with an S1PR3 antagonist. $SPHK1^+/ALDH1^+$cells or $S1PR3^+/ALDH1^+$cells were found in breast cancer patient-derived mammospheres. The SPHK1/S1P/S1PR3/NOTCH pathway was involved in the tumorigenicity of CSCs(Hirata et al., 2014).

5.1.13. The SPHK1/AKT/GSK-3β pathway stabilized HIF-1α in cancer cell lines

SPHK1 activity was stimulated under low oxygen conditions and regulated by reactive oxygen species. Inhibition of SPHK1 activity with siRNA blocked the accumulation of hypoxia inducible factor 1α (HIF-1α) and its transcriptional activity in several human cancer cell lines. The SPHK1-dependent stabilization of HIF-1α levels was regulated by the AKT/GSK-3β signaling pathway which prevented von Hippel-Lindau protein-mediated degradation by the proteasome(Ader, Brizuela, Bouquerel, Malavaud, & Cuvillier, 2008).

5.1.14. SPHK1/S1P/S1PR regulates glioblastoma cell invasiveness through urokinase plasminogen activator (uPA) and CCN1

S1P induced expression of CCN1, a protein predictive of poor prognosis. The uPA-stimulated invasion of GBM cells was also triggered by S1P, and the S1P1, S1P2, and S1P3 receptors were involved in the process. SPHK1 was necessary for basal activity of the uPA system and invasion of glioma cells, whereas S1P/S1PR signaling promoted invasion partially through uPA and CCN1(Young, Pearl, & Van Brocklyn, 2009).

5.2. Regulation of SPHK1 expression in cancer

5.2.1. Regulation of SPHK1 expression by transcription factors

Transcription factors are proteins that bind to specific DNA sequences and regulate transcription of genes from DNA to messenger RNA. Transcription factors play a pivotal role in expression of SPHK1.

There is one specificity protein-1 (Sp-1) and two activator protein-2 (AP-2) binding motifs in the promoter region distal to the first exon of the SPHK1 gene. The expression of SPHK1 mRNA and protein can be increased by treating cells with PMA (phorbol 12-myristate 13-acetate) which acts via the protein kinase C (PKC) pathway. The PMA-induced increase in SPHK1 activity can be prevented with an inhibitor of PKC (Buehrer, Bardes, & Bell, 1996). It was found that Sp1 protein was originally bound to the Sp1 site on the promoter of the SPHK1 gene, but that PMA treatment resulted in the binding of AP-2 to the two AP-2 motifs in MEG-01 cells (human megakaryoblastic leukemia). SPHK1 gene expression was upregulated via the PKC signaling pathway by binding of transcription factors to the AP-2 motifs(Nakade et al., 2003).

A positive correlation was found between the mRNA levels of SPHK1 and the hepatitis B virus X protein (HBx) in thirty-eight cases of hepatocellular carcinoma (HCC). Silencing HBx inhibited SPHK1 expression and activity in the hepatoma cell lines, HepG2-X and HepG2.2.15, while over-expressing HBx in HepG2 cells dose-dependently increased SPHK1 promoter activity and expression. Silencing HBx in HepG2-X cells abolished the HBx-enhanced proliferation and colony formation *in vitro*, and tumor growth *in vivo*. Transcription factor AP2α was found to directly interact with the SPHK1 gene promoter, and silencing AP2α inhibited SPHK1 expression, thus proving its involvement in regulation of SPHK1 expression(Lu et al., 2015).

Platelet-derived growth factor (PDGF) induced expression of SPHK1 and promoted cell proliferation in human pulmonary artery smooth muscle cells (hPASMCs). Selective inhibition of SPHK1 reduced PDGF-mediated cell proliferation. Further studies showed that there were several binding sites for early growth response protein 1 (Egr-1) in the SPHK1 gene, and that SPHK1 expression in hPASMCs was regulated by PDGF via the transcription factor Egr-1(Sysol, Natarajan, & Machado, 2016).

5.2.2. Regulation of SPHK1 expression by epigenetics

Epigenetics refers to the heritable changes in a cellular phenotype that were independent of alterations in the primary DNA sequence. Epigenetic changes include DNA methylation, histone modification, non-coding RNA (ncRNA)-associated gene regulation and chromosome remodeling. Epigenetic modifications are an important mechanism for regulation of gene expression and play an important role in the development of cancer.

MicroRNAs (miRNAs) are a class of endogenous short-chain, non-coding, regulatory RNAs with an average length of 20–25 nucleotides. In recent years, specific miRNAs have proven to be very important in regulating gene expression. SPHK1 was identified as a target of miR-506, the expression of which inhibited the SPHK1/Akt/NF-κB signaling pathway in pancreatic cancer(Li et al., 2016). MiR-124 also reduced expression of SPHK1 by directly binding to its 3′-untranslated region (3′-UTR); and miR-124 expression was inversely correlated with expression of SPHK1 in gastric cancer (J. Xia et al., 2012). In addition, over-expression of miR-124 significantly inhibited proliferation and tumorigenicity of gastric cancer cells both *in vitro* and *in vivo* by targeting the SPHK1/AKT/FOXO1 signal pathway(J. Xia et al., 2012). Luciferase assays showed that miR-125b directly targeted the 3′-UTR of SPHK1 and over-expression of miR-125b inhibited cellular growth, migration and arrested the cell cycle at G1 by reducing the expression of SPHK1(X. Zhao et al., 2015). SPHK1 was also identified as a direct target gene of miR-613 in bladder cancer cells. Over-expression of miR-613 reduced

expression of SPHK1 and inhibited EMT, vimentin, Snail and N-cadherin while inducing expression of E-cadherin(Yu, Duan, Zhu, & Rao, 2017).

Long non-coding RNAs (lncRNAs) are a class of non-coding RNA with an average length >200 nucleotides that are known to be involved in carcinogenesis and cancer progression. LncRNAs can regulate gene expression at epigenetic level, transcriptional level and post-transcriptional level. The lncRNA, Khps1, increased SPHK1 expression by recruiting the histone acetyl-transferase p300/CBP to the SPHK1 promoter, which lead to local changes of the chromatin structure that ensured E2F1 binding and enhanced transcription(Postepska-Igielska et al., 2015). The LncRNA, HULC, induced expression of SPHK1, which can enhance tumor angiogenesis. Inhibiting expression of SPHK1 markedly blocked HULC-induced angiogenesis. Further research showed that HULC activated the SPHK1 promoter by recruiting the transcription factor, E2F1, that bound to the E2F1 element in the SPHK1 promoter in hepatoma cells(Z. Lu et al., 2016). Epigenetic changes including expression of non-coding RNAs and histone acetylation also regulated SPHK1 expression. Micro-RNAs can regulate SPHK1 expression by directly binding to the 3′-UTR of SPHK1 in tumor cells. LncRNAs can increase expression of SPHK1 in tumors by increasing acetylation of histones, but also by promoter activation of SPHK1.

6. Inhibitors of SPHK1

Although studies on SPHK1 inhibitors have made some progress, no SPHK1 inhibitor is currently used for treating cancer in the clinic. An ideal SPHK1 inhibitor should have strong potency at the nanomole level, high selectivity for SPHK1 isoforms (100-fold or more), and metabolic stability, with no toxicity upon daily usage (Santos & Lynch, 2015). In addition, the ideal SPHK1 inhibitor should decrease the level of S1P as a pharmacodynamic marker in the blood and the level of sphingosine or S1P in target tissues(Shida, Takabe, et al., 2008). Up to now, no inhibitors meeting all these criteria have been identified.

6.1. Inhibitors found from 1990 to 2000

6.1.1. FTY720

FTY720 (Fingolimod) (Fig. 6) is now the most studied analog of sphingosine. FTY720 is an immunosuppressant used to treat multiple sclerosis (MS) potentially by interacting with S1P-R1 and regulating immune function(Chun & Hartung, 2010). The main effect of FTY720 is to induce apoptosis, a process in which cleavage of caspase 9 and 3, is followed by PARP cleavage(Bai, Chiu, Chiu, Chu, & Weng, 2017). FTY720 also inhibits the anti-apoptotic proteins, Bcl-2, Bcl-xl and Mcl-1, while increasing the pro-apoptotic protein Bax which changes the permeability of the mitochondrial membrane, allowing cytochrome *c* to be released(Yasui et al., 2005). There is also evidence that FTY720 can inhibit the PI3K/Akt/mTOR signaling pathway which mediates the development of many different types of cancer. Apoptosis triggered by the synergistic combination of metformin and FTY720 can be blocked by activation of Akt(Y. Zhao et al., 2018). FTY720 can be phosphorylated by SPHK2, and P-FTY720 is an agonist of four of the five S1P receptors–1, 3, 4 and 5–(but not S1PR2)(Brinkmann et al., 2002). In September of 2010, FTY720 was approved as a new drug for treating MS by the U.S. Food and Drug Administration (FDA). The clinical success of FTY720 has greatly encouraged researchers to try targeting other proteins involved in S1P metabolism, such as S1P receptors, S1P lyase, and S1P synthase (SPHK).

6.1.2. DMS and DHS

N,N-dimethylsphingosine (DMS) and dihydroxysphingosine (DHS, Safingol) are two SPHKs inhibitors developed in the early stages of SPHK-targeted therapy(Olivera, Kohama, Tu, Milstien, & Spiegel, 1998). They are analogs of Sph (Fig. 6). As a simple derivative of Sph, DHS has one less double bond than Sph. DHS is a competitive inhibitor of SPHK1 with Sph. It can also act as the substrate of SPHK2(Liu et al., 2000). Compared with Sph, DMS has two more methyl groups on the N atom. DMS is a non-selective inhibitor of SPHKs, whose inhibitory effect on SPHK1 is similar to that of DHS(Melendez, Carlos-Dias, Gosink, Allen, & Takacs, 2000). However, the poor selectivity of DMS and DHS can result in off-target effects like the inhibition of other kinases such as PKC(Orr Gandy & Obeid, 2013). Although DMS and DHS have anti-tumor effects in tumor xenograft models, they have severe side effects, such as hemolysis and hepatotoxicity. Safingol is used as a combination drug and has been in two clinical trials (ClinicalTrials.gov identifiers NCT01553071 and NCT00084812).

6.2. SPHK Inhibitors found from 2000 to 2010

6.2.1. SKI-I to IV and derivatives

A group of non-lipid SPHK inhibitors (SKI-I to IV) was found through a series of screenings, among which SKI-II was the most prominent (Fig. 7)(K. French et al., 2003; K. French et al., 2006). SKI-II is a non-selective inhibitor of SPHKs which competitively binds to the Sph binding pocket and inhibits the proliferation of several types of cancer cells (Z. Wang et al., 2013). SKI-II can be orally administered to mice and has a relatively long half-life ($t_{1/2}$–15 h)(K. French et al., 2006). SKI-II's potency *in vivo* was exerted via activation of the cathepsin B-associated lysosomal degradation pathway resulting in the degradation of SPHK1(Ren, Xin, Pfeilschifter, & Huwiler, 2010). Grbčić, et al. found that combined administration of 5-Fu and SKI-II in HepG2 cells significantly inhibited cell proliferation, migration and clonogenic survival, and induced apoptosis(Grbčić et al., 2017). SKI-I is also a potent SPHK1 inhibitor and is more effective than SKI-II, but it can also inhibit

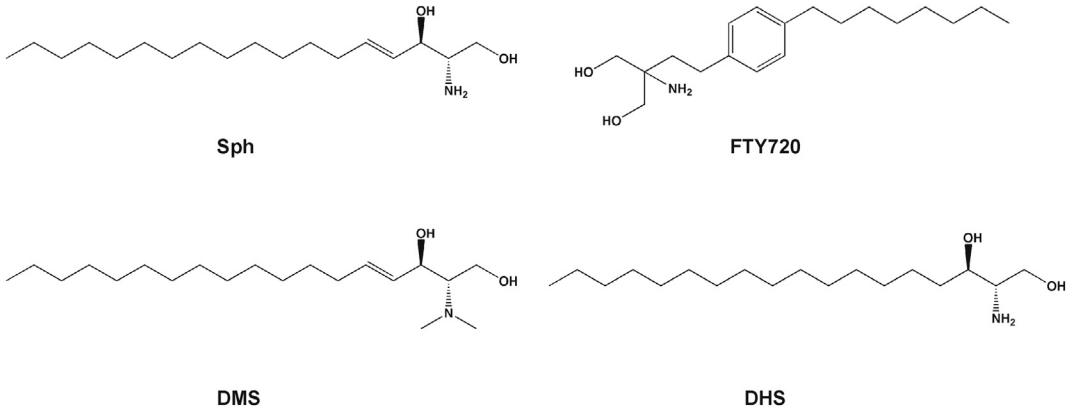
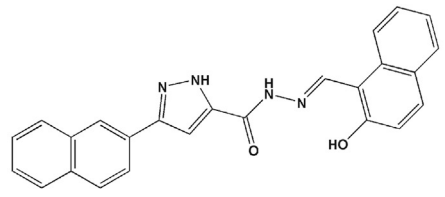
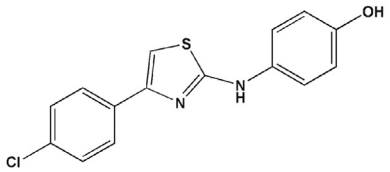


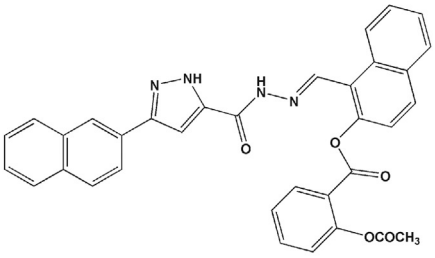
Fig. 6. Structure of sphingosine, FTY720, dimethylsphingosine and dihydroxysphingosine. The SPHK1 inhibitors designed initially were mostly analogs of sphingosine (Sph) with characteristically low effectiveness. Dimethylsphingosine (DMS) and dihydroxysphingosine (DHS) are simple Sph derivatives with two more methyl groups and one less unsaturated bond, respectively, and they are non-selective inhibitors of SPHKs. FTY720 is an S1PR1 agonist used to treat MS.



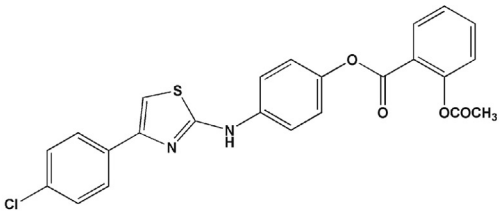
SKI-I



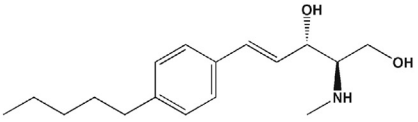
SKI-II



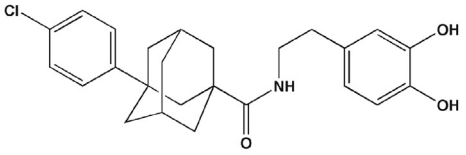
SKI-I-Asp



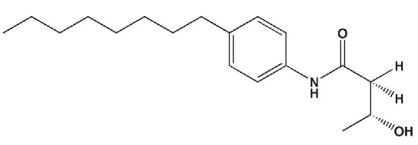
SKI-II-Asp



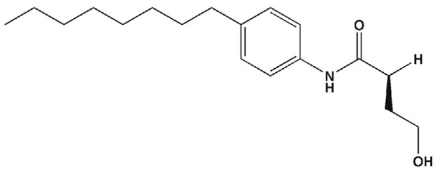
SK1-I



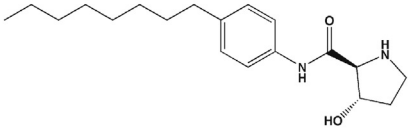
ABC294735



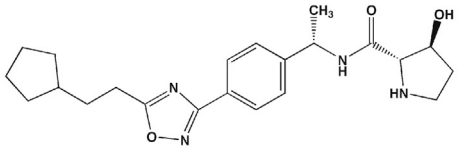
6ag



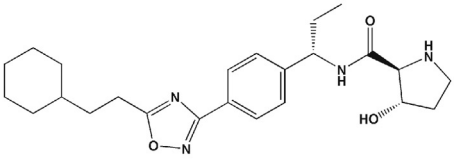
9ab



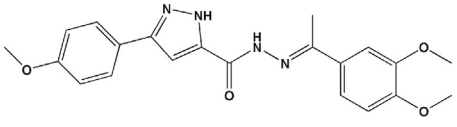
12aa



Genzyme51



Genzyme54



SKI-178

Fig. 7. Structure of SKI-I, SKI-II, SKI-I-Asp, SKI-II-Asp, SK1-I, ABC294735, Genzyme(6ag), Genzyme(9ab), Genzyme(12aa), Genzyme51, Genzyme54 and SKI-178. SKI-I and SKI-II are potent SPHK1 inhibitors while SKI-I-Asp and SKI-II-Asp have higher solubility and bioavailability by adding an aspirinyl group to them. SK1-I is a simple water soluble Sph derivative with low bioavailability. Genzyme(6ag, 9ab, 12aa) are Sph derivatives with high inhibition activity but low bioavailability. Genzyme51 and 54 improve the poor aqueous solubility by changing the hydrophobic part of 12aa. SKI-178 is an analog of SKI-I with a small improvement in its efficiency.

ERK2(K. French et al., 2003; A. Sharma et al., 2010). Poor solubility and minimal bioavailability make these two compounds difficult to use as drugs. To solve this problem, aspirinyl derivatives of SKI-I and SKI-II were designed as pro-drug conjugates. The IC_{50} values of SKI-I-Asp in cell lines from different tissues were similar to or lower than those of SKI-I, and SKI-I-Asp had a lower IC_{50} than SKI-II-Asp (Fig. 7)(A. Sharma et al., 2010).

6.2.2. SK1-I

SK1-I is a water soluble sphingolipid analog (Fig. 7), which can selectively inhibit SPHK1 with a Ki ~10 μmol/L, but not SPHK2 or other protein kinases(Paugh et al., 2008). By reducing the S1P level and increasing the ceramide level *in vitro*, SK1-I exhibits cytotoxic effects on leukemia and malignant glioma cells(Kevin J. French et al., 2006; Paugh et al., 2008). SK1-I can significantly reduce SPHK1 activity in a dose-dependent manner and enhance the cytotoxic effects of doxorubicin in insensitive SCC25 cells(Hazar-Rethinam et al., 2015). However, SK1-I has low bioavailability and does not meet Lipinski's five rules, which means it is a specific inhibitor of SPHK1 but has low efficiency (Plano, Amin, & Sharma, 2014).

6.2.3. ABC294735

ABC294735 is a potent dual inhibitor of SPHKs with IC_{50}~10 μM (Fig. 7)(P. Gao, Peterson, Smith, & Smith, 2012). The combination of ABC294735 with sorafenib has the effect of synergistic cytotoxicity and can cause a strong decrease in ERK phosphorylation in Bxpc-3 and A498 cells. Oral administration of ABC294735 delayed tumor growth in xenograft models, and the effect can be enhanced by adding sorafenib(Beljanski, Knaak, Zhuang, & Smith, 2011).

6.2.4. Genzyme 51 and 54

By modifications to Sph, Genzyme developed three potent compounds N1- (4-octylphenyl)-L-threoninamide (6ag) with IC_{50} = 0.65 μM, N1-(4-octylphenyl)-L- homoserinamide (9ab) with IC_{50} = 50 nM and 3(*S*)-hydroxy-N-(4-octylphenyl)-L- prolinamide (12aa) with IC_{50} = 62 nM in inhibition of SPHK1(Fig. 7)(Xiang et al., 2009). However, the most potent compound with IC_{50} = 50 nM suffered from poor aqueous solubility. To solve this problem, Genzyme optimized a class of 3-hydroxyproline derivatives and designed a series of SPHK1 inhibitors based on an N-(5-alkyloxadiazol-3-yl)benzyl)-3-hydroxypyrrolidine-2-carboxamide scaffold in which compounds 51 and 54 demonstrated modest improvements in oral bioavailability with acceptable half-lives in blood circulation with IC_{50} = 58 nM and 10 nM, respectively (Fig. 7) (Xiang et al., 2010). These compounds are novel and potent SPHK1 inhibitors and further studies are needed to explain the biological process *in vivo*.

6.2.5. SKI-178

After the discovery of SKI-I to IV, SKI-178 with enhanced pharmacological properties was found (Fig. 7). SKI-178 is a SPHK1-specific analog of SKI-I with IC_{50} = 0.1–1.8 μM in different type of cancer cell lines (Hengst et al., 2010). SKI-178 induced cytotoxicity through mitochondria-dependent apoptosis and JNK-mediated, ceramide-induced apoptotic cell death in three acute myeloid leukemia(AML) cell lines(T. Dick et al., 2015). Though the improvement in efficacy is modest, it is specific for SPHK1 and provides a new SPHK1-specific leading compound platform for further drug development.

6.3. Inhibitors found from 2010 to now

6.3.1. (S)FTY720-vinylphosphonate

By optimizing the structure of FTY720, many derivatives were developed that also inhibit SPHKs. One such analog of FTY720, (S)FTY720-vinylphosphonate, is an SPHK1 inhibitor with a K_{iu} = 14.5 ± 4.4 μM (Fig. 8). (S)FTY720- vinylphosphonate can induce proteasomal degradation of SPHK1 by binding to the allosteric site and stabilizing it, which enhances the inhibitory effect on SPHK1 activity(Lim et al., 2011).

6.3.2. PF543

PF543 is an excellent SPHK1 inhibitor designed by Pfizer with IC_{50} = 3.6 nM (Fig. 8)(M. Schnute et al., 2012). PF543 is a competitive inhibitor of Sph, whose inhibitory effect on SPHK1 is 100-fold higher than SPHK2 (Lynch, 2012). Tong Fa Ju et al. found that PF543 significantly inhibited proliferation and was cytotoxic towards a panel of cell lines (HCT-116, HT-29 and DLD-1) and primary human colorectal cancer (CRC) cells both *in vivo* and *in vitro*(Ju, Gao, & Fang, 2016).

6.3.3. Amgen 82

Amgen designed a series of compounds using a structure-based method, in which they merged the structures of Sph and SKI-II to yield a feature structure, which they then modified to develop a further class of compounds, (2*R*,4*S*)-2- (hydroxymethyl)piperidin-4-ol moiety, which is a significant inhibitor of SPHKs(Gustin et al., 2013). Among 91 compounds designed, compound 82 was an inhibitor of both SPHK1 and SPHK2. Amgen82 has a higher selectivity for SPHK1 (IC_{50} = 70 nM) than for SPHK2 (IC_{50}>10 μM) and has good pharmacokinetic properties (Fig. 8)(Gustin et al., 2013). Amgen 82 shows good anti-tumor potency by inhibiting the activity of SPHK and reducing the generation of S1P *in vitro* and *in vivo*.

6.3.4. RB-005 and RB-019

Based on the scaffold of FTY720, a series of SPHKs inhibitors was designed and synthesized. RB-005 and RB-019 showed the highest selectivity for SPHK1 over SPHK2 at 15.0-fold and 6.1-fold, respectively (Fig. 8). RB-005 was the most potent inhibitor with IC_{50} = 3.6uM and induced the proteasomal degradation of SPHK1(Baek, MacRitchie, Pyne, Pyne, & Bittman, 2013). The difference between RB-005 and RB-019 is the location of 4-hydroxy or 3-hydroxy on piperidine which results in less potency of RB-019 than RB-005(Baek, MacRitchie, et al., 2013b).

6.3.5. MP-A08

By virtual screening, the compound MP-A08 was found to bind to the SPHK1 ATP binding site. MP-A08 can inhibit both SPHK1 and SPHK2 (Fig. 8). *In vitro,* MP-A08 can inhibit proliferation and colony formation in many cancer cell lines by inducing mitochondrial-mediated apoptosis. MP-A08 also attenuated growth of the human lung adenocarcinoma cell line, A549, in xenografted mice(M. Pitman et al., 2015).

6.4. Other sources

Natural products are important sources of new drugs. Many scientists look for new anti-tumor drugs by isolating natural products and modifying their structures. It was reported that epigallocatechin gallate (EGCG), resveratrol, green tea and vine polyphenols can inhibit the growth of prostate cancer cells by inhibiting the SPHK1/S1P pathway *in vivo* and *in vitro*(Brizuela et al., 2010). Icaritin, a flavonoid, was found to exert cytotoxic and pro-apoptotic activity in hepatocellular carcinoma cell lines (HepG2, KYN-2 and Huh-7) and to inhibit tumor growth both *in vivo* and *in vitro* by inhibiting SPHK1(P.-H. Lu et al., 2017). Now it is in the phase III clinical trial (NCT03236636).

Another flavonoid, hispidulin, also effectively inhibited proliferation, migration, and invasion and induced apoptosis of renal cell carcinoma, Caki-2 and A498 cell lines by inhibiting SPHK1(M. Gao et al., 2017). Peretinoin is a derivative of vitamin A. It can inhibit expression and activity of SPHK1 in a human hepatoma cell line Huh-7 by reducing SPHK1 promoter activity which can be reversed by over-expression of transcription factor, Sp1(Funaki et al., 2017).

The characteristics of SPHK1 inhibitors are shown in Table 1. Although there still is no effective and selective natural inhibitor of SPHK1 available for use in the clinic, many natural products have been

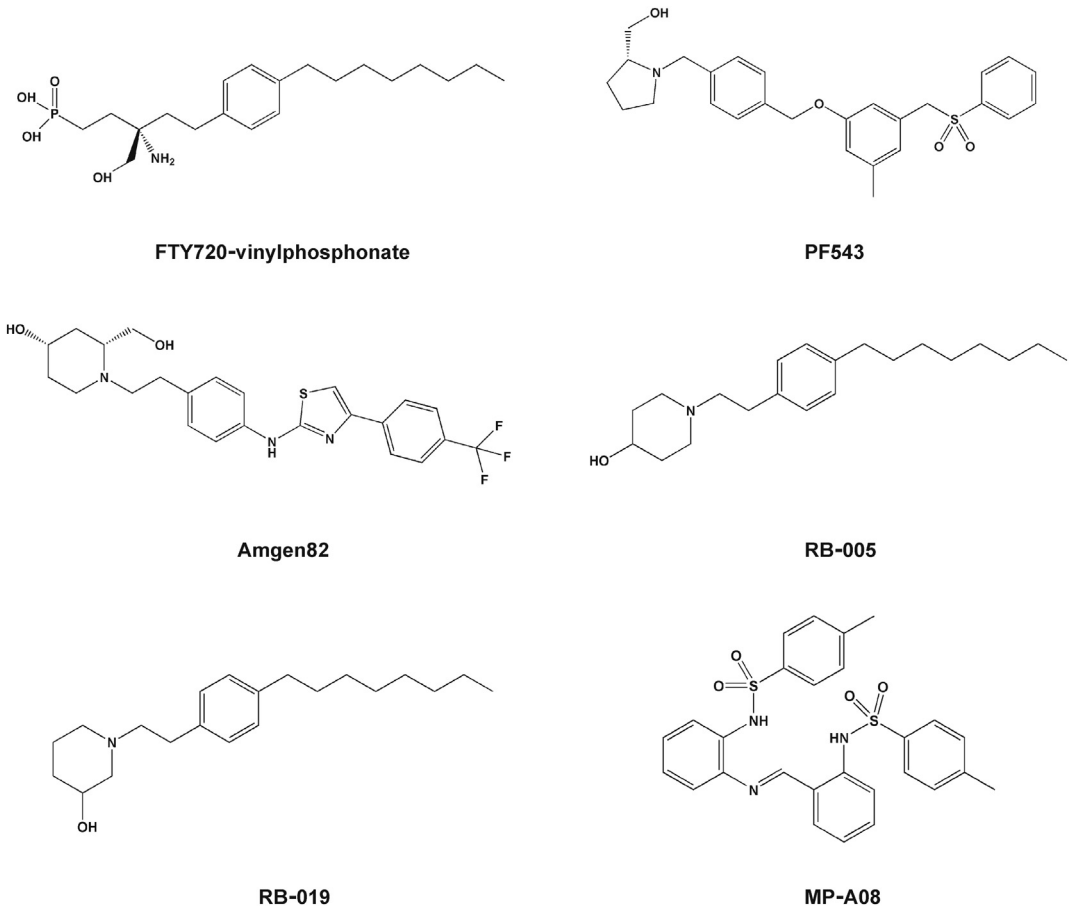


Fig. 8. Structure of (S)FTY720-vinylphosphonate, PF543, Amgen 82, RB-005, RB-019 and MP-A08. (S)FTY720-vinylphosphonate is an analog of FTY720. Amgen 82 is based on the combination of Sph and SKI-II and is a selective inhibitor of SPHK1. RB-005 and RB-019 are based on FTY720 but with higher selectivity for SPHK1.

shown to have potential anti-tumor activity and synergistic effects with various chemotherapeutic agents. In addition, compounds derived from marine organisms, such as pachastrissamine and its derivatives, also exhibit good inhibitory activity against SPHKs (I. Kuroda et al., 2002). Our review may provide the information needed to develop new inhibitors of SPHK1.

7. SPHK1 and drug resistance in cancers

Drug resistance is common in cancer therapy and studies showed that SPHK1 was associated with drug resistance. Over-expression of SPHK1 contributed to cetuximab resistance while inhibition of SPHK1 restored sensitivity to cetuximab in human colorectal cancer (Rosa et al., 2013). The human colon cancer cell line, RKO, with high SPHK1 expression was resistant to treatment with oxaliplatin whereas the human colon cancer cell line, HCT116, with low SPHK1 expression was sensitive to oxaliplatin(Nemoto et al., 2009). SPHK1 expression was correlated with sensitivity to oxaliplatin in human colon cancer cell lines. Up-regulation of SPHK1 significantly inhibited doxorubicin or docetaxel-induced apoptosis by inducing expression of Bcl-xl, c-IAP1, c-IAP2, and TRAF1 in non-small cell lung cancer (NSCLC) cells (Song et al., 2011). Silencing SPHK1 expression or inhibiting SPHK1 activity with the specific inhibitor, SK1-I, significantly enhanced the PI3K/Akt/NF-kB-mediated apoptosis in NSCLC cells induced by chemotherapeutics both *in vitro* and *in vivo* (Song et al., 2011). It was also shown that inhibiting SPHK1 activity sensitized pancreatic cancer cells to gemcitabine whereas up-regulating SPHK1 activity, promoted gemcitabine resistance in these cells(Guillermet-Guibert et al., 2009).

Over-expression of human SPHK1 in MCF-7 human breast cancer cells promoted cell proliferation and contributed to resistance to tamoxifen. SPHK1 expression was found to be higher in tamoxifen-resistant (TamR) MCF-7 cells selected by prolonged exposure to 4-hydroxytamoxifen than in control cells. SPHK1 activity was also associated with endocrine resistance in MCF-7 cells(Sukocheva, Wang, Verrier, Vadas, & Xia, 2009).The SPHK1/S1P pathway played a crucial role in the resistance of prostate cancer cells to chemotherapy. Inhibiting SPHK1 with the compound, B-5354c, sensitized LNCaP and PC-3 cells to docetaxel and camptothecin, respectively. Camptothecin synergized with B-5354c to reduce tumor size in PC-3 cells, which provided a new strategy for treatment of cancers by targeting the SPHK1/S1P pathway(Pchejetski et al., 2008).

All in all, the SPHK1-S1P axis is a key player in the development of drug resistance in cancers of the colon, lung (NSCLC), breast, prostate and pancreas, which provides new potential for more effective combined treatment of these cancers.

8. Conclusion: summary and perspectives

In conclusion, SPHK1 may be a good potential drug target to treat cancers and reverse drug resistance. Sphingolipids and SPHK1 play an important role in tumorigenesis and progression of cancers, and therefore it is reasonable to target SPHK1, which maintains the balance of sphingolipids, as part of a therapeutic regimen. However, there is some controversy about SPHK1's role in cancer. A study from Yugesh Kharel et al. showed that SPHK1 inhibitors effectively lowered S1P levels in cells, but that SPHK1 inhibition wasn't correlated with changes in cell survival(Kharel et al., 2011). Specific inhibition of SPHK1 by PF-543 had no effect on the proliferation and survival of 1483 head and neck carcinoma cell(M. Schnute et al., 2012). Research from Karen Rex et al. also showed that both specific SPHK1/2 inhibitors and knockdown

Table 1
Classification of SPHK Inhibitors.

NO.	Code name	Generic name	Selective	IC_{50}	Cancer type	Highest phase	Indications	Ref
1	DMS	N,N-Dimethylsphingosine	NO	< 1.00 μM	Histiocytic lymphoma	Preclinical	Oncolytic Drugs	(Bandgar et al., 2010; Wong, Tan, Lam, & Melendez, 2009)
2	SPC-100270 (DHS)	Safingol	NO	0.73–9.5 μM	Breast, ovarian, colon	Phase II	Atopic Dermatitis; Agents for Cancer Multidrug Resistance Modulators; Antipsoriatics	(Leong Uung Ling & Chiu, 2007; L. U. Ling, Lin, Tan, & Chiu, 2009)
3	FTY720	Fingolimod	NO	5.0–12.5 μM	Adrenocortical carcinoma, hepatocellular carcinoma, ovarian cancer	Launched-2010	Multiple Sclerosis	(Beider et al., 2017; Hung et al., 2008; Y. Xu et al., 2016; Zhang et al., 2010)
4	(S)FTY720-vinylphosphonate		YES	14.5 ± 4.4 μM	Breast cancer			(Lim et al., 2011)
5	SK1-I	N	YES	10 μM	Leukemia	Preclinical	Antiallergy/Antiasthmatic Drugs Oncolytic Drugs	(Paugh et al., 2008; Price et al., 2013)
6	SKI-I	N	NO	1-5 μM	Brain, ovarian, breast, pancreas, lung cancer	Preclinical	Oncolytic Drugs	(K. J. French et al., 2003; K. J. French et al., 2006; A. Sharma et al., 2010)
7	SKI-II	N	NO	7.7–10.7 μM	Brain, ovarian, breast, pancreas, lung cancer, acute myelogenous leukemia	Preclinical	Oncolytic Drugs	(A. Sharma et al., 2010; A. K. Sharma et al., 2010; Yang et al., 2015)
8	SKI-I-ASP	N	YES	1–5.5 μM	Brain, ovarian, breast, pancreas, lung cancer	Preclinical	Oncolytic Drugs	(A. Sharma et al., 2010)
9	SKI-II-ASP	N	YES	5.7–11.3 μM	Brain, ovarian, lung, breast, pancreatic cancer, lung cancer	Biological Testing		(A. Sharma et al., 2010)
10	N (Genzyme, 6ag)	N	YES	0.65 μM		Biological Testing	Oncolytic Drugs	(Xiang et al., 2009)
11	N (Genzyme, 9ab)	N	YES	0.05 μM		Biological Testing	Oncolytic Drugs	(Xiang et al., 2009)
12	N (Genzyme, 12aa)	N	YES	62 nM		Biological Testing	Oncolytic Drugs	(Xiang et al., 2009)
13	N (Genzyme, 51)	N	YES	58 nM		Preclinical	Oncolytic Drugs	(Xiang et al., 2010)
14	N (Genzyme, 54)	N	YES	10 nM		Preclinical	Oncolytic Drugs	(Xiang et al., 2010)
15	Amgen82	N	NO	70 nM		Preclinical	Oncolytic Drugs	(Gustin et al., 2013)
16	PF543	N	YES	3.6 nM	Colorectal cancer, breast cancer	Preclinical	Oncolytic Drugs; Treatment of Inflammation,	(Jones, Kaiser, & Avery, 2015; Ju et al., 2016; M. E. Schnute et al., 2017; S. Wang, Liang, Chang, Hu, & Zhang, 2018)
17	SKI-178	N	YES	0.1–1.8 μM	Acute myeloid leukemia	Preclinical	Oncolytic Drugs	(T. E. Dick et al., 2015)
18	RB-019	N	YES					(Baek, MacRitchie, et al., 2013b)
19	RB-005	N	YES	3.6 ± 0.36 μM		Biological Testing	Oncolytic Drugs; Pulmonary Hypertension	(Baek, MacRitchie, et al., 2013b)
20	SNG-162	Icaritin	YES	2.7-μM	Prostate cancer, melanoma, breast cancer, liver cancer	Phase 3	Oncolytic Drugs; Breast Cancer Therapy; Liver Cancer Therapy	(Hong et al., 2013; Sun et al., 2015; C. Wang, Wu, Shi, Jiang, & Wei, 2015; Wu et al., 2016)
21	N	Hispidulin	NO	8-20 μM	Pancreatic cancer, renal cell carcinoma,	Preclinical	Hepatoprotectants; Oncolytic Drugs; Bronchodilators	(H. Gao, Jiang, Han, Peng, & Wang, 2015; Han et al., 2018; He et al., 2011; M. Kuroda et al., 2007)
22	ABC-294735	N	NO	14-40μM	Kidney adenocarcinoma, pancreatic adenocarcinoma	Preclinical	Oncolytic Drugs	(Beljanski et al., 2011; P. Gao et al., 2012; Kadam & Chuan, 2016; Vogt et al., 2014)
23	MP-A08	N	NO	8.0 ± 1.0 μM		Preclinical	Myeloid Leukemia Therapy	(M. R. Pitman et al., 2015)
24	N	Pachastrissamine	NO	4.6 μM	Melanoma	Biological Testing	Oncolytic Drugs	(I. Kuroda et al., 2002; Yoo, Lee, Lee, Kim, & Kim, 2012)
25	EGCG	Epigallocatechin gallate	NO	75 μM	Prostate cancer	Phase II/III	Breast neoplasms et al.	(Brizuela et al., 2010)

of SPHK1 and SPHK2 by siRNA didn't reduce growth of tumor cells *in vitro* or *in vivo*(Rex et al., 2013). It is well known that cancers are caused by many factors and mechanisms. Survival of some cancer cells may be sustained by a network of signaling pathways. Although the SPHK-S1P pathway may be blocked, the cells still survive because of other signal pathways. More in-depth experiments on SPHK1 in various cancers need to be done in future.

Up to now, there have been no reports about SPHK1 inhibitors used for treatment of tumors in clinic. More and more investigations should be focused on looking for new inhibitors of SPHK1 which have strong affinity, high selectivity and low toxicity. The SPHK1 structure has been resolved, which will accelerate the development of SPHK1 inhibitors. In addition, the role of the SPHK1-SIP axis in inflammation and other diseases should be studied in the future. All in all, there are important, significant and broad potential applications waiting to be discovered for inhibitors of SPHK1 and researchers need to work strenuously to elucidate the functions and mechanisms of SPHK1 action in cancers and other diseases.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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