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INSIGHT INTO THE ROLE OF SPHINGOSINE KINASE 1 (SK1) AND SPHINGOSINE-1-PHOSPHATE (S1P) IN THE ONSET AND DEVELOPMENT OF CANCER

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ABSTRACT
Sphingolipids are ubiquitous components of cell membranes and their metabolites ceramide (Cer), sphingosine (Sph), and sphingosine-1- phosphate (S1P) have important physiological functions. We present an overview of sphingolipid metabolism and the compartmentalization of various sphingolipid metabolites. In addition, the sphingolipid rheostat, a fine metabolic balance between ceramide and S1P, is discussed. Most notably, the balance of the levels of these three sphingolipids in cells, termed the 'sphingolipid rheostat', can dictate cell fate, where ceramide and sphingosine induce cell growth arrest and apoptosis and S1P promotes cell survival and proliferation. S1P is generated from the conversion of ceramide to sphingosine by ceramidase and the subsequent conversion of sphingosine to S1P, which is catalyzed by sphingosine kinases. In addition, the functions of S1P regulates cellular processes by binding to five specific G protein coupled-receptors (S1PR1–5), are further highlighted. In mammals, two isoforms of SphK (type 1 and type 2) have been identified, they are activated by G-protein-coupled receptors, receptor tyrosine kinases, immunoglobulin receptors, cytokines, and other stimuli, which are critical regulators of the "sphingolipid rheostat", producing pro-survival S1P and decreasing levels of pro-apoptotic Sph. SK1 has been extensively studied and there is a large body of evidence to demonstrate its role in promoting cell survival, proliferation and neoplastic transformation. The regulation and biological functions of SphK isoforms are discussed. The up-regulation of SPHK1 is observed in various cancer types and is also linked to radio- and chemoresistance and poor prognosis in cancer patients. S1P/SphK has been implicated as a signaling pathway to regulate diverse cellular functions, including cell growth; proliferation and survival are discussed in detail. In this review, we discuss the current findings on the SPHK1 and S1P and its association with various human malignancies highlighting their functional role, mechanism of activation and regulation involvement in the Onset and Development of cancer.

Keywords: Sphingolipids, Sphingosine, Sphingosine-1-phosphate (S1P, sphingosine kinase1 (SphK1) , Apoptosis, Cancer

INTRODUCTION

Sphingolipids are structural and functional components of biological membranes **(**Ipatova *et al.*, 2006), which contribute to maintenance of membrane fluidity and subdomain structure. They are also implicated in bioeffector roles in cancer pathogenesis (Ogretmen and Hannun, 2004), and their metabolites, including sphinganine, ceramide, sphingosine, and sphingosine 1-phosphate (S1P), have emerged as critical players in a number of fundamental biological processes. For example, these metabolites act as bioactive mediators in various cellular processes, including survival, proliferation, differentiation, and apoptosis (Hannun and Obeid, 2008;, Ricci *et al.*, 2006). Moreover, sphingolipids are known to be involved in almost every type of disease (Cuvillier, 2002). They are reported to have regulation effect in cancer pathogenesis, progression, angiogenesis, proliferation, migration, inflammation, drug resistance, and cell death (apoptosis, necrosis, autophagy, and anoikis) (Zheng, et al. 2006;, Selvam and Ogretmen 2013).

There are two isoforms of sphingosine kinase (SK1 and SK2) which differ in their subcellular localisations, regulation and functions (Hannun and Obeid, 2008), both isoforms catalyze the same biological reaction, conversion of sphingosine to S1P (Pyne *et al.,* 2009) and are crucial regulators of the balance among ceramides, sphingosine, and S1P (Shida *et al.,* 2008). However, they show distinct subcellular location, tissue distribution, and substrate specificity (Melendez *et al.,* 2000) SphK1 is found in the cytoplasm and translocated to the plasma membrane when activated. However, SphK2 is explicitly present in the nucleus (Pyne *et al.,* 2009). A diverse range of external stimuli act as activators of SphK1 such as tumor necrosis factor α, a proinflammatory cytokine, and various several growth factors, including nerve growth and epidermal growth factors (EGFs (Hannun and Obeid, 2018). Upon stimulation by these agonist molecules, SphK1 is phosphorylated at Ser225 by an extracellular signal-regulated kinase that leads to its activation and translocation to the plasma membrane. (Pitson *et al.,* 2003). Activated SphK1 further catalyzes the phosphorylation of sphingosine, resulting in a transient increase in the intracellular levels of S1P, which acts as a bioactive lipid molecule having both extracellular function and intracellular targets (Payne *et al.,* 2002).
The S1P formed by these enzymes can either be exported from cells (through transporter proteins e.g. Spns2) and acts as a ligand on a family of five S1P-specific G protein coupled receptors (S1P1-5) or can bind to specific intracellular target proteins (Blah and Hla, 2014), and activates several downstream signaling pathways regulating various cellular processes. S1PRs are differentially expressed in various cell types that explain the diverse signaling and hence the biological functions of S1P. In addition to interacting with S1PRs placed on the plasma membrane, S1P also functions as an intracellular messenger and acts on specific intracellular targets (Okamoto *et al.,* 2011). The interconversion of ceramide to sphingosine and S1P has been termed the sphingolipid rheostat (Newton *et al.,* 2015). In this model, shifting the balance toward ceramide induces apoptosis, while predominance of S1P formation promotes cell surviva (Dobrowsky *et al.,* 1993). In general, the effects of S1P (proliferation, migration, cell survival etc.) are largely opposed to those of ceramide (apoptosis, senescence, growth arrest etc.) and the concept of the 'sphingolipid rheostat' was proposed, whereby the inter-conversion of ceramide, via sphingosine, to intracellular S1P contributes to cellular fate (Cuvillier *et al.,* 1996).
S1P can regulate cell fate through intracellular mechanisms that are beginning to be elucidated. In addition, S1P can be released from cells to exert autocrine and paracrine effects on nearby cells expressing any of the five S1P-specific G-protein coupled receptors (Strub *et al.,* 2010). Based on many studies, a model has emerged that proposes that the relative level of ceramide, sphingosine, and S1P can determine cell fate (Hait *et al.,* 2006).
As described above, the cellular levels of S1P are largely controlled through its formation from sphingosine by the activity of sphingosine kinase. Dephosphorylation of S1P is catalysed by S1P phosphatase and the sphingosine formed is then acylated to ceramide catalysed by ceramide synthase isoforms (Stiban *et al.,* 2010). S1P can also be irreversibly cleaved by S1P lyase to produce (E)-2 hexadecenal and phosphoethanolamine (Degagné and Saba, 2014). In most cells this balance between S1P generation and degradation results in low basal levels of S1P in the cell. However, when cells are exposed to specific growth factors and other agonists, S1P levels can increase rapidly and transiently as a direct consequence of rapid changes in sphingosine kinase activity in the cell (Pitson *et al.,* 2000).

1. Sphingolipid Metabolism

Sphingolipids, over the last 3 decades, have come to be recognized as a group of bioactive signaling lipids. These lipids play roles in diverse cell biologies including – cell growth, inflammation, angiogenesis, cell death, survival, and many more (Hannun and Obeid, 2017). Sphingolipids are one of the major components of eukaryotic cell plasma membranes. Aside from their structural role, they have attracted attention as potent second messengers regulating programmed cell death (Proia and Hla, 2015).
Sphingolipid metabolism is a highly coordinated broad-spectrum cellular pathway, where in several pathways are linked together. The synthesis and degradation of bioactive sphingolipids are regulated by several enzymes having fluxes of diverse metabolites (Hannun and Obeid, 2018). Almost all the key enzymes of sphingolipid metabolic pathways have been identified which has unveiled the complexity of

these pathways and their distinct subcellular compartmentalization (Yamaji and Hanada, 2015). An outline of the highly coordinated sphingolipid metabolism connecting several pathways is represented in (Figure 1**).** The bioactive lipid ceramide occupies the central position in the sphingolipid metabolism hub and can be produced through diverse pathways via three primary mechanisms. The **first one** is the de novo generation of ceramide that begins in the endoplasmic reticulum (ER) with 3-ketosphinganine from palmitoyl-CoA and serine units the catalytic action of serine palmitoyltransferase (SPT) (Hannun and Obeid, 2011).
Ceramide synthase (CERS) catalyzes the acylation of dihydrosphingosine to form dihydroceramide which is converted to ceramide by desaturase (Saddoughi and Ogretmen, 2013). Thereafter, ceramide can follow multiple intracellular routes. Ceramide is transported to the Golgi bodies by ceramide transport protein (CERT) where it is metabolically converted to sphingomyelin and various complex sphingolipids (Hannun and Obeid, 2011). The transport of ceramide from ER to the site of sphingomyelin synthesis occurs via both vesicular and non-vesicular pathways (Meer *et al.*, 2004).
In Golgi bodies, ceramide is converted to sphingomyelin, a vital component of the plasma membrane, by incorporating phosphocholine head group by sphingomyelin synthases (SMS). Glycosylation of ceramide by glycosyl or galactosyl CERS results in the formation of complex glycosphingolipids, an integral part of cell membranes and known to confer drug resistance to cancerous cells (Liu *et al.*, 2013). Additionally, ceramide can be directly phosphorylated to form ceramide-1-phosphate (C1P) by the catalytic activity of a specific ceramide kinase (CERK), which plays a crucial role in regulating cell homeostasis as well as in mediating inflammatory responses. C1P is further transported to the plasma membrane or other organelles for various biological signaling cascades by the C1P transfer protein. Conversely, ceramide can be generated through the catabolic degradation of sphingomyelin via sphingomyelinase (SMase), another mechanism (**second mechanism**) of ceramide generation (Gault *et al.*, (2010).

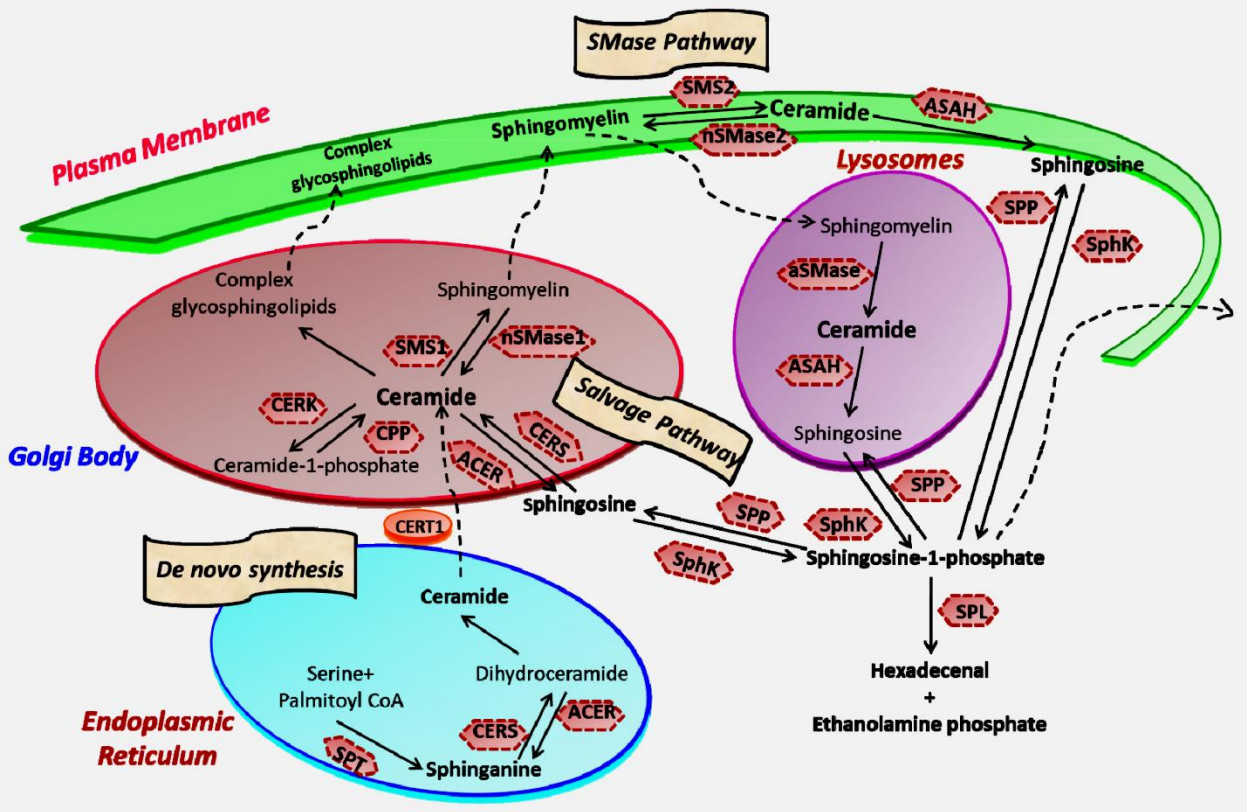

Figure 1. Sphingolipid metabolism and compartmentalization. Three significant mechanisms for ceramide generation are illustrated. Ceramide is synthesized via s "de novo pathway" in the ER from where it is transported to the Golgi bodies through CERT and serves as a substrate for the synthesis of complex

glycosphingolipids (GLSL) and sphingomyelin (SM). GLSL and SM are transported to the plasma membrane through vesicular transport. In another mechanism; ceramide can be generated by the action of neutral or acidic SMases ("SMase pathway") in the plasma membrane. Finally, in the "salvage pathway", ceramide is synthesized from sphingosine released from the lysosome by the catalytic action of CERS (Deevska *et al.,* 2017).

Finally, the third mechanism is the salvage pathway through which ceramide is generated from sphingosine by the action of CERS. Ceramide can be metabolized back to bioactive lipid sphingosine by specific ceramidase (CDase or ACER in Golgi bodies) and ASAH in lysosomes). Subsequently, sphingosine kinase (SphK or SK) catalyzes the phosphorylation of sphingosine to form another potent signaling molecule, sphingosine-1-phosphate (S1P). The S1P can either be degraded to ethanolamine phosphate and fatty aldehyde by lyase (SPL) or dephosphorylated to sphingosine by S1P phosphatase and reacylated back to ceramide (Espaillat *et al.,* 2015).

In general, cleavage of a pro-apoptotic sphingolipid ceramide yields pro-apoptotic sphingosine that is phosphorylated by sphingosine kinases (SKs) to anti-apoptotic sphingosine-1- phosphate (S1P). (Proia and Hla, 2015). Ceramide and S1P are important sphingolipids but have opposite effects. Ceramide promotes cancer cells apoptosis, blocks cell growth; S1P promotes cancer cells survival, enhancing cell proliferation, angiogenesis and autophagy (Gupta *et al.,* 2021).

Therefore, the dynamic balance between S1P and sphingosine/ceramide signalling is referred to as the "sphingolipid rheostat" regulating the cell's fate (Cuvillier *et al.,* 1996) (Figure 2), which suggests that it leads to cell death when this balance moves towards ceramide or sphingosine, but to cell survival or proliferation when S1P levels are increased (Mora *et al.,* 2010). Indeed, several reports have suggested that reduction of S1P level that leads this rheostat towards ceramide/sphingosine might provide a potential target for cancer therapies (Visentin *et al.,* 2006).

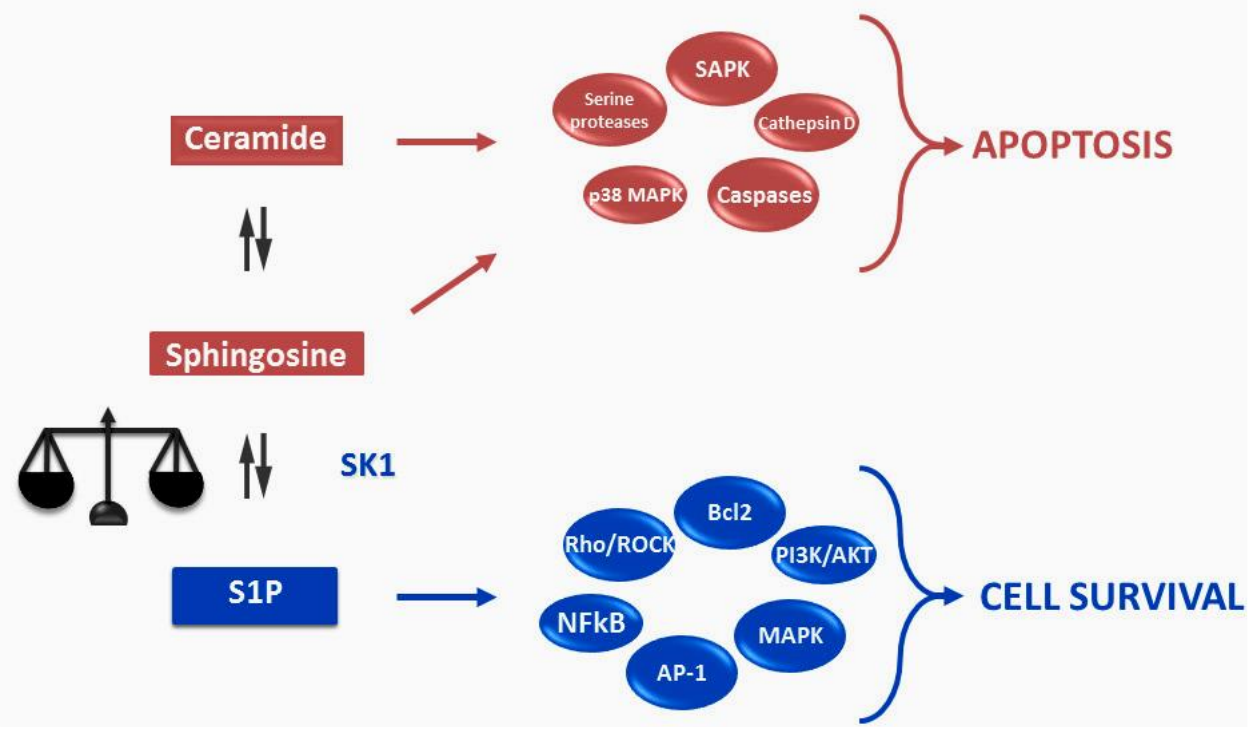

Figure 2. Sphingolipid rheostat. Ceramide and sphingosine are intracellular lipid second messengers, which induce activation of apoptotic pathways. In turn, SK1 can phosphorylate sphingosine to yield S1P, a lipid second messenger that activates anti-apoptotic pathways and antagonises the effects of ceramide and

sphingosine. The intracellular balance between ceramide, sphingosine and S1P determines the cell fate (Alshaker *et al.,* 2013).

2. Sphingosine Kinase

Sphingosine kinase (SphK) is a lipid enzyme central to lipid metabolism, maintaining the cellular lipid homeostatic balance through its pivotal role in the conversion of sphingosine to sphingosine 1 phosphate (S1P) for maintenance of normal cellular function (Hannun and Obeid, 2008). The SphKs are known to play pivotal roles in regulating many physiological pathways, and in the pathophysiology of various diseases, and the complexity of the SphK multi-functional signaling pathways are slowly being unraveled. Key cellular roles for SphK/S1P include the promotion of cell survival and proliferation, prevention of apoptosis, maintenance of vascularization and stimulation of angiogenesis for tissue regeneration in tissue damage, metabolic rewiring, and metabolic stability (Hannun and Obeid, 2008; Pyne *et al.,* 2012).

SphK is a lipid kinase that catalyzes the ATP-dependent phosphorylation of sphingosine to S1P (Lewis *et al.,* 2018). Two isoforms of SphK—SphK1 and SphK2 have been characterized in humans that regulate various cellular processes (Pyne *et al.,* 2009). SphK1 is highly expressed in cells in the lung and spleen, whereas SphK2 is more abundant in liver, kidney, brain and heart· Moreover, the intracellular localization of SphK1 and SphK2 is closely linked to their physiological functions (Melendez *et al.,* 2000). SphK1 and SphK2 have some overlapping but also distinct functions due to their different in substrate affinities, tissue distribution, and intracellular localizations (Strub *et al.,* 2011). Although the two isoenzymes are highly homologous, they have been observed to perform distinct functions. SphK1 shows pro-survival effects, whereas studies point towards a pro-apoptotic role of SphK2 (Gupta *et al.,* 2020). SphK1 is mainly cytosolic and translocated to the plasma membrane upon stimulation with growth factors and cytokines (Pyne and Pyne 2010), SphK1 is activated and translocated from the cytosol to the plasma membrane to carry out its catalytic conversion of sphingosine to S1P.

Activation of SphK1 is associated with promotion of cell proliferation, survival, migration, differentiation, angiogenesis and inflammation (Ogretmen, 2018**).** While SPHK2 is found in nucleus, cytoplasm, and mitochondria (Pyne *et al.,* **2016).** Because of their crucial roles in sphingolipid metabolism, SPHK1 and SPHK2 both determine cell fates by regulating the balance between survival and cell death via S1P and ceramide metabolism (Imbert *et al.,* **2020).**

3. Sphingosine-1-phosphate: Generation and Function

Sphingosine-1-phosphate (S1P) is a bioactive sphingolipid metabolite was discovered by Sarah Spiegel almost 3 decades ago (Olivera and Spiegel, 1993) as a lipid mediator, which acts as a potent activator of cellular signaling pathways (Spiegel *et al.,* 1993). S1P plays a role in various cellular processes including cell proliferation, differentiation, angiogenesis, inflammation and cancer (Maceyka *et al.,* 2012).

Since then, molecules that regulate S1P have been described, including S1P synthetic enzymes, receptors and degrading enzymes, all of which regulate S1P concentration and its signaling inside and outside of cells (Takabe *et al.,* 2008). These discoveries of S1P and related molecules have driven lipid research forward (Spiegel *et al.,* 1993). S1P is produced by two different sphingosine kinases, SphK1 and SphK2 (Maceyka *et al.,* 2012).

As discussed above, S1P is formed in cells by phosphorylation of sphingosine by sphingosine kinases (SphK1 and SphK2). Breakdown of S1P can be achieved by irreversible hydrolysis by S1P lyase or reversible dephosphorylation by S1P phosphatases (SPP1 and SPP2) back to sphingosine (Le Stunff *et al.,* 2004). Intracellular S1P is a second messenger to trigger calcium release from the endoplasmic reticulum (Ghosh, T.K.; Bian, J.; Gill, D.L. (1994). Important intracellular target proteins of S1P such as histone deacetylases (HDACs) and tumor necrosis factor (TNF)-associated factor 2 (TRAF2) have been identified (Hait *et al.,* 2009). These findings further address the molecular mechanisms by which S1P mediates TNF-α signaling and epigenetic regulation. S1P plays an important part in migration, survival, and growth of mammalian cells because of its involvement in multiple signaling cascades. It has been known that S1P exists in the ceramide-sphingosine-S1P rheostat, where S1P promotes cell growth and survival while ceramide and sphingosine exhibit pro-apoptotic properties (Cuvillier *et al.,* 1996).

As mentioned previously like most signaling molecules, intracellular S1P level is tightly regulated by its synthesis and degradation. S1P is exclusively synthesized via phosphorylation of the 1-hydroxyl group on sphingosine either by SphK1 or SphK2 in response to diverse stimuli, including inflammatory cytokines, growth factors, and activation of GPCRs. S1P can be converted back to sphingosine by S1P specific phosphatase in the cytosol or degraded by S1P lyase to ethanolamine phosphate and hexadecanal (Stoffel *et al.*, 1970). Unlike sphingosine, which is sufficiently hydrophobic to diffuse cross membranes, S1P is a more hydrophilic molecule, which requires specific transporters to be exported to the extracellular space. Several membrane-associated transporters have been identified as active S1P transporters, including ATP-binding cassette (ABC) transporters, ABCA1, ABCC1, ABCG2 and spinster homologue 2 (Spns2) (Fukuhara *et al.*, 2012). The exported S1P can activate the S1P-specific GPCRs on the cell membrane to induce various physiological responses (Figure 3) (Strub *et al.*, 2010).

Outside the cell, S1P binds to S1P-specific G-protein- coupled receptors, S1PR1-5, which further evoke cell signaling in an autocrine, paracrine or endocrine manner. The "inside-out signaling" process refers to the signaling pathway by which S1P synthesized in the cell is transported out of the cell to activate S1PRs on the cell surface in an autocrine and paracrine manner (Takabe and Spiegel 2014). The combination and cell-type-specific expression of different S1PRs determines a broad range of biological functions mediated by S1P (Takabe *et al.*, 2008). Owing to its multiple biological functions, S1P is implicated in various physiological and pathological conditions such as inflammation and cancer (Pyne and Pyne 2010). S1P has also been reportedly stored in erythrocytes which act as a buffer system to protect S1P from degradation (Hanel *et al.*, 2007). S1P acts as a tumorigenic growth factor in the tumor microenvironment to promote cancer progression (Li *et al.*, 2008).

In general, S1P cell death-suppressing and cell survival promoting effects are opposite to those typically attributed to ceramide that, almost invariably, induces apoptosis, senescence, autophagy, and growth arrest (Saddoughi and Ogretmen, 2013).

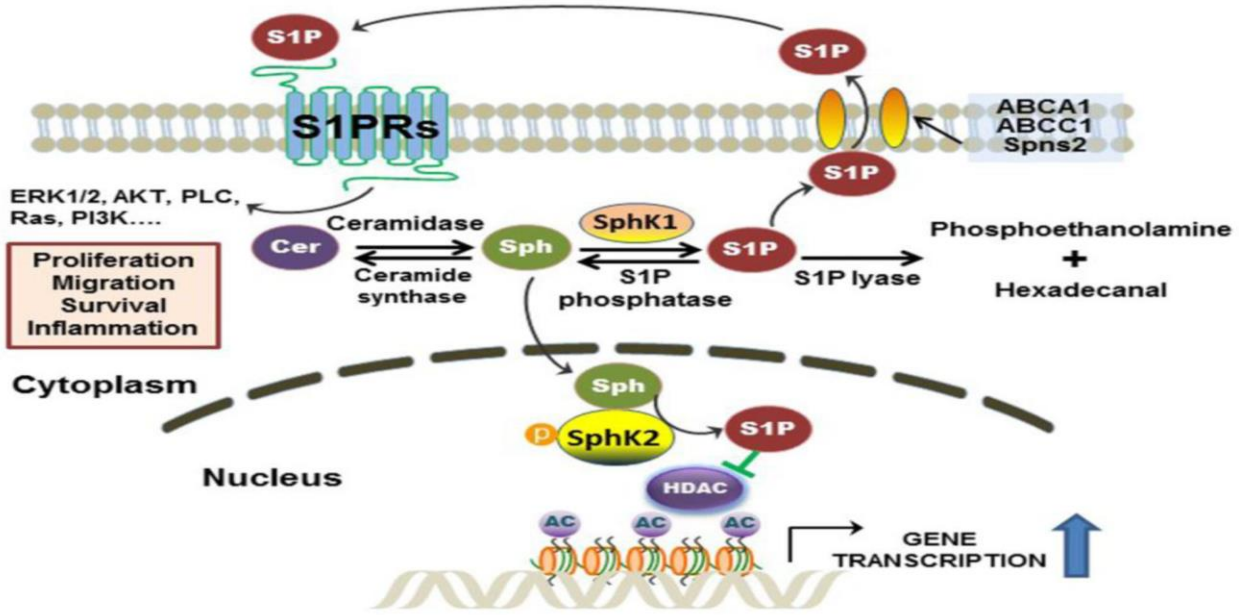

Figure 3. Biosynthetic and signaling pathways of sphingosine 1-phosphate (S1P). S1P is synthesized by phosphorylation of sphingosine (Sph), which is exclusively mediated by sphingosine kinase 1 (SphK1) in the cytosol and Sphk2 in the nucleus. Cytosolic S1P can be exported by transporters (ABCA1, ABCC1, and Spns2) and activates GPCRs (S1PR1-5) on the cell surface. S1P also can be dephosphorylated by S1P phosphatase back to Sph for ceramide (Cer) synthesis or further degraded by S1P lyase into phosphoethanolamine and hexadecenal. Both SphK1 and SphK2 can be activated by ERK. Nuclear S1P

generated by SphK2 inhibits HDAC1/2 activity, which results in increase of histone acetylation and up-regulation of gene transcription **(**Strub *et al.,* 2010).

4. SphK1 Activation and Functions

Since SphK1 is found primarily in the cytosol and its substrate, Sph, is generated in membranes, it is not surprising that translocation of SphK1 to the plasma membrane appears to be an important and common feature of its activation (Spiegel and Milstien 2003). The function of SphK1 and subsequent intracellular levels of S1P is regulated by various mechanisms, including gene transcription, translational regulation and posttranslational modifications (Pulkoski *et al.,* 2018). In particular, the activity of SphK1 is regulated by various agonists and stimuli that facilitate its phosphorylation and translocation to the plasma membrane, where it synthesizes S1P from sphingosine (Figure 4).

Activation of SK1 by agonists of GPCRs, protein kinases, proinflammatory cytokines, and small GTPases leads to its translocation to plasma membrane where it catalyzes the conversion of sphingosine to S1P. Importantly, phosphorylation of SK1 at Ser 225 by ERK1 and ERK2 is important for its activation and translocation to the plasma membrane (Pitson *et al.,* 2003). The membrane affinity and plasma membrane selectivity are determined by Thr54 and Asn89 residues of human SK1, where in these residues interact specifically with phosphatidyl serine in the plasma membrane, thus making sphingosine available to generate S1P, which can be secreted outside the cell and engage with S1PRs to induce prosurvival functions (Stahelin *et al.,* 2005).

A range of external stimuli that induce phosphorylation of SphK1 includes transforming growth factor β (TGF-β), tumor necrosis factor α (TNF-α), pro-inflammatory cytokine, and various growth factors via the activation of receptor tyrosine kinases, G-protein coupled receptors and toll-like receptors (Nishino *et al.,* 2019). TGF-β has been observed to induce SphK1 activity by upregulating its gene expression levels (Ebenezer *et al.,* 2016). Moreover, TGF-β exposure reduces the activity of S1P phosphatase, ultimately leading to a transient and rapid increase in S1P intracellular levels (Nicholas *et al.,* 2017). Another activator of SphK1 is TNFα that enhances SphK1 activity in the human umbilical vein endothelial cell (HUVEC) (Zhang *et al.,* 2013). Other endogenous agonists include prolactin and 17β-estradiol, both upregulate the expression and activity of SphK1 (Maczis *et al.,* 2018).

SK1 is functionally linked with some of the hallmarks of cancer SK1. For instance, the over-expression of SK1 enhances the Ras-dependent transformation of fibroblasts into fibrosarcoma (Xia *et al.,* 2000). Indeed, K-RasG12V is a common mutation in cancer and, through SK1, increases the production of S1P and decreases ceramide levels. Over-expression of the K-RasG12V oncogene signaling promotes translocation of SK1 from the cytoplasm to the plasma membrane via Raf/MEK/ERK signaling. Indeed, constitutively active B-Raf or MEK1 activate SK1 (Gault *et al.,* 2012). Therefore, SK1 can function within the context of oncogenic transformation. SK1 activation and localization to the plasma membrane and subsequent activation of S1P2 by released S1P ('inside-out' signaling) also regulates transferrin receptor 1 (TFR1) expression (Pham *et al.,* 2014). This is important as inhibition of TFR1 prevents SK1-induced cell proliferation, survival and neoplastic transformation of NIH3T3 fibroblasts (Pyne and Pyne 2010).

Previous studies had shown that intracellularly generated S1P acts upstream of ERK1/2 in the signaling pathways initiated by TNF-α and VEGF (Xia *et al.,* 1999). Upon activation, SphK1 interacts with calcium-myristoyl switch protein 1 that further facilitates the phosphorylation of sphingosine to S1P on the plasma membrane (Jarman *et al.,* 2010). Several lines of evidence have consolidated the notion that SphK1 promotes cell survival. The elevated intracellular SphK1 levels appear to play an essential role in uncontrolled cell proliferation and metastasis in various cancer cell types (Ebenezer *et al.,* 2016). A correlation between the expressions of SphK1 with short patient survival has also been observed. Brocklyn *et al.,* 2005 have demonstrated that SphK1 expression is inversely correlated with patient survival in glioblastoma multiform. Multiple studies have illustrated that the targeted inhibition of SphK1 activity can be considered a potential strategy to combat cancer (Hannun and Obeid, 2018).

Likewise, the down-regulation of SphK1 via targeted inhibition induces apoptosis and enhances the sensitivity of cancer cell lines towards chemo- and radiation therapy (Haddadi *et al.,* 2017).

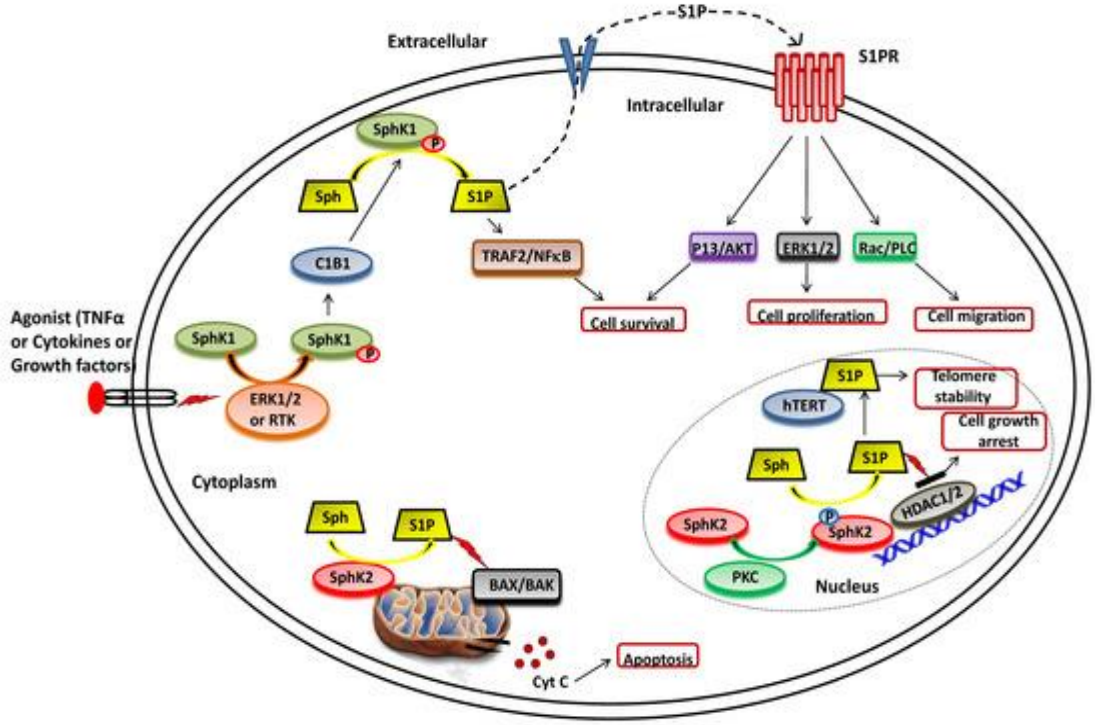

Figure. 4. Functional roles of SphKs and S1P in cells. Upon ERK1/2 mediated phosphorylation/activation in the presence of various agonist (such as TNFα, cytokines and diverse growth factors), SphK1 is translocated to plasma membrane from cytoplasm and interact with calcium-myristoyl switch protein 1 (C1B1). This facilitates the phosphorylation of sphingosine to generate S1P, which can either be secreted out or interacts with intracellular targets (such as TRAF2) to elicit its functions. Once secreted out of the cell, S1P binds to the S1P receptor (S1PR) embedded in the plasma membrane and activates various downstream signaling pathways that control cell survival, proliferation, and migration (Deevska *et al.,* 2017).

5. The role of S1P in cancer Progression

In the **three** decades since the discovery that S1P regulates cell growth **(**Zhang *et al.,* 1991., Olivera and Spiegel, 1993) and suppresses apoptosis (Cuvillier *et al.,* 1996)**,** numerous reports have been published on S1P signaling and its functions as a bioactive lipid mediator. The role of S1P in cancer progression has been established by studies demonstrating that the up regulation/activation of SphK1 and production of S1P inhibits apoptosis and facilitates survival of cancer cells, thus promoting tumor growth, angiogenesis, and metastasis (Pyne and Pyne 2010). These clearly established that S1P regulates many important cellular processes involved in cancer, including growth, survival, migration, and invasion as well as angiogenesis and immune responses **(**Spiegel and Milstien, 2011). Growth factors, cytokines, hormones, and antigenic factors important for cancer, including estradiol (E2) **(**Sukocheva *et al.,* 2003)**,** epidermal growth factor (EGF) **(**Hait *et al.,* 2005)**,** insulin-like growth factor-1 (IGF-1) **(**El-Shewy *et al.,* 2006**),** and VEGF **(**Hayashi *et al.,* 2009)**,** all stimulate sphingosine kinase (SphK), the enzyme that phosphorylates sphingosine to produce S1P.

SphK1 is a critical regulator of the S1P rheostat and produces S1P that functions in inside-out signaling by activation of the S1P receptors (S1PRs). These important functions of SphK1 are described in detail below. SphK1 also produces intracellular S1P that can function as a cofactor for the E3 ubiquitin ligase activity of tumor necrosis factor receptor-associated factor 2 (TRAF2) in nuclear factor-$_k$B (NF-$_k$B) signaling **(**Alvarez

et al., 2010). Numerous studies have shown that SphK1 and production of S1P promotes tumor growth, resistance to apoptosis, tumor angiogenesis and metastasis (Pyne and Pyne 2010). SphK1 message and protein levels are often upregulated in cancerous tissue and expression is correlated with chemo- and radio-resistance and poor prognosis. Consistent with these observations, overexpression of SphK1 promotes, while its inhibition reduces, tumor growth, angiogenesis and chemoresistance in numerous xenograft models (Pyne and Pyne 2010).

S1P secreted from tumor cells can act through S1PRs either in an autocrine manner to promote growth, survival, motility, and metastasis **(**Salas *et al.,* 2011) or in a paracrine manner to induce endothelial adhesion molecules, angiogenesis and regulate tumor-stromal interactions as well as immune cells **(**Anelli V, et al. 2010)**.** As discussed above, cancer cells may adapt both the intracellular actions of S1P and inside-out signaling of S1P to promote their survival and metastasis. S1P may act on intracellular targets such as HDACs and NF-κB to promote cancer progression **(**Alvarez *et al.,* 2010). Finally, S1P can promote resistance of cancer cells to therapy by counteracting the pro-apoptotic effects of ceramide (Cuvillier *et al.*, 1996) (Figure 5).

Over expression of SphK1 has been identified in mRNA screening or immunohistochemistry staining in multiple cancer cells derived from breast, colon, lung, ovary, stomach, uterus, kidney, and rectum(Kohno *et al.*, 2006). Inhibition of SphK1 with its specific inhibitor SK1-I reduces the growth of acute myelogenous leukemia and glioblastoma **(**Kapitonov *et al.,* 2009). Tumor cells export S1P to act through S1PRs to promote growth, survival, motility and metastasis in an autocrine manner **(**Salas *et al.,* 2011). A paracrine action of tumor cells-exported-S1P is to induce the production of endothelial adhesion molecules, angiogenesis, and to regulate tumor–stromal interactions as well as immune cells **(**Anelli *et al.,* 2010)**.** S1PR1 has been shown to mediate persistent activation of signal transducer and activator of transcription-3 (STAT3) in tumor. Activated STAT3 therefore plays two regulatory roles as transcription factor for both S1PR1 and IL-6, which is the most potent oncogenic cytokine **(**Lee *et al.*, 2010).

Thus, this is a new feed forward mechanism that explains persistent STAT3 activation in cancer cells and the tumor microenvironment that is important for malignant progression and metastasis **[(**Michael*et al.,* 2012).

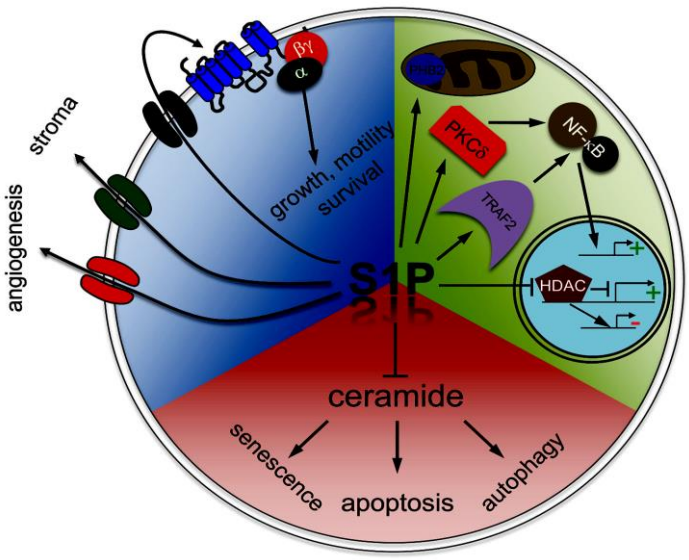

Figure 5. Intracellular and extracellular actions of S1P. S1P produced intracellularly can inhibit functions of its pro-apoptotic precursor ceramide. Ceramide is implicated in growth arrest, apoptosis and autophagy (red quadrant). S1P also has intracellular targets (green quadrant) or can be exported out of cells to act in autocrine and/or paracrine manners through the S1P receptors (blue quadrant) (Guan*et al.*, 2011).

6. Roles of Sphingosine kinase 1 in Cancer Pathogenesis

Given its prime position in sphingolipid metabolism, SK1 expression affects the balance between prodeath and prosurvival sphingolipids to determine cell fate a prime regulatory position for a potential oncogene (Vadas *et al.,* 2008). SK1 has well-established pro-survival functions in various cancers. Indeed, by virtue of its transformation potential, SK1 is considered to be a bona fide oncogene (Xu *et al.,* 2018). There is substantial evidence of a role for SK1 in numerous cancers (Pyne and Pyne 2010). SPHK1 is widely upregulated across a diverse range of human cancers, such as breast cancer, lung cancer, uterine cancer, ovarian cancer, gastric cancer, kidney cancer, liver cancer, prostate cancer, colorectal cancer, small bowel cancer, chronic myeloid leukemia, glioblastoma, and lymphoma (Johnson *et al.,* 2005).

Moreover, a twofold elevation of SK1 mRNA expression was observed in cancer versus normal tissue for several types of solid tumors including breast, uterus, ovary, colon, small intestine, and rectum in addition to lung (Karliner, 2013). Many factors influence SK1 expression, such as hypoxia, growth factors and cytokines, and this has a clear indication concerning prognosis (Pyne and Pyne 2010).

As we learned before, it is generally well-consolidated that SphK1 is a cell survival promoter (Guillermet-Guibert *et al.,* 2009). Elevated cellular SphK1 levels appear to play a major role in enhanced proliferation and metastasis/invasion of several types of cancer cells (Shida *et al.,* 2008). In this context more than one study has demonstrated that inhibition of SphK1 has considerable potential as an anti-cancer strategy (Pyne *et al.,* **2016)**. Similarly, the downregulation of SphK1 has proven able to induce apoptosis and confer sensitivity to chemo- or radiation therapy of cancer cell lines (French *et al.,* 2003).

Interestingly, SK1 expression was found also to be important for Ras-mediated transformation. In cancers, such as breast, lung, ovary, stomach, and kidney, SK1 mRNA increased approximately twofold compared to paired normal tissues (Ruckhaberle *et al.,* 2008). Also immunohistochemical studies with lung, colon, and breast cancer tissues were positive for SK1 expression in tumor tissues and/or carcinoma cells (Nindl *et al.,* 2006). Moreover, microarray data show elevated SK1 in squamous cell carcinoma (Chan *et al.,* 2005). N-methyl-N-nitrosourea-induced rat breast cancer (Ma *et al.,* 2004), Recurrent breast cancer after tamoxifen treatment (Wong *et al.,* 2003), cervical cancer (Andersson *et al.,* 2007), head and neck cancer, and leukemia (Shirai *et al.,* 2011).

There are many examples which provide additional evidence for a role of SK1 in cancer. For instance, SK1 is overexpressed head and neck squamous cell carcinoma (HNSCC) (stages I-IV). The knockout of SK1 reduces S1P generation and decreases tumor incidence, multiplicity, and volume in 4-NQO-induced HNSCC carcinogenesis. This was associated with reduced cell proliferation, increased apoptosis and reduction in phosphorylated AKT levels (Stayrook *et al.,* 2015). TGF-β also induces an increase in SK1 expression and this can be correlated with metastasis and increased viability of MDA-MB-231 cells, suggesting that TGF-β and the SK1/S1P axis might have a critical role in promoting metastasis (Huang *et al.,* 2014). It is worth stressing that one of the proposed mechanism by which SphK1 controls cell death is the regulation of ceramide (Maceyka *et al.,* 2012). In contrast to S1P, the ceramide-signaling molecule is able to exert pro-apoptotic actions. Its synthesis and accumulation are enhanced in cells lacking SphK1, while prevented in presence of high SphK1 levels (Maceyka *et al.,* 2012).

However, although SphK1 appears to play a major role in the regulation of this "rheostat," previous studies suggested that sphingolipid per se are able to influence the whole mechanism, thus including the "inside-out" one. In this context, Huang et al. (2014) have recently shown that S1P can activate a positive feedback amplification loop via S1PRs activation and consequent increase in SphK1 expression.

CONCLUSION

Sphingolipids have a wide range of biological functions. Sphingolipids, as complex biological molecules, have been shown to regulate various biological/cellular processes including tumor cell death and survival. However, recent studies show that sphingolipid enzymes have helped in understanding the mechanisms and functions of sphingolipids, such as ceramides, sphingosine and S1P, in regulating cancer signaling.

Sphingolipids metabolism and signaling within biological membranes have a strong influence on the regulation of cell death and survival.

There is no doubt that excessive activity of SphK-S1P has an essential role in the development of cancer. S1P is a bioactive lipid mediator that is now recognized as a bona fide regulator of many important cellular and biological processes, including proliferation, survival, migration, angiogenesis, and autophagy; all of which are associated with tumor growth, invasion, and metastasis. Given the important roles of S1P in tumorigenesis, targeting S1P signaling may be an adjunct to cancer therapy. SphK1 is a master regulator that determines cell fate. Since S1P is generated from sphingosine by SphKs, the factors that regulate the balance of the ceramide-sphingosine- S1P rheostat towards ceramide and decreasing SphK activity may be candidates for anti-cancer drug development.

There is increasing evidence that altered regulation of S1P levels and expression of SphK1 might play important roles in the abnormal growth of various cancers, and this is consistent with the antiapoptotic functions of S1P. S1P, which is known for its pro-tumorigenic effects and inducing tumor progression, has also emerged as a promising target for cancer treatment with few ongoing clinical trials.

SphK1 is involved in many of the cell biological phenomena associated with cancer, it is overexpressed in cancer, mutates, and inhibition of SphK1 attenuates cancer growth and resistance to chemo- or radio-therapeutic agents. Thus it seems safe to conclude that the SphK1/S1P system is an important signaling pathway in cancer development and progression. Given the critical role of SphK/S1P in cancer progression and development, targeting SphK/S1P signaling pathway is an attractive therapeutic strategy. We report in this recent review findings that have highlighted the possible role of SphK1 and S1P in onset and development of cancer.

Further investigation into the detailed oncogenic mechanism of SPHK1 and the development of potent SPHK1 inhibitors with improved specificity may offer a novel direction in cancer therapy.

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