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Review

Regulation and function of sphingosine kinase 2 in diseases

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Summary. Sphingosine kinase functions to phosphorylate sphingosine to sphingosine 1-phosphate (S1P) to keep balance in the metabolites of sphingolipids. There are two isoforms of sphingosine kinase, sphingosine kinase 1 (SphK1) and sphingosine kinase 2 (SphK2). Although SphK1 and SphK2 share high sequence similarity, SphK2 has distinct distribution, regulation and function. SphK2 is involved in the pathological processes of varieties of diseases including cancer, neurodegenerative disorders, stroke, cardiovascular diseases and inflammation. SphK2 may promote the proliferation of cancer cells and the progression of inflammation. The SphK2/S1P pathway is also involved in the pathogenesis of neurodegenerative disorders and stroke. S1P produced by SphK2 in the nucleus binds to HDACs, which then inhibits histone acetylation and regulates memory. The SphK2 pathway mediates platelet aggregation, thrombosis, cardioprotection and helps to ameliorate hepatic steatosis. This review focuses on the recent advances in research on SphK2 regulation and its potential roles in diseases, highlighting SphK2 may be a novel therapeutic strategy for diseases.

Key words: Sphingosine kinase 2, Sphingosine 1-phosphate, Apoptosis, Autophagy, Cancer, Stroke, Neurodegenerative disorders, Preconditioning

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Introduction

There are three main intermediates in the metabolism of sphingolipid: ceramide, sphingosine and sphingosine 1-phosphate (S1P). It is now generally accepted that ceramide and S1P constitute a rheostat system in response to stress. Ceramide and sphingosine induce cell apoptosis and suppress cell growth , while S1P promotes cell survival and proliferation (Bernacchioni et al., 2012). The ceramide chain length is decisive for its apoptotic role. C16/C18 ceramides are often pro-apoptotic, and very long-chain ceramides, such as C24:0/C24:1, are sometimes pro-proliferative (Grosch et al., 2012). S1P is a bioactive phospholipid that has dual functions as extracellular first messenger and intracellular second messenger. S1P plays a first messenger role through binding to the cell membrane five S1P G protein- coupled receptors, while it is also an intracellular second messenger involved in cell survival, proliferation, immunoregulation carcinogenesis and regulation of cell migration (Bektas et al., 2005; Maceyka et al., 2009; Cyster and Schwab, 2012). S1P, sphingosine and ceramide are reversibly interconverted. Sphingosine kinases (SphK), including SphK1 and SphK2 subtypes, catalyze the phosphorylation of sphingosine to S1P. S1P phosphatase dephosphorylates S1P to sphingosine, which then can be transformed into ceramide by ceamide synthase. Conversely, ceramide is catalyzed by ceramidase to form sphingosine (Fig. 1).

SphK is a lipid kinase family highly conserved in evolution. There are two main isoforms of SphK in mammals, SphK1 and SphK2. Generally, SphK1 promotes cell growth and inhibits apoptosis, while

SphK2 induces apoptosis and autophagy (Maceyka et al., 2005; Sheng et al., 2014). SphK2 plays an essential role in cancer, inflammation, cardiovascular diseases etc. In this review, the structure and function of SphK2 is described. We especially focus on the role of SphK2 in varieties of diseases, as well as the possibilities of SphK2 becoming a potential novel therapeutic target.

Distribution and regulation of SphK2

SphK1 and SphK2 share high amino acid sequence similarity (80%), while the N terminals and central regions of SphK1 and SphK2 are different (Hait et al., 2006; Neubauer and Pitson, 2013). Both SphK1$^{-/-}$ and SphK2$^{-/-}$ mice are phenotypically normal, indicating that SphK1 and SphK2 compensate each other to maintain essential functions (Allende et al., 2004). Lack of SphK2 leads to a compensatory upregulation of SphK1 expression in macrophages (Xiong et al., 2013). However, SphK1 and SphK2 mostly show distinct cellular location, activity and biological functions. SphK1 is expressed in lung, spleen, thymus, heart, renal cortical proximal convoluted epithelial cells (Facchinetti et al., 2008), and endothelial cells (Melendez et al., 2000), while SphK2 is expressed in heart (Karliner, 2009), liver (Nagahashi et al., 2015), and rodent brain (Blondeau et al., 2007). SphK1 is mainly localized in the cytosol (Spiegel and Milstien, 2003; Takabe and Spiegel, 2014). SphK2 can also be expressed in cytosol, but SphK2 additionally has a proline-rich domain and several putative transmembrane domains, and then SphK2 shows different subcellular location as nucleus (Neubauer and Pitson, 2013), endoplasmic reticulum (ER) (Maceyka et al., 2005) and mitochondria (Strub et al., 2011). SphK2 has both nuclear import signal and nuclear export signals and shuttles between the nucleus and cytoplasm depending on cell state (Igarashi et al., 2003).

SphK/S1P pathway is regulated by kinds of extracellular ligands, including cytokines, growth factors such as platelet-derived growth factor (Olivera and Spiegel, 1993), vascular endothelial growth factor (VEGF) (Lee et al., 2014), epidermal growth factor (EGF) (Hait et al., 2005), immune response inducers as tumor necrosis factor (TNF) (Xia et al., 1999), and lipopolysaccharide (Wu et al., 2004). Then ligands bind to their receptors to mediate activation of SphKs and to increase intracellular S1P (Takabe et al., 2008; Gandy and Obeid, 2013). Subsequently, SphKs are regulated by various intracellular signaling pathways, such as protein kinase C (PKC) and extracellular signal-regulated kinase (ERK) (Taha et al., 2006). SphK2 can be activated by EGF and phorbol ester, TNFα and IL-1β, and then is regulated by intracellular ERK and protein kinase D (PKD) (Hait et al., 2005, Mastrandrea et al., 2005, Xiao et al., 2014). EGF and phorbol ester, a PKC activator, induce phosphorylation of hSPHK2 through mitogen-activated protein kinase 1 (MEK1)/ERK pathway. ERK1 forms a complex with hSPHK2 and induces phosphorylation of hSPHK2 on Ser-351 and Thr-578. Then the SphK2 activity is regulated by phosphorylation and participates in human cancer progression (Hait et al., 2007). Phorbol 12-myristate 13-acetate (PMA) treatment leads to a PKD-mediated phosphorylation of hSPHK2, which then regulates hSPHK2's localization. PKD can phosphorylate the Ser-419/Ser-421 residues in the nuclear export signal (NES) of hSPHK2, resulting in the nuclear export of hSPHK2 for subsequent cellular signaling (Ding et al., 2007). Thus ERK/PKD pathway stays upstream of SphK2 by phosphorylation of SphK2

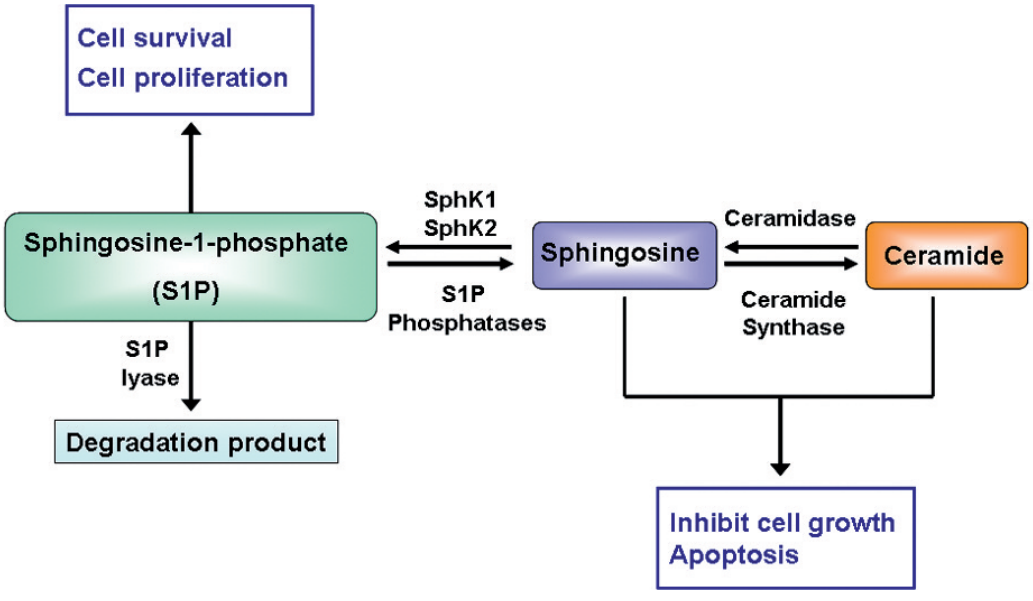

Fig. 1. The schematic diagram depicts the pathway of sphingolipid metabolism. S1P, sphingosine and ceramide could transform reciprocally. Sphingosine is catalyzed by SphK1 and SphK2 into S1P. S1P lyase cleaves S1P; S1P phosphatase dephosphorylates S1P to sphingosine. Sphingosine is metabolized into ceramide by ceramide synthase. Ceramide is in turn cleaved into sphingosine by ceramidase.

to mediate the export of SphK2 from nuclei, leading to suppression of cell apoptosis (Xiao et al., 2014). On the other hand, a recent study suggests SphK2 is also regulated by endoplasmic reticulum (ER) stress-mediated unfolded protein response (UPR) pathways. SphK2 in liver was transcriptionally up-regulated by tunicamycin and lipopolysaccharides through acute ER stress and subsequent activation of activating transcription factor (ATF) 4. Then SphKs helps to correct abnormal lipid biosynthesis and development of insulin resistance (Lee et al., 2015).

The biological functions of SphK2

SphK2 and S1P

The major function of SphK2 is to catalyze sphingosine into S1P, but recent studies show that the pharmacological inhibition or genetic ablation of SphK2 results in increased blood S1P levels, opposite to SphK1 null animals (Kharel et al., 2015, Santos and Lynch, 2015). One possible mechanism of the contradictory results is that SphK2 is involved in S1P transport. The SphK2 null mice display defective S1P transport from blood to tissues and SphK2 seems to regulate cellular uptake of S1P (Sensken et al., 2010). Thus higher S1P levels were found in blood accompanied by lower S1P concentrations in lymphoid tissues of the SphK2 null mice treated with S1P-lyase inhibitor. As S1P is implicated in vascular barrier function, increased S1P gradient (high in blood and low in tissue) plays a role to protect vascular leak during ischemia-reperfusion injury (Camerer et al., 2009).

SphK2 in regulation of apoptosis

SphK1 and SphK2 catalyze the same reaction to produce the same metabolite S1P, but they may have opposite biological functions (Maceyka et al., 2005). SphK1 promotes proliferation and inhibits cell apoptosis, while SphK2 enhances apoptosis in diverse cell types and suppresses cell proliferation. SphK2 instead of SphK1 enhances ceramide biosynthesis and enhances caspase-mediated apoptosis. The mechanism of SphK2-induced apoptosis remains to be elucidated. One possible mechanism is that the location and translocation in cells of S1P produced by SphK1 or SphK2 is different. Serum starvation increases the proportion of SphK2 in the endoplasmic reticulum to enhance cell apoptosis. Consistently, targeting SphK1 to the endoplasmic reticulum converted it from anti-apoptotic to pro-apoptotic (Maceyka et al., 2005). SphK2 distributes in nuclei through its nuclear localization signal (NLS) sequence, and then S1P catalyzed by nuclear SphK2 may induce apoptosis within the nucleus (Igarashi et al., 2003). Another mechanism is that SphK2 contains a 9-amino acid motif similar to the sequence in BH3-only proteins, a pro-apoptotic subgroup of the Bcl-2 family (Chittenden, 2002; Liu et al., 2003). SphK2 interacts with Bcl-XL like the other BH3-only proteins. When the conserved leucine residue present in the BH3 domain (Leu-219) mutates, the apoptosis induced by SphK2 is suppressed, suggesting that the apoptosis induced by SphK2 depends on its BH3 domain (Liu et al., 2003).

On the other hand, paradoxical evidence also shows that SphK2 plays a role in regulating cancer cell proliferation and migration (Gao and Smith, 2011; Venkata et al., 2014) and SphK2 inhibitors may induce apoptosis of cancer cells (Neubauer and Pitson, 2013). In fact, SphK2 activation is one of the endogenous protective mechanisms in both cerebral ischemia and myocardial ischemia (Pfeilschifter et al., 2011; Vessey et al., 2011). SphK2-mediated mechanism is involved in the anti-apoptotic effects of allicin against cerebral ischemia both *in vivo* and *in vitro* (Lin et al., 2015). Overexpressed SphK2 in cardiomyocytes effectively inhibits apoptosis induced by hypoxia/reoxygenation, accompanied by phosphorylation of focal adhesion kinase (FAK) and AKT and decreased activities of caspase-3 and -9. SphK2 induced protection can be abolished by PI3K/AKT inhibitor LY294002, suggesting SphK2 may protect cardiomyocytes against H/R injury via its downstream FAK/AKT signaling pathway (Zhang et al., 2016b). These results suggest the complex regulation of SphK2 on apoptosis under different conditions.

SphK2 in regulation of autophagy

Autophagy is a process that engulfs cell membrane and cytoplasm or other inclusion into the vesicles, which then fuses with lysosome to form autolysosome for degradation. Many components in sphingolipids metabolism such as ceramide, S1P and sphingosine are involved in the regulation of autophagy. As second messengers *in vivo*, ceramide and S1P have been demonstrated to induce autophagy (Pattingre et al., 2009). An increase of S1P level after starvation or overexpression of SphK1 promotes cell survival by inhibiting apoptosis, involving the suppression of mTOR activity and the mild accumulation of Beclin-1 (Scarlatti et al., 2004). Some other studies show that ceramide in the cytoplasm contributes to autophagy activation (Scarlatti et al., 2004; Lavieu et al., 2007). An increase of endogenous ceramide induces a robust accumulation of Beclin-1, resulting in autophagy associated with cell death. Ceramide reverts the IL-13 dependent inhibition of macroautophagy by inhibiting the activity of protein kinase B. Our recent results show that cerebral preconditioning upregulates SphK2 and contributes to autophagy activation to exert neuroprotection (Yung et al., 2012; Sheng et al., 2014). Notably, SphK2-mediated autophagy is not dependent on its catalytic activity, but through the disruption of the Beclin-1/Bcl-2 interaction. As mentioned above, SphK2 is a BH3-only protein that

induces apoptosis (Liu et al., 2003). However, evidence shows that BH3 domain is also involved in autophagy activation. Our further study shows that the BH3 mutation in SphK2 prevents autophagy activation and neuroprotection. SphK2 directly interacts with Bcl-2. Tat-SphK2 BH3 peptide can activate autophagy and protect neural cells from oxygen glucose deprivation (OGD) injury (Song et al., 2017). These findings strongly suggest that SphK2, through its BH3 domain, can dissociate the complex between Beclin-1 and Bcl-2 by interacting with Bcl-2, promoting the release of Beclin-1 to activate autophagy. By contrast, in macrophages, attenuation of SphK activity, particularly SphK2, results in autophagy activation. The contradictory result is partly due to the direct activation of autophagy by the intracellular sphingolipids (Mosser and Edwards, 2008). Exogenous sphingosine can enhance intracellular sphingolipid level, and autophagosome formation. It is assumed that the deactivation of SphK2 may disrupt the network of sphingolipid homeostasis, and then sphingolipid metabolites accumulate to induce compensatory autophagy. All these results imply the complex roles of SphK2 and its metabolites in autophagy activation.

SphK2 in regulation of inflammation

SphK1 is the isoform that can be activated by TNF-α so as to induce the generation of S1P, which plays an essential role in cytokine-induced inflammatory pathways. S1P then binds to TNF-α receptor associated factor (TRAF)-2 and stimulates its E3 ubiquitin ligase activity (Alvarez et al., 2010). S1P is also required for NFκB induction to regulate lymphocyte trafficking and vascular development during inflammation (Tiper et al., 2016).

On the other hand, SphK2 may regulate inflammation in a manner independent of S1P. Interleukin-12 (IL-12) is a critical immunoregulatory cytokine, which plays an important role in cell-mediated immune responses by enhancing differentiation of type 1 T helper (Th-1) cells and promoting interferon-γ (IFN-γ) production. IL-12 shows cytotoxic activity to NK T cells, and facilitates the differentiation of naïve CD4+ T cells into the Th-1 subset of Th cells (Hamza et al., 2010). Mouse SphK2 can bind to IL-12Rβ1 cytoplasmic region and then modulate IL-12 signaling. SphK2 then regulates the tyrosine kinase 2 (TYK2)/signal transducers and activators of transcription 4 (STAT4)/STAT3 pathway, and promotes serine phosphorylation of STAT4 by mitogen-activated protein kinase kinase 6 (MKK6)/p38 mitogen-activated protein kinase (MAPK), which are critical for IFN-γ production. Transient expression of wild-type SphK2 in T cell hybridoma enhanced IL-12-induced STAT4-mediated transcriptional activation and IL-12-induced IFN production, while ectopic expression of dominant-negative SphK2 in Th1 cell clone significantly decreased IL-12-induced IFN-γ production (Yoshimoto et al., 2003).

SphK2 in regulation of mitochondrial function

Some reports imply the regulatory effect of SphK2/S1P on mitochondria. The mitochondrial S1P is mainly produced by SphK2. Prohibitins (PHB) are highly conserved proteins that regulate mitochondrial assembly and function. S1P in the mitochondria binds with high affinity and specificity to PHB2 rather than PHB1. Depletion of SphK2 greatly reduces the interaction between subunit IV of cytochrome-c oxidase and PHB2, leading to a dysfunction in mitochondrial respiration (Strub et al., 2011). S1P also contributes to DNA replication and transcription of mitochondria, and increases mass of mitochondria through S1P receptor 2 (S1PR2) (Shen et al., 2014). In addition, S1P stimulates expression of peroxisome proliferator-activated receptor γ coactivator α (PGC-1α) and its downstream nuclear respiratory factor (NRF) and mitochondrial transcription factor A (TFAM), which are involved in regulation of mitochondrial function. Inhibition of SphK2 decreases the expression of PGC-1α, its downstream NRF and TFAM, leading to reduced intracellular total ATP and superoxide dismutase 2 (SOD2) (Sivasubramanian et al., 2015). Mitochondrial S1P produced by SphK2 regulates the mitochondrial level by mediating permeability transition (PTP) opening and oxidative phosphorylation (Gomez et al., 2011) as well. All these results suggest that SphK2/S1P plays an important role in the maintenance of mitochondrial function.

SphK2 in diseases

From the above, SphK2 is involved in multiple pathways and plays distinct roles in different signals. Consequently, SphK2 may show different effects in varieties of diseases.

SphK2 in cancer

SphK2 in non-small cell lung cancer

Both SphK1 and SphK2 are essential in the pathogenesis of non-small cell lung cancer (Zhu et al., 2015; Wang et al., 2014). SphK2 elevates gradually from normal tissues, dysplasia tissues to non-small cell lung cancer cell (NSCLC) tissues (Wang et al., 2014). Knock down of SphK2 decreases cell proliferation and enhances apoptosis or chemosensitivity of NSCLCs to gefitinib, an inhibitor of epidermal growth factor receptor (EGFR) used in the chemotherapy of lung cancer. By contrast, overexpression of SphK2 promotes chemoresistance and apoptosis resistance in NSCLC (Yang et al., 2015; Liu et al., 2016). Thus, overexpression of SphK2 may be a novel, potent and independent prognostic biomarker of NSCLC patients, while inhibition of SphK2 may become a new strategy for resistant NSCLC cells.

SphK2 in gastrointestinal cancer

Overexpression of SphK2 is observed in colorectal cancer (CRC) cells. SphK2 depletion by specific small interfering RNA (siRNA) reduces cell proliferation and migration in CRC cells (Zhang et al., 2016a). Similarly, the inhibition of SphK2 by ABC294640 suppresses the growth of CRC cells both *in vitro* and *in vivo* and induces apoptosis in transformed and primary CRC cells (Xun et al., 2015). However, SphK1 may be upregulated in SphK2 knockout mice leading to severe chronic intestinal inflammation and colitis-associated cancer in a CRC model *in vivo* (Liang et al., 2013). Thus, inhibition of SphK2 as well as SphK1 may be beneficial for the treatment of CRC. Extracellular signal regulating kinase (ERK)/PKD pathway regulates the export of SphK2 from nuclei to regulate cell apoptosis. Sodium butyrate shows anti-tumor effects in colon cancer cells, but exposure to sodium butyrate in human colon cancer cell (HCT116) can activate the phosphorylation cascade of ERK/PKD/SphK2 to suppress apoptosis (Xiao et al., 2014). Thereby, inhibition of ERK/PKD/SphK2 may sensitize colon cancer cells to sodium butyrate. Similarly, down-regulation of SphK2 enhances the action of all-trans retinoic acid (ATRA) on colon cancer cells, indicating that inhibition of SphK2 may become a synergistic therapeutic strategy when ATRA is used in the treatment of cancer (Chu et al., 2014).

SphK2 in breast cancer

S1P generated by SphK2 can prevent nuclear translocation of c-Src and tyrosine 416 phosphorylation of S1P receptor subtype 2 (S1P2) by binding to S1P4 (Ohotski et al., 2014). SphK2 then promotes growth of estrogen receptor negative breast cancer cells. Insulin can activate phosphorylation of two SphK isoforms simultaneously with similar dose dependent manner. SphK1 and SphK2 show equal effects in insulin induced mitosis in breast cancer cells (Dai et al., 2014b), suggesting that the two isoenzymes have overlapped or complementary functions in the pathophysiology of breast cancer. In MCF-7 and the estrogen (E2) receptor (ER) transfected HEK293 cells (ER-transfected HEK293 cells), SphK2 inhibitor ABC294640 reduces progesterone receptor gene by E2-mediated regulation of ER. ABC294640 binds to the ER antagonist ligand-binding domain, playing a similar role with tamoxifen. After treatment with ABC294640 for 15 days, the tumor volume decreased by 68.4%, showing obvious anti-tumor effects (Antoon et al., 2010).

SphK2 in prostate cancer

Inhibition of SphK2 with ABC294640 can restrain the proliferation and survival of prostate cancer cells with androgen resistance (Gestaut et al., 2014). Low dose of ABC294640 suppresses cell viability without apoptosis response, suggesting the anti- proliferative mechanism is independent of the intrinsic apoptosis pathway. It is likely that either high dose of ABC294640 has an effect on apoptosis or SphK2 does not mediate apoptosis in endocrine therapy resistant prostate cancer cells. Besides, ABC294640 can suppress proliferation of castration-resistant prostate cancer cells via inhibiting SphK2 (Venant et al., 2015).

SphK2 in lymphoblastic leukemia, lymphoma and multiple myeloma

Myelocytomatosis viral oncogene (MYC) plays an important role in hematologic malignancies. SphK2 regulates the expression of MYC to show oncogenesis in acute lymphoblastic leukemia (ALL) (Wallington-Beddoe et al., 2014). Both genetic and pharmacological inhibition of SphK2 can relieve ALL. Besides, sphingosine kinase inhibitors SKi and ROMe reduce cell viability of T-cell acute lymphoblastic leukemia cell and primary cell of human patients (Evangelisti et al., 2014). SphK2 is also overexpressed in myeloma cells, and promotes survival and proliferation of myeloma cells. SphK2 specific inhibitor ABC294640 promotes degradation of Mcl-l and c-Myc protease to suppress the growth of myeloma both *in vivo* and *in vitro* and induces apoptosis of multiple myeloma cells (Venkata et al., 2014). In addition, targeting SphK2 induces apoptosis of primary exudative lymphoma cell lines (PEL) infected by Kaposi's sarcoma-associated herpesvirus (KSHV) and selective apoptosis of endothelial cells infected with KSHV by promoting the expression of viral lytic gene (Dai et al., 2014a). These results suggest that SphK2 plays an important role in the ontogenesis of lymphoblastic leukemia, lymphoma and multiple myeloma, while SphK2 inhibitors might become a novel chemotherapy of these diseases.

SphK2 involved in cancer therapy

From the above mentioned, SphK2/S1P signaling is involved in the malignancy and invasion of a variety of cancers. SphK2/S1P is of great importance in epidermal growth factor (EGF)-mediated ezrin-radixin-moesin (ERM) phosphorylation in Hela cells, essential to EGF-induced invasion. Overexpression of SphK2 increases the invasion of Hela cells 2-fold. Thus, SphK2 presents a new target for cancer malignancies and invasion (Adada et al., 2015). Besides, evidence also shows that SphK2/S1P signaling may decrease the sensitivity of tumor cells to chemotherapy (Yang et al., 2015; Liu et al., 2016). Pan SphK2 inhibitors that competitively inhibit SphK1 and SphK2 are often sphingosine analogues, including D-erythro-, N-dimethylsphingosine (D-erythro-DMS) and D-erythro-dihydrosphingosine (D-erythro-DHS). Both agents show promising anti-tumor effects to inhibit growth and to promote apoptosis of a variety of cancers. SphK2 inhibitors such as ABC294640, SG-12 and K145 may be used in the therapy of cancer by selective reduction of SphK2

activity in malignant tumors. ABC294640, a high micromolar inhibitor of SphK2, which demonstrated anticancer activity in many preclinical models, is now under investigation in clinical trials. However, its off target effects including antagonizing estrogen receptor (Antoon et al., 2010), inhibition of dihydroceramide desaturase (Venant et al., 2015) and inducing the proteasomal degradation of SphK1 (McNaughton et al., 2016) complicate its mechanisms in disease models. SG-12, a synthetic sphingosine analogue, functions as a novel SphK2 inhibitor. SG-12 is able to promote Fas-mediated cell death of the murine B lymphoma-derived A20/2J cell line, which is relieved by transfection of SphK2 rather than SphK1. It has been demonstrated that SG-12 serves as a SphK2 specific inhibitor to induce apoptosis after being phosphorylated by SphK2. Thus, SG-12 provides a novel perspective for the treatment of SphK2 associated tumor (Hara-Yokoyama et al., 2013). Recently, some more potent and selective SphK2 inhibitors such as SLP120701 (Patwardhan et al., 2015) and SLM6031434 (Kharel et al., 2015) have been reported.

SphK2 in brain

SphK2 in neurodegenerative disorders

Disturbance of sphingolipid metabolism plays a key role in the pathophysiology of neurodegenerative disorders including Alzheimer' s disease (AD), Parkinson's disease (PD) and Huntington's disease (HD). In the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced PD rodent and cellular models, the expression of both SphK1 and SphK2 is significantly downregulated (Pyszko and Strosznajder, 2014). SphK2 is predominantly present in the mitochondria of neurons. Depletion of SphK2 reduces PGC-1 α and its downstream NRF-1 and TFAM, leading to a significant reduction in cellular ATP and increased reactive oxygen species (ROS). Exogenous S1P shows neuroprotection against MPP+ models via activation of p - CREB, PGC-1 α, NRF-1, restoration of ATP and reduction of ROS (Sivasubramanian et al., 2015). Similarly, declined SphK1 and SphK2 activity and loss of S1P is shown in AD pathogenesis and prior to AD diagnosis (Couttas et al., 2014). Amyloid-β peptide (Aβ) is the pathogenic protein causative for Alzheimer disease (AD). Endogenously liberated Aβ significantly decreases the expression and activity of SphK1/2. Inhibition of SphK(s) decreases neuronal survival, whereas exogenous S1P shows a cytoprotective effect dependent on Aβ peptides (Gassowska et al., 2014). S1P receptor agonist FTY720 and FTY720-P can protect neurons against oligomeric Aβ-induced neurotoxicity (Doi et al., 2013) and prevent AD rats from impairment of spatial learning and memory (Hemmati et al., 2013). The neuroprotective effects of FTY720 and FTY720-P may be mediated by upregulated neuronal brain-derived neurotrophic factor (BDNF) or decreased production of Aβ (Takasugi et al., 2013). These results suggest that SphK2/S1P plays a protective role in PD and AD. However, SphK2 seems to mediate neurotoxicity in the pathogenesis of HD. Overexpression of SphK2 in the nucleus is neurotoxic to cultured cortical and striatal neurons in a dose- dependent manner and leads to DNA double-stranded breaks. SphK2 inhibitor ABC294640 can mitigate DNA damage and neurotoxicity, and show neuroprotection in neuron models of HD (Moruno-Manchon et al., 2017). One possible mechanism for the contradictory effects of SphK2 in PD and HD may be due to the intracellular localization of SphK2. In neurons, SphK2 can be expressed in the cytoplasm, mitochondria, and nucleus under physiological or pathological conditions (Chakraborty et al., 2012; Hasegawa et al., 2013; Sivasubramanian et al., 2015; Moruno-Manchon et al., 2017). The different intracellular localization of SphK2 may mediate different biological effects through a distinct signaling pathway. It is assumed that SphK2/S1P pathway in mitochondria or cytoplasm may be protective to cell survival, whereas nuclear SphK2 may lead to apoptosis and neurotoxicity.

SphK2 in stroke and preconditioning

SphK2 is the main isoform to synthesize S1P in normal brain parenchyma including cerebellum, cortex and brain stem (Blondeau et al., 2007). The expression of SphK2 mRNA was quickly upregulated (within 30 min) in the cortex instead of striatum after experimental MCAO (Blondeau et al., 2007), then SphK2 was found to be decreased in the striatum (ischemic core) and preserved in the cortex (penumbra) at 24 hours. Besides, S1P1 and SphK1 were also decreased in the infarct cortex but preserved in the periinfarct cortex after tMCAO. FTY720 significantly improved neurological function and brain injury possibly via S1P1 activation, suggesting that activation of the sphingosine metabolic pathway may be neuroprotective in cerebral ischemia (Hasegawa et al., 2013).

On the other hand, cerebral preconditioning provides insights into the mechanisms that protect the brain against ischemic injury. Evidence shows that cerebral preconditioning upregulates SphK2 in the brain. The dysfunction of blood-brain barrier (BBB) and the resultant vasogenic edema is a feature of stroke and protection of BBB is associated with improved outcome in stroke. Giddy's lab found that SphK2 activity plays a critical rolein the BBB protection afforded by preconditioning through S1P in microvascular endothelial cells. SphK2 then regulates adherens junction protein VE-cadherin, tight junction proteins claudin-5, occludin, and zona occludens-1 (ZO-1) to protect BBB damage caused by ischemia (Wacker et al., 2012). In addition, by using genetic and pharmacological approaches, Waeber's group found that SphK2 but not SphK1 is upregulated after isoflurane preconditioning and hypoxic preconditioning in mice brain. SphK2

mediates cerebral preconditioning to protect the neurons against ischemic injury (Yung et al., 2012). These results suggest that the SphK2 isoform plays an important role in cerebral preconditioning.

Autophagy might contribute to SphK2 mediated cerebral preconditioning. Preconditioning induces autophagy activation in primary cultured murine cortical neurons, whereas SphK2 inhibitor (ABC294640 and SKI- II) or knock down of SphK2 prevent preconditioning induced autophagy. SphK2 may activate autophagy in response to preconditioning by a mechanism that interferes with the interaction of Beclin-1/Bcl-2 (Sheng et al., 2014). Another mechanism of SphK2 during preconditioning is related to S1P. Mitochondrial S1P generated by SphK2 could regulate mitochondrial permeability transition pore (MPTP) and oxidative phosphorylation (Gomez et al., 2011). SphK2 knockout mitochondria results in the reduction of oxidative phosphorylation and increased susceptibility to PTP (Waeber and Walther, 2014). The preconditioning induced cardioprotection was abrogated by SphK2 knockdown concomitant with decreased PTP resistance, suggesting mitochondrial SphK2/S1P is required for the protective modulation against MPTP as an effector of preconditioning.

SphK2 in regulation of memories

Both the pharmacological and genetic approaches have demonstrated that histone deacetylases (HDACs) affect memory and learning processes (Stefanko et al., 2009; Morris et al., 2010). SphK2, expressed in the nuclei of various cells, produces nuclear S1P that specifically binds to HDAC1 and HDAC2. Then HDACs' enzymatic activities are inhibited to increase histone acetylation, linking nuclear S1P to epigenetic regulation of gene expression and memory regulation (Hait et al., 2009). In SphK2$^{-/-}$ mice, the levels of S1P and dihydro-S1P in the hippocampus are lower than those in wild type. A lack of SphK2 gene significantly decreased hippocampal histone acetylation on specific lysine residues (H3K9, H4K5) that are linked to the impairment of memory and learning deficits. By contrast, FTY720 can be phosphorylated by SphK2 in the nucleus to inhibit hippocampal HDACs. FTY720 then enhance histone acetylation to facilitate fear extinction in severe combined immunodeficiency (SCID) mice. Thus, SphK2 plays a specific role in the functions of memory and FTY720 might provide an effective complementary therapy to eliminate horrific memories (Hait et al., 2014). Similarly, a higher level of SphK2 is expressed in spinal cord compared to SphK1. SphK2$^{-/-}$ mice shows substantially lower spinal S1P levels. Deficiency of SphK2 not only facilitates nociceptive transmission during the late response, but also causes bilateral increase in mechanical sensitivity by chronic peripheral inflammation. These results suggest that SphK2 is also involved in pain memory formation and facilitation of nociceptive circuits in the central nervous system (Canlas et al., 2015).

SphK2 in cardiovascular diseases

SphK2 in atherosclerosis

High-density lipoprotein (HDL) is a significant lipoprotein showing cardioprotection in atherosclerosis. HDL activates prostacyclin I-2 (PGI-2) release via inducing the expression of cyclooxygenase-2 (COX-2) in endothelial cells to exert antiatherogenic effects (Bolli et al., 2002; Liu et al., 2011). Gene silence or pharmacological inhibition of SphK2 inhibits the activation of COX-2/PGI-2 induced by HDL in human umbilical vein endothelial cells (HUVECs), suggesting that HDL induces the expression of COX-2 and release of PGI-2 via activating PKC/ERK/SphK2 pathway. SphK2 activated by HDL is accompanied by increased nuclear S1P level, and HDL-bounded S1P elicits survival and migration of endothelial cells. Chip analysis demonstrates SphK2 is associated with the c-AMP response element binding (CREB) protein in COX-2 promoter region to induce COX-2 expression and PGI-2 release in endothelial cells (Xiong et al., 2014). These findings implicate novel anti-atherothrombotic effects of SphK2.

SphK2 in platelet activation and thrombosis

SphK2 is the main sphingosine kinase isoform in human megakaryocyte (MKs) and provides the resource of intracellular S1P that controls platelet generation and aggregation. S1P generated by SphK2 induces aggregation of platelets via S1PR1. SphK2 also upregulates the expression and activation of Src family kinase (SFKs) (Zhang et al., 2013). Loss of SphK2 and inhibition of SFK activity results in defective thrombopoiesis. Consequently, loss of SphK2 partially protects against arterial thrombosis after vascular injury (Urtz et al., 2015). Thus targeting SphK2 pathway could present a novel therapy to prevent thrombosis.

SphK2 in hepatic nutrient metabolism and hepatocyte regeneration

Bile acids are important hormones that allow the coordinate regulation of liver to nutrient metabolism. Hepatic SphK2 is markedly upregulated in mice fed with high-fat diet (HFD) or rats with chronic biliary fistula treated with taurocholate. The overexpression of gene encoding S1P recptor 2 (S1PR2) in murine hepatocyte can also upregulate SphK2. Conjugated bile acid (CBA) returning from the intestines activates S1PR2, which in turn activates nuclear SphK2 and then elevates S1P in nucleus. Nuclear S1P subsequently upregulates genes encoding nuclear receptors and enzymes associated with nutrient metabolism. Thereby, CBA and S1PR2 are the key regulators of SphK2 and hepatic genetic expression (Nagahashi et al., 2015), and nuclear SphK2/S1P plays a

important role in the epigenetic mechanism that underlie the sterol and lipid metabolism. Further study shows that hepatic overexpression of SphK2 in mice fed with HFD not only increases S1P, but also results in the reduction of ceramide, sphingomyelin and glucosylceramide in plasma and liver. SphK2 also up-regulates the fatty acid (FA) oxidizing genes, increases FA oxidation and reduces lipid droplets accumulation in liver induced by HFD feeding. In addition, SphK2 ameliorates glucose intolerance and insulin resistance by improved hepatic insulin signaling. These results suggest that SphK2 helps to ameliorate hepatic steatosis and insulin resistance (Lee et al., 2015). Exosomes are small membrane vesicles involved in intercellular communication. Recent studies shows that exosome derived from hepatocyte transports neutral SphK2 and ceramidase to increase the synthesis of S1P in target hepatocytes, contributing to cell proliferation and liver regeneration during ischemia/reperfusion (I/R) injury. These data implicate that SphK2/S1P signaling is involved in hepatocyte regeneration and hepatocyte-derived exosomes may become a novel therapeutic method for liver ischemia/reperfusion (Nojima et al., 2016).

SphK2 in inflammatory disease

As mentioned above, sphingosine kinase is of great importance to activate immune cells and to induce inflammation, so it is involved in many inflammatory diseases such as lupus nephritis, glomerular disease and arthritis. ABC294640, a selective inhibitor of SphK2, decreases liver injury and prevents acute liver failure after hepatic ischemia/reperfusion via inhibition of inflammation (Liu et al., 2012). Inhibition of SphK also potentially protects against lupus nephritis (LN). Dihydro-S1P (dh-S1P) obviously increases in the serum and kidney of MRL/MpJ-Faslpr/2J (MRL/lpr) mice (a murine model of LN). Dh-S1P can be phosphorylated by SphK2 more easily than SphK1. The elevations of S1P and dh-S1P in circulation are obviously decreased in

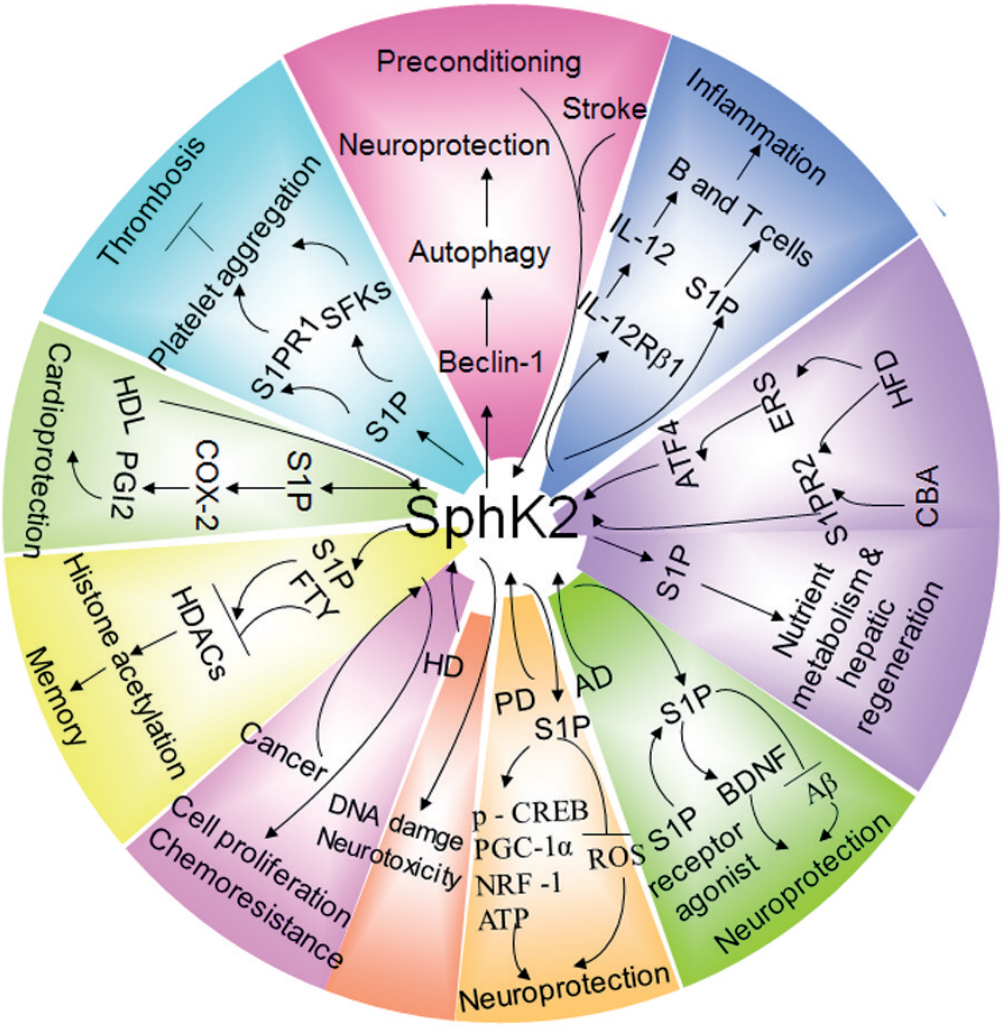

Fig. 2. The function and regulation of SphK2 in diseases. Preconditioning upregulates SphK2 to activate autophagy and to protect against ischemic injury. The SphK2 pathway is involved in cancer cell proliferation and chemoresistance. SphK2 might induce inflammation by activating immune cells. SphK2 participates in platelet aggregation and thrombosis. HDL induces the expression of COX-2 and release of PGI-2 in endothelial cells via activating SphK2 pathway to mediate cardioprotection. S1P produced by SphK2 in the nucleus binds to HDACs, which then inhibit histone acetylation and regulate memory. HFD and CBA can activate SphK2, resulting in the increase of S1P, and then helps to ameliorate hepatic steatosis. S1P or S1P receptor agonist exerts neuroprotection in PD or AD, while overexpression of SphK2 in nucleus can induce DNA damage and neurotoxicity.

mice treated with ABC294640 in murine LN model. ABC294640 also effectively reduces progression of glomerular disease, and reduces B and T cells accumulated in spleen. These results suggest that inhibition of SphK2 isoform could prevent the increase in S1P and dh-S1P and potentially protect against LN (Snider et al., 2013). Besides, selective inhibition or knockout of SphK2 also effectively blocks kidney fibrosis induced by folic acid (FA) or unilateral ischemia-reperfusion injury, partly due to decreased immune cell infiltration and expression of inflammatory markers in kidney. SphK2 deficiency results in more IFN-γ production to upregulate IFN-γ-responsive genes (Cxcl9 and Cxcl10) in kidney to protect fibrosis. Thus SphK2 inhibition may serve as a novel therapeutic approach for attenuating kidney fibrosis via IFN-γ (Bajwa et al., 2017). However, in TNF-α induced arthritis, pharmacological inhibition of SphK2 can deteriorate arthritis, while genetic inhibition of SphK2 makes no influence on the progression of rheumatoid arthritis (Baker et al., 2013; Xu et al., 2014). The difference may be due to a compensatory mechanism, since the mice with genetic deficiency of SphK2 may compensate for a SphK2 deficiency with additional sphingosine kinases. For example, genetic compensation is shown in SphK2 null mice that are able to phophorylate S1P pro-drugs and to cause lymphopenia, in the absence of SphK2 (Kharel et al., 2005). All these data suggest the controversial roles of SphKs in inflammatory diseases.

Conclusion and future perspectives

Mounting evidence implies that SphK2, present in different tissues with distinct subcellular location, is involved in the regulation of multiple signaling pathways. Thus SphK2 is likely to take part in the pathogenesis of multiple diseases including cancer, cardiovascular diseases, neurodegenerative disorders, preconditioning and inflammation. SphK2 promotes apoptosis or cancer proliferation, while inhibition of SphK2 might suppress the growth or induce apoptosis of cancer cells to restrict cancer development and progression. SphK2/S1P pathway is involved in the pathogenesis of neurodegenerative disorders and stroke, while SphK2 can also participate in preconditioning to protect the heart or brain from ischemic injury. SphK2 may induce inflammation by activating immune cells. SphK2 pathway mediates platelet aggregation, thrombosis and cardioprotection (Fig. 2). Thus SphK2 may provide novel therapeutic strategies for various diseases. For example, SphK2 inhibitors such as ABC294640, SG-12 and K145 may be used in the therapy of malignant tumors, lupus nephritis or arterial thrombosis. By contrast, agents that activate or mimic the function of SphK2 such as S1P, FTY720, FTY720-P or bioactive peptide might elicit protection to prevent cadiocerebrovascular diseases or neurological disorders. In summary, how the SphK2 pathway participates in diseases still needs to be further elucidated and this is an expanding area of research with increasing significance.

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