



# Recent advances in the role of sphingosine 1-phosphate in cancer

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**Sphingosine 1-phosphate (S1P) is a bioactive lipid that binds to a family of G protein-coupled receptors (S1P<sub>1-5</sub>) and intracellular targets, such as HDAC1/2, that are functional in normal and pathophysiologic cell biology. There is a significant role for sphingosine 1-phosphate in cancer underpinning the so-called hallmarks, such as transformation and replicative immortality. In this review, we survey the most recent developments concerning the role of sphingosine 1-phosphate receptors, sphingosine kinase and S1P lyase in cancer and the prognostic indications of these receptors and enzymes in terms of disease-specific survival and recurrence. We also provide evidence for identification of new therapeutic approaches targeting sphingosine 1-phosphate to prevent neo-vascularisation, to revert aggressive and drug-resistant cancers to more amenable forms sensitive to chemotherapy, and to induce cytotoxicity in cancer cells. Finally, we briefly describe current advances in the development of isoform-specific inhibitors of sphingosine kinases for potential use in the treatment of various cancers, where these enzymes have a predominant role. This review will therefore highlight sphingosine 1-phosphate signalling as a promising translational target for precision medicine in stratified cancer patients.**

**Keywords:** cancer stem cells; EMT; metastasis; sphingosine 1-phosphate; sphingosine 1-phosphate lyase; sphingosine 1-phosphate phosphatase; sphingosine 1-phosphate receptors; sphingosine kinase; STAT; YAP

Sphingosine 1-phosphate (S1P) is a quantitatively minor sphingolipid that exerts a multitude of physiological effects through both receptor-mediated

signalling pathways and by regulation of intracellular target proteins. Two isoforms of sphingosine kinase (SK1 and SK2), which differ in their tissue expression,

## Abbreviations

ALDH1, alcohol dehydrogenase 1; AML, acute myeloid leukaemia; B-ALL, B-cell acute lymphoblastic leukaemia; BC, bladder cancer; Brms1, breast carcinoma metastasis suppressor; ccRCC, clear cell renal cell carcinoma; CIB, calcium and integrin binding protein; CSCs, cancer stem cells; CTGF, connective tissue growth factor; Cul3, cullin 3; DLBCL, diffuse large B-cell lymphoma; DYNC111, dynein 1; EDAC, epithelial defence against cancer; EGF, epidermal growth factor; ER $\alpha$ , oestrogen receptor alpha; GC, germinal cell; HBEGF, heparin-binding epidermal growth factor; HCC, hepatocellular carcinoma; HDAC1/2, histone deacetylase 1/2; HDL, high-density lipoprotein; HGF, hepatocyte growth factor; HIF1, hypoxia-inducible factor 1; hTERT, human telomerase reverse transcriptase; IBD, inflammatory bowel disease; KLHL5, Kelch-like protein 5; MCL, mantle cell lymphoma; MFN2, mitofusin 2; MM, multiple myeloma; MMP-9, matrix metalloproteinase-9; MRTF-A, myocardin-related transcription factor A; MSCs, mesenchymal stem cells; NKT, natural killer T cells; PD-1, programmed cell death 1; PGAM1, phosphoglycerate mutase; PHB2, prohibitin 2; PPAR, peroxisome proliferator-activated receptor gamma; PTC, papillary thyroid carcinoma; PTEN, phosphatase and tensin homologue; S1P, sphingosine 1-phosphate; S1P<sub>1-5</sub>, sphingosine 1-phosphate receptor 1-5; SFMBT1, Scm-like with four malignant brain tumour domains 1; SGPL, sphingosine 1-phosphate lyase; SGPP, sphingosine 1-phosphate phosphatase; SK, sphingosine kinase; SNX1, sorting nexin-1; STAT, signal transducer and activator of transcription; TFR1, transferrin receptor 1; TRAF2, TNF receptor-associated factor; VEGF, vascular endothelial growth factor; VHL, Von Hippel-Lindau; YAP, Yes-associated protein 1.

subcellular localisation and biochemical properties, catalyse the phosphorylation of sphingosine (derived by deacylation of ceramide) to produce S1P. Degradation of S1P is either by irreversible cleavage at the C<sub>2</sub>–C<sub>3</sub> bond to hexadecenal and phosphoethanolamine, catalysed by S1P lyase (SGPL) or by dephosphorylation to sphingosine, catalysed by two isoforms of S1P phosphatase (SGPP1 and SGPP2) and nonspecific lipid phosphate phosphatases. In general, the biological actions of S1P at a cellular level are to promote proliferation and survival and opposed to the effects of ceramide which typically induces apoptosis, growth arrest or senescence; this balance has been termed the ‘sphingolipid rheostat’. A more nuanced view of this concept encompasses the influence of these two sphingolipids and their interconversion on cellular fate together with the receptor-mediated (autocrine, paracrine and signal amplification loops) and intracellular target protein-mediated effects of S1P in counterbalance with the effects of ceramide. S1P can affect cellular transformation, epigenetic regulation, migration, angiogenesis, lymphangiogenesis etc. and an imbalance, with excessive S1P-driven signalling, can contribute to disease pathologies, including cancer [1]. Thus, the deregulation of enzymes that control the synthesis and removal of S1P can underlie certain cancers and may provide opportunities for therapeutic intervention to indirectly influence the receptor-mediated or intracellular target-mediated effects of S1P.

Five differentially expressed G protein-coupled receptors (GPCR), named S1P<sub>1–5</sub>, mediate many of the physiological roles of S1P, such as trafficking of lymphocytes, regulation of vascular barrier integrity and modulation of vascular tone [2]. S1P receptors are successfully targeted for therapeutic benefit: for example, Gilenya™ (a formulation of fingolimod/FTY720) is the first oral medicine for treatment of relapsing and remitting multiple sclerosis. This sphingosine-like prodrug is phosphorylated by SK2 to FTY720-phosphate, which is then exported from cells to agonise and chronically downregulate S1P<sub>1</sub>. This limits the S1P<sub>1</sub>-mediated invasion of inflammatory T cells into the CNS and, together with a reduction in astrogliosis and support for nerve remyelination and recovery, relieves symptoms in this autoimmune and neurodegenerative condition [3]. In the context of cancer, there are correlations between S1P receptor expression in tumours and clinical prognosis [4]. Signalling through S1P receptors contributes to, for example, signal amplification loops that drive cancer and associated preceding inflammatory disease as well as epithelial–mesenchymal transition (EMT), metastasis and angiogenesis within the tumour. However,

there are no current cancer treatments targeting S1P receptors.

Extracellular S1P is derived from a number of sources. Erythrocytes are the major sources of S1P in the blood; S1P is constitutively released through active transport by the mfsd2B2 transporter. Activated platelets, which lack S1P lyase, contribute lesser amounts of S1P through both calcium- and ATP-dependent transporters, including mfsd2B2 [5,6]. Vascular and lymphatic endothelial cells also passively release S1P through the Spns2 transporter [7]. Carrier proteins, such as albumin and high-density lipoprotein (HDL), are associated with released S1P, and this can influence S1P receptor signalling. For example, S1P<sub>1</sub> signalling is more sustained for HDL-S1P compared with albumin-S1P [8]. On the other hand, S1P may access the binding pocket of S1P<sub>1</sub> by lateral movement between two transmembrane helices and though the lipid bilayer of the plasma membrane [9]. The release of S1P through transporter proteins into the tumour microenvironment influences stromal cells, promotes inflammation, alters immune cells and induces angiogenesis and lymphangiogenesis [10]. Therefore, targeting S1P transporters also has potential for novel therapeutics to combat cancer.

The intracellular targets of S1P produced by SK1 and SK2 differ, and this might be a consequence of the distinct subcellular localisation of SK1 and SK2 and target effector proteins. SK1 is predominantly cytoplasmic and translocates to the plasma membrane to access sphingosine, whereas SK2 shuttles to and from the nucleus [11–13]. SK1-derived S1P binds to the RING domain of TNF receptor-associated factor 2 (TRAF2), an E3 ligase which associates with SK1, thus acting as a cofactor in the TRAF2-catalysed Lys63-polyubiquitination of RIP1, a protein kinase in the NF-κB pathway regulating cell survival and inflammation [14]. However, others report that elimination of SK1 has no effect on NF-κB signalling [15]. SK2-derived S1P binds prohibitin 2 (PHB2), a regulator of mitochondrial assembly and electron transport chain function at complex IV (cytochrome *c* oxidase) [16]. In contrast, proapoptotic BAK cooperates with SK2-derived S1P in apoptosis, affecting cytochrome *c* release upon altered mitochondrial outer membrane potential [17]. The catalytic subunit of telomerase, human telomerase reverse transcriptase (hTERT), is stabilised by binding SK2-derived S1P, preventing its interaction with the E3 ligase makorin ring finger protein (MKRN1), which ubiquitinates hTERT and targets it for proteasomal degradation. The stabilisation of telomerase enhances proliferation and tumour growth [18]. Gene expression is also affected by

intracellular S1P when nuclear SK2 occurs in a repressor complex with histone deacetylase 1 and 2 (HDAC 1/2) and histone H3, as is the case for p21 (a cyclin-dependent kinase inhibitor) and c-fos (a regulator of transcription) [19]. Inhibition of HDAC1/2 by S1P sustains lysine acetylation of histone, thereby enhancing gene expression. Additionally, cytoplasmic S1P binds to peroxisome proliferator-activated receptor gamma (PPAR $\gamma$ ) to enhance the expression of genes regulated by this transcription factor [20]. Interestingly, SK2-derived S1P is required for epidermal growth factor (EGF)-stimulated phosphorylation of ezrin (an adapter molecule of the ezrin–radixin–moesin family), which participates in cancer cell invasion [21]. This is an example of intracrine signalling where S1P might be delivered to S1P<sub>2</sub> via close proximity with Spns2. SK1 is also required for endosomal signalling, being recruited to early endosomes, and may contribute significantly to the molecular and cellular mechanisms in cancer [22]. Therefore, SK inhibitors have the potential to reduce inflammation, counter replicative immortality and alter mitochondrial function, gene expression and S1P receptor-mediated signalling.

The aim of this review is to focus on the latest advances concerning the role of S1P in cancer and to identify new potential signalling networks and targets for therapeutic intervention in cancer.

## Role of S1P in tumour neovascularisation and metastasis

There is a wealth of evidence to support the involvement of deregulated production and removal of S1P in the ‘hallmarks of cancer’ [1,2,23,24]. Recent reviews which focus on the role of S1P in specific cancer types are available (e.g., breast [25], ovarian [26], gastrointestinal [27], hepatocellular carcinoma [28], glioblastoma [29]). Regardless of the cancer type, S1P is involved in tumour/stromal cell communication, the migration and invasiveness of cancer cells into the niche microenvironment, neovascularisation and metastasis, which are hallmarks of cancer that lead to patient mortality. Stromal cell/tumour cell communication involving S1P in the tumour microenvironment is exemplified by the observation that local tumour growth and dissemination of cancer cells is compromised *in vivo* when proximal nontumour cells lack SK1 [30]. Cooperating signalling pathways and S1P receptors involved in the microenvironmental niche vary by tumour type. A recent example is S1P<sub>1</sub> and IL-22R1, which are overexpressed in invasive and bone metastatic breast cancer. In this case, IL-22 stimulates the expression of IL-22R1 and S1P<sub>1</sub> in triple-negative

MDA-MB-231 breast cancer cells and increases SK1 expression and S1P production in mesenchymal stem cells (MSCs) to promote migration of MDA-MB-231 cells. Increased IL-22R1 and S1P<sub>1</sub> expression are associated with increased matrix metalloproteinase-9 (MMP-9) levels and breast cancer cell invasion. Moreover, IL-22 also induces MCP1, IL-22R and S1P<sub>1</sub> expression in MSCs to facilitate macrophage infiltration [31]. Thus, the signalling interplay between S1P and other factors needs to be considered in the development of potential cancer treatments.

Mutation and deregulation of S1P receptors can also be a significant factor in relation to tumour cell/stromal cell interaction in the microenvironment. For example, S1P<sub>1</sub>, which is involved in recirculation of B lymphocytes from lymph nodes, is one of several receptor types regulating mantle cell lymphoma (MCL) localisation in the microenvironment. Analysis of 200 MCL patient biopsies reveal that mutation of S1P<sub>1</sub> is more prevalent in stage 4 lymphoma and is associated with relapse. Various frameshift insertion/deletion and other mutations have been identified and predicted to diminish S1P<sub>1</sub> expression or function, which may trap MCL cells in lymph nodes. Thus, retention of MCL in the supportive microenvironment could represent a residual reservoir of cancer cells linked to relapse. It remains to be determined whether S1P<sub>1</sub> inactivating mutations can reduce ibrutinib sensitivity in MCL [32]. However, chemotherapeutic resistance is associated with enhanced adhesion of MCL to the stroma and ibrutinib increases expression of S1P<sub>1</sub>, while decreasing CCR7 levels in chronic lymphocytic leukaemia.

In the context of solid tumours, antagonism of S1P<sub>1</sub> may hold potential for cancer treatment. In this regard, it is recognised that S1P and its receptors are linked with the neovascularisation of tumours in cooperation with vascular endothelial growth factor (VEGF) [33,34]. In a further recent study, it was reported that VEGF-A-VEGFR2 pathway promotes tumour vascularisation by stimulating proangiogenic endothelial cell signalling. Activation of endothelial S1P<sub>1</sub> receptors by tumour-derived S1P amplifies VEGFR2-dependent c-Abl1 and Rac activation and endothelial cell migration to enhance tumour growth. On the other hand, endothelial cell-specific deletion of S1P<sub>1</sub> receptors was shown to limit vascularisation and reduce tumour growth [35]. However, contrasting observations have been made by others. For example, elimination of S1P<sub>1</sub> receptors from the vascular endothelium promotes excessive sprouting and branching and this was ablated by overexpression of S1P<sub>1</sub> receptors in vascular endothelial cells. Combined

knockout of *S1pr1-S1pr3* worsened the sprouting/branching phenotype, suggesting some functional redundancy. The consequence of this is that endothelial cell-specific *S1pr1* knockout animals develop significantly larger tumours with increased vascular leak and more metastatic foci. The opposite was seen when S1P<sub>1</sub> was overexpressed in endothelial cells, accompanied by increased efficacy of antitumour therapies. Thus, expression of S1P<sub>1</sub> in endothelial cells induces vascular normalisation and suggests that enhancing S1P<sub>1</sub> function in the tumour vasculature may improve the efficacy of anticancer therapies [36]. Similarly, poor functionality of tumour vessels compromises effective chemotherapy in Ewing sarcoma. An imbalance of S1P<sub>1</sub> and S1P<sub>2</sub> function may contribute to tumour vessel hyperpermeability in this case. Indeed, pharmacological activation of S1P<sub>1</sub>, using SEW2871, or antagonism of S1P<sub>2</sub>, using JTE-013, enhances the organisation and integrity of tumour vessels and improves antitumour efficacy. However, a potential involvement of tumour, rather than vessel, S1P<sub>1</sub> and/or S1P<sub>2</sub> was not excluded. Despite this, there may be potential for adjuvant S1P<sub>1</sub> agonists and/or S1P<sub>2</sub> antagonists with standard chemotherapy for Ewing sarcoma patients [37]. In contrast, the S1P<sub>1</sub> functional antagonist, Siponimod, reduces angiogenesis and tumour growth in a mouse model of diffuse large B-cell lymphoma (DLBCL). Interestingly, tumour angiogenesis meta-signature genes are enriched and correlated with SK1 mRNA expression in a meta-analysis of over 2000 cases of DLBCL (both cell of origin and stromal subtypes). Moreover, S1P induces angiogenic signalling and gene expression programmes that are common to the vasculature of SK1-expressing DLBCL tumours [38]. Therefore, the potential therapeutic benefit of S1P<sub>1</sub> agonists, competitive antagonists and functional antagonists may depend upon the cancer type and further research is warranted.

The role of S1P<sub>2</sub> in cancer is also somewhat controversial. A body of evidence suggests that S1P<sub>2</sub> is protective against cancer. An example of this is its role in epithelial defence against cancer (EDAC) whereby epithelial cells sense and actively eliminate neighbouring transformed epithelial cells. This involves an S1P<sub>2</sub>-induced activation of Rho in normal cells adjacent to RasV12-transformed cells, thereby promoting Rho-kinase-mediated accumulation of filamin, which is a critical regulator of EDAC. Thus, JTE-013, an S1P<sub>2</sub> antagonist, or S1P<sub>2</sub> knockdown reduces apical extrusion of RasV12-transformed cells *in vitro*. Moreover, S1P<sub>2</sub> stimulation with exogenous S1P is required for EDAC, whereas inhibition of S1P production by RasV12-transformed cells and surrounding epithelial

cells has no effect on extrusion [39]. In addition, S1P<sub>2</sub> inhibits the motility of, for example, gastric cancer cells [40]. In a further example, hepatocyte growth factor (HGF)-induced migration of human hepatocellular carcinoma (HCC) cells (HuH7 cells) is reduced by S1P and this is replicated by a selective S1P<sub>2</sub> agonist, CYM5520, but not by other S1P receptor subtype-selective agonists. Moreover, the selective antagonist for S1P<sub>2</sub>, JTE-013 or knockdown of S1P<sub>2</sub> with siRNA reduces the inhibitory effect of S1P on HCC migration [41]. This highlights the possibility that S1P<sub>2</sub> receptor-selective agonists might be usefully employed to inhibit metastasis of HCC. In regard to a limiting effect of S1P<sub>2</sub>, high nuclear expression of this receptor in tumours from ER<sup>+</sup> breast cancer patients is associated with improved prognosis [42]. In addition, multiple somatic mutations of *S1PR2* are detected in ~26% of patients with DLBCL [43], supportive of a protective role for S1P<sub>2</sub>. Indeed, aged *S1pr2*<sup>-/-</sup> mice develop germinal centre (GC)-derived DLBCL [43] where S1P<sub>2</sub> participates in homeostasis and niche confinement of GC B cells through AKT inhibition [44]. In addition, more recent studies have shown that the overexpression of wild-type S1P<sub>2</sub>, but not a signalling deficient mutant, induces apoptosis in DLBCL cells and reduces tumour growth but this was independent of AKT. In addition to the recognised multiple mutations in the *S1PR2* locus, the tumour suppressor activity of S1P<sub>2</sub> can be lost through transcriptional silencing by FOXP1. Thus, S1P<sub>2</sub> expression was repressed in GC-DLBCL cell lines with aberrantly high levels of the haematopoietic oncoprotein FOXP1. Moreover, low S1P<sub>2</sub> expression was prognostic for reduced patient survival, alone and especially in combination with high FOXP1 expression [45]. In normal B cells, S1P<sub>2</sub> expression is regulated through the TGF- $\beta$ /TGF- $\beta$ RII/SMAD1 signalling pathway. However, this pathway may be ablated in DLBCL patients; DLBCL cell lines deficient in S1P<sub>2</sub>, TGFBR2 or SMAD1 exhibit enhanced growth. SMAD1 expression is limited due to hypermethylation of CpG-rich regions surrounding its gene transcription start site. Indeed, decitabine, a demethylating agent, restores SMAD1 expression and resensitises cells to TGF- $\beta$ -induced apoptosis [46].

In contrast to the preceding examples, a deleterious role for S1P<sub>2</sub> is evident from other studies. For example, SK1 activation and S1P release with subsequent activation of S1P<sub>2</sub> upregulate transferrin receptor 1 (TFR1) expression, which contributes to SK1-mediated transformation [47]. In addition, S1P<sub>2</sub> is shed in hsp70<sup>+</sup> and CD63<sup>+</sup> containing exosomes from MDA-MB-231 breast cancer cells. When these exosomes are added to fibroblasts, S1P<sub>2</sub> is taken up and



N-terminally processed to a constitutively active shorter form that activates the ERK-1/2 pathway and DNA synthesis. An N-terminally truncated form of S1P<sub>2</sub>, which might correspond to the processed form generated in fibroblasts, is also constitutively active in transfected HEK293 cells [48]. S1P<sub>2</sub> is also involved in metastatic spread. For example, lung colonisation by tumour cells is promoted by systemic S1P, formed by host SK1, through a S1P<sub>2</sub>/Brms1 (breast carcinoma metastasis suppressor 1) axis; systemic S1P increases S1P<sub>2</sub> expression in cancer cells and activation of tumour S1P<sub>2</sub> reduces Brms1 expression, thereby facilitating metastasis [49].

Studies of S1P transporters, other S1P receptors and S1P lyase also link S1P to metastasis. For instance, metastatic burden decreases upon deletion of the S1P transporter, *Spns2*, either globally or in a lymphatic endothelial-specific manner. *Spns2* deletion induces lymphopenia that is accompanied by the localisation of effector T cells and natural killer (NK) cells in the lung, thereby improving tumour cell killing and limiting the metastatic burden [50]. The ABCC1 transporter also releases S1P and disease-specific survival is reduced in patients whose breast tumours express both ABCC1 and activated SK1. In breast cancer models, overexpression of ABCC1 in human MCF7 and murine 4T1 breast cancer cells increases S1P release and promotes proliferation and migration of breast cancer cells. Exported S1P also induces SK1 expression, suggesting a positive feedback amplification mechanism for increasing the bioavailability of S1P. Moreover, orthotopic implantation of ABCC1 overexpressing breast cancer cells promotes tumour growth, angiogenesis, lymph node and lung metastases and the survival time of mice is decreased [51]. Others have reported a role for S1P/S1P<sub>3</sub>-dependent activation of Notch signalling in the migration of triple-negative breast cancer cells where elevated levels of phosphorylated SK1 are associated with high S1P content [52]. In addition to overproduction of S1P, its aberrant removal is also implicated in metastasis. Significantly, an oncogenic role for mutated *SGPL1* (homozygous A to G point mutation at position 321) has been identified in paediatric alveolar rhabdomyosarcoma (RMA) cells. This mutation reduces enzymatic activity and causes mislocalisation of the protein from the ER; however, complementation with wild-type *SGPL1* restores ER localisation and limits S1P-induced migration and colony formation [53].

Finally, SK1 modulates Ca<sup>2+</sup> handling by mitochondria, affecting downstream cellular responses. This is significant in the context of oncogenesis where Ca<sup>2+</sup> microdomains in mitochondrial associated ER

membranes (MAMs) are important and SK1 is often upregulated. Moreover, deregulation of mitofusin 2 (MFN2) affects ER-mitochondria contacts that are associated with malignancy. Thus, overexpression of SK1 enhances Ca<sup>2+</sup> exchange from ER to the mitochondria and calpain-induced cleavage of MFN2 in HeLa cells. N- and C-terminal fragments of MFN2, predicted to be formed through calpain activity, recapitulate the exchange of Ca<sup>2+</sup> between the ER and mitochondria and this is linked with increased cellular respiration and enhanced cell migration [54].

Therefore, evidence linking S1P signalling to the cancer hallmarks of neovascularisation and metastasis is clearly a prevalent mechanism that has significant causal effect.

### Role of S1P in protumorigenic inflammation and immune signalling

S1P is also involved in regulating inflammation-induced oncogenesis and modulation of immune-based signalling. For example, an axis of SK1/S1P/S1P<sub>1</sub> is at the nexus between NF- $\kappa$ B, IL-6 and STAT3 signalling and increased S1P<sub>1</sub> expression in a persistent amplification loop that links chronic inflammation with colitis-associated colon cancer [55]. In addition, mice deficient in intestinal *Sgpl1* have greater disease activity. This includes colon shortening, suppression of miR-targeted antioncogene products, tumour formation, changes in cytokine expression, accumulation of S1P and stimulation of STAT3 and STAT3-activated micro-RNAs (miRNAs). The significance of STAT3 is underscored by the fact that STAT3 inhibition attenuates the phenotype and enhanced S1P/STAT3 signalling is evident in patients with inflammatory bowel disease (IBD). Tumorigenic transformation in response to silencing *Sgpl1* involves S1P receptor-dependent activation of JAK2/STAT3, thereby inducing miR-181b-1 which silences cylindromatosis (CYLD). Interestingly, dietary sphingadienes reduce tumorigenesis and this is accompanied by increased colonic SGPL expression and reduced S1P, STAT3 signalling and cytokine levels. Thus, SGPL prevents transformation and carcinogenesis [56]. Further evidence for a link between SGPL1 activity, inflammation/tumorigenesis and STAT3 signalling was revealed by *Sgpl1* knockout in either immune cells (I- *Sgpl1*<sup>-/-</sup>) or tissue (T- *Sgpl1*<sup>-/-</sup>). In both cases, local sphingolipid accumulation leads to the development of colitis-associated cancer, although the pathophysiology differs depending upon the source of S1P. I- *Sgpl1*<sup>-/-</sup> enhance immune cell infiltration, thereby initiating colitis. Formation of tumours is delayed due to pathological crypt

remodelling and S1P signalling associated with increased S1P<sub>1</sub>/STAT3 mRNA, expression of programmed cell death ligand 1 and a counter regulatory phosphorylation of STAT1<sup>S727</sup>. In contrast, epithelial-driven tumours develop immediately in T- *Sgpl1*<sup>-/-</sup> mice. These tumours exhibit increased SK1, S1P<sub>2</sub> and EGF receptor signalling. Tumour formation is accompanied by an IL-12 to IL-23 shift leading to a Th2/GATA3-dependent tumour-supportive microenvironment. Therefore, distinct mechanisms of inflammation-associated cancer and cancer-associated inflammation are evident and dependent on the source of S1P [57].

In colitis-associated cancer, S1P<sub>1</sub> participates in inflammation/oncogenesis. However, other S1P receptor types are also involved in other cancers. For instance, Gram-negative bacteria have been implicated in prostatitis and prostate cancer tissues and LPS is involved in prostate cancer cell invasion. Activation of Toll-like receptor 4 (TLR4) by LPS promotes Ser225 phosphorylation of SK1, resulting in its translocation from the cytoplasm to the plasma membrane, release of S1P and S1P<sub>4</sub>-induced activation of matriptase. A similar phenomenon occurs in tumour explants from prostate cancer patients where poor survival is correlated with increased SK1 expression and tumour Gleason grade [58]. However, S1P<sub>2</sub> agonists might be useful in the management of chemotherapy-induced neuropathy since the selective S1P<sub>2</sub> agonist, CYM-5478, reduces allodynia in cisplatin-induced neuropathy and attenuates the associated inflammatory processes in the dorsal root ganglia *via* the transcription factor ATF3 and haeme oxygenase 1 (HO-1) [59].

In terms of immune signalling, regulatory T cells (Tregs) have an important role in mediating immune evasion by cancer cells. Bladder cancer (BC) patients were found to have more CD4<sup>+</sup>Foxp3<sup>+</sup> Tregs in circulating and tumour-infiltrating lymphocytes and increased tumour-infiltrating Foxp3<sup>+</sup> Tregs that is correlated with increased tumour S1P<sub>1</sub> expression. In addition, S1P<sub>1</sub> and Tregs are associated with poor patient survival. *In vitro* data support a mechanism of S1P<sub>1</sub>-mediated Treg formation from CD4<sup>+</sup>CD25<sup>-</sup> cells involving TGF- $\beta$  and IL-10 release from BC cells. Tumour S1P<sub>1</sub> also promotes Treg migration, and therefore, S1P<sub>1</sub> is linked with tumour-derived Treg expansion and might serve as a biomarker and potential therapeutic target in BC [60]. In addition to S1P receptor-mediated effects on the differentiation of Treg and Th17 cells, SK1-derived intracellular S1P also plays a role. In this regard, *Sphk1*-deficient T cells maintain a central memory phenotype (due to nuclear retention of Foxo1) with higher lipolysis and mitochondrial activity and reduced differentiation of Tregs

due to decreased PPAR $\gamma$  expression and activity. In addition, S1P formed in T cells by SK1 directly activates PPAR $\gamma$ , whereas PPAR $\gamma$ -deficient T cells exhibit enhanced antitumour activity. Thus, genetic deletion or pharmacological inhibition of SK1 improves the metabolic fitness of T cells and promotes their antitumour activity. Moreover, simultaneous inhibition of SK1 and programmed cell death 1 (PD-1) enhances antitumour adoptive T-cell therapy [61]. Tumour SK1 also plays a role in antitumour immunity. For example, increased expression of SK1 in tumour cells is associated with shorter survival times in patients with metastatic melanoma. Silencing SK1 decreased TGF $\beta$ , IL10, CCL17 and CCL22 levels in the tumour microenvironment to limit Treg infiltration, accompanied by downregulation of prostaglandin E synthase and PGE2 formation. Furthermore, SK1 silencing markedly enhances responses to anti-PD-1 and to other immune checkpoint inhibitors (ICIs) in murine models of melanoma, breast and colon cancer, thereby reducing tumour growth [62]. The activation of natural killer T (NKT) cells (by glycolipid antigens on CD1d) is also increased by knockdown of SK1 or antagonism of S1P<sub>1</sub> in mantle cell lymphoma, an aggressive subtype of non-Hodgkin's lymphoma that is associated with increased S1P levels. Activated NKT cells reduce tumour burden. Interestingly, the level of cardiolipin (which can bind CD1d) is increased upon SK1 knockdown and activates NKT hybridomas, as evidenced by the formation of IL-2 and IFN $\gamma$  [63]. Therefore, targeting S1P signalling holds potential for reducing inflammation-induced cancer and enhancing the immune response to counter oncogenesis.

### Cancer stem cells: emerging roles for S1P signalling

Cancer stem cells (CSCs) are a subpopulation of tumour cells that are more resistant to chemo- and radiotherapies and may underlie disease recurrence and metastasis. The role of sphingolipids and their altered metabolism in stem cell biology has recently been reviewed [64]. Recently, a novel functional interaction has been identified between  $\beta$ 3 adrenergic receptors ( $\beta$ 3-AR) and SK2/S1P<sub>2</sub> in neuroblastoma where the regulation between stemness and differentiation is particularly important. In this regard,  $\beta$  adrenergic receptors are established players in the pathogenesis of multiple cancers, including neuroblastoma. Importantly, antagonism of  $\beta$ 3-AR with SR59230A switches the stemness/proliferative capacity of human neuroblastoma cell lines to differentiation *in vitro* and reduces murine neuroblastoma tumour growth and progression

*in vivo*. This occurs *via* a mechanism that involves reduced expression of SK2 and S1P<sub>2</sub> (i.e. blockade of SK2/S1P<sub>2</sub> signalling) whereas the S1P<sub>2</sub> agonist, CYM5520, counters the effects of  $\beta$ 3-AR antagonism [65]. In human breast CSCs, overexpression of SK1 enhances survival and mammosphere formation but does not affect EMT. Conversely, knockdown of SK1 expression with siRNA increases apoptosis and reduces cell proliferation of both breast CSCs and non-CSCs. In addition, SK1-mediated suppression of STAT1 was identified as a mechanism that promotes cancer cell survival, with STAT1 and IFN signalling being novel regulatory targets of SK1 [66]. Overexpression of SK1 also enhances stemness and self-renewal of ovarian cancer cells, *via* a SOX2-dependent mechanism, thereby enhancing tumour clonogenicity. This is accompanied by increased proliferation, migration and invasion. Interestingly, ovarian cancer patients treated with metformin, which has anticancer effects, have reduced serum S1P levels and the cytotoxic effect of metformin is enhanced in ovarian cancer cells with high SK1 expression. Metformin reduces hypoxic (HIF1 $\alpha$  and HIF2 $\alpha$ )-induced expression of SK1 in TYKnu and CAVO3 cells and induces caspase-3-mediated apoptosis in the presence of SK1 but not after its knockdown by SK1 siRNA. These findings suggest that metformin targets SK1 and therefore the sphingolipid rheostat. Thus, tumours with high SK1 may be more sensitive to the cytotoxic effect of metformin [67].

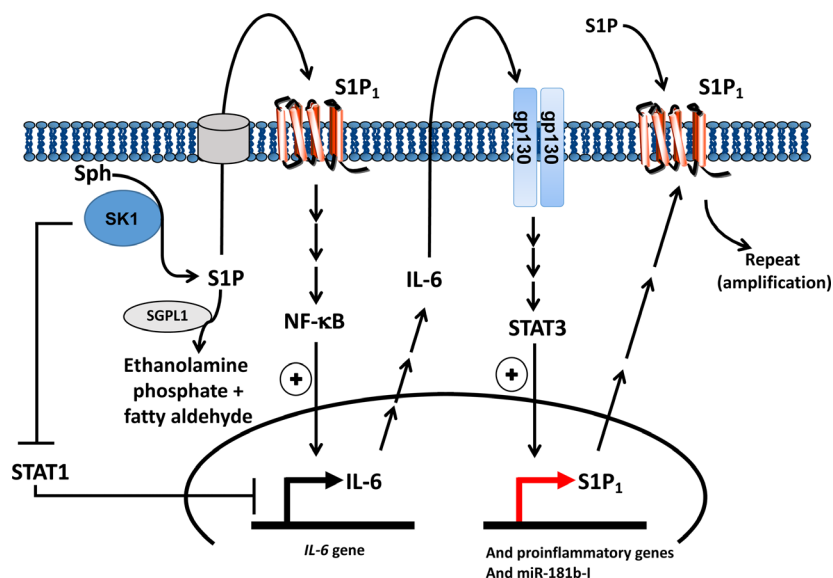
The S1P-JAK2-STAT3 axis of regulation represents a recent novel signalling network that has a significant role in regulating oncogenesis and cancer/stem cell survival and is therefore a potential target for therapeutic intervention in cancer. The possible mechanism by which S1P regulates IL-6-mediated STAT3 signalling and interaction with negative regulators such as STAT1 is summarised in Fig. 1.

### S1P: novel approaches and targets for therapeutic intervention

Pharmacological or biological targeting of S1P signalling in cancer cells is established experimentally to limit cancer progression and sensitise tumours to established anticancer agents. Despite this, very few such agents have been assessed in clinical trials for cancer treatment. Examples of agents (alone or in combination) reaching phase I/II trials for cancers include the SK2 inhibitor, ABC294640 (NCT01488513, NCT02229981, NCT02757326, NCT02939807, NCT03377179, NCT03414489) and the S1P-specific monoclonal antibody, sonopizumab (ASONEP)

(NCT00661414, NCT01762033), whereas phase I trials have been conducted for safingol (*L-threo*-dihydrosphingosine, which is also a PKC inhibitor) (NCT01553071, NCT00084812) and the prodrug and functional antagonist of S1P<sub>1, 3-5</sub>, FTY720 (Fingolimod) (NCT02490930 and, to counter chemotherapy-induced neuropathy, NCT03941743, NCT03943498). Of these, FTY720 is already licensed for therapeutic use in relapsing and remitting multiple sclerosis. It is also recognised to have multiple molecular targets and therefore actions.

An example of the potential for FTY720 in cancer treatment is its ability to suppress oncogenesis and tumour progression and reverse high-fat diet-induced loss of progesterone and oestrogen receptors (ER) in advanced breast carcinoma. Biotransformation to FTY720-phosphate (produced by SK2-catalysed phosphorylation of FTY720) results in inhibition of HDAC1/2 and enhanced histone acetylation leading to the regulation of a specific subset of genes. In this regard, FTY720 reactivates expression of ER $\alpha$  in ER $\alpha$ -negative human and murine breast cancer cells, which become sensitive to tamoxifen. Moreover, FTY720 re-establishes ER $\alpha$  expression in ER $\alpha$ -negative syngeneic breast tumours and confers sensitivity to tamoxifen *in vivo* [68]. Tamoxifen is an antagonist of ER $\alpha$ 66 but an agonist of the splice variant ER $\alpha$ 36. Notably, tamoxifen resistance correlates with increased SK1 and ER $\alpha$ 36 expression in tamoxifen-resistant breast cancer cells and in patient-derived xenografts. Moreover, stimulation of ER $\alpha$ 36 by either 17 $\beta$ -estradiol or tamoxifen activates SK1 and promotes release of S1P from triple-negative breast cancer cells. Therefore, targeting the ER $\alpha$ 36/SK1 axis might represent a novel therapeutic approach to treat tamoxifen-resistant breast cancer [69]. Another approach to combating triple-negative breast cancer is to sensitise tumours to Herceptin as reported recently using Compound 2 (Targaprimir-515), a designed small molecule inhibitor of noncoding RNA. The hairpin precursor of miR-515 is targeted by Compound 2, thereby inhibiting production of miR-515 which normally represses SK1 expression. Therefore, Compound 2 enhances SK1 expression, S1P levels and HER2 expression, which then provides sensitivity to Herceptin. However, this would need to be carefully balanced against the increased breast cancer cell migration that is also observed [70]. In addition, very high levels of SK1 expression reduce HER2 expression, in a negative feedback loop [71] and this would require consideration if using Compound 2 in any therapeutic approach. Pancreatic cancer is also difficult to treat and prognosis is poor. Persistent activation of STAT3



**Fig. 1.** Role of sphingosine kinase 1 and sphingosine 1-phosphate in regulating STAT signalling in cancer. Schematic showing a possible mechanism for the role of SK1 in regulating inflammation-associated cancer and cancer cell survival. S1P formation catalysed by SK1 is released from cancer cells via specific transporters to act in an autocrine manner ('inside-out' signalling) on S1P<sub>1</sub>. This results in the activation of NF-κB-induced IL-6 gene expression. The subsequently formed IL-6 acts on cell surface IL-6 receptor to promote STAT3-induced gene expression, including that of S1P<sub>1</sub> receptors, which in turn participate in an amplification loop to promote oncogenesis. In addition, SK1 represses the STAT1 signalling pathway, which normally opposes STAT3 signalling, thereby providing a negative regulatory node.

in KRAS-dependent cancers contributes to gemcitabine resistance and fibrous/connective tissue growth around the cancer. However, FTY720 may hold promise as a therapeutic agent, either alone or in combination with gemcitabine. FTY720 inhibits proliferation and increases apoptosis of pancreatic cancer cell lines; S1P<sub>1</sub>/STAT3 signalling is reduced and EMT prevented using a gemcitabine/FTY720 combination. Moreover, FTY720 enhances the cytotoxicity of gemcitabine in an orthotopic mouse model of pancreatic cancer, accompanied by a reduction in tumour size, increased apoptosis, inhibited NF-κB signalling, altered expression of gemcitabine-metabolising transport enzymes and restoration of the expression of the tumour suppressor protein PP2A [72].

Recent studies have identified Yes-associated protein 1 (YAP) signalling as a pathway regulated by S1P receptor activation, via Rho, and which may provide novel avenues for therapeutic intervention in cancer. YAP is an oncoprotein that is phosphorylated and inactive in the cytoplasm but can move to the nucleus and act as a transcriptional coactivator by relieving repression of subsets of genes. For example, in 1321N1 glioblastoma cells and patient-derived explants, S1P induces YAP activation to promote migration/invasion and MRTF-A (myocardin-related transcription factor

A) to enhance adhesion, while both YAP and MRTF-A cooperate to stimulate proliferation. S1P-treated YAP or MRTF-A knockout cells and gene expression analysis identified 44 genes that are induced through RhoA and highly dependent on one or other or both transcriptional regulators. Tissue factor F3 has been identified as a YAP-regulated gene and its transcription is required for cell invasion and migration, whereas MRTF-A-regulated expression of heparin-binding EGF-like growth factor (HBEGF) is essential for cell adhesion in response to S1P. In addition, both YAP- and MRTF-AA-regulated genes are linked to proliferation in response to S1P [73]. S1P signalling via YAP is also associated with the Warburg effect. In this case, the activation of S1P<sub>3</sub> receptors by S1P induces YAP signalling in osteosarcoma cells and YAP forms a complex with c-Myc to enhance transcription of phosphoglycerate mutase (PGAM1) of the glycolytic pathway, that is linked with the c-Myc-dependent increase in aerobic glycolysis of tumours. Moreover, the growth suppressive effect of methotrexate is potentiated by the S1P<sub>3</sub> antagonist TY52156 both *in vitro* and *in vivo* [74]. Therefore, antagonism of S1P<sub>3</sub> could hold therapeutic potential by limiting the YAP/myc-dependent upregulation of the glycolytic pathway to indirectly reduce tumour growth. S1P binding to S1P<sub>2</sub>



also activates YAP in both human and mouse HCC cells to stimulate proliferation *via* a connective tissue growth factor (CTGF)-dependent mechanism. In this case, YAP signalling upregulates CTGF expression. Activation of YAP by S1P<sub>2</sub> is also independent of MST1/2 suggesting that the canonical Hippo pathway is not involved. These findings are consolidated by the fact that hepatocytes of liver-specific YAP-overexpressing transgenic mice exhibit increased expression of S1P<sub>2</sub> and CTGF, thereby suggesting the presence of an amplification loop. Indeed, the transcription factor HNF4a has been identified as a negative regulator of S1P-induced CTGF expression, which is significant as its chromatin binding is influenced by YAP [75]. S1P binding to S1P<sub>2</sub> and S1P<sub>3</sub> also induces the rapid upregulation of SNAI2 in breast cancer cells *via* activation of YAP and MRTF-A, respectively. This is linked with increased invasiveness of MCF-7 breast cancer cells. Finally, SK1 expression correlates with SNAI2 in breast tumours of patients and with EMT score (critical for metastasis) in breast cancer cells [76]. Thus, S1P<sub>2</sub>/YAP/SNAI2 and S1P<sub>3</sub>/MRTF-A/SNAI2 may represent novel points for therapeutic intervention to limit metastasis in breast cancer.

### SGPP1/SGPP2 and cancer

S1P is dephosphorylated by two endoplasmic reticulum-localised phosphatases, SGPP1 and SGPP2 [77]. There is some evidence for the role of SGPP1 in cancer. For instance, SGPP1 expression levels are reduced in radiation resistance of tumours suggesting that the consequential increase in S1P levels might account for the resistance. Ionising radiation increases miR-95 levels, which reduces *SGPP1* transcription in PC3 prostate cancer cells. Moreover, the overexpression of miR-95 promotes PC3 xenograft tumour growth *in vivo* consistent with S1P being tumorigenic. In addition, miR-95-overexpressing tumours are more resistant to radiation-induced cell death compared with control tumours. A similar radio-resistant effect is evident in MDA-MB-231 breast cancer cells when miR-95 is overexpressed. Significantly, miR-95 levels are upregulated in prostate and breast tumours compared with normal tissues although a statistical significance with survival was not achieved [78]. In addition, a stem-like (ALDH1<sup>+</sup>CD133<sup>+</sup>) subpopulation of lung cancer cells exhibit elevated miR-95 and miR-21 levels (compared to ALDH1<sup>-</sup>CD133<sup>-</sup> cells). Indeed, combined anti-miR95 and anti-miR21 delivery *in vivo* reduces xenograft tumour growth and sensitises tumours to radiation. This was accompanied by an increase in the expression of SGPP1, SNX1 (sorting

nexin-1, involved in intracellular trafficking) and PTEN (phosphatase and tensin homologue) with an associated reduction in prosurvival AKT phosphorylation [79]. Thus, SGPP1 appears to exhibit a tumour suppressor function. Indeed, this is supported by earlier studies, which reported that siRNA knockdown of SGPP1 confers resistance to TNF and daunorubicin [80] and promotes an ER stress-induced autophagic survival response, associated with an increase in AKT phosphorylation [81]. There is also a functional link between Runx and SGPP1. In this case, Runx transcription factors (Runx1, 2 and 3) have previously been shown to repress transcription of *Sgpp1*, whereas they promote transcription of *Ugcg* (UDP-glucose ceramide glycosyltransferase) and *St3gal5* (ganglioside GM3 synthase). In addition, overexpression of Runx1 reduces certain ceramide species and promotes cell survival in fibroblasts [82]. In combination with overexpressed Myc or in the absence of p53, Runx1 functions as an oncogene to promote lymphoma and to confer resistance to glucocorticoids. This might involve the repression of dexamethasone-induced *Sgpp1* expression in T lymphoma to prevent cell death. Indeed, ectopic expression of Runx1 to reduce *Sgpp1* levels or shRNA knockdown of *Sgpp1* is protective against cell death. Thus, Runx-directed lymphomagenesis appears to involve increase flux through the sphingolipid rheostat as a consequence of *Sgpp1* transcriptional repression [83].

### SK1/SK2 and cancer

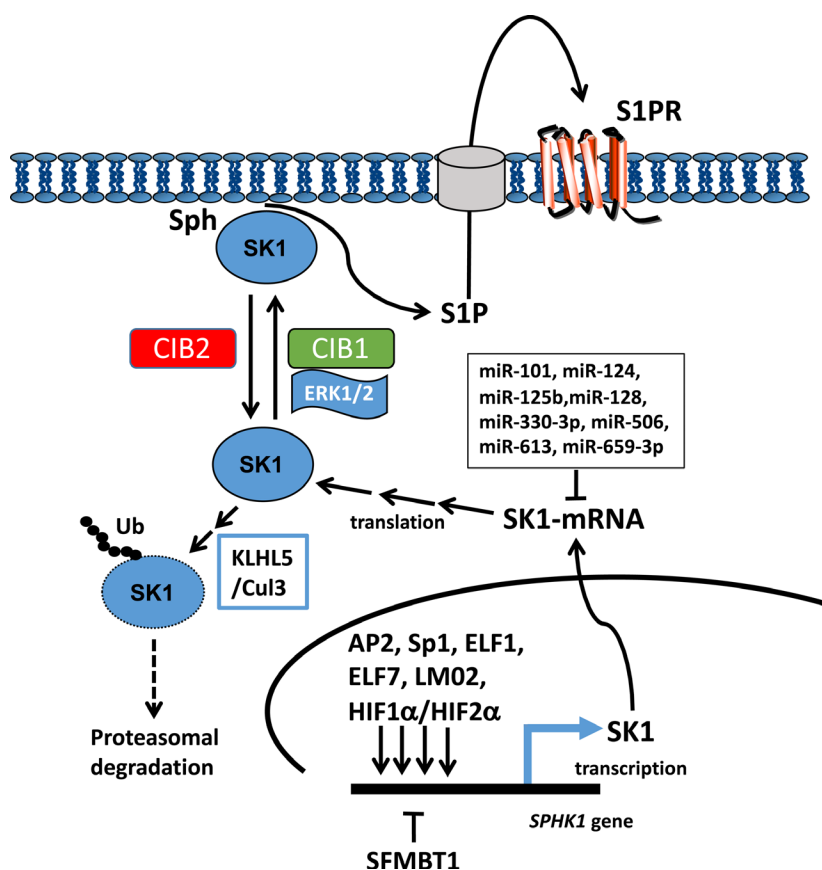
The molecular mechanisms regulating SK1 and SK2 are summarised in Figs 2 and 3, respectively. There are many examples of SK1 upregulation at the mRNA and protein level, which is often associated with poor prognosis including reduced survival and earlier disease recurrence in cancer patients [2]. Recent examples include melanoma [62], papillary thyroid carcinoma [84], non-small cell lung cancer [85], triple-negative breast cancer [86] and colorectal cancer [87]. This is consistent with the ability of SK1 to promote cell survival, proliferation and neoplastic transformation and supports the therapeutic potential of SK1 inhibitors. SK2 expression levels also exhibit prognostic significance in some cancer types. However, while some evidence supports a prosurvival role of SK2, including the anticancer effect of SK2 selective inhibitors, other evidence suggests SK2 has an antiproliferative/proapoptotic function. Examples of cancers with increased SK2 mRNA or protein in patient tumours include large granular lymphocyte leukaemia [88], papillary thyroid carcinoma [89], cholangiocarcinoma [90],

primary glioblastoma [91] and non-small cell lung cancer [92]. In contrast, high SK2 mRNA is associated with increased survival of patients with non-small cell lung carcinoma [93], while SK2 mRNA was reduced in oral cancer [94]. An analysis of available human cancer datasets indicate generally modest upregulation (up to 2.5-fold) of SK2 in many cancers. For instance, the expression levels of SK2 are increased in NK- and T-LGL (large granular lymphocyte) leukaemia and SK2 is involved in regulating cell survival, chemotherapeutic resistance and apoptosis. Thus, siRNA knockdown of SK2 reduces LGL proliferation and pharmacological inhibitors (ABC294640 and K145) decrease the prosurvival protein MCL-1 *via* proteasomal degradation and reduce cell viability [88].

The contrasting reports on the role of SK2 in cancer might be due to differing effects that are dependent on SK2 expression in various tumours. For instance, a comparison of low and high overexpression of SK2 revealed that high overexpression reduced cell proliferation and survival (and increased cellular ceramide levels), while low overexpression promoted cell survival and proliferation. Low overexpression of SK2 also induced neoplastic transformation *in vivo* together with

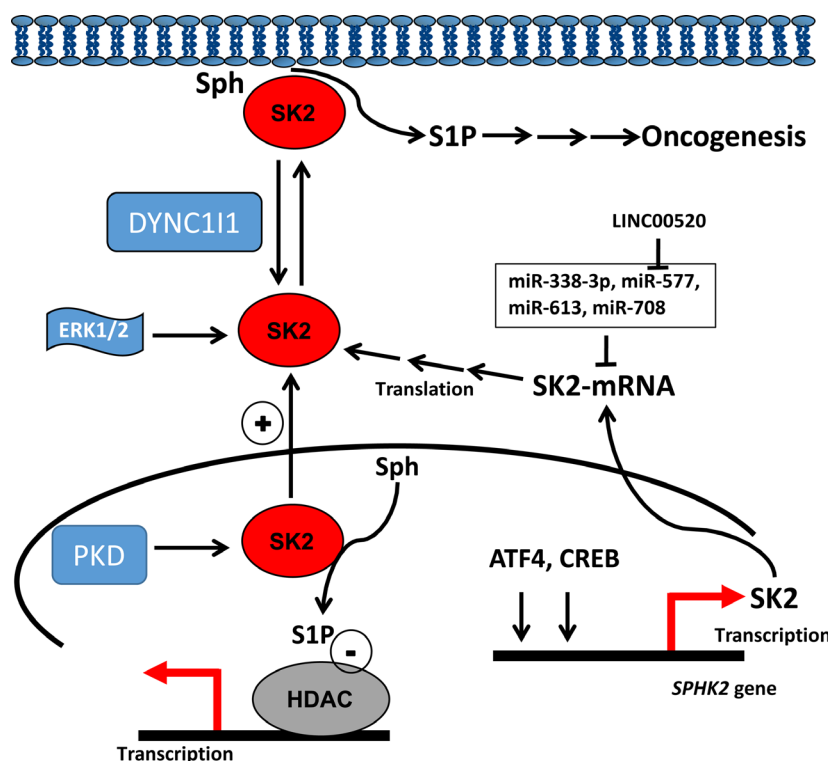
a redistribution of SK2 from a nuclear to plasma membrane localisation, which was accompanied by increased extracellular S1P formation. These findings suggest SK2-specific inhibitors hold therapeutic potential in the treatment of cancer [95]. Moreover, the findings suggest that SK2 might form competent signalling complexes with other proteins at low levels to promote survival (termed combinatorial signalling), but can function to form incompetent complexes dependent on the abundance of other binding proteins. This might effectively confer a dominant negative effect of high levels of SK2 due to competition of incompetent and competent SK2 complexes for the same effector. This might lead to enhanced apoptosis as a consequence of loss of protection by competent SK2 signalling complexes.

The level of SK1 or SK2 expression is determined by regulation of their transcription, translation and degradation. In this regard, transcriptional regulators which increase SK1 expression include AP2, Sp1 [96], E2F1 [97], E2F7 [98], LIM-domain-only protein 2 (LMO2) [99] and the hypoxia-inducible HIF1 $\alpha$ /HIF2 $\alpha$  [100], whereas SFMBT1 (Scm-like with four malignant brain tumour domains 1) has recently been shown to



**Fig. 2.** Regulation of sphingosine kinase 1 in cancer. Schematic showing the transcriptional and post-translational mechanisms regulating SK1 in cancer. Stimulated transcriptional regulation of SK1 gene expression involves AP2, Sp1, ELF1, ELF7, LMO2 and HIF1 $\alpha$ /HIF2 $\alpha$ , while inhibition involves SFMBT2. SK1 is post-translationally modified by ERK-2 (phosphorylation) and translocated to the plasma membrane; translocation is positively regulated by CIB1 and inhibited by CIB2. Localisation at the plasma membrane enables SK1 to access its substrate thereby leading to the production of S1P, which is then released to act on S1P receptors. SK1 is subject to regulation by KLH5-Cul3, thereby promoting ubiquitin-proteasomal degradation of SK1.

**Fig. 3.** Regulation of sphingosine kinase 2 in cancer. Schematic showing the transcriptional and post-translational mechanisms regulating SK2 in cancer. Transcriptional regulation of SK2 involves ATF4 and CREB, and noncoding RNAs, such as miR-338-3p, miR-577, miR-613 and miR-708, limit its translation. SK2 can translocate to and function at the plasma membrane to promote oncogenesis; this is negatively regulated by dynein (DYNC111). Nuclear SK2 produces S1P to inhibit HDAC1/2 and induce epigenetic regulation and the expression of immediate early genes. Post-translation regulation of SK2 involves phosphorylation by ERK-1/2 and PKD that can affect the subcellular localisation of SK2 and therefore cellular responses. For example, PKD promotes exit of SK2 from the nucleus and would therefore prevent regulation of HDAC1/2.



limit transcription of *SPHK1* [101]. The latter is a histone binding protein that mediates recruitment of corepressor proteins to target genes. At the post-transcriptional level, a number of micro-RNAs (miRNA) limit the translation of mRNA to SK1 protein. These include miRNA-101 (colorectal cancer), miRNA-124 (osteosarcoma), miRNA-125b, miRNA-128 (thyroid carcinoma), miRNA-330-3p (gastric cancer), miRNA-506 (liver cancer), miRNA-613 (bladder cancer) and miRNA-659-3p (colorectal cancer) and are reduced in cancer so may have potential for diagnosis, prognosis and therapeutics [102]. SK1 is subject to proteasomal degradation following its ubiquitination at K183. SK1 ubiquitination involves the Kelch-like protein 5 (KLHL5), which functions as an adaptor/linker between SK1 and the cullin 3 (Cul3) ubiquitin ligase complex [103]. Notably, SK1 inhibitors and chemotherapeutic agents induce the proteasomal degradation of SK1 which contributes to the anti-cancer activity of these compounds [104–106].

Transcription factors which increase SK2 expression include the ER stress marker ATF4 [107] and CREB [108], whereas miRNAs which normally limit SK2 expression but which are reduced in cancers include miR-338-3p (non-small cell lung carcinoma), miR-613 (papillary thyroid carcinoma) and miR-708 (glioma) [109–111] whereas miR-92b is increased and associated

with upregulation of SK2 (cholangiocarcinoma) [112]. In addition, the long noncoding RNA (lncRNA) LINC00520 modulates oncogenesis in several cancers, including papillary thyroid carcinoma (PTC) where high expression is associated with poor prognosis. Silencing of LINC00520 reduces growth and enhances apoptosis of PTC cells. A novel LINC00520/miR-577/SK2 axis in which LINC00520 neutralises miR-577 and thereby increases SK2 expression might be a viable target for therapeutics in PTC [89]. A further link is between S1P and PIWI-interacting RNA-004800 (piR-004800). PIWI-interacting RNAs (piRNAs) are noncoding single-stranded RNAs which exhibit altered expression in cancer. piR-004800 is overexpressed in bone marrow supernatant exosomes and primary cells from multiple myeloma (MM) patients and associated with MM stage. Interference of piR-004800 induces autophagic/apoptotic death of MM cells; this is significant as S1P receptor signalling pathways regulate the PI3K/Akt/mTOR pathway by modulating the expression of piR-004800 [113].

Both SK isoforms are also subject to post-translational modifications which affect their subcellular localisation and regulation through protein/protein interaction [2,114]. In this regard, SKIP has previously been identified as an inhibitor of SK1 in fibroblasts [115]. Recently, it has been shown that the SKIP gene

is silenced by hypermethylation of the gene promoter in acute myeloid leukaemia (AML). Primary AML cells have lower levels of SK1 and intracellular S1P and this can be reversed by re-expression of SKIP, concomitant with increased ceramide levels, and reduced ERK and increased apoptosis. Therefore, contrary to previous findings the downregulation of SKIP reduces SK1 activity in AML [116].

In addition to the level of expression, the intracellular localisation of SK1 and SK2 is also key. A Ras-driven upregulation of calcium and integrin binding 1 protein (CIB1) has been shown to mediate the translocation of SK1 from the cytoplasm to the plasma membrane and overexpression of CIB1 induces transformation in a SK1-dependent manner but without affecting SK1 expression [117]. CIB1 is a  $\text{Ca}^{2+}$ -myristoyl switch protein, required for agonist-stimulated translocation of SK1 to the plasma membrane and which interacts with SK1 independently of Ser225 phosphorylation [11], likely through helix  $\alpha 8$  [118]. In contrast, CIB2 (which lacks the  $\text{Ca}^{2+}$ -myristoyl switch function) opposes CIB1, blocks SK1 translocation to the plasma membrane and inhibits Ras-driven transformation [119]. CIB1 expression is upregulated in various cancer types and CIB2 downregulated [117,119]. Thus, sustained, aberrant localisation of SK1 at the plasma membrane promotes transformation. It remains to be determined whether recently identified CIB1 peptide inhibitors [120] that affect SK1 translocation have efficacy *in vivo* and whether they have therapeutic potential in cancers where CIB1 is upregulated.

SK2 is found in the nucleus (where S1P exerts epigenetic regulation), endoplasmic reticulum (where it is involved in regulating ER stress [121,122]) and mitochondria (where it is involved in cell death mechanisms) [12]. However, SK2 also localises to the plasma membrane where it has recently been implicated in cancer initiation and progression. An interaction of SK2 with the intermediate chain subunits of the retrograde-directed transport motor complex, cytoplasmic dynein 1 (DYNC1H1 and -2) facilitates SK2 movement away from the plasma membrane. This is important since low expression of DYNC1H1 is associated with reduced survival in glioblastoma patients and possibly increased plasma membrane localisation of SK2. Indeed, DYNC1H1 re-expression reduces plasma membrane-localised SK2, S1P release, tumour growth and progression *in vivo*. Pharmacological inhibition of SK2 similarly decreased tumour growth *in vivo*. Thus, DYNC1H1 is tumour-suppressive and its regulation of SK2 may provide new opportunities for therapeutic intervention in glioblastoma [123].

There are many examples of interplay between SK1 or SK2, oncogenes and tumour suppressors. For example, Ras proteins are commonly mutated in cancers [124] and S1P increases (while ceramide decreases) in K-RasG12V overexpressing cells where SK1 translocation to the plasma membrane (and therefore access to sphingosine) is increased [125]. This may be due to the upregulation of CIB1 [117] rather than ERK-1/2-catalysed phosphorylation of Ser225 of SK1 to promote its membrane recruitment [126]. Indeed, K-Ras-driven oncogenic transformation is independent SK1 phosphorylation [125].

SK2 is involved in regulating expression of c-Myc, a prognostic marker of B-cell acute lymphoblastic leukaemia (B-ALL) progression and severity. This is likely through S1P-dependent inhibition of HDAC1/2 activity. Thus, pharmacological inhibition of SK2 extends survival of mice in xenograft models and knockout of *Sphk2* reduces leukaemia development in a mouse model of ALL. In both cases, c-Myc expression is reduced; this is significant as Myc is a prognostic marker of B-ALL disease progression and severity [127]. Decreased c-Myc expression is also reported in *Sphk1*<sup>-/-</sup> mice where fewer and smaller liver tumours are induced by diethylnitrosamine treatment [128]. SK1 inhibition also induces a p53-dependent autophagic death of cancer cells. Indeed, the SK1 selective inhibitor, SK1-I, reduces cancer cell growth and induces apoptosis of wild-type TP53 cells, but not TP53 null cells. This is associated with phosphorylation of p53 at Ser15 and transcriptional activation of BAX, BAK1 and BID in wild-type TP53 cells. Inhibition of BECN1 and ATG5 reduces the cytotoxicity of SK1-I; SK1-I also induces formation of autophagic vesicles and large vacuoles in a p53-dependent manner [129]. A novel Von Hippel-Lindau (VHL)-SFMBT1-SK1 axis has also recently been identified. The absence or mutation of the VHL tumour repressor protein is associated with increased expression of SK1 in clear cell renal cell carcinoma (ccRCC) [130]. In this regard, VHL facilitates ubiquitination and degradation of SFMBT1 but ccRCC patients with VHL loss-of-function mutations display elevated SFMBT1 protein levels; depletion of SFMBT1 inhibits orthotopic tumour growth *in vivo* and cell proliferation *in vitro*. This is important as *SPHK1* has been identified as a SFMBT1-regulated gene contributing to its oncogenic phenotype [101].

## SK inhibitor development

The most important future development concerns the design of novel nanomolar potent SK inhibitors with



isoform selectivity. This would allow targeting cancers where one or other isoform has a predominant role. In addition, isoform selective inhibitors would maintain host S1P thereby avoiding deleterious side effect. Although there are a number of nanomolar potent inhibitors of SK1, for example PF-543 [131], there has been slower development of nanomolar potent SK2 selective inhibitors. In this regard, mapping SK2 amino acid differences onto the SK1 crystal structure indicates subtle differences in the 'foot' of 'J-channel' (which accommodates sphingosine) of the two isoforms. Probing these isoform-specific differences with a chemical series (derived from the potent SK1-selective inhibitor, PF-543) demonstrated that it was possible to systematically turn a 100-fold SK1-selective inhibitor, through chemical modification, to an equipotent SK1/SK2 inhibitor (Compound 49,  $pIC_{50}$  7.8) and, with further modification, to a 100-fold SK2 selective inhibitor, with nanomolar potency (HWG-35D (Compound 55),  $pIC_{50}$  7.4) [132]. In addition, structure–activity relationship profiling has identified a side cavity in SK2 that can be exploited to increase inhibitor potency, with relatively small hydrophobic moieties preferred (e.g., SLM6071469,  $K_i = 89$  nM, 73-fold SK2 selective) [133]. The utility of SK2 inhibitors is exemplified by the synergy observed between bortezomib and the micromolar potent SK2 inhibitor, K145. Each of these compounds induces ER stress and an unfolded protein response to induce apoptosis in myeloma cells *in vitro*. Their synergistic effect was replicated *in vivo* where survival was extended in a murine myeloma model [121]. Future challenges involve the development of drug-like SK isoform selective inhibitors that exhibit good pharmacokinetic and pharmacodynamic properties with limited side effects.

## Conclusion

We have surveyed the most recent observations concerning the role of S1P in cancer, which highlights several significant advances. These include evidence for mutations of S1P receptors and metabolising enzymes, such as S1P lyase, that impact cancer progression and prognosis; identification of the involvement of S1P with novel signalling networks that are inextricably linked with oncogenesis, such as JAK2/STAT3 and YAP; and the development of highly potent SK isoform selective inhibitors and S1P receptor modulators. Our understanding of the role of S1P in cancer stem cell biology is also improving, with respect to the regulation of fundamental biology and chemotherapeutic resistance, the latter being a major problem in terms of effective treatment for cancer patients. In this

regard, modulation of the S1P signalling axis to revert triple-negative aggressive breast cancer to ER<sup>+</sup> or HER2<sup>+</sup> cancer that can be treated with established medicines, such as tamoxifen and Herceptin, represents a very important advance and paradigm shift in stratified medicine approaches. However, there are still many unresolved controversies, such as the role of S1P<sub>1</sub> and S1P<sub>2</sub> in cancer, which require more clarity in order to inform on their potential as therapeutic targets using precision medicine approaches. Nevertheless, there is much optimism that effective S1P-directed therapeutics for cancer treatment will be developed in the future.

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