The role of sphingolipid signalling in diabetes-associated pathologies (Review)

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Abstract. Sphingosine kinase (SphK) is an important signalling enzyme that catalyses the phosphorylation of sphingosine (Sph) to form sphingosine-1-phosphate (S1P). The multifunctional lipid, S1P binds to a family of five G protein-coupled receptors (GPCRs). As an intracellular second messenger, S1P activates key signalling cascades responsible for the maintenance of sphingolipid metabolism, and has been implicated in the progression of cancer, and the development of other inflammatory and metabolic diseases. SphK and S1P are critical molecules involved in the regulation of various cellular metabolic processes, such as cell proliferation, survival, apoptosis, adhesion and migration. There is strong evidence supporting the critical roles of SphK and S1P in the progression of diabetes mellitus, including insulin sensitivity and insulin secretion, pancreatic β-cell apoptosis, and the development of diabetic inflammatory state. In this review, we summarise the current state of knowledge for SphK/S1P signalling effects, associated with the development of insulin resistance, pancreatic β-cell death and the vascular complications of diabetes mellitus.

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1. Introduction

Diabetes mellitus is one of the most prevalent metabolic diseases, affecting 347 million individuals worldwide (1). This heterogeneous disease ultimately arises from either the failure of pancreatic β cells to produce insulin, and/or the development of insulin resistance in peripheral tissues (2). Type 1 diabetes (T1D; juvenile-onset, approximately 10% of all patients with diabetes) is often caused by an autoimmune attack on pancreatic β cells, resulting in the loss of insulin secretion. T1D represents the insulin-dependent form of diabetes, requiring daily insulin therapy. Type 2 diabetes (T2D; adult-onset, 90% of all patients with diabetes) is caused by insulin resistance, associated with relative hyperinsulinemia. T2D is usually a non-insulin-dependent form of diabetes. Nevertheless, it requires active and often complex therapeutic interventions. Obesity and associated inflammation are common risk factors for T2D (3,4). Aberrant lipid metabolism and signalling are tightly interconnected with the pathogenesis of obesity, inflammation and diabetes. In this review, we highlight the mechanisms through which the key signalling sphingolipid molecule, sphingosine-1-phosphate (S1P), and S1P-producing enzyme sphingosine kinase (SphK) have been shown to affect diabetes-related pathologies.

There are two major isoforms of SphK (SphK1 and SphK2), each having diverse and compensatory biological functions (Figs. 1 and 2) (5). Both SphKs can phosphorylate sphingosine (Sph) to form S1P, thus activating a variety of extracellular and intracellular signalling mechanisms. Controlled by SphKs, the conversion of pro-apoptotic Sph into the pro-survival molecule, S1P, maintains the equilibrium amid opposing cellular functionsm, such as cell growth, proliferation, secretion and migration on the one side; and apoptosis, senescence, autophagy and growth arrest on the other (6,7). This balance, known as the 'sphingolipid rheostat', has been suggested to

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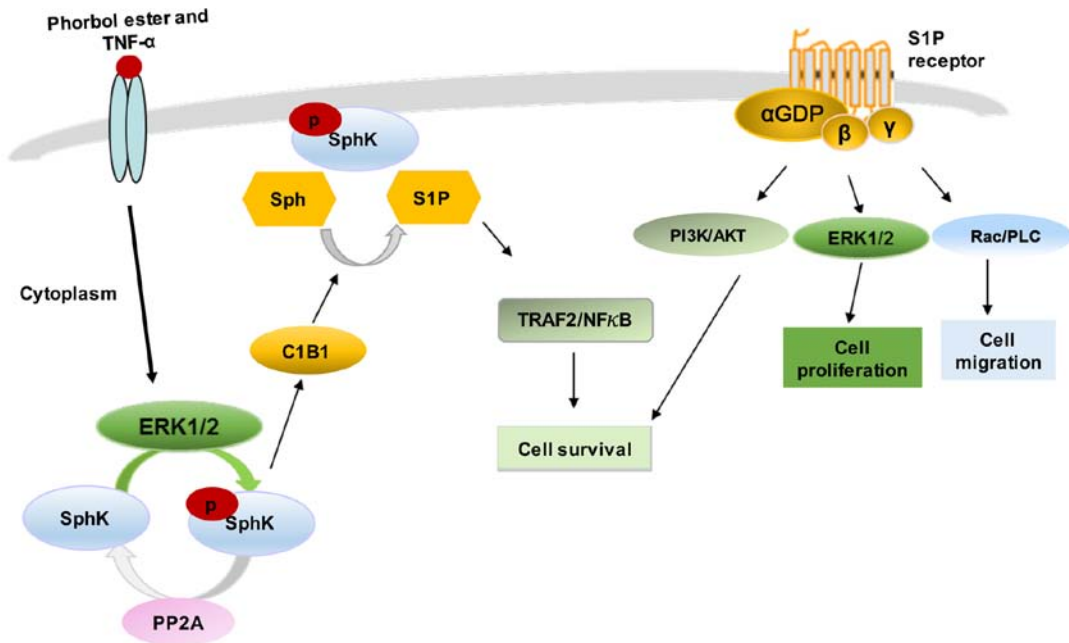E-mail: nmeili@usm.my

Figure 1. Regulation of SphK1 signalling. SphK1 is located mainly in the cytoplasm. Various agonists (such as phorbol ester and TNF-α) induce activation of SphK1 phosphorylation, activation and translocation to plasma membrane. This relocation is mediated by interaction with CIB1. S1P is exported from the cells and binds to S1P receptors (S1P$_{1-5}$) to activate classical GPCR signalling pathways, leading to control of cell survival, proliferation and migration. Alternatively, S1P binds to TRAF2 intracellularly and activates the NF-κB pathway and its downstream targets. SphK, sphingosine kinase; CIB1, calcium and integrin-binding protein 1; S1P, sphingosine-1-phosphate; GPCR, G protein-coupled receptor; TRAF2, TNF receptor-associated factor 2; NF-κB, nuclear factor κ-light-chain-enhancer of activated B cells; PLC, phospholipase C; PP2A, protein phosphatase-2A.
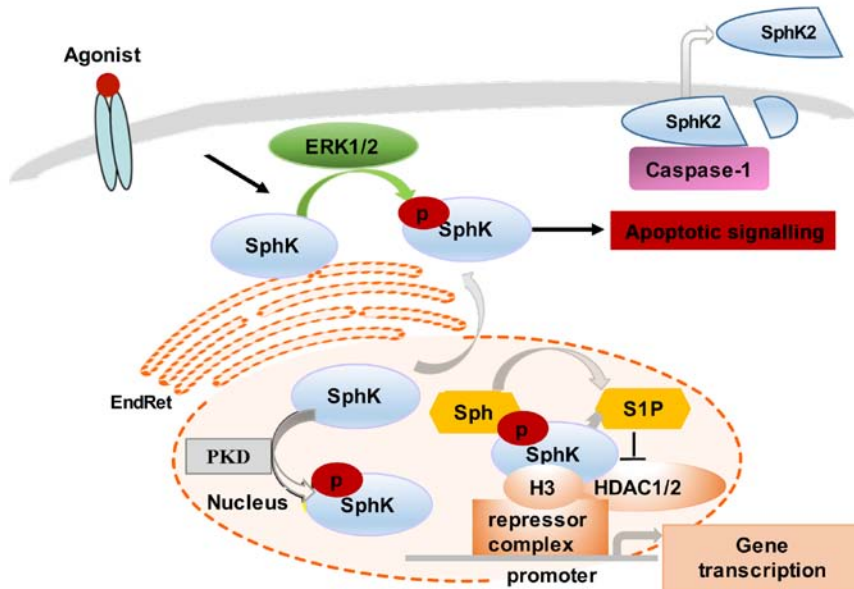

Figure 2. Regulation and SphK2 signalling. Several agonists (such as EGF and cross-linking of FcεRI) stimulate SphK2 activation via ERK1/2-mediated phosphorylation. SphK2 in the EndRet induces apoptotic signalling through localised generation of S1P. The release of active SphK2 can also occur via caspase-1-mediated cleavage and allows extracellular generation of S1P. SphK2 localization in the nucleus is regulated via PKD-mediated activation of nuclear export signals. Nuclear SphK2 generates S1P that regulates histone acetylation. S1P specifically binds to the HDAC1/2 and inhibits their enzymatic activity. SphK2 associates with HDAC1/2 in repressor complex at promoters of the genes, where it enhances local H3 acetylation and promotes gene transcription. SphK, sphingosine kinase; EGF, epidermal growth factor; EndRet, endoplasmic reticulum; S1P, sphingosine-1-phosphate; PKD, protein kinase D; HDAC1/2, histone deacetylase 1 and 2; H3, histone 3; Sph, sphingosine.

be critical to cell fate (8,9). For example, tipping the balance in favour of Sph accumulation may cause insulin resistance, whereas an increased S1P level has been shown to promote insulin action (10-12).

The highly bioactive lipid, S1P, is involved in maintaining metabolic stability; however, it can also mediate the development of serious pathological conditions (13-15). S1P binds specifically to five (S1P$_{1-5}$) transmembrane G protein-coupled

receptors (GPCRs) (16) and activates cellular responses via S1P receptor-mediated mechanisms and/or by targeting a complex network of intracellular messengers. The biological actions of S1P are cell type- and receptor subtype-specific as reviewed previously (17). In this review, we summarise the recent evidence implicating SphKs/S1P signalling in diabetes-associated intracellular abnormalities and metabolic aberrations.

2. Divergence of SphK isoforms

The SphK isoforms (Sphk1 and SphK2) are structurally related, with five highly conserved domains (C1 to C5), although they differ in size, intracellular localization and function. SphK isoforms are encoded by two different genes, *SPHK1* (chromosome 17, cytoband q25.1) and *SPHK2* (chromosome 19, cytoband q13.33). SphK1 is a 48 kDa protein first purified from rat kidney cells (18). There are three splice isoforms of SphK1 (1a, b and c); all are cytosolic proteins differing slightly in subcellular distribution. SphK2 is larger in size (69 kDa) and has sequence homology to SphK1. There are two recently discovered splice isoforms of SphK2 (a and b) (18). SphK2 contains an extended N-terminal region with a proline-rich polypeptide insertion and several other unique sites within the N-terminal sequence. The N-terminal region of SphK2 includes a nuclear export sequence (NES), important for shuttling the enzyme between the nucleus and cytoplasm. The SphK2 sulphite-binding site facilitates the membrane localization of SphK2 (19), while the caspase-1 cleavage site regulates SphK2 maturation and secretion from cells during the induction of apoptosis (21).

Furthermore, SphK isoforms differ in developmental expression, tissue distribution and subcellular localization (5,6). SphK1 predominates in the lungs and spleen (7,8,11), whereas SphK2 is more common in the heart, brain and liver (9,10,12,13). Notably, SphK1 and SphK2 have been shown to regulate different intracellular processes. For instance, SphK1 promotes cell survival and proliferation, whereas SphK2 is involved in the induction of apoptosis and cell growth arrest (22). The divergent roles of SphK isoforms in diabetes-related pathologies will be discussed in greater detail below.

3. Mechanisms of SphK activation and subcellular localization

The different steady-state localization of the SphK isoforms corresponds to the specific intracellular functioning of the enzymes. SphK1/2 are redistributed to distinct intracellular sites in an agonist-dependent manner. SphK1/2 substrates (Sph and dihydrosphingosine) and product (S1P) are lipids and therefore, the subcellular membrane localization of SphK in close proximity to substrates is necessary for the enzyme to fulfill its housekeeping and signalling functions.

SphK localization to specific intracellular compartments is critical to the functional consequences of signalling, such as the stimulation of cancer cell growth (23). Under basal conditions, SphK1 predominates in the cytosol where it maintains low levels of intracellular S1P required for normal cell metabolism (24). It has been documented that the translocation of SphK1 to the plasma membrane is required for its oncogenic effect (23). However, the targeting of SphK1 to a specialised

subcellular compartment enables its regulation of different functions. For example, the translocation of SphK1 to the endoplasmic reticulum (EndRet) promotes cell apoptosis (25), whereas the translocation of SphK1 to the nuclear envelope promotes G1/S transition during cell division (22).

Various stimuli, such as growth factors and cytokines can activate SphK1 by phosphorylation of the enzyme at Ser-225, mediated by mitogen-activated protein kinase (MAPK) ERK1/2 (20,21,26,27). This phosphorylation promotes SphK1 to undergo conformational changes accompanied by a rapid increase in the catalytic activity of the enzyme and its subcellular translocation from the cytosol to plasma membrane (26). The continuous retention of SphK1 at the plasma membrane requires binding to phosphatidylserine or phosphatidic acid (20,21,27). In addition to phosphorylation, SphK1 membrane translocation can be also induced by protein-protein interactions (28). SphK1 contains a calmodulin-binding site that binds calcium and integrin-binding protein 1 (CIB1) in a calcium-dependent manner. CIB1 functions as a calcium-myristoyl switch, providing a novel mechanism for SphK1 translocation to the plasma membrane (28).

In comparison to SphK1, SphK2 function-associated localization is less well understood. However, it has been shown that SphK2 can be found both in the nucleus and the cytoplasm, shuttling between these two compartments (28). Similar to SphK1, SphK2 cellular levels and distribution are cell type-specific, agonist-dependent and modifiable by cell culture conditions. For example, SphK2 translocates to the EndRet following serum starvation, which coincides with the induction of apoptosis (29-31). Notably, SphK2 can be released from apoptotic cells by caspase-1-mediated cleavage at its amino terminus (30) (Fig. 2).

Several stimuli, such as epidermal growth factor (EGF) and phorbol ester (PMA) (protein kinase C stimulant) activate SphK2 through MAPK ERK1-mediated phosphorylation at Ser-351 and/or Thr-578 (32). The EGF-induced phosphorylation and activation of SphK2 has been linked to breast cancer cell migration and to the increased invasive capacity of tumor cells (32). The nuclear localization of SphK2 is required for the epigenetic regulation of specific target genes. For instance, SphK2 produces nuclear S1P that binds and, thus, inhibits histone deacetylase 1 and 2 (HDAC1/2) activity, preventing the deacetylation of histone 3. Nuclear SphK2 may also regulate cyclin-dependent kinase inhibitor p21 and transcription regulator c-*fos* activity (15,29). The overexpression of SphK2 has been shown to stimulate the PMA-induced expression of c-*fos* mRNA, and, thus, indirectly influence a large group of genes controlled by c-*fos* (15,29). The SphK2 regulation of HDAC1-dependent deacetylation of histone H3 also results in repression of *p21* gene transcription, thus interfering with cell cycle progression and cellular senescence. The nuclear signalling of SphK is regulated by protein kinase D (PKD)-induced phosphorylation, which promotes its export from the nucleus (30).

4. Dual mechanisms of S1P signalling: extracellular and intracellular effects

The multifunctional signalling lipid, S1P, mediates the effects of numerous biological stimuli, including cytokines, growth factors and hormones (8,33). S1P regulates diverse signalling
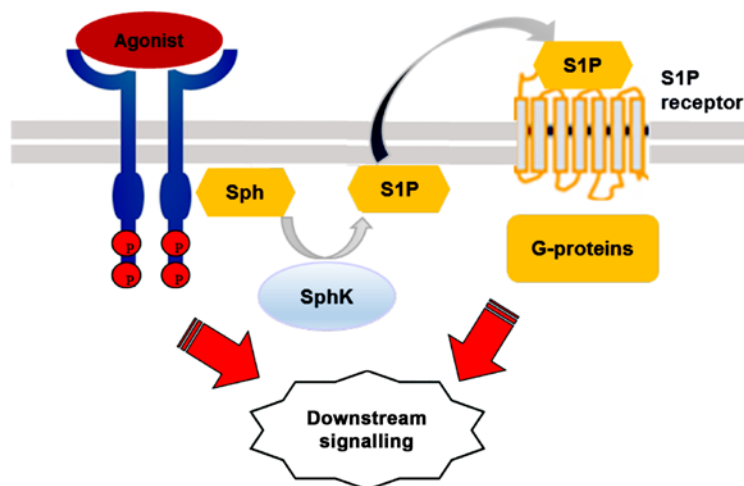
Figure 3. SphK/S1P pathway 'inside-out' signalling model. Various biologically stimulatory molecules bind and activate receptor tyrosine kinase that, in turn, induces the translocation of SphK1 from the cytosole to the plasma membrane. S1P is then locally produced and secreted from the cells. Extracellular S1P binds and activates S1P receptors to initiate further intracellular downstream signaling cascades such as cell proliferation, migration, and invasion. SphK, sphingosine kinase; S1P, sphingosine-1-phosphate; Sph, sphingosine.

via five transmembrane GPCRs, $S1P_{1-5}$, originally known as endothelial differentiation gene (EDG) receptors (34). S1P may bind its receptors in a paracrine or autocrine manner, followed by differential coupling to specific G proteins (34).

According to the 'inside-out' signalling model, activated SphK1 is translocated to the plasma membrane where it generates S1P. The lipid is then released locally and, in an autocrine manner, binds to one or more S1P receptor subtypes on the same cell, or, in a paracrine manner, activates the receptors on neighbouring cells (33). Activated SphK1 may also be secreted from cells to produce S1P from extracellular Sph (35).

Amongst its intracellular targets, S1P has been shown to interact with several cytoplasmic and nuclear proteins (33), including HDAC1/2, which is involved in regulating histone acetylation and the epigenetic regulation of specific target genes, such as p21 and c-fos (36). S1P can also act as a co-factor, stimulating E3 ligase activity in TNF receptor-associated factor 2 (TRAF2) and controlling the survival response (37). Moreover, S1P interacts with inner mitochondrial membrane protein prohibitin 2 (PHB2) to regulate cytochrome *c* oxidase assembly and mitochondrial respiration (38). Finally, S1P has been shown to bind the transcription factor peroxisome proliferator-activated receptor γ (PPARγ) (39), human telomerase reverse transcriptase (hTERT) (40) and beta-site amyloid precursor protein-cleaving enzyme 1 (BACE1) (41). The essential cellular effects of S1P are summarised in Fig. 3 and were recently reviewed in full detail elsewhere (42).

5. The role of SphK/S1P signalling in the development of diabetes and related pathologies

SphK signalling and hepatic insulin resistance. Reduced sensitivity to insulin of the hormone target tissues, such as skeletal muscle, liver and adipose deposits is defined as insulin resistance. Hepatic insulin resistance has been confirmed as the major risk factor for T2D onset. Previous studies have shown that SphK activation improves hepatic insulin signalling in obesity and diabetes (43-45,47). Notably, in a previous

study, low total SphK activity was detected in the livers of mice fed a high-fat diet (HFD). The mice also had elevated liver triacylglycerol (TAG) and diacylglycerol (DAG) levels, and demonstrated glucose intolerance. SphK1 overexpression in the liver reduced hepatic TAG synthesis and the total TAG content in the HFD-fed mice (46). However, these SphK1-overexpressing mice exhibited no changes in glucose metabolism, including the states of gluconeogenesis, glycogen synthesis and glucose tolerance (46), suggesting it has minimal influence on carbohydrate metabolism.

It was recently found that SphK2 is the major SphK isoform in the liver. The overexpression of the *SphK2* gene has been shown to elevate hepatic S1P expression and improve glucose/lipid metabolism in KK/Ay diabetic mice. The adenoviral-mediated expression of SphK2 activated the Akt pathway, a key signalling mechanism in the insulin-induced regulation of glucose metabolism, thus, confirming an important role of SphK2 in regulating hepatic insulin signalling (47).

Insulin resistance is also associated with the pathological transformation of proteins and lipid biosynthesis in the EndRet. The perturbation of specific EndRet functions, the so-called EndRet stress, can be caused by excess nutrient intake (one of the major causes of obesity) that further induces the activation of multiple pathological chain-reaction mechanisms, including the unfolded protein response (UPR) and aberrant lipid biosynthesis (48). Notably, SphK/S1P-signalling activation ameliorates hepatic insulin resistance induced by EndRet stress. Accordingly, SphK2 activation improves insulin signalling and its metabolic actions under conditions of EndRet stress in HFD-fed mice. Activated SphK2 also reduces hepatic lipid accumulation, thus improving the effects of insulin in these mice *in vivo*, an effect confirmed *in vitro* in primary hepatocytes (47).

SphK signalling and pancreatic β-cell death. Decreased insulin secretion and T2D can be triggered by pancreatic β-cell death that is often caused by excessive levels of circulating lipids (lipotoxicity) in obese or overweight patients. Increased

sphingolipid metabolites that are observed during lipotoxicity also induce β-cell dysfunction, leading to apoptosis (49). For example, increased intracellular ceramide promotes an apoptotic cascade and initiates β-cell death in diabetic fatty rodent models of T2D *in vivo* and human β-cells *in vitro* (50-52).

Caspase activation mediates ceramide-induced apoptosis (53). Studies using the MIN6 insulinoma cell line and INS-1 cells, which are used as cell models of glucolipotoxicity, have demonstrated that caspases-3/7 are activated during apoptosis initiated by increased levels of intracellular ceramide (54,55). In addition to triggering apoptosis, ceramide has been shown to induce EndRet stress followed by insulin resistance in MIN6 cells *in vitro* (54). Insulin resistance initiated by ceramide treatment has been shown to be accompanied by reduced proinsulin mRNA levels in INS-1 cells and isolated rat pancreatic islets *in vivo* (56,57). The process is marked by attenuated ERK1/2 signalling (57). Thus, elevated ceramide accumulation in pancreatic cells promoted insulin resistance and β-cell death. However, the detailed mechanisms of ceramide signalling and the development of associated pancreatic pathologies require further investigation.

Contrary to the deleterious effect of ceramide, SphK/S1P have been shown to promote insulin release, to stimulate the development of intra-islet vasculature, improve glucose sensing and prevent inflammation-linked attacks of the immune system (58). The relative intracellular balance of sphingolipid species, such as ceramide and S1P critically determines the direction of β-cell fate; deciding between activating apoptosis or proliferation, or stimulating insulin secretion, and/or islet-cell inflammatory responses (49).

There is abundant evidence demonstrating the pro-survival role of S1P in pancreatic β-cells. S1P has been shown to improve β-cell function in the HIT-T15 cell line and isolated mouse islets, through phospholipase C (PLC) activation (59). S1P has also been shown to protect pancreatic islet cells from IL-1β-induced apoptosis (60). The exposure of INS-1 cells and isolated pancreatic islets to IL-1β and TNF-α has been shown to activate SphK2 as a self-protective mechanism, reducing β-cell inflammatory damage (61). Furthermore, SphK1 activation promotes β-cell survival in diabetic obese mice *in vivo* (10). The roles of SphK/S1P in β-cell survival processes have also been addressed in several cell lines and animal models following lipotoxicity-induced β-cell damage. It was previously demonstrated that the inhibition of SphK/S1P signalling, activated in INS-1 β-cells by palmitate treatment, potentiated β-cell apoptosis; however, SphK1 overexpression significantly mitigated β-cell apoptosis under lipotoxic conditions (62). In another study, the assessment of SphK1(-/-) and wild-type HFD-fed mice demonstrated that HFD-fed SphK1(-/-) mice developed evident diabetes, accompanied by reduced β-cell mass and a 3-fold decrease in insulin secretion (10). Furthermore, the oral administration of FTY720 (a S1P receptor agonist) to diabetic (db/db) mice facilitated β-cell mass preservation and normalised fasting blood glucose (48,63). In addition to its pro-survival effect, SphK activation has been shown to promote glucose-stimulated insulin secretion (GSIS) in MIN6 cells and mouse pancreatic islets (64).

SphK activation and S1P formation have recently been linked to endogenic adiponectin signalling during the induction of β-cell survival. The pro-survival effect of adiponectin has been shown to be modulated by increased S1P formation, employing the AMP-activated protein kinase (AMPK)-dependent pathway in obese mice (44,65). Supporting these findings, S1P2 receptor inhibition has been shown to attenuate streptozotocin (STZ)-induced β-cell apoptosis in T1D models (66). Collectively, current studies indicate divergent signalling mechanisms and positive involvement of the different SphK isoforms and S1P receptor subtypes in protecting pancreatic β cells from apoptosis and malfunction.

Sphingolipid signalling and the development of peripheral insulin resistance. Skeletal muscles consume energy and provide a sink for insulin-stimulated glucose disposal and glycogen formation, thus contributing to the regulation of whole body metabolism. Skeletal muscle insulin resistance is often considered to be the initiating event for T2D, evident prior to β-cell failure and overt hyperglycaemia. Previous studies have implicated SphK/S1P signalling in skeletal muscle insulin resistance, followed by decreased whole-body insulin sensitivity (67,68). Notably, SphK1 overexpression promotes basal and insulin-stimulated glucose uptake in C2C12 cells (67) and a remarkable reduction in blood glucose in diabetic mice (12,67). In support of this, pharmacological SphK inhibition reduces insulin-stimulated glucose disposal (69).

Rapizzi *et al* reported that S1P induced ligand-independent trans-phosphorylation of the insulin receptor and increased glucose uptake in C2C12 myoblasts (70). The same group illustrated the involvement of SphK in a positive feedback loop during the sustained activation of insulin receptor involvement (71). Taking into account the negative effects of ceramide in the development of insulin resistance, it is important to determine whether improved insulin signalling in skeletal muscle is a consequence of SphK overexpression/activation or the result of reduced ceramide intracellular levels.

Bruce *et al* demonstrated that the overexpression of SphK improved skeletal muscle insulin sensitivity and decreased intracellular ceramide in transgenic mice, although S1P abundance was only moderately increased, or remained unaltered (12). Moreover, Takuwa *et al* reported that SphK overexpression only moderately enhanced S1P in the tissues of transgenic mice, suggesting that decreased ceramide is the main mechanism for SphK-dependent regulation of insulin sensitivity (72).

The adiponectin receptor is another alternative mechanism potentially explaining the protective effects of SphK. The overexpression of the adiponectin receptor AdipR1 improves local insulin sensitivity in rat skeletal muscle, at the same time reducing the concentration of both S1P and ceramide (73). Overall, experimental findings suggest a fundamental role of SphK signalling as a tool for ceramide utilisation and the modulation of skeletal muscle insulin resistance. However, the contribution of S1P to skeletal muscle insulin resistance requires further clarification.

Intriguingly, despite intensive research in the field, the role of SphK/S1P signalling in regulating insulin sensitivity remains controversial, as alternative studies demonstrate the pro-inflammatory effects of the SphK/S1P pathway. However, it is well established that obesity and adipocyte-triggered inflammation give rise to insulin resistance in peripheral tissues, and that SphK1 signalling mediates lipolysis-associated inflamma-

tion in adipocytes. Excessive lipolysis can induce inflammation via the increased production of inflammatory cytokines, such as IL-6 and/or the acute activation of β3-adrenergic receptors. The pharmacological inhibition of SphK1 activity blocks ADRB3-induced IL-6 production in adipocytes, both *in vitro* and *in vivo* (74). Furthermore, the selective inhibition of SphK1 protects adipocytes from lipopolysaccharide (LPS)-induced inflammation in Zucker diabetic fatty rats (75). SphK1 deficiency upregulates the gene expression of the anti-inflammatory molecules (IL-10 and adiponectin) and improves overall insulin sensitivity in the adipose and muscle tissues of SphK1 knockout mice *in vivo* (68). Further detailed investigations of the involvement of SphK in inflammatory processes are required. However, taken together, the accumulating evidence indicates that the inhibition of the SphK/S1P axis is a potential therapeutic target for the treatment of insulin resistance.

6. The role of SphKs in the progression of diabetes-related complications

SphK/S1P signalling has been linked to several diabetic microvascular complications, such as neuropathy (76-78), retinopathy (79,80) and nephropathy (81).

SphK signalling and diabetic nephropathy (DN). Aberrant sphingolipid metabolism and/or generation of specific sphingolipid metabolites are thought to aggravate diabetic complications, including the pathogenesis of DN. DN is characterised by a series of pathological events, such as early glomerular proliferation and hypertrophy, accumulation of extracellular matrix (ECM) components and renal fibrosis that may progress to end-stage renal disease. The incidence of DN accounts for 30% of diabetic patients diagnosed with glomerular sclerosis and/or tubulointerstitial (renal) fibrosis (82). S1P stimulates the survival, proliferation and migration of renal mesangial cells (83,84), and induces the upregulation of the pro-fibrotic growth factors, collagen and fibronectin synthesis (81,85). Phosphorylated Smads, secreted phospholipase A2 and matrix metalloproteinase-9 mediate the effect of S1P in renal cells (81,85).

SphK/S1P signalling has previously been linked to glomerular proliferation. However, S1P can promote not only renal mesangial cell proliferation, but also renal inflammation and fibrosis (86). The stimulatory effect of S1P on renal mesangial cell proliferation was first demonstrated in Swiss 3T3 fibroblasts (87). In a previous study, activated SphK and 10-fold upregulated S1P levels stimulated the proliferation of glomerular mesangial cells in rats with STZ-induced diabetes *in vivo* (88). Further studies have confirmed an association of SphK1 activation and S1P production with renal hypertrophy and increased levels of fibronectin (81,89). SphK1/S1P promotes glomerular mesangial cell proliferation via increased fibronectin production, but also through the activation of transforming growth factor-β1 (TGF-β1) and AP-1 signalling (90).

Expressed in glomerular mesangial cells, S1P2 and S1P3 receptors mediate renal fibrosis, glomerular cell proliferation and pathological angiogenesis (91,92). Lymphocyte migration to the site of inflammation is mediated through binding to the S1P1 receptor (93), indicating the involvement of this receptor subtype in potentially damaging immune reactions

in kidneys and other organs (94-96). However, an inflammation-associated role of the SphK/S1P pathway remains controversial, as other authors have demonstrated that S1P can reduce inflammatory signals in cultured renal mesangial cells by downregulating prostaglandin E2 synthesis and the expression of pro-inflammatory mediators, such as cytokine-triggered secretory phospholipase A2 and inducible nitric oxide (NO) synthase (97).

The relevance of SphK/S1P signalling to DN progression was investigated in SphK-deficient mice *in vivo* (35). One study on SphK2-deficient mice showed reduced plasma creatinine concentrations, suggesting that SphK2 protected cells from renal ischaemia (98), and another study detected the worsening of nephropathy conditions in SphK1-deficient mice (86). The loss of SphK1 activity has been shown to aggravate cytogenesis in a mouse model of polycystic kidney disease and renal injury (35). Similarly, the lentiviral-mediated overexpression of human SphK1 in mice subjected to ischaemia-perfusion injury demonstrated less tubular necrosis and reduced inflammation (98). Overall, the current evidence suggests the involvement of both SphK isoforms, probably to a different degree and affecting distinct targets, in regulating microvascular complications, such as DN. Further studies are warranted in order to clarify which SphK isoform is involved in inflammation-associated signalling and whether S1P receptors should be targeted for nephropathy drug design.

Involvement of the SphK/S1P signalling in diabetes-related vascular complications. The risks of cardiovascular disease, atherosclerosis (99,100) and heart failure are critically elevated in patients with diabetes (99-104). Multiple studies have indicated a cardioprotective role of SphK/S1P pathway activation *in vivo* (67,105-107). In a diabetic mouse model with increased myocardial glycogen accumulation leading to cardiomyopathy, SphK1 overexpression was shown to improve heart function (67). With regard to atherosclerotic complications, S1P reduces glucose-stimulated monocyte-endothelial interaction in the non-obese diabetic mouse model NOD/LtJ (108,109).

Diabetic patients commonly present with silent myocardial ischaemia (110). SphK1 activation has been shown to protect isolated mouse hearts against ischaemia-associated injury (94,111,112) and promotes the recovery of haemodynamic function following ischaemic injury (94). Pharmacological SphK inhibition with *N,N*-dimethylsphingosine (DMS) confirms the SphK-linked protection of murine hearts against ischemia/reperfusion injury employing protein kinase Cε-dependent mechanisms (95). The overexpression of SphK1 mediates myocardial ischaemic preconditioning-induced cardioprotection in murine hearts (96). SphK1 is also important maintaining the blood vessel integrity (113) and the wound healing process in diabetic rats (114). On the other hand, SphK1 inhibition ameliorates angiotensin II-induced acute hypertension, suggesting a negative influence of SphK on vascular health (115).

However, several recent investigations have indicated a positive effect of S1P receptor signalling in maintaining vascular health. The transactivation of S1P1/3 receptors stimulates eNOS, increasing NO production and vasodilation (116,117). The increased expression of S1P1/3 receptors improves recovery following cardiac microvascular dysfunc-

tion associated with diabetes (118). The S1P1 receptor also mediates estrogen-induced activation of Akt/eNOS signalling in endothelial cells (119). Estrogen replacement therapy in post-menopausal women prevents diabetes-associated cardiovascular complications (120), thus indicating that S1P receptors should be further explored as potential drug targets for the treatment of diabetes-associated vascular pathologies.

7. Summary

The complex interactions among members of the sphingolipid signalling pathway, insulin signalling and diabetic pathologies have been extensively investigated. Yet the role of SphK/S1P signalling in the development of diabetes mellitus remains unclear. The divergence of SphK/S1P signalling seems to be dependent on cell type, the expression pattern of S1P receptor subtypes and the relative expression of the specific SphK isoforms. In diabetes mellitus, SphK activation has been known to promote β-cell survival and insulin secretion, prevent vascular pathologies related to diabetes (39,116,119), ameliorate peripheral insulin resistance and obesity in diabetic patients and animal models (121,122). A low S1P content has been linked to increased incidence of coronary artery disease (97,122-124) and diabetes mellitus (97,125-127).

In addition, emerging evidence has also linked SphK/S1P signalling to the development of diabetes-related vascular complications, such as neuropathy (76-78), retinopathy (79,80,128,129) and nephropathy (81). However, the roles of the different S1P receptor subtypes and two SphK isoforms in the pathogenesis of diabetes remain to be confirmed *in vivo* and in future clinical trials. SphK therefore represents a potential therapeutic target for diabetes mellitus. The FTY720 (fingolimoid), an S1P receptor agonist has demonstrated promising therapeutic effects, including the stimulation of lipolysis, decreased accumulation of skeletal muscle ceramide, improved systemic glucose homeostasis, and β-cell survival in diabetic mouse models (63,118,130). Combination therapy with FTY720 plus insulin glargine has been shown to be a promising therapeutic strategy for the treatment of diabetes mellitus (130). A second generation of S1P3 modulators (siponimod, ponesimod, KRP-203, ONO-4641, RPC1063, CS-0777 and GSK2018682) targets different S1P receptor subtypes (131). These substances promise to elucidate the mechanisms underlying the divergent outcomes of S1P signalling, and consequently, the potential efficacy of targeting the SphK/S1P pathway for the treatment of diabetes and associated pathologies.

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