FEBS Letters

FEBS PRESS science publishing by scientists

REVIEW ARTICLE

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

Nigel J. Pyne and Susan Pyne

Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde, Glasgow, UK

Correspondence
S. Pyne, Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde, 161 Cathedral Street, Glasgow G4 0RE, UK
Tel: +44 141 548 2012
E-mail: susan.pyne@strath.ac.uk

(Received 22 August 2020, revised 5 September 2020, accepted 7 September 2020)

doi:10.1002/1873-3468.13933

Edited by Christopher Clarke

Sphingosine 1-phosphate (S1P) is a bioactive lipid that binds to a family of G protein-coupled receptors ($S1P_{1–5}$) 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 neovascularisation, 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; DYNC1I1, dynein 1; EDAC, epithelial defence against cancer; EGF, epidermal growth factor; ERα, 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; $S1P_{1–5}$, 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_2–C_3 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 $S1P_{1–5}$, 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 $S1P_1$. This limits the $S1P_1$-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, $S1P_1$ signalling is more sustained for HDL-S1P compared with albumin-S1P [8]. On the other hand, S1P may access the binding pocket of $S1P_1$ 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 (PPARγ) 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 $S1P_2$ *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 $S1P_1$ 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 $S1P_1$ 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 $S1P_1$ 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 $S1P_1$ 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, $S1P_1$, 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 $S1P_1$ 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 $S1P_1$ 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 $S1P_1$ 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 $S1P_1$, while decreasing CCR7 levels in chronic lymphocytic leukaemia.

In the context of solid tumours, antagonism of $S1P_1$ 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 $S1P_1$ 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 $S1P_1$ receptors was shown to limit vascularisation and reduce tumour growth [35]. However, contrasting observations have been made by others. For example, elimination of $S1P_1$ receptors from the vascular endothelium promotes excessive sprouting and branching and this was ablated by overexpression of $S1P_1$ 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 $S1P_1$ was overexpressed in endothelial cells, accompanied by increased efficacy of antitumour therapies. Thus, expression of $S1P_1$ in endothelial cells induces vascular normalisation and suggests that enhancing $S1P_1$ 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 $S1P_1$ and $S1P_2$ function may contribute to tumour vessel hyperpermeability in this case. Indeed, pharmacological activation of $S1P_1$, using SEW2871, or antagonism of $S1P_2$, using JTE-013, enhances the organisation and integrity of tumour vessels and improves antitumour efficacy. However, a potential involvement of tumour, rather than vessel, $S1P_1$ and/or $S1P_2$ was not excluded. Despite this, there may be potential for adjuvant $S1P_1$ agonists and/or $S1P_2$ antagonists with standard chemotherapy for Ewing sarcoma patients [37]. In contrast, the $S1P_1$ 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 $S1P_1$ agonists, competitive antagonists and functional antagonists may depend upon the cancer type and further research is warranted.

The role of $S1P_2$ in cancer is also somewhat controversial. A body of evidence suggests that $S1P_2$ 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 $S1P_2$-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 $S1P_2$ antagonist, or $S1P_2$ knockdown reduces apical extrusion of RasV12-transformed cells *in vitro*. Moreover, $S1P_2$ 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, $S1P_2$ 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 $S1P_2$ agonist, CYM5520, but not by other S1P receptor subtype-selective agonists. Moreover, the selective antagonist for $S1P_2$, JTE-013 or knockdown of $S1P_2$ with siRNA reduces the inhibitory effect of S1P on HCC migration [41]. This highlights the possibility that $S1P_2$ receptor-selective agonists might be usefully employed to inhibit metastasis of HCC. In regard to a limiting effect of $S1P_2$, high nuclear expression of this receptor in tumours from ER^+ 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 $S1P_2$. Indeed, aged $S1pr2^{-/-}$ mice develop germinal centre (GC)-derived DLBCL [43] where $S1P_2$ 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 $S1P_2$, 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 $S1P_2$ can be lost through transcriptional silencing by FOXP1. Thus, $S1P_2$ expression was repressed in GC-DLBCL cell lines with aberrantly high levels of the haematopoietic oncoprotein FOXP1. Moreover, low $S1P_2$ expression was prognostic for reduced patient survival, alone and especially in combination with high FOXP1 expression [45]. In normal B cells, $S1P_2$ expression is regulated through the TGF-β/TGF-βRII/SMAD1 signalling pathway. However, this pathway may be ablated in DLBCL patients; DLBCL cell lines deficient in $S1P_2$, TGFBRII 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-β-induced apoptosis [46].

In contrast to the preceding examples, a deleterious role for $S1P_2$ is evident from other studies. For example, SK1 activation and S1P release with subsequent activation of $S1P_2$ upregulate transferrin receptor 1 (TFR1) expression, which contributes to SK1-mediated transformation [47]. In addition, $S1P_2$ is shed in $hsp70^+$ and $CD63^+$ containing exosomes from MDA-MB-231 breast cancer cells. When these exosomes are added to fibroblasts, $S1P_2$ 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 $S1P_2$, which might correspond to the processed form generated in fibroblasts, is also constitutively activity in transfected HEK293 cells [48]. $S1P_2$ is also involved in metastatic spread. For example, lung colonisation by tumour cells is promoted by systemic S1P, formed by host SK1, through a $S1P_2$/Brms1 (breast carcinoma metastasis suppressor 1) axis; systemic S1P increases $S1P_2$ expression in cancer cells and activation of tumour $S1P_2$ 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 $S1P/S1P_3$-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^{2+} handling by mitochondria, affecting downstream cellular responses. This is significant in the context of oncogenesis where Ca^{2+} 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^{2+} 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^{2+} 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 $SK1/S1P/S1P_1$ is at the nexus between NF-κB, IL-6 and STAT3 signalling and increased $S1P_1$ 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- $Sgpl1^{-/-}$) or tissue (T- $Sgpl1^{-/-}$). 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- $Sgpl1^{-/-}$ enhance immune cell infiltration, thereby initiating colitis. Formation of tumours is delayed due to pathological crypt

remodelling and S1P signalling associated with increased $S1P_1$/STAT3 mRNA, expression of programmed cell death ligand 1 and a counter regulatory phosphorylation of $STAT1^{S727}$. In contrast, epithelial-driven tumours develop immediately in T- *Sgpl1*$^{-/-}$ mice. These tumours exhibit increased SK1, $S1P_2$ 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, $S1P_1$ 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 $S1P_4$-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, $S1P_2$ agonists might be useful in the management of chemotherapy-induced neuropathy since the selective $S1P_2$ 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 $CD4^+Foxp3^+$ Tregs in circulating and tumour-infiltrating lymphocytes and increased tumour-infiltrating $Foxp3^+$ Tregs that is correlated with increased tumour $S1P_1$ expression. In addition, $S1P_1$ and Tregs are associated with poor patient survival. *In vitro* data support a mechanism of $S1P_1$-mediated Treg formation from $CD4^+CD25^-$ cells involving TGF-β and IL-10 release from BC cells. Tumour $S1P_1$ also promotes Treg migration, and therefore, $S1P_1$ 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 PPARγ expression and activity. In addition, S1P formed in T cells by SK1 directly activates PPARγ, whereas PPARγ-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 TGFβ, 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 $S1P_1$ 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 IFNγ [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 β3 adrenergic receptors (β3-AR) and SK2/$S1P_2$ in neuroblastoma where the regulation between stemness and differentiation is particularly important. In this regard, β adrenergic receptors are established players in the pathogenesis of multiple cancers, including neuroblastoma. Importantly, antagonism of β3-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 $S1P_2$ (i.e. blockade of $SK2/S1P_2$ signalling) whereas the $S1P_2$ agonist, CYM5520, counters the effects of β3-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 (HIF1α and HIF2α)-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, sonepcizumab (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 $S1P_{1,\ 3–5}$, 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 ERα in ERα-negative human and murine breast cancer cells, which become sensitive to tamoxifen. Moreover, FTY720 re-establishes ERα expression in ERα-negative syngeneic breast tumours and confers sensitivity to tamoxifen *in vivo* [68]. Tamoxifen is an antagonist of ERα66 but an agonist of the splice variant ERα36. Notably, tamoxifen resistance correlates with increased SK1 and ERα36 expression in tamoxifen-resistant breast cancer cells and in patient-derived xenografts. Moreover, stimulation of ERα36 by either 17β-estradiol or tamoxifen activates SK1 and promotes release of S1P from triple-negative breast cancer cells. Therefore, targeting the ERα36/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

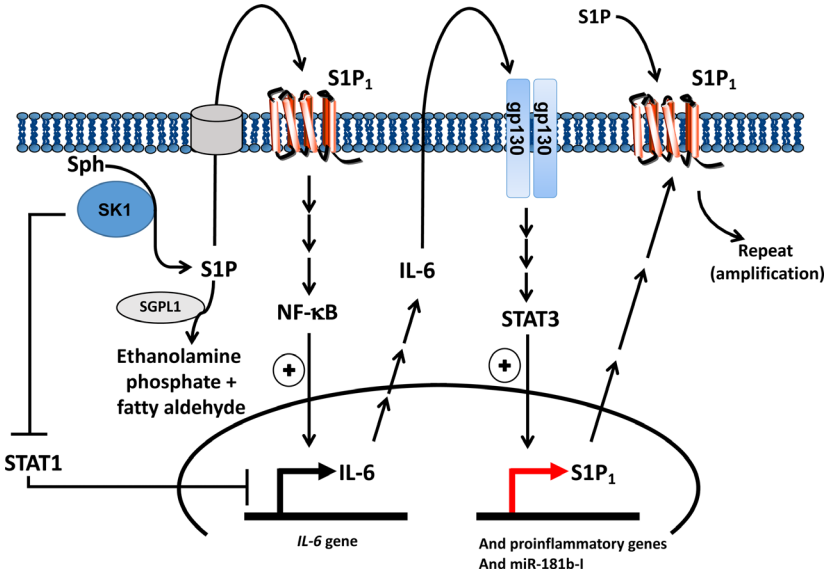

Fig. 1. Role of sphingosine kinase 1 and sphingosine 1-phosphate in regulating STAT signalling in cancer. Schematic showing a possible mechanism for the role of SK1 in regulating inflammation-associated cancer and cancer cell survival. S1P formation catalysed by SK1 is released from cancer cells *via* specific transporters to act in an autocrine manner ('inside-out' signalling) on $S1P_1$. 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 $S1P_1$ 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; $S1P_1$/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 $S1P_3$ 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 $S1P_3$ antagonist TY52156 both *in vitro* and *in vivo* [74]. Therefore, antagonism of $S1P_3$ could hold therapeutic potential by limiting the YAP/myc-dependent upregulation of the glycolytic pathway to indirectly reduce tumour growth. S1P binding to $S1P_2$

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 $S1P_2$ 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 $S1P_2$ 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 $S1P_2$ and $S1P_3$ 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, $S1P_2$/YAP/SNAI2 and $S1P_3$/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 ($ALDH1^+CD133^+$) subpopulation of lung cancer cells exhibit elevated miR-95 and miR-21 levels (compared to $ALDH1^-CD133^-$ 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 dexamethasome-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 HIF1α/HIF2α [100], whereas SFMBT1 (Scm-like with four malignant brain tumour domains 1) has recently been shown to

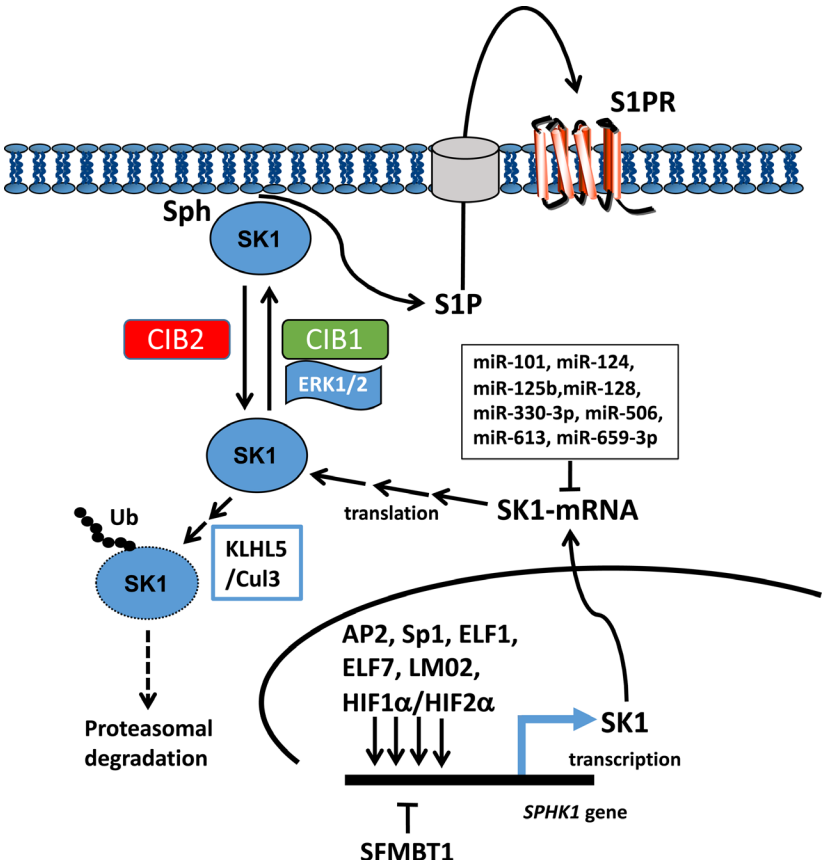

Fig. 2. Regulation of sphingosine kinase 1 in cancer. Schematic showing the transcriptional and post-translational mechanisms regulating SK1 in cancer. Stimulated transcriptional regulation of SK1 gene expression involves AP2, Sp1, ELF1, ELF7, LM02 and HIF1α/HIF2α, while inhibition involves SFMBT2. SK1 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, there by promoting ubiquitin-proteasomal degradation of SK1.

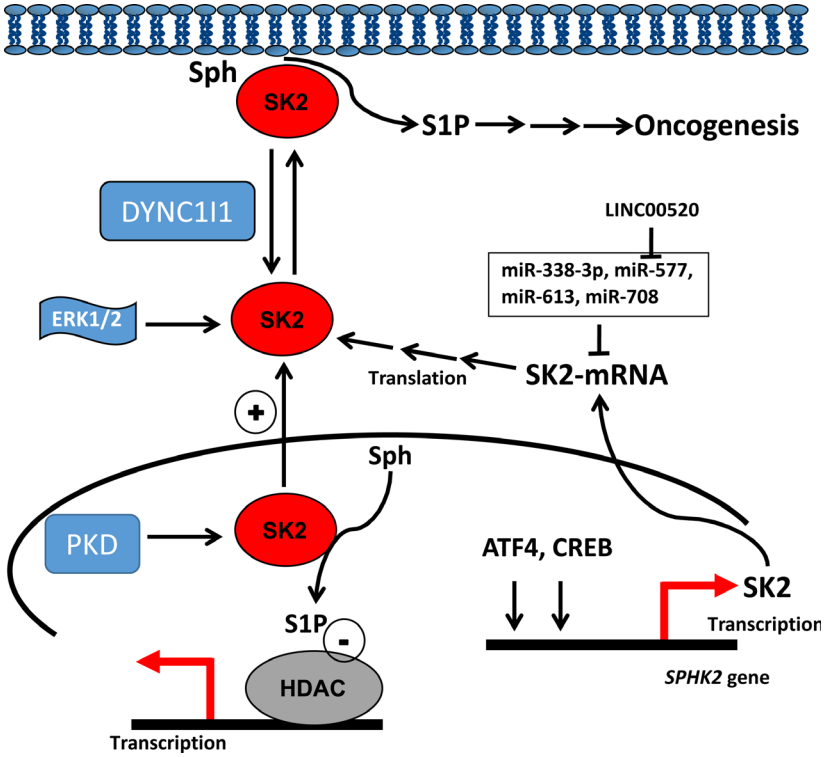

Fig. 3. Regulation of sphingosine kinase 2 in cancer. Schematic showing the transcriptional and post-translational mechanisms regulating SK2 in cancer. Transcriptional regulation of SK2 involves ATF4 and CREB, and noncoding RNAs, such as miR-338-3p, miR-577, miR-613 and miR-708, limit its translation. SK2 can translocate to and function at the plasma membrane to promote oncogenesis; this is negatively regulated by dynein (DYNC1I1). 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 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 helx α8 [118]. In contrast, CIB2 (which lacks the 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 (DYNC1I1 and -2) facilitates SK2 movement away from the plasma membrane. This is important since low expression of DYNC1I1 is associated with reduced survival in glioblastoma patients and possibly increased plasma membrane localisation of SK2. Indeed, DYNC1I1 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, DYNC1I1 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 $Sphk1^{-/-}$ 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 SKI-1; 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^+ or $HER2^+$ 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 $S1P_1$ and $S1P_2$ 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.

References

1 Pyne NJ and Pyne S (2010) Sphingosine 1-phosphate and cancer. *Nat Rev Cancer* **10**, 489–503.
2 Pyne S, Adams DR and Pyne NJ (2016) Sphingosine 1-phosphate and sphingosine kinases in health and disease: recent advances. *Prog Lipid Res* **62**, 93–106.
3 Brinkmann V, Cyster JG and Hla T (2004) FTY720: sphingosine 1-phosphate receptor-1 in the control of lymphocyte egress and endothelial barrier function. *Am J Transplant* **4**, 1019–1025.
4 Watson C, Long JS, Orange C, Tannahill CL, Mallon E, McGlynn LM, Pyne S, Pyne NJ and Edwards J (2010) High expression of sphingosine 1-phosphate receptors, S1P1 and S1P3, sphingosine kinase 1, and extracellular signal-regulated kinase-1/2 is associated with development of tamoxifen resistance in estrogen receptor-positive breast cancer patients. *Am J Pathol* **177**, 2205–2215.
5 Nishi T, Kobayashi N, Hisano Y, Kawahara A and Yamaguchi A (2014) Molecular and physiological functions of sphingosine 1-phosphate transporters. *Biochem Biophys Acta* **1841**, 759–765.
6 Vu TM, Ishizu AN, Foo JC, Toh XR, Zhang F, Whee DM, Torta F, Cazenave-Gassiot A, Matsumura T, Kim S *et al.* (2017) Mfsd2b is essential for the sphingosine-1-phosphate export in erythrocytes and platelets. *Nature* **550**, 524–528.
7 Hisano Y, Kobayashi N, Yamaguchi A and Nishi T (2012) Mouse SPNS2 functions as a sphingosine-1-phosphate transporter in vascular endothelial cells. *PLoS One* **7**, e38941.
8 Galvani S, Sanson M, Blaho VA, Swendeman SL, Obinata H, Conger H, Dahlbäck B, Kono M, Proia RL, Smith JD *et al.* (2015) HDL-bound sphingosine 1-phosphate acts as a biased agonist for the endothelial cell receptor S1P1 to limit vascular inflammation. *Sci Signal* **8**, ra79.
9 Hanson MA, Roth CB, Jo E, Griffith MT, Scott FL, Reinhart G, Desale H, Clemons B, Cahalan SM,

Schuerer SC *et al*. (2012) Crystal structure of a lipid G protein-coupled receptor. *Science* **335**, 851–855.
10 Spiegel S, Maczis MA, Maceyka M and Milstien S (2019) New insights into functions of the sphingosine-1-phosphate transporter SPNS2. *J Lipid Res* **60**, 484–489.
11 Jarman KE, Moretti PA, Zebol JR and Pitson SM (2010) Translocation of sphingosine kinase 1 to the plasma membrane is mediated by calcium- and integrin-binding protein 1. *J Biol Chem* **285**, 483–492.
12 Maceyka M, Sankala H, Hait NC, Le Stunff H, Liu H, Toman R, Collier C, Zhang M, Satin LS, Merrill AH Jr *et al*. (2005) SphK1 and SphK2, sphingosine kinase isoenzymes with opposing functions in sphingolipid metabolism. *J Biol Chem* **280**, 37118–37129.
13 Ding G, Sonoda H, Yu H, Kajimoto T, Goparaju SK, Jahangeer S, Okada T and Nakamura S (2007) Protein kinase D-mediated phosphorylation and nuclear export of sphingosine kinase 2. *J Biol Chem* **282**, 27493–27502.
14 Alvarez SE, Harikumar KB, Hait NC, Allegood J, Strub GM, Kim EY, Maceyka M, Jiang H, Luo C, Kordula T *et al*. (2010) Sphingosine-1-phosphate is a missing cofactor for the E3 ubiquitin ligase TRAF2. *Nature* **465**, 1084–1088.
15 Etemadi N, Chopin M, Anderton H, Tanzer MC, Rickard JA, Abeysekera W, Hall C, Spall SK, Wang B, Xiong Y *et al*. (2015) TRAF2 regulates TNF and NF-κB signalling to suppress apoptosis and skin inflammation independently of Sphingosine kinase 1. *eLife* **4**, e10592.
16 Strub GM, Paillard M, Liang J, Gomez L, Allegood JC, Hait NC, Maceyka M, Price MM, Chen Q, Simpson DC *et al*. (2011) Sphingosine-1-phosphate produced by sphingosine kinase 2 in mitochondria interacts with prohibitin 2 to regulate complex IV assembly and respiration. *FASEB J* **25**, 600–612.
17 Chipuk JE, McStay GP, Bharti A, Kuwana T, Clarke CJ, Siskind LJ, Obeid LM and Green DR (2012) Sphingolipid metabolism cooperates with BAK and BAX to promote the mitochondrial pathway of apoptosis. *Cell* **148**, 988–1000.
18 Panneer Selvam S, De Palma RM, Oaks JJ, Oleinik N, Peterson YK, Stahelin RV, Skordalakes E, Ponnusamy S, Garrett-Mayer E, Smith CD *et al*. (2015) Binding of the sphingolipid S1P to hTERT stabilizes telomerase at the nuclear periphery by allosterically mimicking protein phosphorylation. *Sci Signal* **8**, ra58.
19 Hait NC, Allegood J, Maceyka M, Strub GM, Harikumar KB, Singh SK, Luo C, Marmorstein R, Kordula T, Milstien S *et al*. (2009) Regulation of histone acetylation in the nucleus by sphingosine-1-phosphate. *Science* **325**, 1254–1257.
20 Parham KA, Zebol JR, Tooley KL, Sun WY, Moldenhauer LM, Cockshell MP, Gliddon BL, Moretti PA, Tigyi G, Pitson SM *et al*. (2015) Sphingosine 1-phosphate is a ligand for peroxisome proliferator-activated receptor-γ that regulates neoangiogenesis. *FASEB J* **29**, 3638–3653.
21 Adada MM, Canals D, Jeong N, Kelkar AD, Hernandez-Corbacho M, Pulkoski-Gross MJ, Donaldson JC, Hannun YA and Obeid LM (2015) Intracellular sphingosine kinase 2-derived sphingosine-1-phosphate mediates epidermal growth factor-induced ezrin-radixin-moesin phosphorylation and cancer cell invasion. *FASEB J* **29**, 4654–4669.
22 Shen H, Giordano F, Wu Y, Chan J, Zhu C, Milosevic I, Wu X, Yao K, Chen B, Baumgart T *et al*. (2014) Coupling between endocytosis and sphingosine kinase 1 recruitment. *Nat Cell Biol* **16**, 652–662.
23 Maczis M, Milstien S and Spiegel S (2016) Sphingosine-1-phosphate and estrogen signalling in breast cancer. *Adv Biol Regul* **60**, 160–165.
24 Saba JD (2019) Fifty years of lyase and a moment of truth: sphingosine phosphate lyase from discovery to disease. *J Lipid Res* **60**, 456–463.
25 Singh SK and Spiegel S (2020) Sphingosine-1-phosphate signalling: a novel target for simultaneous adjuvant treatment of triple negative breast cancer and chemotherapy-induced neuropathic pain. *Adv Biol Reg* **75**, 100670.
26 Hernández-Coronado CG, Guzmán A, Castillo-Juárez H, Zamora-Gutiérrez D and Rosales-Torres AM (2019) Sphingosine-1-phosphate (S1P) in ovarian physiology and disease. *Ann Endocrinol* **80**, 263–272.
27 Sukocheva OA, Furuya H, Ng ML, Friedemann M, Menschikowski M, Tarasov VV, Chubarev VN, Klochkov SG, Neganova ME, Mangoni AA *et al*. (2020) Sphingosine kinase and sphingosine-1-phosphate receptor signalling pathway in inflammatory gastrointestinal disease and cancers: a novel therapeutic target. *Pharmacol Ther* **207**, 107464.
28 Maceyka M, Rohrbach T, Milstien S and Spiegel S (2020) Role of sphingosine kinase 1 and sphingosine-1-phosphate axis in hepatocellular carcinoma. *Handb Exp Pharmacol* **259**, 3–17.
29 Tea MN, Poonnoose SI and Pitson SM (2020) Targeting the sphingolipid system as a therapeutic direction for glioblastoma. *Cancers* **12**, 111.
30 Albinet V, Bats ML, Huwiler A, Rochaix P, Chevreau C, Ségui B, Levade T and Andrieu-Abadie N (2014) Dual role of sphingosine kinase-1 in promoting the differentiation of dermal fibroblasts and the dissemination of melanoma cells. *Oncogene* **33**, 3364–3373.
31 Kim EY, Choi B, Kim JE, Park SO, Kim SM and Chang EJ (2020) Interleukin-22 mediates the chemotactic migration of breast cancer cells and macrophage infiltration of the bone microenvironment by potentiating S1P/SIPR signalling. *Cells* **9**, 131.

32 Wasik AM, Wu C, Mansouri L, Rosenquist R, Pan-Hammarström Q and Sander B (2018) Clinical and functional impact of recurrent *S1PR1* mutations in mantle cell lymphoma. *Blood Adv* **2**, 621–625.
33 Visentin B, Vekich JA, Sibbald BJ, Cavalli AL, Moreno KM, Matteo RG, Garland WA, Lu Y, Yu S, Hall HS *et al.* (2006) Validation of an anti-sphingosine-1-phosphate antibody as a potential therapeutic in reducing growth, invasion, and angiogenesis in multiple tumour lineages. *Cancer Cell* **9**, 225–238.
34 Taniguchi K, Ishizaki T, Ayada T, Sugiyama Y, Wakabayashi Y, Sekiya T, Nakagawa R and Yoshimura A (2009) Sprouty4 deficiency potentiates Ras-independent angiogenic signals and tumour growth. *Cancer Sci* **100**, 1648–1654.
35 Balaji Ragunathrao VA, Anwar M, Akhter MZ, Chavez A, Mao Y, Natarajan V, Lakshmikanthan S, Chrzanowska-Wodnicka M, Dudek AZ, Claesson-Welsh L *et al.* (2019) Sphingosine-1-phosphate receptor 1 activity promotes tumour growth by amplifying VEGF-VEGFR2 angiogenic signalling. *Cell Rep* **29**, 3472–3487.e4.
36 Cartier A, Leigh T, Liu CH and Hla T (2020) Endothelial sphingosine 1-phosphate receptors promote vascular normalization and antitumour therapy. *Proc Natl Acad Sci USA* **117**, 3157–3166.
37 Marmonti E, Savage H, Zhang A, Bedoya C, Morrell MG, Harden A, Buzbee M and Schadler K (2020) Modulating sphingosine-1-phosphate receptors to improve chemotherapy efficacy against Ewing sarcoma. *Int J Cancer* **147**, 1206–1214.
38 Lupino L, Perry T, Margielewska S, Hollows R, Ibrahim M, Care M, Allegood J, Tooze R, Sabbadini R, Reynolds G *et al.* (2019) Sphingosine-1-phosphate signalling drives an angiogenic transcriptional programme in diffuse large B cell lymphoma. *Leukemia* **33**, 2884–2897.
39 Yamamoto S, Yako Y, Fujioka Y, Kajita M, Kameyama T, Kon S, Ishikawa S, Ohba Y, Ohno Y, Kihara A *et al.* (2016) A role of the sphingosine-1-phosphate (S1P)-S1P receptor 2 pathway in epithelial defense against cancer (EDAC). *Mol Biol Cell* **27**, 491–499.
40 Yamashita H, Kitayama J, Shida D, Yamaguchi H, Mori K, Osada M, Aoki S, Yatomi Y, Takuwa Y and Nagawa H (2006) Sphingosine 1-phosphate receptor expression profile in human gastric cancer cells: differential regulation on the migration and proliferation. *J Surg Res* **130**, 80–87.
41 Matsushima-Nishiwaki R, Yamada N, Fukuchi K and Kozawa O (2018) Sphingosine 1-phosphate (S1P) reduces hepatocyte growth factor-induced migration of hepatocellular carcinoma cells via S1P receptor 2. *PLoS One* **13**, e0209050.
42 Ohotski J, Edwards J, Elsberger B, Watson C, Orange C, Mallon E, Pyne S and Pyne NJ (2013) Identification of novel functional and spatial associations between sphingosine kinase 1, sphingosine 1-phosphate receptors and other signalling proteins that affect prognostic outcome in estrogen receptor-positive breast cancer. *Int J Cancer* **132**, 605–616.
43 Cattoretti G, Mandelbaum J, Lee N, Chaves AH, Mahler AM, Chadburn A, Dalla-Favera R, Pasqualucci L and MacLennan AJ (2009) Targeted disruption of the S1P2 sphingosine 1-phosphate receptor gene leads to diffuse large B-cell lymphoma formation. *Can Res* **69**, 8686–8692.
44 Green JA, Suzuki K, Cho B, Willison LD, Palmer D, Allen CD, Schmidt TH, Xu Y, Proia RL, Coughlin SR *et al.* (2011) The sphingosine 1-phosphate receptor $S1P_2$ maintains the homeostasis of germinal center B cells and promotes niche confinement. *Nat Immunol* **12**, 672–680.
45 Flori M, Schmid CA, Sumrall ET, Tzankov A, Law CW, Robinson MD and Müller A (2016) The hematopoietic oncoprotein FOXP1 promotes tumour cell survival in diffuse large B-cell lymphoma by repressing S1PR2 signalling. *Blood* **127**, 1438–1448.
46 Stelling A, Wu CT, Bertram K, Hashwah H, Theocharides A, Manz MG, Tzankov A and Müller A (2019) Pharmacological DNA demethylation restores SMAD1 expression and tumour suppressive signalling in diffuse large B-cell lymphoma. *Blood Adv* **3**, 3020–3032.
47 Pham DH, Powell JA, Gliddon BL, Moretti PA, Tsykin A, Van der Hoek M, Kenyon R, Goodall GJ and Pitson SM (2014) Enhanced expression of transferrin receptor 1 contributes to oncogenic signalling by sphingosine kinase 1. *Oncogene* **33**, 5559–5568.
48 El Buri A, Adams DR, Smith D, Tate RJ, Mullin M, Pyne S and Pyne NJ (2018) The sphingosine 1-phosphate receptor 2 is shed in exosomes from breast cancer cells and is N-terminally processed to a short constitutively active form that promotes extracellular signal regulated kinase activation and DNA synthesis in fibroblasts. *Oncotarget* **9**, 29453–29467.
49 Ponnusamy S, Selvam SP, Mehrotra S, Kawamori T, Snider AJ, Obeid LM, Shao Y, Sabbadini R and Ogretmen B (2012) Communication between host organism and cancer cells is transduced by systemic sphingosine kinase 1/sphingosine 1-phosphate signalling to regulate tumour metastasis. *EMBO Mol Med* **4**, 761–775.
50 van der Weyden L, Arends MJ, Campbell AD, Bald T, Wardle-Jones H, Griggs N, Velasco-Herrera MD, Tüting T, Sansom OJ, Karp NA *et al.* (2017) Genome-wide in vivo screen identifies novel host regulators of metastatic colonization. *Nature* **541**, 233–236.

51 Yamada A, Nagahashi M, Aoyagi T, Huang WC, Lima S, Hait NC, Maiti A, Kida K, Terracina KP, Miyazaki H *et al.* (2018) ABCC1-exported sphingosine-1-phosphate, produced by sphingosine kinase 1, shortens survival of mice and patients with breast cancer. *Mol Cancer Res* **16**, 1059–1070.
52 Wang S, Liang Y, Chang W, Hu B and Zhang Y (2018) Triple negative breast cancer depends on sphingosine kinase 1 (SphK1)/sphingosine-1-phosphate (S1P)/sphingosine 1-phosphate receptor 3 (S1PR3)/Notch signalling for metastasis. *Med Sci Monit* **24**, 1912–1923.
53 Adamus A, Engel N and Seitz G (2020) $SGPL1_{321}$ mutation: one main trigger for invasiveness of pediatric alveolar rhabdomyosarcoma. *Cancer Gene Ther* **27**, 571–584.
54 Pulli I, Löf C, Blom T, Asghar MY, Lassila T, Bäck N, Lin KL, Nyström JH, Kemppainen K, Toivola DM *et al.* (2019) Sphingosine kinase 1 overexpression induces MFN2 fragmentation and alters mitochondrial matrix Ca^{2+} handling in HeLa cells. *Biochim Biophys Acta Mol Cell Res* **1866**, 1475–1486.
55 Liang J, Nagahashi M, Kim EY, Harikumar KB, Yamada A, Huang WC, Hait NC, Allegood JC, Price MM, Avni D *et al.* (2013) Sphingosine-1-phosphate links persistent STAT3 activation, chronic intestinal inflammation, and development of colitis-associated cancer. *Cancer Cell* **23**, 107–120.
56 Degagné E, Pandurangan A, Bandhuvula P, Kumar A, Eltanawy A, Zhang M, Yoshinaga Y, Nefedov M, de Jong PJ, Fong LG *et al.* (2014) Sphingosine-1-phosphate lyase downregulation promotes colon carcinogenesis through STAT3-activated microRNAs. *J Clin Investig* **124**, 5368–5384.
57 Schwiebs A, Herrero San Juan M, Schmidt KG, Wiercinska E, Anlauf M, Ottenlinger F, Thomas D, Elwakeel E, Weigert A, Farin HF *et al.* (2019) Cancer-induced inflammation and inflammation-induced cancer in colon: a role for S1P lyase. *Oncogene* **38**, 4788–4803.
58 Lee CF, Dang A, Hernandez E, Pong RC, Chen B, Sonavane R, Raj G, Kapur P, Lin HY, Wu SR *et al.* (2019) Activation of sphingosine kinase by lipopolysaccharide promotes prostate cancer cell invasion and metastasis via SphK1/S1PR4/matriptase. *Oncogene* **38**, 5580–5598.
59 Wang W, Xiang P, Chew WS, Torta F, Bandla A, Lopez V, Seow WL, Lam B, Chang JK, Wong P *et al.* (2020) Activation of sphingosine 1-phosphate receptor 2 attenuates chemotherapy-induced neuropathy. *J Biol Chem* **295**, 1143–1152.
60 Liu YN, Zhang H, Zhang L, Cai TT, Huang DJ, He J, Ni HH, Zhou FJ, Zhang XS and Li J (2019) Sphingosine 1 phosphate receptor-1 (S1P1) promotes tumour-associated regulatory T cell expansion: leading to poor survival in bladder cancer. *Cell Death Dis* **10**, 50.
61 Chakraborty P, Vaena SG, Thyagarajan K, Chatterjee S, Al-Khami A, Selvam SP, Nguyen H, Kang I, Wyatt MW, Baliga U *et al.* (2019) Pro-survival lipid sphingosine-1-phosphate metabolically programs T cells to limit anti-tumour activity. *Cell Rep* **28**, 1879–1893.e7.
62 Imbert C, Montfort A, Fraisse M, Marcheteau E, Gilhodes J, Martin E, Bertrand F, Marcellin M, Burlet-Schiltz O, Peredo AG *et al.* (2020) Resistance of melanoma to immune checkpoint inhibitors is overcome by targeting the sphingosine kinase-1. *Nat Commun* **11**, 437.
63 Lee MS, Sun W and Webb TJ (2020) Sphingosine kinase blockade leads to increased natural killer T cell responses to mantle cell lymphoma. *Cells* **9**, 1030.
64 Lewis AC, Powell JA and Pitson SM (2017) The emerging role of sphingolipids in cancer stem cell biology. In Lipidomics of Stem Cells. Stem Cell Biology and Regenerative Medicine (Pébay A and Wong R, eds). Humana Press, Cham. 151–170. https://doi.org/10.1007/978-3-319-49343-5_8.
65 Bruno G, Cencetti F, Pini A, Tondo A, Cuzzubbo D, Fontani F, Strinna V, Buccoliero AM, Casazza G, Donati C *et al.* (2020) β3-adrenoreceptor blockade reduces tumour growth and increases neuronal differentiation in neuroblastoma via $SK2/S1P_2$ modulation. *Oncogene* **39**, 368–384.
66 Hii LW, Chung FF, Mai CW, Yee ZY, Chan HH, Raja VJ, Dephoure NE, Pyne NJ, Pyne S and Leong CO (2020) Sphingosine kinase 1 regulates the survival of breast cancer stem cells and non-stem breast cancer cells by suppression of STAT1. *Cells* **9**, 886.
67 Hart PC, Chiyoda T, Liu X, Weigert M, Curtis M, Chiang CY, Loth R, Lastra R, McGregor SM, Locasale JW *et al.* (2019) SPHK1 is a novel target of metformin in ovarian cancer. *Mol Cancer Res* **17**, 870–881.
68 Hait NC, Avni D, Yamada A, Nagahashi M, Aoyagi T, Aoki H, Dumur CI, Zelenko Z, Gallagher EJ, Leroith D *et al.* (2015) The phosphorylated prodrug FTY720 is a histone deacetylase inhibitor that reactivates ERα expression and enhances hormonal therapy for breast cancer. *Oncogenesis* **4**, e156.
69 Maczis MA, Maceyka M, Waters MR, Newton J, Singh M, Rigsby MF, Turner TH, Alzubi MA, Harrell JC, Milstien S *et al.* (2018) Sphingosine kinase 1 activation by estrogen receptor α36 contributes to tamoxifen resistance in breast cancer. *J Lipid Res* **59**, 2297–2307.
70 Costales MG, Hoch DG, Abegg D, Childs-Disney JL, Velagapudi SP, Adibekian A and Disney MD (2019) A Designed small molecule inhibitor of a non-coding RNA sensitizes HER2 negative cancers to herceptin. *J Am Chem Soc* **141**, 2960–2974.

71 Long JS, Edwards J, Watson C, Tovey S, Mair KM, Schiff R, Natarajan V, Pyne NJ and Pyne S (2010) Sphingosine kinase 1 induces tolerance to human epidermal growth factor receptor 2 and prevents formation of a migratory phenotype in response to sphingosine 1-phosphate in estrogen receptor-positive breast cancer cells. *Mol Cell Biol* **30**, 3827–3841.
72 Lankadasari MB, Aparna JS, Mohammed S, James S, Aoki K, Binu VS, Nair S and Harikumar KB (2018) Targeting S1PR1/STAT3 loop abrogates desmoplasia and chemosensitizes pancreatic cancer to gemcitabine. *Theranostics* **8**, 3824–3840.
73 Yu OM, Benitez JA, Plouffe SW, Ryback D, Klein A, Smith J, Greenbaum J, Delatte B, Rao A, Guan KL *et al.* (2018) YAP and MRTF-A, transcriptional co-activators of RhoA-mediated gene expression, are critical for glioblastoma tumourigenicity. *Oncogene* **37**, 5492–5507.
74 Shen Y, Zhao S, Wang S, Pan X, Zhang Y, Xu J, Jiang Y, Li H, Zhang Q, Gao J *et al.* (2019) S1P/S1PR3 axis promotes aerobic glycolysis by YAP/c-MYC/PGAM1 axis in osteosarcoma. *EBioMedicine* **40**, 210–223.
75 Cheng JC, Wang EY, Yi Y, Thakur A, Tsai SH and Hoodless PA (2018) S1P stimulates proliferation by upregulating CTGF expression through S1PR2-mediated YAP activation. *Mol Cancer Res* **16**, 1543–1555.
76 Wang W, Hind T, Lam B and Herr DR (2019) Sphingosine 1-phosphate signalling induces SNAI2 expression to promote cell invasion in breast cancer cells. *FASEB J* **33**, 7180–7191.
77 Le Stunff H, Peterson C, Liu H, Milstien S and Spiegel S (2002) Sphingosine-1-phosphate and lipid phosphohydrolases. *Biochem Biophys Acta* **1582**, 8–17.
78 Huang X, Taeb S, Jahangiri S, Emmenegger U, Tran E, Bruce J, Mesci A, Korpela E, Vesprini D, Wong CS *et al.* (2013) miRNA-95 mediates radioresistance in tumors by targeting the sphingolipid phosphatase SGPP1. *Can Res* **73**, 6972–6986.
79 Zhang J, Zhang C, Hu L, He Y, Shi Z, Tang S and Chen Y (2015) Abnormal expression of miR-21 and miR-95 in cancer stem-like cells is associated with radioresistance of lung cancer. *Cancer Invest* **33**, 165–171.
80 Johnson KR, Johnson KY, Becker KP, Bielawski J, Mao C and Obeid LM (2003) Role of human sphingosine-1-phosphate phosphatase 1 in the regulation of intra- and extracellular sphingosine-1-phosphate levels and cell viability. *J Biol Chem* **278**, 34541–34547.
81 Lépine S, Allegood JC, Park M, Dent P, Milstien S and Spiegel S (2011) Sphingosine-1-phosphate phosphohydrolase-1 regulates ER stress-induced autophagy. *Cell Death Differ* **18**, 350–361.
82 Kilbey A, Terry A, Jenkins A, Borland G, Zhang Q, Wakelam MJ, Cameron ER and Neil JC (2010) Runx regulation of sphingolipid metabolism and survival signaling. *Can Res* **70**, 5860–5869.
83 Kilbey A, Terry A, Wotton S, Borland G, Zhang Q, Mackay N, McDonald A, Bell M, Wakelam MJ, Cameron ER *et al.* (2017) Runx1 orchestrates sphingolipid metabolism and glucocorticoid resistance in lymphomagenesis. *J Cell Biochem* **118**, 1432–1441.
84 Li J, Zhang B, Bai Y, Liu Y, Zhang B and Jin J (2019) Upregulation of sphingosine kinase 1 is associated with recurrence and poor prognosis in papillary thyroid carcinoma. *Oncol Lett* **18**, 5374–5382.
85 Gachechiladze M, Tichý T, Kolek V, Grygárková I, Klein J, Mgebrishvili G, Kharaishvili G, Janíková M, Smičková P, Cierna L *et al.* (2019) Sphingosine kinase-1 predicts overall survival outcomes in non-small cell lung cancer patients treated with carboplatin and navelbine. *Oncol Lett* **18**, 1259–1266.
86 Acharya S, Yao J, Li P, Zhang C, Lowery FJ, Zhang Q, Guo H, Qu J, Yang F, Wistuba II *et al.* (2019) Sphingosine kinase 1 signalling promotes metastasis of triple-negative breast cancer. *Can Res* **79**, 4211–4226.
87 Bae GE, Bae S-I, Kim K, Park JH, Cho S and Kim H-S (2019) Increased sphingosine kinase 1 expression predicts distant metastasis and poor outcome in patients with colorectal cancer. *Anticancer Res* **39**, 663–670.
88 LeBlanc FR, Pearson JM, Tan SF, Cheon H, Xing JC, Dunton W, Feith DJ and Loughran TP Jr (2020) Sphingosine kinase-2 is overexpressed in large granular lymphocyte leukaemia and promotes survival through Mcl-1. *Br J Haematol* **190**, 405–417.
89 Sun Y, Shi T, Ma Y, Qin H and Li K (2020) Long noncoding RNA LINC00520 accelerates progression of papillary thyroid carcinoma by serving as a competing endogenous RNA of microRNA-577 to increase Sphk2 expression. *Cell Cycle* **19**, 787–800.
90 Ding X, Zhang Y, Huang T, Xu G, Peng C, Chen G, Kong B, Friess H, Shen S, Lv Y *et al.* (2019) Targeting sphingosine kinase 2 suppresses cell growth and synergizes with BCL2/BCL-XL inhibitors through NOXA-mediated MCL1 degradation in cholangiocarcinoma. *Am J Cancer Res* **9**, 546–561.
91 Quint K, Stiel N, Neureiter D, Schlicker HU, Nimsky C, Ocker M, Strik H and Kolodziej MA (2014) The role of sphingosine kinase isoforms and receptors S1P1, S1P2, S1P3, and S1P5 in primary, secondary, and recurrent glioblastomas. *Tumour Biol* **35**, 8979–8989.
92 Wang Q, Li J, Li G, Li Y, Xu C, Li M, Xu G and Fu S (2014) Prognostic significance of sphingosine kinase 2 expression in non-small cell lung cancer. *Tumour Biol* **35**, 363–368.

93 Wang Y, Shen Y, Sun X, Hong TL, Huang LS and Zhong M (2019) Prognostic roles of the expression of sphingosine-1-phosphate metabolism enzymes in non-small cell lung cancer. *Transl Lung Cancer Res* **8**, 674–681.
94 Vishwakarma S, Agarwal R, Goel SK, Panday RK, Singh R, Sukumaran R, Khare S and Kumar A (2017) Altered expression of sphingosine-1-phosphate metabolizing enzymes in oral cancer correlate with clinicopathological attributes. *Cancer Invest* **35**, 139–141.
95 Neubauer HA, Pham DH, Zebol JR, Moretti PA, Peterson AL, Leclercq TM, Chan H, Powell JA, Pitman MR, Samuel MS *et al.* (2016) An oncogenic role for sphingosine kinase 2. *Oncotarget* **7**, 64886–64899.
96 Murakami M, Ichihara M, Sobue S, Kikuchi R, Ito H, Kimura A, Iwasaki T, Takagi A, Kojima T, Takahashi M *et al.* (2007) RET signalling-induced SPHK1 gene expression plays a role in both GDNF-induced differentiation and MEN2-type oncogenesis. *J Neurochem* **102**, 1585–1594.
97 Lu Z, Xiao Z, Liu F, Cui M, Li W, Yang Z, Li J, Ye L and Zhang X (2016) Long non-coding RNA HULC promotes tumour angiogenesis in liver cancer by up-regulating sphingosine kinase 1 (SPHK1). *Oncotarget* **7**, 241–254.
98 Hazar-Rethinam M, de Long LM, Gannon OM, Topkas E, Boros S, Vargas AC, Dzienis M, Mukhopadhyay P, Simpson F, Endo-Munoz L *et al.* (2015) A novel E2F/sphingosine kinase 1 axis regulates anthracycline response in squamous cell carcinoma. *Clin Cancer Res* **21**, 417–427.
99 Matrone G, Meng S, Gu Q, Lv J, Fang L, Chen K and Cooke JP (2017) Lmo2 (LIM-domain-only 2) modulates Sphk1 (sphingosine kinase) and promotes endothelial cell migration. *Arterioscler Thromb Vasc Biol* **37**, 1860–1868.
100 Anelli V, Gault CR, Cheng AB and Obeid LM (2008) Sphingosine kinase 1 is up-regulated during hypoxia in U87MG glioma cells. Role of hypoxia-inducible factors 1 and 2. *J Biol Chem* **283**, 3365–3375.
101 Liu X, Simon JM, Xie H, Hu L, Wang J, Zurlo G, Fan C, Ptacek TS, Herring L, Tan X *et al.* (2020) Genome-wide screening identifies SFMBT1 as an oncogenic driver in cancer with VHL loss. *Mol Cell* **77**, 1294–1306.e5.
102 Khoei SG, Sadeghi H, Samadi P, Najafi R and Saidijam M (2020) Relationship between Sphk1/S1P and microRNAs in human cancers. *Biotechnol Appl Biochem*. https://doi.org/10.1002/bab.1922. Advance online publication.
103 Powell JA, Pitman MR, Zebol JR, Moretti P, Neubauer HA, Davies LT, Lewis AC, Dagley LF, Webb AI, Costabile M *et al.* (2019) Kelch-like protein 5-mediated ubiquitination of lysine 183 promotes proteasomal degradation of sphingosine kinase 1. *Biochem J* **476**, 3211–3226.
104 Loveridge C, Tonelli F, Leclercq T, Lim KG, Long JS, Berdyshev E, Tate RJ, Natarajan V, Pitson SM, Pyne NJ *et al.* (2010) The sphingosine kinase 1 inhibitor 2-(p-hydroxyanilino)-4-(p-chlorophenyl) thiazole induces proteasomal degradation of sphingosine kinase 1 in mammalian cells. *J Biol Chem* **285**, 38841–38852.
105 Tonelli F, Lim KG, Loveridge C, Long J, Pitson SM, Tigyi G, Bittman R, Pyne S and Pyne NJ (2010) FTY720 and (S)-FTY720 vinylphosphonate inhibit sphingosine kinase 1 and promote its proteasomal degradation in human pulmonary artery smooth muscle, breast cancer and androgen-independent prostate cancer cells. *Cell Signal* **22**, 1536–1542.
106 McNaughton M, Pitman M, Pitson SM, Pyne NJ and Pyne S (2016) Proteasomal degradation of sphingosine kinase 1 and inhibition of dihydroceramide desaturase by the sphingosine kinase inhibitors, SKi or ABC294640, induces growth arrest in androgen-independent LNCaP-AI prostate cancer cells. *Oncotarget* **7**, 16663–16675.
107 Lee SY, Hong IK, Kim BR, Shim SM, Sung Lee J, Lee HY, Soo Choi C, Kim BK and Park TS (2015) Activation of sphingosine kinase 2 by endoplasmic reticulum stress ameliorates hepatic steatosis and insulin resistance in mice. *Hepatology* **62**, 135–146.
108 Mizutani N, Omori Y, Tanaka K, Ito H, Takagi A, Kojima T, Nakatochi M, Ogiso H, Kawamoto Y, Nakamura M *et al.* (2015) Increased SPHK2 transcription of human colon cancer cells in serum-depleted culture: the involvement of CREB transcription factor. *J Cell Biochem* **116**, 2227–2238.
109 Zhang G, Zheng H, Zhang G, Cheng R, Lu C, Guo Y and Zhao G (2017) MicroRNA-338-3p suppresses cell proliferation and induces apoptosis of non-small-cell lung cancer by targeting sphingosine kinase 2. *Cancer Cell Int* **17**, 46.
110 Qiu W, Yang Z, Fan Y and Zheng Q (2016) MicroRNA-613 inhibits cell growth, migration and invasion of papillary thyroid carcinoma by regulating SphK2. *Oncotarget* **7**, 39907–39915.
111 Chen Y, Deng X, Chen W, Shi P, Lian M, Wang H, Wang K, Qian D, Xiao D and Long H (2019) Silencing of microRNA-708 promotes cell growth and epithelial-to-mesenchymal transition by activating the SPHK2/AKT/β-catenin pathway in glioma. *Cell Death Dis* **10**, 448.
112 Zhou MH, Zhou HW, Liu M and Sun JZ (2018) The role of miR-92b in cholangiocarcinoma patients. *Int J Biol Markers* **33**, 293–300.
113 Ma H, Wang H, Tian F, Zhong Y, Liu Z and Liao A (2020) PIWI-interacting RNA-004800 is regulated by

S1P receptor signalling pathway to keep myeloma cell survival. *Front Oncol* **10**, 438.
114 Neubauer HA and Pitson SM (2013) Roles, regulation and inhibitors of sphingosine kinase 2. *FEBS J* **280**, 5317–5336.
115 Lacaná E, Maceyka M, Milstien S and Spiegel S (2002) Cloning and characterization of a protein kinase A anchoring protein (AKAP)-related protein that interacts with and regulates sphingosine kinase 1 activity. *J Biol Chem* **277**, 32947–32953.
116 Ghazaly EA, Miraki-Moud F, Smith P, Gnanaranjan C, Koniali L, Oke A, Saied MH, Petty R, Matthews J, Stronge R *et al*. (2020) Repression of sphingosine kinase (SK)-interacting protein (SKIP) in acute myeloid leukemia diminishes SK activity and its re-expression restores SK function. *J Biol Chem* **295**, 5496–5508.
117 Zhu W, Gliddon BL, Jarman KE, Moretti P, Tin T, Parise LV, Woodcock JM, Powell JA, Ruszkiewicz A, Pitman MR *et al*. (2017) CIB1 contributes to oncogenic signalling by Ras via modulating the subcellular localisation of sphingosine kinase 1. *Oncogene* **36**, 2619–2627.
118 Adams DR, Pyne S and Pyne NJ (2016) Sphingosine kinases: emerging structure-function insights. *Trends Biochem Sci* **41**, 395–409.
119 Zhu W, Jarman KE, Lokman NA, Neubauer HA, Davies LT, Gliddon BL, Taing H, Moretti P, Oehler MK, Pitman MR *et al*. (2017) CIB2 negatively regulates oncogenic signalling in ovarian cancer via sphingosine kinase 1. *Can Res* **77**, 4823–4834.
120 Puhl AC, Bogart JW, Haberman VA, Larson JE, Godoy AS, Norris-Drouin JL, Cholensky SH, Leisner TM, Frye SV, Parise LV *et al*. (2020) Discovery and characterization of peptide inhibitors for calcium and integrin binding protein 1. *ACS Chem Biol* **15**, 1505–1516.
121 Wallington-Beddoe CT, Bennett MK, Vandyke K, Davies L, Zebol JR, Moretti P, Pitman MR, Hewett DR, Zannettino A and Pitson SM (2017) Sphingosine kinase 2 inhibition synergises with bortezomib to target myeloma by enhancing endoplasmic reticulum stress. *Oncotarget* **8**, 43602–43616.
122 Bennett MK, Wallington-Beddoe CT and Pitson SM (2019) Sphingolipids and the unfolded protein response. *Biochim Biophys Acta Mol Cell Biol Lipids* **1864**, 1483–1494.
123 Neubauer HA, Tea MN, Zebol JR, Gliddon BL, Stefanidis C, Moretti P, Pitman MR, Costabile M, Kular J, Stringer BW *et al*. (2019) Cytoplasmic dynein regulates the subcellular localization of sphingosine kinase 2 to elicit tumour-suppressive functions in glioblastoma. *Oncogene* **38**, 1151–1165.
124 Simanshu DK, Nissley DV and McCormick F (2017) RAS proteins and their regulators in human disease. *Cell* **170**, 17–33.
125 Gault CR, Eblen ST, Neumann CA, Hannun YA and Obeid LM (2012) Oncogenic K-Ras regulates bioactive sphingolipids in a sphingosine kinase 1-dependent manner. *J Biol Chem* **287**, 31794–31803.
126 Pitson SM, Moretti PA, Zebol JR, Lynn HE, Xia P, Vadas MA and Wattenberg BW (2003) Activation of sphingosine kinase 1 by ERK1/2-mediated phosphorylation. *EMBO J* **22**, 5491–5500.
127 Wallington-Beddoe CT, Powell JA, Tong D, Pitson SM, Bradstock KF and Bendall LJ (2014) Sphingosine kinase 2 promotes acute lymphoblastic leukemia by enhancing MYC expression. *Can Res* **74**, 2803–2815.
128 Chen J, Qi Y, Zhao Y, Kaczorowski D, Couttas TA, Coleman PR, Don AS, Bertolino P, Gamble JR, Vadas MA *et al*. (2018) Deletion of sphingosine kinase 1 inhibits liver tumourigenesis in diethylnitrosamine-treated mice. *Oncotarget* **9**, 15635–15649.
129 Lima S, Takabe K, Newton J, Saurabh K, Young MM, Leopoldino AM, Hait NC, Roberts JL, Wang HG, Dent P *et al*. (2018) TP53 is required for BECN1- and ATG5-dependent cell death induced by sphingosine kinase 1 inhibition. *Autophagy* **14**, 942–957.
130 Salama MF, Carroll B, Adada M, Pulkoski-Gross M, Hannun YA and Obeid LM (2015) A novel role of sphingosine kinase-1 in the invasion and angiogenesis of VHL mutant clear cell renal cell carcinoma. *FASEB J* **29**, 2803–2813.
131 Schnute ME, McReynolds MD, Kasten T, Yates M, Jerome G, Rains JW, Hall T, Chrencik J, Kraus M, Cronin CN *et al*. (2012) Modulation of cellular S1P levels with a novel, potent and specific inhibitor of sphingosine kinase-1. *Biochem J* **444**, 79–88.
132 Adams DR, Tawati S, Berretta G, Rivas PL, Baiget J, Jiang Z, Alsfouk A, Mackay SP, Pyne NJ and Pyne S (2019) Topographical mapping of isoform-selectivity determinants for J-channel-binding inhibitors of sphingosine kinases 1 and 2. *J Med Chem* **62**, 3658–3676.
133 Sibley CD, Morris EA, Kharel Y, Brown AM, Huang T, Bevan DR, Lynch KR and Santos WL (2020) Discovery of a small side cavity in sphingosine kinase 2 that enhances inhibitor potency and selectivity. *J Med Chem* **63**, 1178–1198.