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Role of Sphingosine Kinase 1 and Sphingosine-1-Phosphate Axis in Hepatocellular Carcinoma

Michael Maceyka

Timothy Rohrbach
Richard Bland College of the College of William and Mary, trohrbach@rbc.edu

Sheldon Milstein

Sarah Spiegel

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Michael Maceyka, **Timothy Rohrbach**, **Sheldon Milstien**, **Sarah Spiegel**

Department of Biochemistry and Molecular Biology, Massey Cancer Center, Virginia Commonwealth University School of Medicine, Richmond, VA, USA

Abstract

Hepatocellular carcinoma (HCC) is primarily diagnosed in the latter stages of disease progression and is the third leading cause of cancer deaths worldwide. Thus, there is a need to find biomarkers of early HCC as well as the development of more effective treatments for the disease. Sphingosine-1-phosphate (S1P) is a pleiotropic lipid signaling molecule produced by two isoforms of sphingosine kinase (SphK1 and SphK2) that is involved in regulation of many aspects of mammalian physiology and pathophysiology, including inflammation, epithelial and endothelial barrier function, cancer, and metastasis, among many others. Abundant evidence indicates that SphK1 and S1P promote cancer progression and metastasis in multiple types of cancers. However, the role of SphK/S1P in HCC is less well studied. Here, we review the current state of knowledge of SphKs and S1P in HCC, including evidence for the correlation of SphK1 expression and S1P levels with progression of HCC and negative outcomes, and discuss how this information could lead to the design of more effective diagnostic and treatment modalities for HCC.

Keywords

Hepatocellular carcinoma; S1P receptors; Sphingosine kinase; Sphingosine-1-phosphate (S1P)

1. Introduction

Hepatocellular carcinoma (HCC) is cancer of the liver arising from malignant hepatocytes and accounts for nearly 90% of all primary liver cancers. HCC has a high level of morbidity, being the third leading cause of cancer deaths (El-Serag 2011). Due in part to both delayed diagnosis of HCC as well as its general aggressiveness, the median 5-year survival rate is less than 7%. Major risk factors for HCC include hepatitis virus B and C infections, overconsumption of alcohol, and nonalcoholic fatty liver disease (NAFLD), particularly when it progresses to nonalcoholic steatohepatitis (NASH). NAFLD/NASH are often associated with obesity and type 2 diabetes, and the rapid increase in the occurrence of these two disorders may contribute to the rise in non-viral associated HCC observed in the industrialized countries (Satapathy and Sanyal 2015). The hallmarks of NAFLD progression

sarah.spiegel@vcuhealth.org.

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including insulin resistance, oxidative stress, and inflammation are all factors that also promote cancer initiation and progression (Cohen et al. 2011).

Sphingolipids, including sphingomyelin and glycosphingolipids, are essential lipid components of mammalian membranes. Sphingolipids consist of various head groups attached to ceramide, which is structurally analogous to the glycerolipid backbone diacylglycerol and, like diacylglycerol, is a second messenger involved in signaling pathways, typically promoting apoptosis and suppressing cell growth (Newton et al. 2015; Coant et al. 2017; Ogretmen 2018). Deacylation of ceramide yields sphingosine that has also been implicated in cell signaling. Sphingosine can be reacylated back to ceramide by a salvage pathway or phosphorylated by one of two sphingosine kinases (SphK1 and SphK2) forming sphingosine-1-phosphate (S1P). There are two fates for S1P: irreversible degradation by S1P lyase or dephosphorylation back to sphingosine. The metabolism of S1P is critical because S1P is a potent pleiotropic signaling molecule that regulates many physiological and pathological processes that are important for cancer including cell growth, proliferation, and cell motility, immune cell recruitment, epithelial and endothelial barrier function, and angiogenesis and lymphangiogenesis, among many others (Ogretmen 2018; Pyne and Pyne 2010; Kunkel et al. 2013; Maceyka and Spiegel 2014). Multiple stimuli, including growth factors, cytokines, and hormones, stimulate phosphorylation and activation of cytosolic SphK1 leading to its translocation to the plasma membrane where its substrate sphingosine resides and/or is generated (Maceyka and Spiegel 2014). S1P can be transported out of the cell, either by a specific S1P transporter called spinster 2 (Spns2), a member of the major facilitator superfamily of transporters, or via a subset of ATP-binding cassette (ABC) transporters, including ABCC1 and ABCG2 (Takabe and Spiegel 2014). This S1P can activate a family of five, S1P-specific G protein-coupled receptors (S1PR1–5) that mediate many of its known actions in an autocrine or paracrine manner, termed "inside-out signaling by S1P" (Takabe et al. 2008). These receptors are differentially expressed and couple to a wide array of heterotrimeric G proteins, leading to a diverse, and at times opposing, range of cellular and physiological responses (Pyne and Pyne 2010; Maceyka and Spiegel 2014). S1P produced inside cells also has intracellular actions; however, only a handful of intracellular targets and pathways have been identified so far. For example, S1P produced by activation of SphK1 binds to and activates proteins such as TNF receptor-associated factor 2 (TRAF2) (Alvarez et al. 2010; Park et al. 2015, 2016; Liu et al. 2017), a key adaptor molecule in TNFR signaling complexes that promotes downstream signaling cascades leading to activation of the master transcription factor NF-κB (Alvarez et al. 2010; Park et al. 2015). S1P can also activate NF-κB through formation of a signaling complex, consisting of S1P, TRAF2, and RIP1 that further associates with heat shock proteins GRP94 and HSP90α and IRE1α (Park et al. 2016). S1P produced in the nucleus by SphK2 is an endogenous histone deacetylase inhibitor (Hait et al. 2009, 2014; Nguyen-Tran et al. 2014; Nagahashi et al. 2015; Gardner et al. 2016) and also binds to hTERT and increases telomerase activity and enhances cancer cell growth (Panneer Selvam et al. 2015).

S1P is present at high levels in the blood and lymph, and the functions of S1P and its receptors as well as Spns2 in the regulation of immune cell trafficking have recently been implicated in tumor immunology and metastasis (Fang et al. 2017; van der Weyden et al. 2017). Moreover, numerous studies have also shown that expression of SphK1 and S1P

promotes progression and metastasis of many types of cancers and correlates with poor prognosis (Ogretmen 2018; Pyne and Pyne 2010; Maceyka and Spiegel 2014). However, while the SphK1/S1P/S1PR signaling axis has been associated with initiation, progression, and chemoresistance of a variety of cancers, much less is known about its role in HCC. SphK1 and S1P expression have been correlated with initiation and progression of NAFLD, which is a risk factor for HCC, and thus, it is not surprising that they would also play a role in HCC. Here we discuss current evidence indicating that SphK1 and S1P are upregulated in human HCC patients and that they may serve as valuable biomarkers for early stage disease detection. We also examine the molecular mechanisms in HCC leading to SphK1/S1P upregulation, evidence that their upregulation promotes epithelial-mesenchymal transition and HCC progression, and the participation of downstream effectors of SphK1/S1P in HCC progression.

2. SphK1 and S1P Are Upregulated in HCC

Several studies have suggested that the SphK1/S1P axis could play a role in the promotion of HCC based on the observations that SphK1 is overexpressed in HCC cell lines and in patient samples and that S1P is increased in both patient serum and HCC tumors (Shi et al. 2015; Cai et al. 2017). For example, using immunohistochemistry techniques, both SphK1 and S1P levels were observed to be upregulated in HCC (Reynolds et al. 2017). Moreover, expression of SphK1 in HCC tumors was higher than in normal adjacent tissues, and SphK1 expression positively correlated with tumor size, tumor stage, and histological differentiation and negatively correlated with overall survival (Cai et al. 2017). Similarly, it was shown that SphK1 levels correlated with increased recurrence and decreased survival of patients with portal vein tumor thrombosis after primary tumor resection (Shi et al. 2015). However, in one study, though HCC tissues had elevated levels of SphKs, they had reduced levels of S1P compared to normal adjacent tissues (Uranbileg et al. 2016), findings that were linked to the elevated expression of the S1P-degrading enzyme, S1P lyase. These results highlight the importance of localized coupling of S1P synthesis, release, and S1PR activation that may not correlate with bulk S1P levels but are still sufficient to promote S1P signaling and HCC progression. Importantly, however, several studies have shown by mass spectrometry that serum levels of S1P are elevated in HCC compared to patients with cirrhosis (Ressom et al. 2012) and significantly elevated compared to normal controls (Grammatikos et al. 2016; Zeng et al. 2016). These results suggest that serum S1P may be a useful diagnostic marker for patients with liver disease that has progressed to HCC, particularly for patients at risk for liver cancer given its typically late stage of diagnosis.

3. Inflammation and Fibrosis

Many studies have linked SphK1 and S1P to the promotion of inflammatory responses, and it is well known that inflammation can promote the initiation and progression of cancer (Kunkel et al. 2013; Pyne et al. 2016). Indeed, several studies have also linked SphK1/S1P signaling to liver inflammation, fibrosis, and NAFLD/NASH (Rohrbach et al. 2017). For example, SphK1 is elevated in NAFLD patients and in obese mice that have many of the hallmarks of the disease, including NF-κB activation, elevated cytokine production, and immune cell infiltration (Geng et al. 2015) (Fig. 1). SphK1 null mice are resistant to these

obesity-driven effects, and inflammatory mediator production in hepatocytes is reduced when S1PR1 is knocked down. Similarly, liver fibrosis in humans and in CCl4-induced fibrosis in mice have been correlated with increased levels of S1P, which stimulate fibrotic angiogenesis through activation of S1PR1 and S1PR3 (Yang et al. 2013).

Myeloid cells, particularly tumor-associated macrophages (TAMS), are frequently increased in the HCC microenvironment and are associated with worse prognosis (Wan et al. 2015). Although the role of the SphK/S1P axis in recruitment and function of TAMS has not been investigated in HCC, in many other cancers, S1P secreted by the tumor due to elevation of SphK1 plays a key role in the recruitment and phenotypic shift of the tumor macrophages from pro-inflammatory (M1) to anti-inflammatory (M2) that promotes tumorigenesis and angiogenesis (Rodriguez et al. 2016; Mrad et al. 2016) (Fig. 1).

4. Regulation of the S1P Signaling Axis in HCC

The observations that SphK1 and S1P are elevated in HCC tumor samples suggest that the tumors have mechanisms for increasing S1P signaling. One group has shown that the HCC cell line HepG2 secretes S1P and that inhibition of this secretion blocks cell proliferation that can be rescued by exogenous S1P, indicative of an autocrine S1P signaling loop (Jin et al. 2016). They linked the secretion of S1P to AKR1B10, a protein involved in lipid metabolism that is elevated in HCC, as siRNA against AKR1B10 reduced S1P levels and cell growth. They further showed that high expression of AKR1B10 correlated with both more negative outcomes and higher levels of S1P in tumor but not peripheral liver tissue. This study utilized an ELISA method to measure S1P that is not as sensitive as mass spectrometry methods and did not detect any changes in S1P synthesis or degradation that could affect S1P levels, although other studies have implicated pathways that control SphK1 expression in HCC. For example, similar to many other growth factors, hepatocyte growth factor (HGF) stimulates SphK activity, S1P production, and motility in HCC cells that can be blocked by downregulating SphK1. HGF induced translocation of SphK1, the S1P transporter Spns2, and the S1P receptor S1PR1 to the lamellipodia at the leading edge of moving lung vascular cells (Fu et al. 2016). This not only reaffirms the inside-out signaling hypothesis, but it also suggests that the pro-motility signaling occurs at distinct regions of the plasma membrane to promote directed motility up a chemoattractant gradient. Similarly, motility and invasion of several HCC cell lines were dependent on overexpression of SphK1 and signaling via S1PR1 (Bao et al. 2012), supporting a role for the SphK1/S1P/S1PR1 axis in liver metastasis and suggesting that this axis might be an attractive therapeutic target for the development of new anti-HCC drugs.

Expression of highly upregulated in liver cancer (HULC), which was originally identified as a very highly overexpressed long noncoding RNA in HCC, was suggested to correlate with both SphK1 mRNA and ELISA-measured S1P levels in patient samples (Lu et al. 2016). Functionally, HULC-induced angiogenesis in situ and in HCC xenografts depended on SphK1 expression. Mechanistically, HULC sequestered miR-107, increasing the transcription of E2F1, which activated the transcription of SphK1. Together, these results suggest that HULC promotes tumor angiogenesis in liver cancer in part by upregulation of SphK1 (Lu et al. 2016). Another miR, miR-506, was also claimed to downregulate SphK1

mRNA and protein, to reduce secreted S1P in vitro, and to negatively correlate with mRNA levels of SphK1 in clinical HCC samples (Lu et al. 2015). Thus, it was proposed that miR-506 depresses angiogenesis of liver cancer by targeting the 3′UTR of SphK1 mRNA. Interestingly, a second group has found a similar downregulation of miR-506 in pancreatic cancer that correlated with the upregulation of SphK1/AKT/NF-κB signaling (Li et al. 2016). Further studies are needed to support the significance of these miRs in HCC and conclusively demonstrate their specific targets.

5.   Downstream Effectors/Pathways of SphK1/S1P that Promote HCC

5.1.   S1PRs

The most obvious and druggable proximal targets of the S1P signaling axis are the cell surface S1P receptors, which have been linked to progression of many cancers (Ogretmen 2018; Pyne and Pyne 2010; Maceyka and Spiegel 2014; Patmanathan et al. 2017). Indeed, several studies have correlated expression of S1PR1 to HCC progression and poor prognoses. Consistent with previous studies suggesting that SphK1 produced S1P promotes tumor cell migration and invasion by activating S1PR1 (Bao et al. 2012), in diethylnitrosamine-induced HCC in mice, SphK1, SphK2, S1PR1, and S1PR3 were upregulated, while the S1P-degrading enzyme S1P lyase was downregulated (Sanchez et al. 2018). Other studies also suggested that S1P-S1PR1 signaling is involved in the progression of HCC in patients. miR-148a dysregulation discriminated overall survival and recurrence-free survival rates of HCC. In human HCC samples, both ubiquitin-specific protease 4 (USP4) and S1PR1 were identified as targets of miR-148a. USP4 and S1PR1 were upregulated in mesenchymal-type liver tumor cells with miR-148a dysregulation, facilitating migration and proliferation of tumor cells, though a significant negative correlation was not found between miR148a and S1PR1 in three heterotopic patient-derived HCC xenografts (Heo et al. 2014). Another putative tumor-suppressing microRNA, miR-363, decreased S1PR1 expression by binding its 3′UTR and also inhibited the proliferation of HCC cells (Zhou et al. 2014).

5.2.   The S1P Signaling Axis Promotes EMT in HCC

The epithelial-mesenchymal transition (EMT), a process by which epithelial cells dedifferentiate to multipotent mesenchymal stem cells, is a hallmark of cancer initiation (Kalluri and Weinberg 2009). Cells that undergo EMT lose the expression of epithelial markers, polarity, and become more motile. Several studies suggest that the S1P signaling axis promotes EMT in HCC. For example, inhibition of SphK activity with N,N-dimethylsphingosine in HCC cells was originally shown to upregulate liver-specific markers of differentiation, including albumin and alpha-fetoprotein (AFP) (Osawa et al. 2001). Intriguingly, in a set of 77 HCC patient samples, not only did expression of SphK1 and SphK2 correlate with decreased differentiation so did expression of S1P lyase. However, in this sample set, there was no correlation between SphK1/2 expression and S1P levels in the tumor (Uranbileg et al. 2016). Once again, these results suggest that localized S1P production rather than absolute levels may be critical to its function in promoting HCC.

It was recently shown that SphK1 induces the EMT in HepG2 cells by accelerating lysosomal degradation of the cell-cell adhesion molecule E-cadherin (CDH1), thus facilitating their invasion and metastasis (Liu et al. 2017). Of note, in HepG2 cells, SphK1-produced S1P bound to TRAF2 and stimulated lysine 63-linked ubiquitination and activation of beclin1 that in turn stimulated autophagic degradation of E-cadherin (Liu et al. 2017). These intriguing findings define a novel mechanism for the regulation of the EMT via SphK1-TRAF2-beclin1-E-cadherin signaling cascade in HCC cells. Moreover, this study suggests that inhibition of SphK1 to attenuate autophagy may be a promising strategy for treatment of HCC. In agreement, a non-isozyme-specific SphK inhibitor promoted the Wnt5a-dependent degradation of the tumor-promoter β-catenin and inhibited HepG2 cell proliferation (Liu et al. 2016). Another group put forward a different mechanism to explain how S1P induces the EMT in HCC showing that signaling through S1PR1 stimulated the MMP7-dependent shedding of syndecan-1, leading to the activation of the TGF-β signaling pathway and the promotion of the EMT phenotype (Zeng et al. 2016) (Fig. 2). Although there is strong evidence for EMT in HCC patients, so far, data are missing that demonstrate that the process of EMT is reversible after intrahepatic or even distal metastatic colonization. Future studies are needed to clarify whether targeting the SphK/S1P axis and inhibition of EMT could lead to development of effective therapies against HCC.

6. Inhibition of the S1P Signaling Axis as Treatment for HCC

The studies discussed clearly demonstrate that SphK1 and S1P signaling axis are associated with and likely promote the initiation and progression of HCC. Therefore, it has been suggested that inhibiting SphK1 might be a useful approach for treatment of HCC. Preclinical studies have suggested that effects of drugs that reduce HCC tumor cell growth, such as melatonin (Sanchez et al. 2018) or the natural prenylflavonoid icaritin (Lu et al. 2017), might be due to inhibition of SphK1 and decreased activation of S1PRs. Several groups have investigated the efficacy of inhibiting S1P signaling more directly in vitro and for treatment of tumor-bearing mice. It has been shown that the dual SphK1/2 inhibitor SKI-II acts synergistically with 5-fluorouracil (5-FU), a common drug used for the treatment of advanced HCC, to both promote HCC cell death and inhibit migration of HCC cells through downstream inhibition of FAK, ERK, and NF-κB (Grbcic et al. 2017). Similar effects were obtained when SKI-II was used in combination with the MEK1/2 inhibitor U0126 (Zhang et al. 2013) (Table 1). It has also been shown that SKI-II, alone or in combination with the ROS-generating drug selenite, is effective at killing Huh7 HCC cells, but not non-tumorigenic hepatocyte cells (Chatzakos et al. 2012). Interestingly, ABC294640, which was developed as a SphK2 inhibitor and decreases serum S1P levels, was found to be effective in two HCC xenograft models when used in combination with sorafenib, a multikinase inhibitor and emerging drug for treatment HCC (Beljanski et al. 2011). It was further shown that ABC294640 decreased the level of S1P in the xenograft tumors themselves (Table 1). Furthermore, ABC294640 is currently in a phase II clinical trial for the treatment of patients with sorafenib-resistant HCC (https://clinicaltrials.gov/ct2/show/NCT02939807).

Several studies examined the potential of FTY720 as a therapeutic option for HCC. FTY720 is a prodrug that is phosphorylated by SphK2 to the S1P analog FTY720-phosphate, which acts a functional antagonist for S1PR1 (Brinkmann et al. 2010) and is also a class I HDAC

inhibitor (Hait et al. 2015). FTY720 is orally available, generally well tolerated, and already widely used for treatment of multiple sclerosis. As such, it is an attractive drug for cancer chemotherapy, and it has been found to effectively inhibit proliferation of many types of cancer cells. In HCC xenograft models, FTY720 administration inhibited tumor growth, tumor microvessel density, and metastases (Ho et al. 2005), effects that were linked to inhibition of AKT activation (Lee et al. 2004). In an orthotopic rat model, FTY720 was shown to reduce both intrahepatic and lung metastases as well as reducing tumor angiogenesis, likely through its function as a S1PR1 modulator (Li et al. 2012) (Table 1). Consistent with these results, FTY720 also decreased circulating endothelial progenitor cells. In this model, FTY720 suppressed tumor proliferation index, as well as activation of AKT and FAK (Ng et al. 2007). FTY720 also effectively downregulated S1PR1, decreased cell migration and ERK activation, and markedly extended life span in rats whose cancerous liver was transplanted with a non-cancerous one (Ushitora et al. 2009). This result is important as liver transplantation after cancer resection can provide long-term survival if recurrence can be avoided. While the clinical translation of FTY720 for cancer treatment is currently limited due to its immune suppression effects, future studies aimed at more complete understanding of the mechanism of action of FTY720, developing approaches for its specific delivery, and reducing effective concentrations and its immunosuppressive effects by using it in synergistic combinations with other drugs could enhance its rapid acceptance as an important anti-HCC drug.

7. Future Perspectives

That multiple mechanisms that promote HCC converge on the activation of the S1P signaling axis suggests that targeting this axis may prove therapeutically useful. S1P is elevated in patient tumors, as is the enzyme that produces it, SphK1, indicating it may be a node for HCC progression and thus a promising drug target. Tumor profiling and published preclinical studies with the functional S1PR1 antagonist FTY720 suggest that S1PR1 may also be a target for HCC treatment. The results discussed here indicate the need for further studies of inhibition of the SphK/S1P/S1PR signaling axis as one arm of a combination therapy for the treatment of this deadly disease.

Acknowledgments

Abbreviations

HCC	Hepatocellular carcinoma
NAFLD	Nonalcoholic fatty liver disease
NASH	Nonalcoholic steatohepatitis
S1P	Sphingosine-1-phosphate
S1PR	S1P receptor

| **SphK**	Sphingosine kinase
Spns2	Spinster 2

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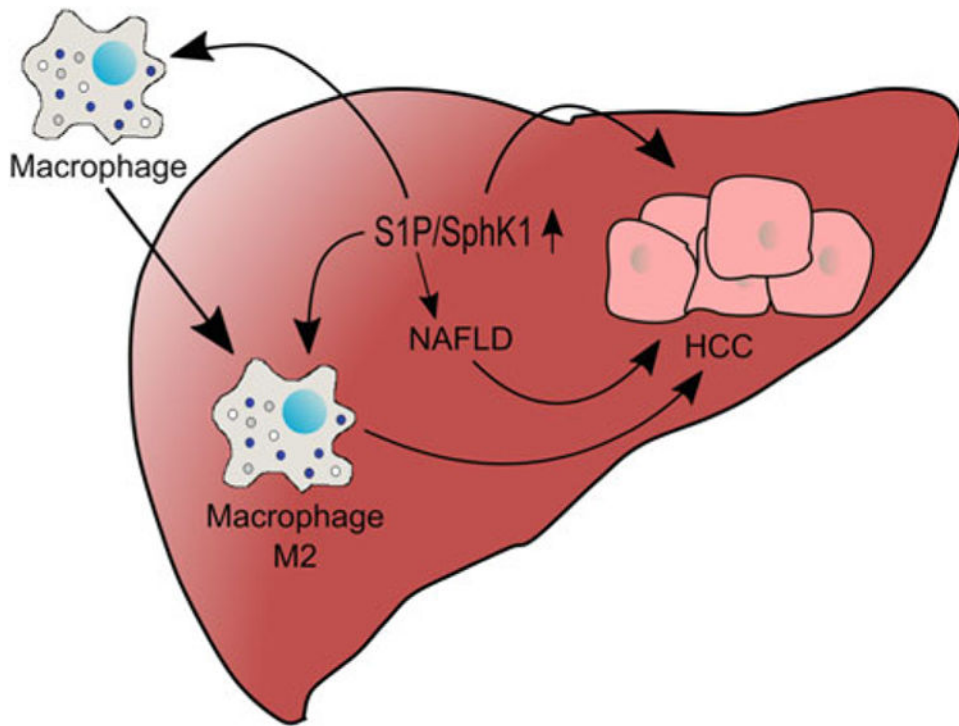
Fig. 1.
Role of S1P in promotion of liver cancer. SphK1 and S1P have been shown to promote
NAFLD, which is a risk factor for HCC development. Moreover, increased SphK1 and S1P
production by the liver can enhance recruitment of macrophages that migrate to the tumor
site, remain there, and aid in angiogenesis, termed tumor-associated macrophages (TAMs),
and are thought to express an M2 phenotype
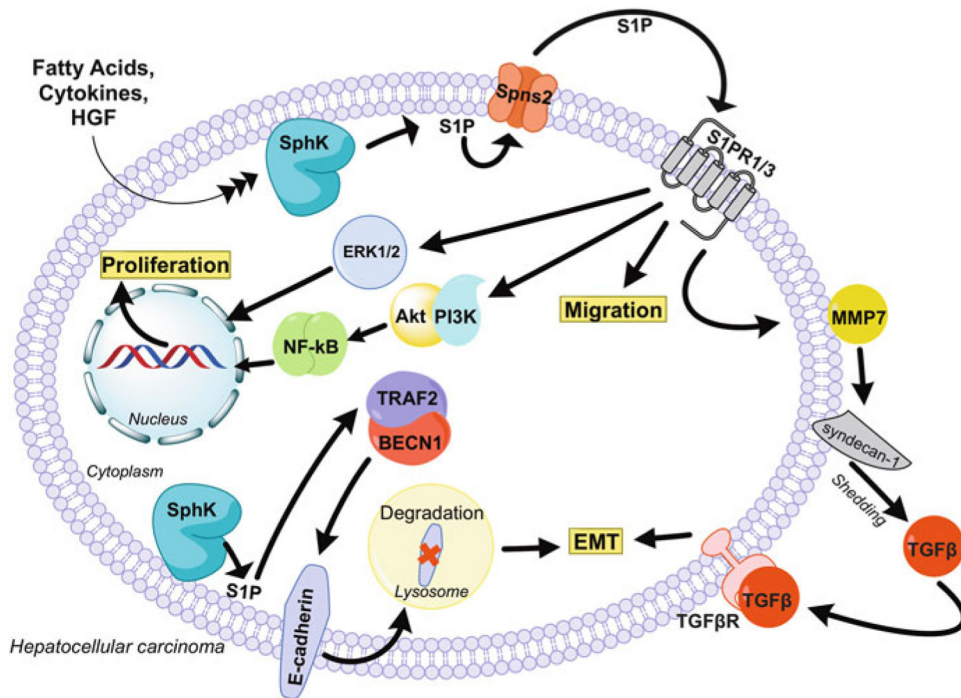
Fig. 2.
S1P signaling in HCC progression. S1P can regulate signaling and physiological processes in several pathways to promote HCC. As indicated in the text, these include increasing cell motility and proliferation through S1PR1/3 activation, S1PR1/3-mediated activation of ERK and AKT, degradation of E-cadherin to promote EMT, and proteolysis of syndecan-1 to induce HGF signaling. The EMT can be regulated by a unique pathway involving the SphK1-TRAF2-beclin1-E-cadherin signaling cascade in HCC cells

Table 1

Compounds that target the S1P axis in liver fibrosis and in preclinical HCC animal models

Compound	Target(s)	Mechanism	Effects	References
N,N-dimethylsphingosine	SphK1/SphK2	SphK1 (Ki = 5 μM) and PKC inhibitor	Blocked bone marrow-derived mesenchymal stem cell differentiation to myofibroblasts during liver injury	Yang et al. (2012)
PF-543	SphK1	SphK1-specific inhibitor (Ki = 3.6 nM)	Reduced liver fibrosis	Gonzalez-Fernandez et al. (2017)
SEW2871	S1PR1	Agonist (EC50 = 14–140 nM)	Reduced hepatic parenchymal damage and fibrosis	Ding et al. (2016)
VPC23019	S1PR1/3	Antagonist (pKi = 7.9/5.9 for S1PR1/3)	Reduced liver fibrosis	Yang et al. (2013)
W146	S1PR1	Antagonist (Ki = 10–20 nM)	Reduced liver fibrosis	Yang et al. (2012)
JTE-013	S1PR2	Antagonist (Ki = 17 nM)	Reduced hepatic inflammation and fibrosis in mice with BDL ligation	Yang et al. (2015)
			Reduced bile acid-induced cholangiocyte proliferation	Wang et al. (2017)
SKI-II	SphK1/SphK2	SphK inhibitor (IC50 = 16 μM for SphK1; 8 μM for SphK2)	Reduced survival and migration of HCC cells	Lu et al. (2017) and Zhang et al. (2013)
			Reduced liver injury	Yang et al. (2013)
FTY720/FTY720-P	S1PR1,3,4,5	S1PR1 agonist/functional antagonist of S1PR1, 3, 4, 5 HDAC inhibitor	Reduced intrahepatic and lung metastases in orthotopic rat model	Li et al. (2012)
			Extended life span of tumor-bearing rats	Ushitora et al. (2009)
ABC294640	SphK2	SphK2 inhibitor	Reduced HCC xenograft in mice	Beljanski et al. (2011)
ABC294640	SphK2	SphK2 inhibitor	Phase II clinical trial for sorafenib-resistant HCC patients	https://clinicaltrials.gov/ct2/show/NCT02939807