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Bioactive sphingolipids: Advancements and contributions from the laboratory of Dr. Lina M. Obeid

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Abstract

Sphingolipids and their synthetic enzymes have emerged as critical mediators in numerous diseases including inflammation, aging, and cancer. One enzyme in particular, sphingosine kinase (SK) and its product sphingosine-1-phosphate (S1P), has been extensively implicated in these processes. SK catalyzes the phosphorylation of sphingosine to S1P and exists as two isoforms, SK1 and SK2. In this review, we will discuss the contributions from the laboratory of Dr. Lina M. Obeid that have defined the roles for several bioactive sphingolipids in signaling and disease with an emphasis on her work defining SK1 in cellular fates and pathobiologies including proliferation, senescence, apoptosis, and inflammation.

1. Introduction

The concept of bioactive lipids has emerged and developed over the last three decades with the identification of several bioactive sphingolipids as key players in important biologic and pathophysiologic processes. Many of the advancements in the field have been driven by an ever-increasing appreciation of the pleotropic functions of this pathway and the potential for therapeutic targeting. Research in the laboratory of Dr. Lina M. Obeid has been critical in discovering and defining the role of bioactive sphingolipids, including roles of ceramide in apoptosis and senescence, and in particular the regulation and roles of sphingosine kinase 1 (SK1) and its lipid product sphingosine-1-phosphate (S1P) in numerous biologic processes.

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Appendix A. Supplementary data

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This review will discuss the significant contributions made by Dr. Obeid in these discoveries and advancements, especially those associated with the structure and function of SK1 as well as the role of this enzyme in cell signaling, inflammation and cancer.

1.1. Sphingolipid metabolism

Sphingolipid metabolism constitutes an elaborate network of interconnected metabolic pathways [1]. The *de novo* generation of sphingolipids begins with the condensation of palmitoyl Co-A and serine via serine palmitoyl transferase (SPT). The amino acid alanine, can also be utilized by SPT to form deoxysphinganine (dSA), a novel sphingoid base lacking the hydroxyl group at the first carbon [2]. Dihydrosphingosine is generated via the reduction of 3-ketosphinganine, which then can be acylated via ceramide synthases (CerS) to form dihydroceramide and desaturated to form ceramide. Ceramide can then be utilized to form sphingomyelin via sphingomyelin synthases (SMSs), ceramide-1-phosphate (C1P) via ceramide kinase (CerK), or glycosphingolipids via glucosylceramide synthase (GCS) and galactosylceramide synthase. Thus, ceramide sits at the hub of this intricate metabolic pathway with multiple forms of ceramide and many compartments in regulation of its metabolism [3]. The generation of S1P first requires the de-acylation of ceramide and the production of sphingosine by one of five ceramidases. Either SK1 or SK2 can then phosphorylate sphingosine generating S1P. S1P can be dephosphorylated by S1P phosphatases (SPP) to generate sphingosine which can then be recycled back to ceramide via CerS, or S1P can be irreversibly broken down by S1P lyase to ethanolamine phosphate and hexadecenal. The latter represents the only known metabolic ‘exit’ pathway for sphingolipids.

1.2. Sphingosine Kinase 1

SK1 has often been associated with “tipping the balance” from apoptosis and differentiation to proliferation by increasing levels of S1P and decreasing levels of ceramide and sphingosine (Fig. 1); therefore, it is not surprising that SK1 is highly regulated. SK1 expression is regulated at the transcriptional level by several transcription factors, many of which are involved in hypoxia and angiogenesis, including hypoxia inducible factor 2 alpha (HIF2α) [4,5], LIM-domain-only protein 2 (LM02) [6], and E2F transcription factors [7–9]. SK1 activity is regulated by numerous growth factors (TGFβ, VEGF, NGF, IGF) and cytokines (TNFα and IL1β) leading to proliferation, migration, inflammation and angiogenesis in several cell types [10–20]. SK1 protein levels are also downregulated by proteolysis following exposure to DNA damage agents [21] and TNFα [22]. These studies, together with the critical contributions made by Dr. Obeid’s laboratory, demonstrate the importance of SK1 regulation in cellular fates and biologies including proliferation, senescence, apoptosis and inflammation (Fig. 2 and 3).

2. SK1: Mechanisms and structure

SK1 is a highly regulated enzyme at multiple levels, most likely due to its central role in metabolism and interconversion of many important bioactive sphingolipids [23].

2.1. Membrane targeting of SK1

To generate S1P, SK1 most likely accesses sphingosine directly from membranes. Therefore, several studies have been conducted to better understand how SK1 associates with the membrane and how different types of membranes and membrane lipids affect the enzyme. Thus, much attention has been paid to translocation of SK1 to the plasma membrane [20,24–27]. Early work from the Obeid lab showed that SK1 translocates to the plasma membrane after stimulation with the protein kinase C (PKC) activator, phorbol 12-myristate 13-acetate (PMA) [24]. Furthermore, PMA-induced SK1 translocation was accompanied by increased SK1 activity and by secretion of S1P into the media, suggesting autocrine/paracrine signaling. Inhibition of PKC with known inhibitors bisindolylmaleimide, calphostin-C, or the indirect inhibitor C_6-ceramide blunted PMA-induced translocation of SK1 to the plasma membrane. PMA also induced phosphorylation of green fluorescent protein (GFP)-tagged SK1, raising the possibility that SK1 is a direct substrate of PKC. However, subsequent studies suggested a key role for phospholipase D (PLD) [20], which is a major enzyme in the regulated production of phosphatidic acid (PA), and also a known target for PKC. Studies have shown that the translocation of SK1 to membrane compartments is dependent on the interaction of SK1 with PA, where specific and direct interactions of PA were studied at the C-terminus of recombinant SK1 [20,28]. In other studies, SK1 has been shown to be activated *in vitro* by anionic phospholipids, especially phosphatidylserine (PS) and PA [29]. PS and PA have been shown to enhance SK1 enzymatic activity in mixed micelle and liposome-based cell-free assays [25,27]. These studies raised the possibility that SK1 may be regulated in cells by interaction with membranes rich in anionic phospholipids (e.g. inner leaflet of the plasma membrane which is rich in PA).

Different molecular mechanisms have been proposed to explain SK1 translocation and interaction with membranes, where two specifically have identified certain residues that mediate membrane localization and have implicated these residues in mediating endocytosis and neurotransmission [25,30]. Stahelin et al. identified Thr54 and Asn89, as amino acid residues that interact with PS in membrane [31]. A biophysical cell study of SK1 and selected mutants was performed to understand the origin and structural determinant of the specific subcellular localization of SK1, where *in vitro* measurements showed that SK1 selectively bound PS over other anionic phospholipids and strongly preferred the plasma membrane-mimicking membrane to other cellular membrane mimetics. Specifically, mutational analysis indicated that conserved residues Thr54 and Asn89 were necessary for lipid selectivity and membrane targeting both *in vitro* and in the cell. Interestingly, phosphorylation of Ser225 enhances the membrane affinity and plasma membrane selectivity of human SK1, presumably by modulating the interaction of Thr54 and Asn89 with the membrane [25].

The ability to predict the potential binding interfaces of human SK1 was facilitated by the publication of the crystal structure [32]. The residues identified by Shen et al. are part of a small hydrophobic patch on the surface of SK1 [30]. This hydrophobic patch was shown to mediate SK1 membrane recruitment by directly interacting with the lipid bilayer. The patch corresponds to one of the two helices that were proposed to function as a gate to control the flux of the substrate sphingosine into the interior of the protein. Since some positively

charged residues surround this hydrophobic patch, it may represent a lipid bilayer-binding interface with hydrophobic residues partially penetrating the membrane. This interpretation is supported by the diffuse cytosolic distribution of SK1 Leul94Gln and of a SK1 mutant that harbors two other mutations in the same hydrophobic patch, SK1 Phel97Ala/Leul98Gln. While both mutants folded correctly, they failed to bind to membranes, especially the tubular invaginations induced by cholesterol extraction, *in vitro* or in cell. The knockdown of SKs results in endocytic recycling defects, and a mutation that disrupts the hydrophobic patch of *C. elegans* SK fails to rescue the neurotransmission defects in loss-of-function mutants of this enzyme.

The Obeid lab subsequently identified a small highly positively charged three-residue motif, composed of the residues Lys27, Lys29, and Arg186, on the surface of SK1, adjacent to the substrate binding sites for both ATP and sphingosine (Fig. 4) [33]. This motif was identified using the Adaptive Poisson-Boltzman Solver in the Chimera software and studied for its electrostatic interactions with membranes, where hydrogen/deuterium exchange MS was used to study interactions of SK1 with membrane vesicles. This study demonstrated that SK1 interacts with membrane-associated anionic phospholipids using a single contiguous interface, containing both an electrostatic interaction site and a hydrophobic interaction site. Cellular studies demonstrated that mutation of the two sites causes decreased membrane association and SK1 activity. To disrupt the electrostatic site, Lys27 and Lys29 were mutated to glutamate residues and Arg186 to an aspartate residue, leaving the hydrophobic site intact. To disrupt the hydrophobic patch, Leul94 was mutated to a glutamine, leaving the electrostatic site intact, this resulted in the loss of SK1-dependent cell invasion and endocytosis. Therefore, both the hydrophobic patch and the electrostatic motif are essential for allowing SK1 binding to membranes *in vitro* and in cells. Altogether, these results from the Obeid lab defined a composite domain in SK1 that regulates its intrinsic ability to bind membranes and indicated that this binding is critical for proper SK1 function.

2.2. Proteolysis of SK1

Several studies have been conducted to show degradation of SK1 can occur by ubiquitination and subsequent proteasomal degradation. Studies from the Obeid lab originally demonstrated that cells undergo a loss of the SK1 protein in response to DNA damage. Actinomycin treatment in MOLT4 cells resulted in the loss of SK1 protein. Interestingly, this loss of SK1 in response to DNA damage was prevented by transfection of cell with the E6 protein, which targets p53 for ubiquitination and subsequent proteasomal degradation, thus demonstrating the role of p53 in the loss of SK1. Furthermore, the knockdown of SK1 by small interfering RNA (siRNA) in MCF-7 cells resulted in a significant reduction in cell viability [21]. Subsequent studies focused on the mechanism of p53-mediated SK1 proteolysis, and it was shown that SK1 proteolysis occurred downstream of the tumor suppressor p53 in response to several DNA-damaging agents, by proteases including cathepsins. Furthermore, Dr. Obeid's work showed that prolonged cytokine (TNFα) treatment of MCF7 breast cancer cells induced the loss of SK1 protein. Knock-down of cathepsin B by siRNA rescued the TNF-induced SK1 loss implying this process was dependent on cathepsin B [22]. Multiple cathepsin B cleavage sites in SK1 were identified by Obeid and colleagues [34]. Two cleavage sites of SK1 were identified, Hisl22

and Arg199. A Hisl22Tyr SK1 mutant showed significant reduction of cleavage at the first site but did not affect cleavage at the Arg199 site, while it maintained enzyme activity.

A subsequent study conducted by the Obeid lab concluded p53-mediated activation of caspase-2 was required for SK1 proteolysis [35]. Caspase-2 was not activated in triple-negative breast cancer (TNBC) cells harboring a mutation in p53, and interestingly SK1 was not degraded. Additionally, caspase-2 activity significantly altered the levels of endogenous sphingolipids (especially sphingosine, S1P, and ceramides). Further work in the Obeid Lab by Carroll et al. demonstrated that activation of the CHK1-suppressed pathway in TNBC resulted in the activation of caspase-2 and subsequent proteolysis of SK1 in response to doxorubicin. This work suggested that SK1 may be the first identified effector of the CHK1-suppressed pathway.

Development of SK1 inhibitors has been pursued as an attractive approach to inhibit tumor growth and metastasis formation. Many SK1 inhibitors, including the highly potent and selective inhibitor PF-543 [36], have dual mechanisms of action: 1) Sphingosine-competitive inhibition of kinase activity and 2) induction of proteolysis of SK1. It appears that substrate-based inhibitors of SK1 such as SKi, ABC294640, and FTY720 induce its ubiquitination and subsequent proteolysis [37,38]. The inhibitor SKI II was found to downregulate SK1 protein expression without affecting SK1 mRNA expression, and this degradation occurred through a lysosomal pathway and involved cathepsin B [39]. The Obeid lab collaborated on the study of LCL351 for its efficacy as a novel SK1 selective inhibitor in a murine model of inflammatory bowel disease (IBD). LCL351 was found to selectively inhibit SK1 both *in vitro* and in cells. LCL351, which accumulates in relevant tissues such as colon, did not have any adverse side effects *in vivo* [40].

3. S1P interaction with receptors: studies on the ERM family

The ERM (ezrin, radixin and moesin) proteins are involved in many important cellular events and link cortical actin to the plasma membrane and coordinate cellular events that require cytoskeletal rearrangement. Ceramide-induced activation of PP1α leads to dephosphorylation and inactivation of ERM proteins while S1P results in phosphorylation and activation of ERM proteins [41]. The Obeid lab has made significant contributions in elucidating the activities of S1P regulating phosphorylation of the ERM family of cytoskeletal proteins [42]. Most S1P-induced biologies are mediated by the interaction of S1P with high affinity G-protein-coupled receptors (GPCRs), namely, S1PR1–5 [43]. Intracellular S1P, derived from SK2 [44], was identified as an acute ERM activator in epidermal growth factor (EGF)-induced cancer cell invasion [44]. Specifically, S1P2R was shown to be necessary to induce phosphorylation of ERM proteins and subsequent filopodia formation [45]. EGF led to cell polarization in the form of lamellipodia, and it also induced cellular invasion. Both responses to EGF were shown to be dependent on SK2, S1P, and SlPR2-mediated phosphorylation of ezrin Thr567, and the S1PR2 antagonist, JTE-013, was specifically effective in preventing these responses. These contributions by the Obeid lab unveiled a novel mechanism of EGF-stimulated invasion, whereby S1P-mediated activation of S1PR2 and phosphorylation of ezrin Thr567 is required [46].

4. SK1: Senescence

Senescence is a state of stable cell cycle arrest triggered by diverse internal and/or external stress stimuli, including telomere shortening, hyper-proliferative signaling and epigenomic or genomic damage [47]. The senescent state is associated with increased expression of tumor suppressor proteins, dephosphorylation of the retinoblastoma protein (Rb), altered cellular morphology, increased senescence-associated ß-galactosidase activity (SA-ß-gal), and development of a secretory phenotype, termed senescence-associated secretory phenotype (SASP) [48]. Paradoxically, senescence is believed to be both a protective mechanism, preventing the proliferation of damaged cells [49], as well as a contributor to disease and dysfunction through accumulation with age and secretion of bioactive molecules into the local tissue microenvironment [50].

Work from Dr. Obeid first uncovered a link between sphingolipids and senescence [51]. Her laboratory observed elevated ceramide levels in high passage WI-38 human fibroblasts [52], which was subsequently observed by other groups [53] and in other human fibroblasts such as IMR-90 [54]. Her group was also the first to show that exogenous C6-ceramide induced senescence in WI-38 fibroblasts [51], via inhibition of cell cycle progression, increased SA-ß-gal, and dephosphorylation of Rb [55,56]. The mechanisms involved the activation of PP1 and PP2A, the induction of p21, and CDK2 inhibition, leading to cell cycle arrest and senescence [57]. Additional studies by Dr. Obeid's group also identified a disruption in the PLD/PKCα pathway in senescence [52] and demonstrated that ceramide is an inhibitor of PLD [58] and of PKCα [59], implicating ceramide as a disruptor of this mitotic signaling pathway in senescence [60]. Furthermore, ceramide was identified as an upstream negative regulator of telomerase [61], a key enzyme driving immortalization of cancer cells and mediating escape from replicative senescence. Mechanistically, ceramide was demonstrated to reduce expression of telomerase by enhancing the degradation of the transcription factor c-Myc [62]. Several other groups demonstrated that exogenous ceramide induces senescence [63,64] in many cell lines [65,66], including cancer cells [67]. Ceramide was also shown to mediate senescence induced by chemotherapeutic drugs in cancer cells [68,69].

4.1. SK1 in aging and age-related diseases

Sphingolipids are also strongly implicated in aging and age-related diseases [70,71]. Genetic studies have buttressed the role of sphingolipids, especially ceramide, in aging. First, studies in *Saccharomyces cerevisiae* have shown that LAG1 (longevity assurance gene) regulates replicative capacity. This gene is the homolog of CerS2 gene in mammalian cells and functions as a CerS in yeast. The deletion of LAG1 induces an increase of yeast longevity, showing that decrease of ceramide synthesis slows down the aging process. Additional studies in *Caenorhabditis elegans* have revealed complex roles for ceramide in aging. In this system, 3 CerSs have been identified, HYL-1, HYL-2 and LAGR-1, synthesizing ceramide of variable chain length. Interestingly, these CerSs have distinct roles in aging. While loss of HYL-1 or LAGR-1 was shown to have no effect on lifespan [72] or decrease of some signs of aging [73], deletion of HYL-2 strongly decreased lifespan [72], suggesting that long-chain ceramide delays aging whereas longer-chain ceramide induces aging.

Clinical studies have also addressed potential roles and changes in sphingolipids, demonstrating that the levels of sphingolipids and their metabolic enzymes are altered during aging and age-related diseases. Very old subjects such as centenarians have higher serum levels of ceramide compared to younger subjects [74]. Positive correlation between the levels of ceramide in blood or in tissue was made in many diseases such as neurodegenerative, cardiovascular and metabolic diseases [75–78]. Patients with cardiovascular diseases have lower serum levels of S1P compared to heathy donors [79]. The challenge has been to evaluate if sphingolipids could be used as diagnostic or prognostic markers or therapeutic targets in those diseases. They may be particularly relevant in atherosclerosis and in Alzheimer's disease.

Dr. Obeid's laboratory was also the first to identify SK1 as a potential therapeutic target in p53-null cancers through the discovery that deleting SK1 induces senescence [80]. Genetic deletion of SK1 in a p53-deficient mouse model induced SA-ß-gal and p21 expression in the thymus, contributing to prevention of thymic lymphoma and extension of life span. These double KO mice exhibited significant increases in sphingosine levels in the thymus, suggesting that the biological response may be driven by this sphingolipid.

Expanding on this initial discovery, the genetic deletion of SK1 was reported to induce senescence in both primary liver cancer cells as well as MEFs, as determined by increased SA-ß-gal and p21 expression [81]. Molecular approaches targeting SK1 can also induce senescence in cancer cells. Pharmacological inhibition of SK activity with the inhibitors SKi or ABC294640, or combined siRNA knockdown of SK1 and dihydroceramide desaturase (Des1), increased p21 expression and induced cell cycle arrest in prostate cancer cells [38]. *In vivo*, SK1 deletion protects from chemically-induced liver tumorigenesis through inhibition of tumor growth and induction of tumor senescence along with apoptosis [81]. In this model, there was no difference in S1P levels. Rather, sphingosine levels were increased in the tumor. These studies demonstrate SK1 may be a powerful therapeutic target in multiple cancer types, through the induction of senescence.

There is also strong evidence that SK1 may be downregulated with aging and may contribute to age-related pathology by disrupting the balance between pro- and anti-proliferative sphingolipids. Dr. Obeid's laboratory first determined that SK1 is negatively regulated by DNA damage and p53, key components of senescence activation [21,80]. In MEFs exposed to ultraviolet-B radiation, which induces senescence, SK1 was downregulated and associated with over 2-fold increase in Cl 6-ceramide level [80]. Also, treatment of MOLT-4 leukemia cells with the DNA damaging agent Actinomycin D, significantly reduced SK1 protein and increased sphingosine and ceramide [21]. In yeast, the expression of the SK homologs (LCB4 and LCB5) was significantly downregulated with age, contributing to an overall increase in sphingoid bases, which were also associated with reduced chronological lifespan [82]. Furthermore, the loss of SK1 was demonstrated to accelerate the deterioration of retinal function with age [83] and shortened lifespan, disrupted neuromuscular function, and locomotor behavior in *Caenorhabditis elegans* [73]. In human adipose-derived stromal cells, SK1 was reportedly downregulated in replicative senescence, and the downregulation of SK1 by shRNA induced a senescence phenotype and increased levels of C16-ceramide, sphingosine and dihydrosphingosine [84]. SK1 expression was also reported to be

downregulated in neurons and in the frontal cortex and hippocampus of human patients with Alzheimer's disease [85,86]. Based on these studies, the downregulation of SK1 disrupts the balance of pro- and anti-proliferative sphingolipids and accumulation of specific species may play a role in age-related diseases.

5. SK1: Inflammation

Inflammation is the body's response to tissue injury and pathogens. This process involves increased cytokines and chemokines leading to the recruitment of leukocytes to the site of injury. Multiple studies have demonstrated that SK1 is regulated by a myriad of growth factors and cytokines, such as TNFα [18,87], IL-1β [18], lipopolysaccharide (LPS) [19] or VEGF [88]. Work in the laboratory of Dr. Obeid in cellular and *in vivo* models has revealed critical and potentially therapeutic roles for SK1 in the regulation of inflammation.

5.1. SK1 in inflammatory signaling

Several studies have demonstrated the importance of SK1 activity in the inflammatory response in cellular models. SK1 has been shown to be regulated by several cytokines, including TNFα and IL1β [89]. Conversely, SK1 has also been implicated in the regulation of specific cytokines and cell signaling pathways associated with inflammation [90,91]. SK1 has been shown to directly interact with, and be activated by, tumor necrosis associated factor 2 (TRAF2), stimulating NF-kB and JNK activity in a HEK-293T cell model [92]. Interestingly, interaction with SK1 is itself necessary to activate the E3 ubiquitin ligase activity of TRAF2, as this allows S1P to act as a cofactor to TRAF2, according to the same model [93]. Dr Obeid's laboratory demonstrated that SK1 is also a key component in the interaction of p38 MAPK and TNFα signaling pathways, whereby SK1 has been shown to regulate this pathway by activating p38 MAPK. This leads to the suppression of the cytokine RANTES, generating a novel anti-inflammatory response [94]. In a collaborative publication from the laboratories of Drs. Obeid and Hannun, it was shown that TNFα is also implicated in the induction of cyclooxygenase 2 (COX2) and production of prostaglandin E2 (PGE2) in L929 fibroblasts [89] as well as in macrophages in colon cancer [95]. There is also evidence in the literature from Pitson and others demonstrating that SK1 activity is required for TNFα-induced activation of integrin, via intracellular signaling pathways [96]. In addition to TNFα, SK1 was activated by IL1β in astrocytes leading to interactions between SK1 and cellular inhibitor of apoptosis protein 2 (cIAP2) [97]. The activation and upregulation of SK1 via cytokines has been implicated in several pathways important in both healthy and diseased states; suggesting that future work aimed at determining the role of SK1 in inflammation is essential.

In addition to activation and upregulation by pro-inflammatory cytokines, SK1 has also been implicated in inflammatory signaling in response to growth factors, LPS, and fatty acids. TGFβ has also been shown to directly upregulate SK1 [98,99], with several downstream effects. In a collaboration involving Dr. Obeid's laboratory, it was shown that TGFβ upregulated SK1, and this upregulation was required for subsequent induction of TIMP-1, and a more specific function for dhS1P as opposed to S1P [98,100]. In pulmonary arterial smooth muscle cells, Notch3 activation and increased proliferation of these cells also

required TGFβ-induced SK1 expression [99]. Early work from Dr. Obeid demonstrated that SK1 provides macrophages with a pro-survival response through S1P production. First, in a collaborative effort using human U937 monocytic cells, it was shown that oxidized LDL-containing immune complexes caused translocation of SK1 to the plasma membrane and subsequent increased in S1P production. Additionally, treatment with these immune complexes increased cell survival over time, survival that was further increased with S1P co-treatment [101]. Subsequent work by Dr. Obeid's group showed that LPS treatment induced SK1 expression and S1P production in RAW 264.7 mouse macrophages. The protective role of SK1 in this cell line was manifested when SK1 downregulation resulted in increased apoptosis after LPS or TNFα treatment [19]. Further studies delved into the role of SK1, not only in macrophages [95], but also in the function of other immune cells, such as neutrophils [102] and dendritic cells [103].

More recent collaborative work has demonstrated a role for SK1 in obesity and high fat diet (HFD). Co-treatment of macrophages with LPS and palmitate increased expression of SK1, and that SK1 was required for increased levels of IL6 [104]. Collaborative work with Dr. Cowart's lab demonstrated that *SK1*$^{-/-}$ mice fed a HFD exhibit increased markers of adipogenesis and IL-10 and decreased levels of TNF and IL6 as compared to wildtype (WT) mice [105]. SK1-deficient mice also exhibit increased insulin sensitivity and glucose tolerance upon HFD challenge. In a cell culture model, palmitate treatment increased SK1 expression via PPARα leading to IL-6 production in in C2C12 myotubes [106]. Together these studies clearly indicate a critical role for SK1 in several inflammatory signaling pathways.

5.2. SK1 in inflammatory diseases

SK1 and S1P have been implicated in human inflammatory diseases and in several inflammatory diseases in mouse models. Inhibition of SK1 using pharmacologic inhibitors or genetic deletion of SK1 has been suggested to decrease disease in animal models of asthma and lung inflammation. These studies have implicated SK1 in the recruitment of immune cells in the lungs [107], as well as in the generation of inflammatory cytokines, and activation of NF-κB in the lungs [108]. SK1 and S1P have also been implicated extensively in multiple sclerosis (MS). The SK1 activator (compound 5) protected from experimental autoimmune encephalomyelitis (EAE) development; however, the mechanisms are not well established yet [109]. In addition, transplantation of mesenchymal stem cells overexpressing SK1 reduced the symptoms of EAE [110]. The sphingosine analog, FTY720/Fingolimod, acts as a substrate for SK2, and the phosphorylated product acts as a functional antagonist to S1PRs, resulting in sequestration of lymphocytes. This molecule is in clinical use in MS [111]. Together these studies implicate critical roles for SKs and S1P in inflammation and autoimmunity.

5.3. SK1 in arthritis

Rheumatoid arthritis (RA) is a chronic inflammatory and destructive joint disease, characterized by elevated levels of inflammatory mediators such as TNFα and IL-1β, resulting in joint pain and disability [112]. S1P levels have been shown to be elevated in the synovial fluid of RA patients as compared to osteoarthritis (OA) [113]. In collaborative

work, Dr. Obeid's group demonstrated a role for SK1 in RA using the transgenic TNFα-mice (hTNFα). Signs of RA, such as joint swelling and deviation, were apparent in both *hTNFα*/*SK1*$^{+/+}$ or *hTNFα*/*SK1*$^{-/-}$ mice at 4 months of age. However, articular/periarticular inflammation and erosive disease were significantly decreased in *hTNFα*/*SK1*$^{-/-}$ mice. These studies implicated SK1 and S1P in the development of Th17 T cells, and in the expression of SOCS3 (suppressor of cytokine signaling) in RA [114–116].

The pro-inflammatory role of SK1 in arthritis has also been supported by other cellular and *in vivo* models. B lymphoblastoid cell lines from peripheral blood of RA patients were resistant to apoptosis when compared to healthy controls [101]. SK1 siRNA reversed this resistance suggesting that SK1 inhibition could be a strategy to induce autoreactive B cell death in RA. Lai et al. demonstrated a pro-inflammatory role of SK1 in a collagen-induced arthritis (CIA) mouse model. The authors showed that N,N-dimethylsphingosine (DMS), an SK1 inhibitor, as well as SK1 siRNA suppressed pro-inflammatory cytokine expression (IL-6, TNFα) and reduced articular inflammation and joint destruction in a CIA model. This work also demonstrated elevations in synovial S1P from RA patients as compare to OA patients [113]. Most recently, Inoue et al. have unveiled the potential role the for S1PR3 in the CIA mouse model as SlPR3-deficient mice exhibited decreased clinical and histological severity of disease as well as significantly lower expression of IL6 in the synovial membrane [117]. Another group has demonstrated that S1P signaling via S1PR1 enhances inflammatory cytokine production in human fibroblast-like synoviocytes derived from RA patients [118]. Together, these studies clearly outline the importance of SK1 in *in vivo* arthritis models.

5.4. SK1 in inflammatory bowel disease

IBD includes ulcerative colitis (UC) and Crohn's Disease (CD) and affects an estimated 3 million Americans. IBD is characterized by chronic inflammation of the gastrointestinal tract [119] and increases risk for the development of colorectal cancer [120]. Dr. Obeid's group was the first to demonstrate increased expression of SK1 in the intestinal epithelium from colitis and colon cancer patients [121,122]. Snider et. al also uncovered a crucial role for SK1 in the development and progression of IBD in an in *vivo* model, namely dextran sulfate sodium (DSS)-induced colitis. SK1, but not SK2, expression and activity were elevated in wildtype (WT) mice treated with DSS. S1P levels were also elevated in the circulation of WT mice with DSS-induced colitis (also reported by Maines and colleagues [123]). Furthermore, *SK1*$^{-/-}$ mice were significantly protected from colitis as demonstrated by decreased intestinal damage, decreased recruitment of neutrophils, as well as decreased COX-2 expression as compared to WT mice [124]. These studies were bolstered by subsequent work from Liang et al. demonstrating that fingolimod (FTY720), an inhibitor of S1PR1, significantly protected from colorectal tumorigenesis associated with chronic colitis in a murine model [125].

Dr. Obeid's group further demonstrated the importance of SK1 in colitis, indicating specific roles for SK1 in hematopoietic cells vs. non-hematopoietic cells in DSS-induced colitis. Bone-marrow chimeric mice were used to demonstrate the critical role for extra-hematopoietic SK1 in the induction of COX-2 in colon tissues in response to DSS-induced

colitis [126]. These studies also highlighted the cross talk of the SK1/S1P pathway with that of STAT3, as loss of SK1 in either the hematopoietic or extra-hematopoietic compartments resulted in decreased phospho-STAT3 in DSS-induced colitis [126]. Similar links between SK1 and STAT3 have also been demonstrated in animal models of colitis-associated colon cancer (CAC) [125]. Use of the SK1 inhibitor LCL351 reduced the inflammatory responses (e.g. weight loss, splenomegaly, and loss of red blood cells) in acute DSS-induced colitis. Interestingly, in DSS-induced colitis, mice treated with LCL351 presented decreased levels of S1P in colon tissue as compared to vehicle treated mice. LCL351 also decreased expression of TNFα, CXCL1 and CXCL2, as well as neutrophil infiltration into colon tissues, suggesting that SK1 as a viable therapeutic target in IBD [40]. These findings were reinforced by work demonstrating that SK1 inhibition with PF543 also exerts a protective effect against colitis in mice treated with DSS [127]. Additional studies from Dr. Obeid and her collaborators have also defined the roles for ceramidases in IBD and colon cancer [128–131]. Together, these studies demonstrate the significant contributions by Dr. Obeid's laboratory on the roles of SK1, and other sphingolipid enzymes, in intestinal inflammation.

6. SK1: Cancer

Cancer cells are characterized by uncontrolled division and the abilities to disseminate and avoid immune responses [132]. Bioactive sphingolipids are now understood to play essential roles in multiple aspects of cancer pathogenesis and therefore represent exciting and therapeutic targets. Whereas ceramide and sphingosine regulate cell death, senescence, and cell cycle arrest, S1P induces proliferation, inflammation and angiogenesis [133–135], and regulates immune responses [136,137]. Regulation of SK1 affects the levels and balance between ceramide, sphingosine, and S1P and has been demonstrated to impact cancer cell fate. Increasing lines of evidence, both *in vitro* cellular models as well as *in vivo* animal research, are unveiling the potential clinical significance of targeting SK1 in cancer [138].

Throughout her research career, Dr. Obeid's studies and findings were crucial to the sphingolipid field and its implication in cancer cells. A large body of work in the Obeid laboratory shed light on the relevance of sphingolipids in cancer tumorigenicity and progression, angiogenesis, migration, inhibition of apoptosis, and chemotherapy resistance, as briefly reviewed below.

6.1. Ceramide and CerS in apoptosis and cancer

Dr. Obeid was the first to discover that ceramide induces programmed cell death. Her landmark study was published in Science in 1993 [139]. Her contribution to this topic, which has been extensively expanded on the sphingolipid field, continued for three decades. Many of her studies defined novel connections in apoptosis [140]; for example, work from her lab demonstrated that the anti-apoptotic Bcl2 interrupted death pathways downstream of initiating caspases [141], and her group defined regulation of release of apoptotic bodies and devised a simple assay to measure that process [142–144]. Her group also showed that Bcl2 acts downstream of ceramide in the apoptotic pathways [145], whereas Bcl-x acts upstream of ceramide generation. Other studies defined roles of ceramide in cytokine-induced apoptosis (and lack of role in activation of NF-kB) [146]. In related studies, her group

implicated regulation of dihydroceramide desaturase (Des) in the regulation of the cell cycle [147].

Mechanistically, Obeid's group defined compartmentalization of ceramide action and discovered a role for mitochondrial ceramide in induction of apoptosis [148–150] especially with activation of sphingomyelinases, and this led to the development of a mitochondrially-targeted ceramide analog that was sufficient to induce apoptosis [151]. Additional studies showed that long chain endogenous ceramides inhibit the mitochondrial permeability pore [152] and that neutral ceramidase may function in reverse to generate ceramide in mitochondria [153]. In a recent study, her group showed that dysregulation of mitochondrial ceramide metabolism contributes to pathogenesis of Charcot-Marie-Tooth type 2F, a neuropathic disorder [154].

The work in her lab also addressed the role of ceramide synthases in apoptosis and related biology and the mechanisms involved [155,156]. Her group showed that disruption of CerS2 induced autophagy and the unfolded protein response [157], whereas *in vivo* disruption of CerSl induced neurodegeneration [158]. On the other hand, CerSs modulated apoptosis downstream of the mitochondrial pathway [159], and were shown to be involved in regulation of membrane leakiness through regulation of focal adhesion kinases [160]. Biochemically, her group was the first to demonstrate that the LAG domain in CerS was required for activity of the enzymes [161]. Her laboratory established that the pro-apoptotic protein BAK was required for CerS activity [162]. Collaborative studies based on this finding also demonstrated the activation of BAK/BAX and mitochondrial out membrane permeabilization MOMP) required S1P and hexadecenal [163]. Work from Dr. Obeid's lab published in 2017 in *Cell Metabolism* described the sequestration of ceramides as acylceramides via a novel interaction between DGAT2 and CerS that lead to protection of cells from apoptosis, thus defining a new pathway for clearance of pro-apoptotic ceramide. Together these studies defined CerS and specific ceramides as critical regulators in apoptosis and other regulator pathways in cancer signaling.

To advance ceramide-based therapeutics, her group collaborated with that of Dr. Sitharaman to promote graphene-based nanoparticles of ceramide [164,165].

These studies thus not only defined this critical function for ceramide but also advanced the cell biologic and biochemical understanding of the enzymes involved and the specific cellular contexts.

6.2. SK1 in carcinogenesis

Dr. Obeid's laboratory was the first to demonstrate the elevation of SK1 in multiple cancers [166]. This has now been supported by several significant studies, and it ushered the specific focus on SK1 in carcinogenesis. Work in the Obeid laboratory demonstrated that SK1 is upregulated in the azoxymethane (AOM)-induced model of colon cancer [167]. These finding were then extended to a murine model for CAC where genetic loss of SK1 led to reduced formation of aberrant crypt foci and decreased cancer development [121]. The same study also found a much higher prevalence of SK1 in human tumor samples than in normal

colon tissues, indicating its potential in human tumor formation. The roles of SK1 and S1P in colon cancer have been further developed by other studies [125,168].

Additional studies have supported roles for SK1 in tumorigenesis [169]. Overexpression of SK1 in MCF-7 breast cancer cells increased tumor growth in xenograft models [170]. Additionally, SK1 in tumor cells has been shown to stimulate metastasis, migration, invasion, angiogenesis, and chemoresistance [167,169,171,172]. SK1 and S1P receptor signaling are required for the invasiveness of glioblastoma cells through the urokinase plasminogen activator pathway [173]. Moreover, SK1 activity has recently been shown to be necessary for the invasion of triple negative breast cancer cells, through NF-kB activation and regulation of the protein fascin [174]. Furthermore, higher SK1 expression in colon cancer has been shown to increase invasiveness via Erkl/2 pathways, thus predicting metastasis and poor prognosis in patients [175,176]. Some work has also demonstrated that metastases of triple negative breast cancer are especially sensitive to SK1 /S1P signaling, showing that inhibition of SK1 leads to reduced growth, survival, and motility [177]. Deletion or inhibition of SK1 reduced both EGF and hormone-induced migration of breast cancer cells [178,179].

SK1 is also involved in angiogenesis and lymphangiogenesis, critical processes in both normal and cancer physiology. Knockdown of SK1 in glioblastoma (U87) as well as in triple negative breast cancer (MDA-MB-231) cells, significantly reduced vessel formation *in vitro* [180]. Interestingly, the reduction of tube formation was independent of the activity of VEGF, a cytokine commonly associated with angiogenesis. This is indicative of the importance of SK1/S1P signaling to angiogenesis independent of other pathways. Similar results have been shown *in vivo*, where SK1 inhibition reduced metastasis and angiogenesis in a syngeneic murine model of breast cancer metastasis using 4T1 cells [181]. Expression of SK1 and efficient signaling by S1P have been shown to stimulate angiogenesis in B-cell lymphoma [166]. Interestingly, endogenous genetic regulation of SK1 via micro-RNAs (miRs) has also been demonstrated to affect angiogenesis. miR-506 decreased SK1 expression in HepG2 cells and reduced angiogenesis in HUVEC cells [182]. SK1 expression was shown to negatively correlate with miR-506 expression in patient liver tissues. The emerging angiogenic properties of SK1 point to its importance for vascularization and tumors expansion.

6.3. SK1 as a prognostic marker

Much evidence has shown that SK1 is upregulated in a myriad of solid tumors, conferring resistance to chemotherapy and leading to poor prognosis to patients. Early research performed by the Obeid laboratory found that both gene and protein expression of SK1 is upregulated in lung cancer. SK1 expression was increased in non-small-cell lung cancer (NSCLC) tissue as compared with patient-matched normal tissue. This increase in expression also correlated with increased SK1 mRNA expression [183]. It was later demonstrated that overexpression of SK1 in NSCLC was associated with tumor progression, metastasis and low survival rate in patients [184]. Similar results have been published in several other human cancers, including breast, uterine, ovarian, colon and small intestine, where elevated expression of SK1 resulted in reduced patient survival [185–187]. Together,

these findings positioned SK1 as a potential therapeutic target and its high expression being a negative prognosis marker.

6.4. SK1 in chemotherapy resistance

A growing body of work suggests that higher expression of SK1 also correlates with resistance to common chemotherapies. Manuscripts from 2004 and 2006 from Dr. Obeid's group demonstrated that overexpression of SK1 in MCF-7 cells induced cell proliferation and protected from cell death. Conversely, downregulation of SK1 decreased cell viability and increased apoptosis in ER-positive MCF-7 breast cancer cells upstream of Bax oligomerization, cytochrome c release and caspase activation [188]. In addition, Watson and colleagues found that expression of SK1 was increased in tamoxifen-resistant MCF-7 cells as compared to sensitive cells [189]. Similarly, high expression of SK1 was shown to correlate with poor response to doxorubicin in NSCLC [184] and breast cancer cells [190], which was reversed upon co-treatment with an SK1 inhibitor (SK1–5C). Furthermore, the sensitivity of pancreatic cancer cells to gemcitabine was improved via either inhibition or knockdown of SK1, and the amount of S1P present in cells was shown to be key to resistance [191]. Song et al. demonstrated that silencing of SK1 by shRNAs or treatment with the inhibitor SK1-I decreased tumor growth in a xenograft model of NSCLC, both basally and in doxorubicin-treated mice, by increasing apoptosis. Thus, targeting SK1 sensitized NSCLC to chemotherapy *in vitro* and *in vivo* [184]. Most importantly, higher levels of SK1 mRNA were observed in patients who did not respond to doxorubicin and docetaxel treatment compared to partial or complete responders [190]. Activation of SK1 and S1P production has been demonstrated to occur downstream of the estrogen receptor-GPR30 axis [192]. Targeted therapies have also been shown to be less effective in instances of high SK1 expression. In chronic myeloid leukemia (CML), SK1 expression is correlated with resistance to imatinib, whereas inhibition or knockdown of SK1 restored imatinib-induced apoptosis [193,194]. The importance of SK1 in drug resistance highlights the need to better understand the regulatory mechanisms behind its expression.

6.5. Regulation of SK1 in cancer

SK1 is now recognized to be regulated at multiple levels and in response to many stimuli and mediators. The Obeid laboratory was one of the first to show the importance of the subcellular localization of SK1 for its kinase activity. As discussed above, in HEK293 cells, it was demonstrated that the PKC activator PMA induced SK1 translocation to the plasma membrane and a consequent increase in S1P secretion [24]. Other studies implicated PLD acting between PKC and SK1 through the generation of PA. The Obeid lab also showed that the oncogenic K-Ras regulates metabolism of bioactive sphingolipids in an SK1-dependent manner [195].

6.6. Role in p53-mediated biology

Also as discussed above, Dr. Obeid's studies were the first to demonstrate that DNA damage resulted in the degradation of the SK1 protein in a p53-dependent manner. The significance of this interaction was then extended to an *in vivo* model where the combined genetic deletion of SK1 and p53 in mice prevented the development of thymic lymphoma and reduced the development of other tumors, resulting in increased survival [80]. Moreover,

loss of SK1 in heterozygous *p53* mice protected from the development of sarcomas, lymphoma and lung adenocarcinoma potentially via the induction of senescence and increased C16-ceramide [80]. Future studies on SK1 as a novel therapeutic target in tumors with loss or mutant *p53* function will lend insight into the therapeutic potential for SK.

6.7. Regulation by hypoxia

Regulation of SK1 expression at the mRNA level has been also studied by the Obeid group. In glioma-derived U87MG cells, hypoxia increased SK1 mRNA levels, protein expression, and enzymatic activity. Under $CoCI_2$ treatment (a known hypoxia mimetic model), the transcription factor HIF2α, but not HIFlα, increased SK1 expression, and the knock-down of HIF2α prevented SK1 expression under hypoxic conditions [196]. In clear cell renal cell carcinoma (ccRCC) cells, which lack the von Hippel-Lindau (VHL) protein, SK1 was upregulated due to increases in HIF2α. Furthermore, knock-down of HIF2α by siRNA caused a consequential decrease in SK1 at the mRNA and protein level [197]. Similar studies by others have reported that SK1 regulates the expression and activity of HIF2α in response to hypoxia in a number of lung and ccRCC cell lines [198], suggesting a potential feedback loop between SK1 and HIF2α in response to oxygen levels.

Several studies have shown SK1 expression to be regulated by different transcription factors in various cancers. In head and neck squamous cell carcinomas (HNSCCs), E2F7 directly increased the transcription of SK1 resulting in activation of AKT and subsequent drug resistance in these cells [9]. In hepatoma cells, E2F1 was found to directly bind the SK1 promoter and induced tumor angiogenesis in liver cancer via the action of the long non-coding RNA (IncRNA) HULC [7]. In HeLa cells, Khpsl, another IncRNA, was found to drive expression of the SK1 gene in an E2F1 dependent manner. Upregulation of SK1 counteracted the apoptotic function of E2F1 and promoted cell proliferation [8]. At the post-transcriptional level, a growing number of studies have implicated various microRNA, with critical roles in cancer initiation, progression and metastasis, in regulation of SK1 expression [199–201].

7. Conclusions

Dr. Obeid, and the contributions of her lab, have propelled sphingolipids and in particular SK1 to the forefront of biomedical research. Her studies have defined fundamental features for the structure and function of SK1, established SK1 and S1P as key mediators in cell signaling pathways, and established SK1 as a critical modulator in several disease states. Completion of ongoing studies from her lab will further define significant roles for SK1 in cancer and metabolism. These studies will highlight the roles of SK1 in serine deprivation of cancer cells, the emerging functions of dSa (deoxysphinganine) as a novel bioactive sphingolipid, the roles of SK1 in senescence, and roles of SK1 as a therapeutic target. These discoveries will fuel the next generation of research and promote SK1 as a valid therapeutic target in multiple human diseases.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations:

AOM	Azoxymethane
cIAP2	Cellular inhibitor of apoptosis protein 2
CerK	Ceramide kinase
CerS	Ceramide synthase
C1P	Ceramide-l-phosphate
CML	Chronic myeloid leukemia
ccRCC	Clear cell renal cell carcinoma
CAC	Colitis-associated cancer
CIA	Collagen-induced arthritis
CD	Crohn’s Disease
COX2	Cyclooxygenase 2
dSA	Deoxysphinganine
DGAT2	Diacylglycerol O-acyltransferase 2
Des1	Dihydroceramide desaturas
EGF	Epidermal growth factor
EAE	Experimental autoimmune encephalomyelitis
GCS	Glucosylceramide synthase
GFP	Green fluorescent protein
HNSCC	Head and neck squamous cell carcinoma
HFD	High fat diet
HIF2α	Hypoxia inducible factor 2 alpha
IBD	Inflammatory bowel disease
IGF	Insulin-like growth factor

IL1β	Interleukin 1 beta
IL6	Interleukin 6
LM02	LIM-domain-only protein 2
LPS	Lipopolysaccharide
Lag	Longevity assurance gene
LDL	Low density lipoprotein
miR	microRNA
MS	Multiple sclerosis
DMS	N,N-dimethylsphingosine
NGF	Nerve growth factor
NSCLC	Non-small-cell lung cancer
OA	Osteoarthritis
PS	Phosphatidylserine
PMA	Phorbol 12-myristate 13-acetate
PA	Phosphatidic acid
PLD	Phospholipase D
PKC	Protein kinase C
PP	Protein phosphatase
RA	Rheumatoid arthritis
S1PR	Sphingosine-1-phosphate receptor
SASP	Senescence-associated secretory phenotype
SPT	Serine palmitoyltransferase
siRNA	Small interfering ribonucleic acid
SMS	Sphingomyelin synthase
SK	Sphingosine kinase
S1P	Sphingosine-1-phosphate
SOCS3	Suppressor of cytokine signaling 3
TGFβ	Transforming growth factor beta
TNBC	Triple-negative breast cancer

TRAF2	Tumor necrosis associated factor 2
TNFα	Tumor necrosis factor alpha
UC	Ulcerative colitis
VEGF	Vascular endothelial growth factor
VHL	Von Hippel-Lindau
WT	Wildtype

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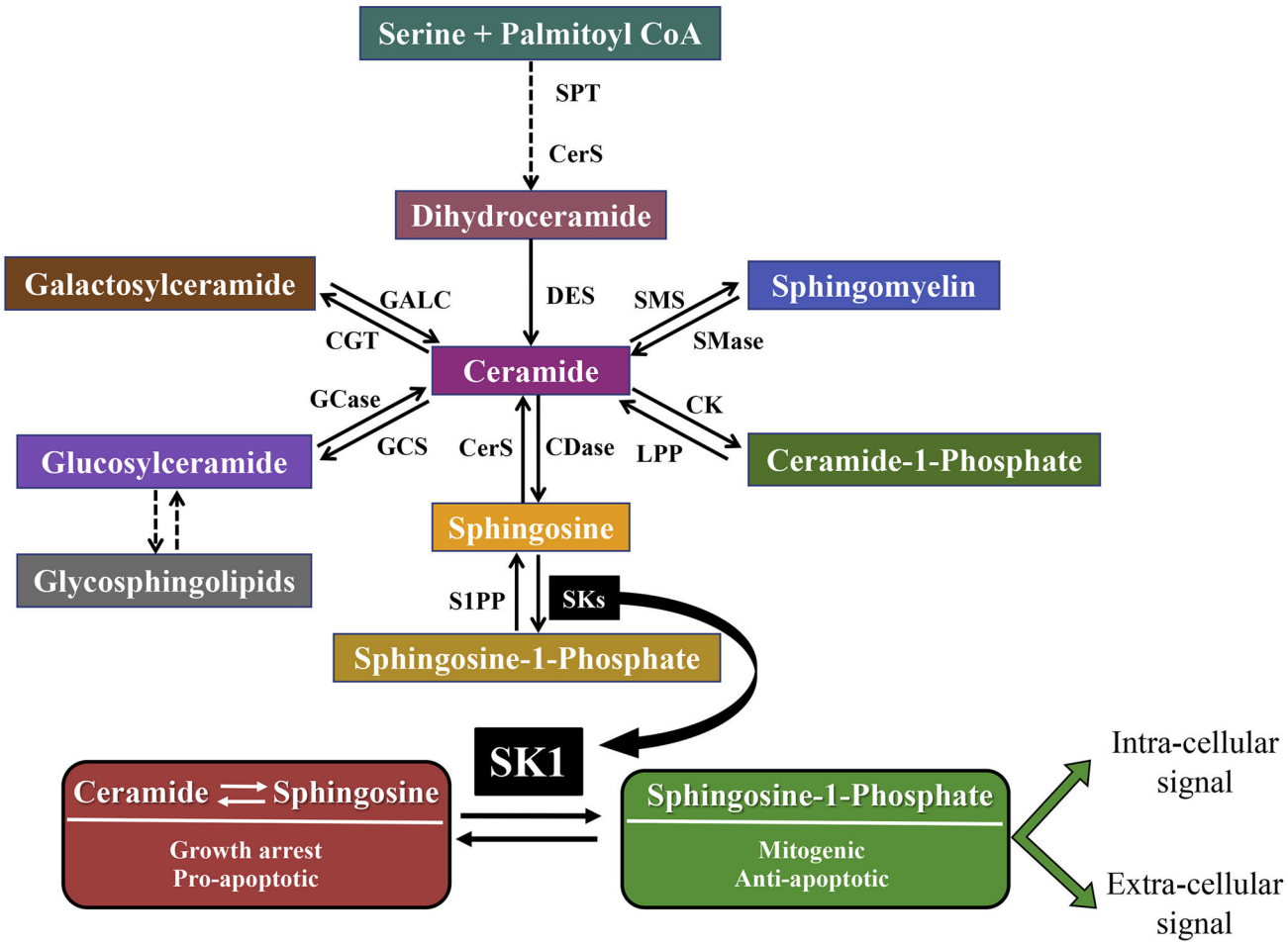

Fig. 1.

SK1: a critical point in sphingolipid metabolism. Sphingolipids are generated *de novo* via the condensation of serine and palmitoyl-CoA. Ceramide is formed after stepwise reduction, acylation and desaturation processes and can be used to generate many different sphingolipids. Ceramidases are required to generate sphingosine, which can then be phosphorylated by one of two sphingosine kinases (SK1 and SK2) to form S1P. SK1 activity is critical in regulating the balance between two groups of sphingolipids with opposite functions. Ceramide and sphingosine have been shown to cause growth arrest and to activate pro-apoptotic signaling, while S1P has been demonstrated to exert mitogenic and anti-apoptotic properties. S1P can act at the intracellular level, through the binding with different proteins, or upon export, can function as a ligand for one of 5 S1PRs triggering intracellular signaling cascades. SK1 participates in several processes such as cell survival, angiogenesis, lymphocyte trafficking, migration, inflammation and chemotherapeutic resistance, through its product S1P (or the alteration of ceramide and/or sphingosine levels). SPT, serine palmitoyltransferase; CerS, ceramide synthase; DES, desaturase; CK, ceramide kinase; LPP, lipid phosphate phosphatase, CDase, ceramidase, SMS, sphingomyelin synthase; SMase, sphingomyelinase; CGT, ceramide galactosyl transferase; GALC, galactosyl ceramidase; GCS, glucosylceramide synthase; GCase, glucocerebrosidase; S1PP, S1P phosphatase.

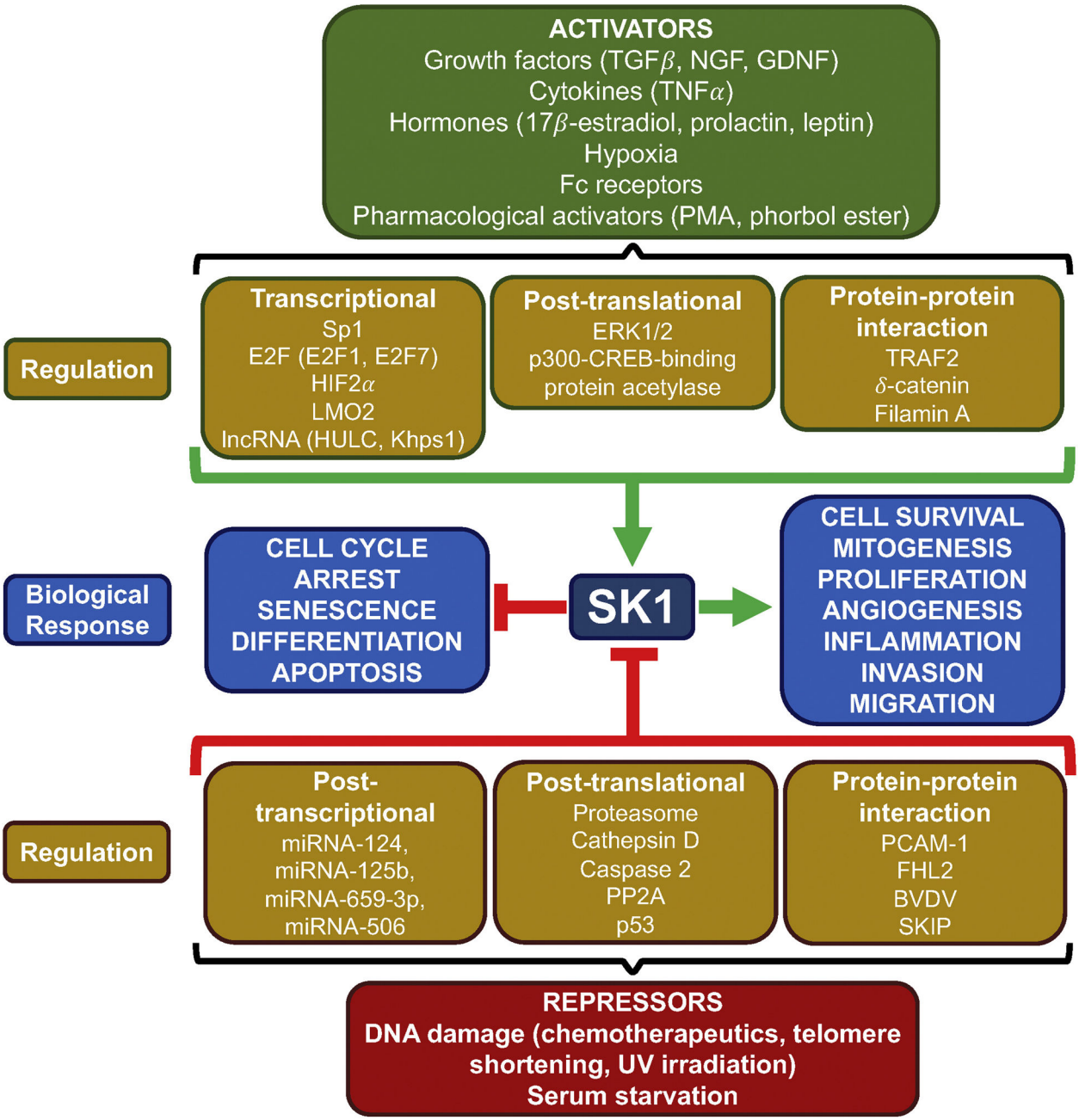

Fig. 2.

Regulation of SK1. SK1 is positively regulated by a wide range of signaling molecules including growth factors, cytokines and hormones as well as by pharmacological compounds, Fc receptors and hypoxia. These activators drive SK1 activity through various signaling pathways resulting in transcriptional upregulation, post-translational modifications including phosphorylation, or by protein-protein interactions. Increased SK1 activity is primarily associated with cell survival, mitogenesis, proliferation, angiogenesis, inflammation, invasion, or migration in a context-dependent manner. Alternatively, SK1 is negatively regulated by DNA damage induced by chemotherapeutics, UV irradiation,

telomere shortening as well as serum starvation. Suppression of SK1 activity can be mediated through post-transcriptional repression by miRNA, post-translational proteasomal degradation, proteolysis via other proteases, dephosphorylation, or through interaction with proteins. Loss of SK1 activity is primarily associated with cell cycle arrest, senescence, apoptosis, and differentiation. PMA, phorbol 12-myristate 13-acetate; ERK, extracellular regulated kinase; TRAF2, TNF receptor-associated factor 2; PP2A, Protein phosphatase 2A; PECAM-1, platelet endothelial cell adhesion molecule 1; FHL2, four-and-a-half LIM domain 2; BVDV, bovine viral diarrhea virus.

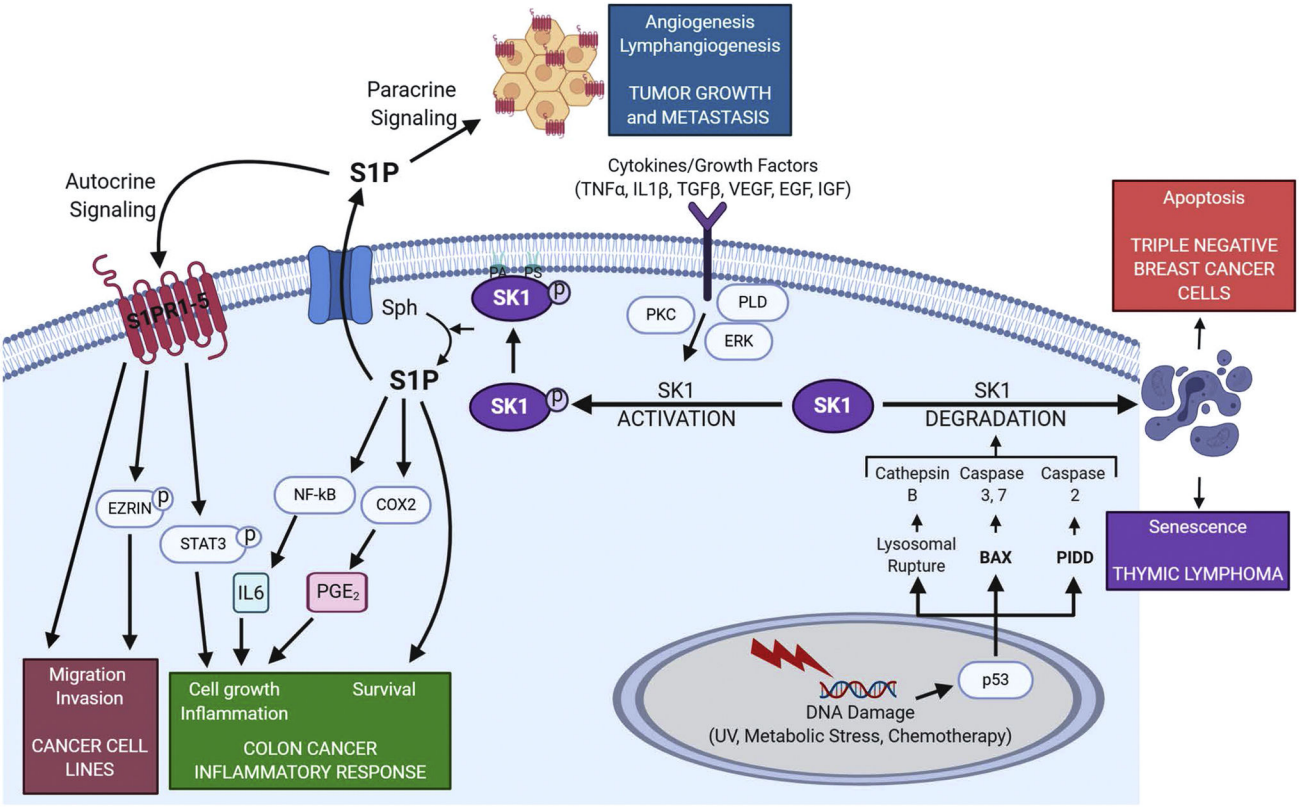

Fig. 3.

SK1 signaling and associated biological responses. SK1 is activated by a myriad of extra cellular signals, such as growth factors and cytokines. This in turn generates S1P which can be transported out of the cell to act in an autocrine manner where it can modulate many signaling pathways and responses, including increased migration and invasion of cancer cells through the phosphorylation of Ezrin and ERM (ezrin, radixin, moesin) proteins. S1P can also act in paracrine manner driving angiogenesis and lymphangiogenesis leading to tumor growth and metastasis. SK1 and S1P also activate inflammatory signaling via NF-kB, STAT3 and COX2, signals that are critical for colitis and colon cancer. Intracellular signaling via effectors such as PKC, PLD and ERK can result in the phosphorylation and membrane association of SK1, resulting in increased cell survival and proliferation. PLD also produces phosphatidic acid (PA) which helps anchor SK1 in the membrane. DNA damaging agents can also lead to the degradation of SK1 via proteases leading to apoptosis and senescent in cancer cells. *Figure created with BioRender.com.

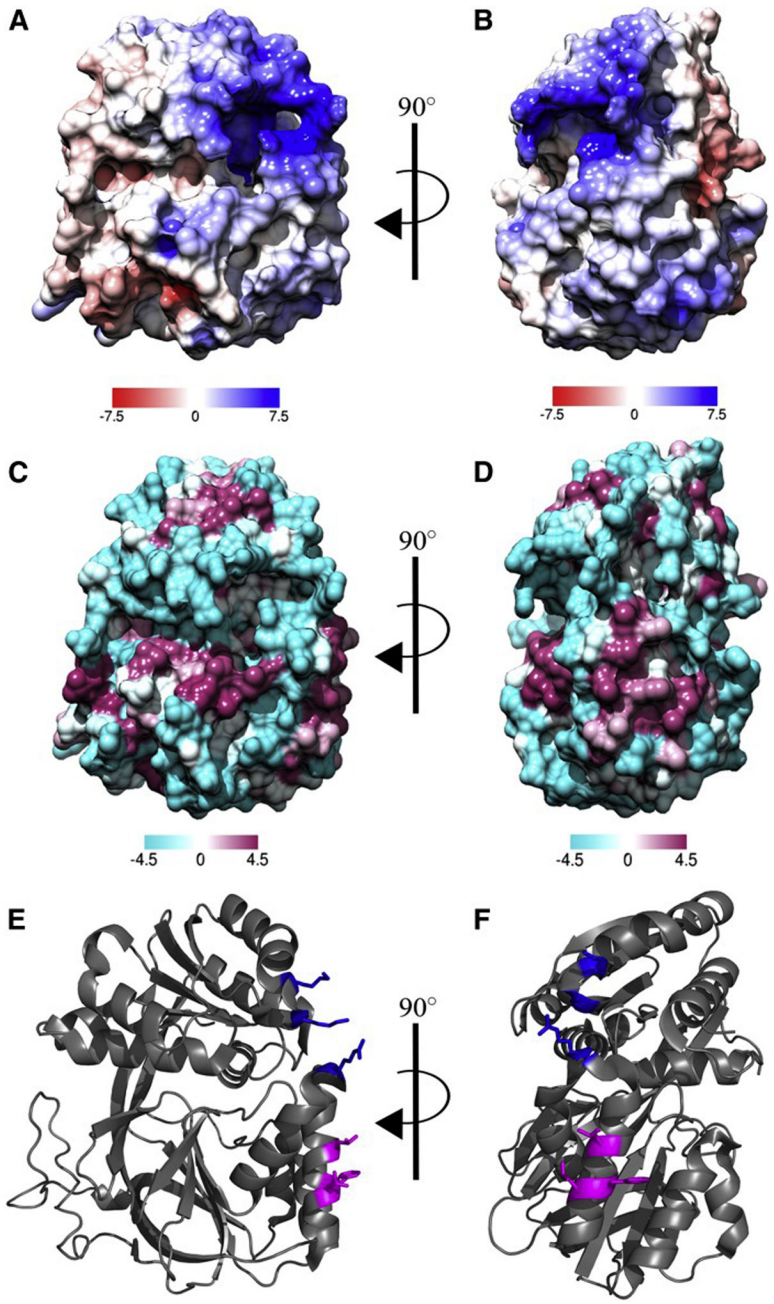

Fig. 4.

In silico surface binding analysis of SK1. A and B: Electrostatic potential maps were generated using Chimera software using the Adaptive Poisson-Boltzman Solver. The scale represents kcal·mol^{-1} where blue represents positively charged areas and red represents negatively charged areas. C and D: The Kyte-Doolittle scale of hydrophobicity was used to predict hydrophobicity and was mapped to the surface of SK1 where cyan represents hydrophilic areas and magenta represents hydrophobic areas. E and F: Cartoon representation of SK1 with residues of the hydrophobic patch and electrostatic patch represented as magenta and blue sticks, respectively. Protein Data Bank identification

number 3VZB [32] used for all analyses. This figure was originally published in the Journal of Lipid Research. Pulkoski-Gross M. J., Jenkins, M.L., Truman, J.P., Salama, M.F., Clarke, C. J., Burke, J.E., Hannun, Y.A., Obeid L.M. An intrinsic lipid-binding interface controls sphingosine kinase 1 function. J Lipid Res. 2018; 59:462–474. © the American Society for Biochemistry and Molecular Biology.