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Nuclear Lipid Mediators: Role of Nuclear Sphingolipids and Sphinosine-1-Phosphate Signaling in Epigenetic Regulation of Inflammation and Gene Expression

Panfeng Fu[1,#], **David L. Ebenezer**[2,#], **Alison W. Ha**[2], **Vidyani Suryadevara**[3], **Anantha Harijith**[4], and **Viswanathan Natarajan**[1,5]

[1]Department of Pharmacology, University of Illinois, Chicago, IL, USA

[2]Department of Biochemistry & Molecular Genetics, University of Illinois, Chicago, IL, USA

[3]Department of Bioengineering, University of Illinois, Chicago, IL, USA

[4]Department of Pediatrics, University of Illinois, Chicago, IL, USA

[5]Department of Medicine, University of Illinois, Chicago, IL, USA

Abstract

Phospholipids, sphingolipids, and cholesterol are integral components of eukaryotic cell organelles, including the nucleus. Recent evidence shows characteristic features of nuclear lipid composition and signaling, which are distinct from that of the cytoplasm and plasma membrane. While the nuclear phosphoinositol lipid signaling in cell cycle regulation and differentiation has been well described, there is a paucity on the role of nuclear sphingolipids and sphingolipid signaling in different physiological and pathophysiological human conditions. In this prospective, we describe the role of sphingolipids and specifically focus on the sphingoid bases, such as sphingosine, ceramide, and sphingosine-1-phosphate (S1P) generation and catabolism in nuclear signaling and function. Particularly, S1P generated in the nucleus by phosphorylation of SphK2 modulates HDAC activity either by direct binding or through activation of nuclear reactive oxygen species and regulates cell cycle and pro-inflammatory gene expression. Potential implication of association of SphK2 with the co-repressor complexes and generation of S1P in the nucleus on chromatin remodeling under normal and pathological conditions is discussed. A better understanding of sphingolipid signaling in the nucleus will facilitate the design and development of new and novel therapeutic approaches to modulate expression of pro-inflammatory and cell cycle dependent genes in human pathologies such as cancer, bacterial lung infection, neurodegeneration and cystic fibrosis.

Correspondence: Viswanathan Natarajan, PhD, Department of Pharmacology, University of Illinois at Chicago, Room # 3137, COMRB Building, 909, South Wolcott Avenue, Chicago, IL 60612, visnatar@uic.edu, Telephone: 312-355-5896, Fax: 312-996-7193.
[#]Contributed equally to the work

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests relating to this manuscript.

Keywords

Nuclear Signaling; Lipids; Phosphoinositides; Epigenetics; Sphingolipids; Sphingosine-1-Phosphate; Sphingosine kinase 2; Inflammation; Histone acetylation and deacetylation

INTRODUCTION

The nucleus of a prokaryotic cell is a highly compartmentalized and a functionally distinct organelle; within its well-defined nuclear envelope (inner and outer membrane) are several distinct endonuclear domains including nuclear matrix, chromatin, and nucleolus. Lipids comprising of phospholipids, sphingolipids, and cholesterol (CHOL) are integral components of all eukaryotic cellular organelle membranes. While the composition and role of plasma membrane and cytosolic organelle membrane lipids have been extensively investigated, only in the last two decades, studies related to eukaryotic nuclear lipids and its role in nuclear signaling and function have gained momentum. The phospho- and sphingo-lipids within the nucleus are distinct from those present in the nuclear envelope. The nuclear envelope is a lipid bilayer that exhibits unique lipid composition between the outer and the inner membranes. The outer membrane of the nuclear envelope is contiguous with the endoplasmic reticulum (ER) and shares some lipidomics characteristics with the ER, while the inner membrane is associated with the nuclear lamina and chromatin architecture and has certain unique lipoid signatures. Several of the phospho- and sphingo-lipid metabolizing enzymes have been identified in the nucleus, suggesting its ability to generate and metabolize lipids independent of other cellular organelles. The nuclear membranes also express a number of receptors including those for platelet activating factor (PAF), prostaglandin (PG) E2, thromboxane (TX) 2, inositol (1,4,5) trisphosphate (IP3), PPARγ, retinoic acid, and G-protein coupled receptors (GPCRs) that transduce nuclear signaling and initiate potential cross-talk between the nucleus and other organelles in the cell. The lipid signaling cross-talk between the nucleus and other organelles under normal and pathological conditions can influence the inter- and intra-cellular functions and cellular physiology. While several studies have elucidated the potential importance of nuclear phospholipids and in particular inositol phospholipid signaling in gene expression, only limited information is available on nuclear sphingolipids, sphingolipid signaling in the nucleus, and its role in regulation of gene expression and chromatin modifications. This perspective focuses on recent revelations in nuclear sphingosine-1-phosphate (S1P) metabolism and activities of S1P metabolizing enzymes that regulate cell cycle and pro-inflammatory gene expression via chromatin modifications.

PHOSPHOLIPIDS AND PHOSPHOINOSITIDES IN NUCLEAR SIGNALING

Similar to other cellular organelles, the nucleus is rich in phospholipids, sphingolipids, and cholesterol (Irvine, 2003; Farooqui, 2009; Ledeen and Wu, 2008; Lucki and Sewer, 2012). Majority of the lipids are localized in the nuclear envelope and provide structural support, as well as participate in signal transduction. Nuclei isolated from different cell types and tissues contain high levels of phosphatidylcholine (PC), sphingomyelin (SM), and CHOL that interact with nuclear proteins to form lipid-protein complexes (Albi et al., 1994; Albi et al.,

2003b; Albi and Magni, 2004;. Recent studies suggest that organization of nuclear PC, SM, CHOL in lipid microdomains could regulate nuclear processes such as transcription, proliferation, differentiation, and apoptosis (Cascianelli et al., 2008). In addition to the major lipids, the nucleus also contains bioactive lipids that are locally generated by enzymes specifically localized in nuclear compartments such as nuclear matrix, chromatin, and nuclear speckles (Ledeen and Wu, 2006; Ledeen and Wu, 2008; Farooqui, 2009; Lucki and Sewer, 2012). Nuclear lipid levels and lipid composition are in a dynamic state, and are regulated by altered metabolic fluxes in response to plasma membrane/cytoplasmic and nuclear signaling cascades.

A well-studied example of modulation of phospholipid signal transduction cascade in the nucleus is turnover of inositol phospholipids, especially phosphatidylinositol-4,5-bisphosphate (PIP_2). Extracellular stimulation of hepatocytes and other cell types by insulin growth factor (IGF) results in enhanced nuclear diacylglycerol (DAG) production via hydrolysis of nuclear PIP_2 by phospholipase C (PLC) β (Payrastre et al., 1992) and modulation of nuclear processes such as proliferation and gene expression (Alessenko and Burlakova, 2002). Interestingly, generation of nuclear DAG also promotes PKC translocation to the nucleus (Divecha et al., 1991) and initiates DNA synthesis (Martelli et al., 1991) (Raben and Baldassare, 2000). In the nucleus, DAG is also generated by a second pathway involving hydrolysis of PC or phosphatidylethanolamine (PE) by nuclear phospholipase D (PLD) to form phosphatidic acid (PA) that is subsequently converted to DAG by a nuclear lipid phosphate phosphatase (LPP) (Gaveglio et al., 2011). Nuclear DAG has a short half-life as it is rapidly converted to PA by nuclear DAG kinase θ ;Tu-Sekine et al., 2016) that is stimulated by a variety of agonists (Martelli et al., 2000). Further, PA produced in the nucleus can function as a second messenger. PA interacts with a variety of cellular and cytoskeletal proteins and these interactions may modulate enzyme activities, spatio-temporal localization of the protein, and cellular functions such as migration, vesicular trafficking, cytoskeletal organization, secretion, and proliferation (Cummings et al., 2002; Liu et al., 2013). PA in the nucleus can also generate another bioactive signaling lipid mediator, lysophosphatidic acid (LPA), by the action of PA specific phospholipase A1/A2 (Fourcade et al., 1995; Sonoda et al., 2002; Aoki et al., 2008). LPA mediates cellular functions by signaling extracellularly via G-protein coupled $LPA_{1–6}$ receptors and intracellularly by binding to target proteins such as PPARγ (McIntyre et al., 2003; Tsukahara et al., 2013). No definitive measurements of LPA levels in nuclear preparations have been reported; however, LPA_1 is known to be constitutively expressed in the nucleus of porcine cerebral microvascular endothelial, rat hepatoma HTC4, (Gobeil et al., 2003) and human bronchial epithelial and neuronal PC12 cells (Waters et al., 2006). Further, in human bronchial epithelial cells (HBECs) and PC12 cells, trafficking of LPA_1 to the nucleus was influenced by cell-matrix interactions via integrin signaling, and nuclear LPA_1 may be involved in regulating intra-nuclear protein phosphorylation (Waters et al., 2006). PLC-β mediated hydrolysis of PIP_2 in the nucleus, in addition to DAG, also generates the second messenger, inositol IP_3 that binds to its cognate receptor present on the inner nuclear membrane to release calcium that is essential for transcriptional regulation of calcium dependent enzymes such as calmodulin kinase (Bootman et al., 2009). In the nucleus, IP_3 is phosphorylated by inositol kinases localized in the nucleus (Nalaskowski et al., 2002; Seeds

and York, 2007; Kim et al., 2017) resulting in the generation of inositol polyphosphates that regulate chromatin remodeling, histone modifications, transcription and mRNA export (Monserrate and York, 2010; Burton et al., 2013). In addition to PIP_2, phosphatidylinositol-5 phosphate (PI5P) was shown to be necessary for the binding of Ubiquitin-like with PHD and RING finger domain 1 (UHRF1) to tri-methylated residues of histone3 (H3K9me3) (Arita et al., 2008). UHRF1 is involved in DNA replication and repair and also associated with targeting HDACs to actively transcribed chromatin via interaction with PI5P. Nuclear PIP_2 also mediates interaction between BASP-1 and WILMS'TUMOR 1 protein in association with HDAC1 (Toska et al., 2012) further implicating a role for polyphosphoinositides and other phospholipid-derived mediators in gene regulation and chromatin structure. Thus, there is compelling evidence that mammalian cells have the ability to synthesize and metabolize phospholipids and generate lipid mediators within the nucleus that signal both intra- and extra-nuclear for cellular processes (Fig. 1).

SIGNALING NUCLEAR SPHINGOLIPIDS

Sphingolipids comprising of sphingophospholipids, sphingoglycolipids and neutral sphingolipids play important roles in several cellular functions. A common feature of all sphingolipids is the long chain sphingosine base (usually C18) that is modified by long-chain fatty acids linked to the amino group and phosphate, phophosphorylcholine, or sugar moieties such as glucose and/or galactose attached to the primary hydroxyl group. Nuclear sphingolipid levels have been partially quantified and different sphingolipid species have been identified in different nuclear domains including nuclear envelope, matrix and chromatin (Lucki and Sewer, 2012; Ledeen and Wu, 2008). While SM is the major sphingolipid in the nucleus and associated with the nuclear matrix and chromatin (Neitcheva and Peeva, 1995; Albi et al., 1994; Lucki and Sewer, 2012), other sphingoid bases such as sphingosine, ceramide and sphingosine-1-phosphate (S1P) are present in the nuclear and cytosolic preparations from lung epithelial cells (Table 1). Many of the nuclear sphingolipids are in a dynamic state and exhibit turnover suggesting localization of sphingolipid metabolizing enzymes such as SM synthase, sphingomyelinase (SMase) ceramidase and sphingosine kinases (SphKs) in the nucleus and its sub-compartments. The nucleus is also rich in glycosphingolipids such as GM_1, GM_3, GD_1, and GD_3 gangliosides (Ledeen and Wu, 2008). Nuclear GM_1 is enriched in the inner membrane of NE and in tight association with Na^+/Ca^{2+} exchanger (Xie et al., 2002), and was shown to modulate nuclear calcium levels (Gerasimenko and Gerasimenko, 2004; Ledeen and Wu, 2007). Sphingolipid (Table 2) and its metabolizing enzymes (Table 3) are components of chromatin and recent evidence points to interaction between sphingolipids/sphingolipid metabolizing enzymes and HDACs in co-repressor complexes such as Sin3 and NuRD (Hait et al., 2009; Ebenezer et al., 2017). Thus, nuclear sphingolipids function as membrane components, signaling lipid mediators, and modulators of calcium and double stranded RNA (Ledeen and Wu, 2008; Micheli et al., 1998).

Sphingomyelin in Nuclear Signaling and Function

SM is the most abundant sphingophospholipid in the nucleus and a major component of chromatin (Ledeen and Wu, 2008; Lucki and Sewer, 2012). Additionally, SM is also

enriched in nuclear matrix (Albi et al., 2003b). Nuclear SM metabolism is independent from the Golgi/ER; its levels are in a dynamic state in the nucleus and modulated by high fat diet, stress, cell cycle and tissue regeneration. Rats on high fat diet revealed elevated SM and ceramide levels in both liver homogenates and hepatocellular nuclei with a shift towards greater fatty acid saturation status in SM and ceramide (Cer) (Chocian et al., 2010). Enzymes regulating SM biosynthesis and catabolism have been identified in the nucleus and nuclear fractions. In the nucleus, the primary pathway involved in generation of SM requires de novo biosynthesis of the Cer intermediate from palmitoyl-CoA and L-serine. Condensation of palmitoyl CoA and L-serine to 3-ketosphinganine is mediated by serine palmitoyl transferase, the rate limiting enzyme in SM biosynthesis, while other enzymes involved in Cer generation are 3-ketosphinganine reductase, Cer synthase and Cer desaturase (Fig. 2). Conversion of Cer to D-erythro-N-palmitoyl-sphingosine-1-phosphocholine (SM) requires SM synthase 1 or 2, which utilize phosphocholine derived from nuclear PC, which is present in both the chromatin and NE (Albi et al., 2003a). SM generated in the nucleus is metabolized by neutral sphingomyelinase (nSMase), first detected in the nuclear matrix of rat ascites hepatoma cells (Tamiya-Koizumi et al., 1989) and subsequently in NE (Alessenko and Chatterjee, 1995), chromatin (Albi and Magni, 1997), and nuclear matrix (Neitcheva and Peeva, 1995). Another SM metabolizing enzyme that carries out the reverse reaction of SM-synthase, namely transfer of phosphocholine from SM to DAG with the formation of PC was detected in rat liver chromatin (Albi et al., 2003a). The role of SM-synthase and reverse SM-synthase in chromatin is unclear; however these two enzymes may modulate Cer/DAG ratio in the chromatin and thus regulate proliferation or apoptosis (Albi and Magni, 2004). Recent studies on intra-nuclear SM localization in various cell types were carried out using nSMase conjugated to colloidal gold particles and transmission electron microscopy, which revealed preferential localization of SM within the peri-chromatin region (Scassellati et al., 2010). Further, microinjection of SMase into living cell nucleus led to deterioration of nuclear structures supporting the intra-nuclear SM association with transcriptionally active ribosomal genes within the dense fibrillar component of the nucleolus and possible involvement of SM in internal nuclear architecture and mRNA processing (Scassellati et al., 2010). However, it is unclear if the resultant loss of SM or accumulation of Cer is responsible for the rapid degradation of intra-nuclear structure. It is not only SM, but also nSMase and SM synthase that appear to be associated with intra-nuclear complexes containing STAT3, a transcriptional factor, showing SM and its metabolizing enzymes to be part of the newly synthesized double stranded RNA in active chromatin (Rossi et al., 2007). Nuclear SM also interacts with CHOL and PC in nuclear lipid microdomains (NLMs) (Slotte, 2013; Cascianelli et al., 2008) and the ratio of CHOL/SM/PC is higher in nuclear matrix during rat liver regeneration suggesting an inverse relationship between nuclear matrix fluidity and DNA synthesis (Albi et al., 2003b). Thus, NLMs may facilitate cross-talk between glycerolipids, sphingolipids, CHOL and lipid mediators generated by metabolic enzymes, which regulate cellular processes such as proliferation, apoptosis and cell cycle in normal and pathological situations. Interestingly, NLMs isolated from hepatocytes and hepatoma cells showed a shift in the ratio between very long chain fatty acid (C24:0) and long chain fatty acid (C16:0). In hepatoma cells, NLMs had a higher proportion of C16:0 fatty acid compared to C24:0 in SM fraction, an increase in the expression of STAT3, Raf1 and PKC δ and a decrease in relative levels of vitamin D receptors (Lazzarini et al., 2015)

indicating a potential link between enhanced dynamic properties of NLMs with increasing shuttling of signaling proteins in the nucleus of cancer cells. Increased expression of PKC δ and SM synthase and decreased SMase activities in murine melanoma cell line compared to the wild type resulted in elevated DAG and decreased ceramide pools in the nucleus, (Albi et al., 2005). The inhibition of SMase, in turn, may increase nuclear SM-synthase activity and enrichment of nuclear SM pool or alter the ratio of Cer to DAG levels in the nucleus to modulate nuclear signaling. Interestingly, exposure of non-Hodgkin lymphoblastic lymphoma cells to gentamycin, an aminoglycoside antibiotic, stimulated cytosolic SMase, inhibited nuclear SMase, increased nuclear SM-synthase activity with elevated nuclear SM levels and induced cell death (Codini et al., 2015). Further analysis of nuclear fractions and NLMs using lipidomics and proteomics should allow identification, quantification and association between SM and its metabolites with proteins in the nucleus, chromatin and nuclear matrix.

Nuclear Ceramide and Ceramide-1-Phosphate Metabolism and Signaling

Cer is central to sphingolipid metabolism as it is not only the building block for all the major sphingophospholipids and sphingoglycolipids in the biosynthetic pathway but also serves as an immediate intermediate of SM degradation by SMase in cells including the nucleus (Fig. 2). In the nucleus, Cer is converted to sphingosine (Sph) by ceramidase (Shiraishi et al., 2003). Cells generate ceramide-1-phosphate (C1P) from Cer in endoplasmic reticulum/golgi organelles through ceramide kinase (CERK) (Sugiura et al., 2002), and there is evidence for CERK to be associated in the nucleus that could generate nuclear C1P (Rovina et al., 2009). In addition to Sph and Cer, nucleus also has dihydro (DH) Sph and DH Cer. Lipidomics of sphingoid bases from mouse lung epithelial cell line, MLE-12, revealed high levels of Cer in the nuclear fraction (Table 3). Further, nuclear Cer were enriched in C16:0, C22:0, C24:0 and C24:1 and C28:0 fatty acids linked to the amino group of Cer (Ebenezer, Fu and Natarajan unpublished data). Nuclear Cer levels are modulated by extracellular agonists, bacterial infections, high fat diet, serum-deprivation and apoptosis inducing agent such as Fas ligand (Watanabe et al., 2004). Hepatic vein ligation in rats enhanced Cer accumulation in hepatocyte nucleus through activation of neutral SMase (Tsugane et al., 1999) that correlated with DNA fragmentation and cell death. Serum-deprivation increased nuclear Cer levels during the early phase of apoptosis while late phase apoptosis correlated with extranuclear stimulation of SMase activity and accumulation of ceramide in the cytoplasmic compartments (Albi et al., 2006). High fat diet increased Cer levels in rat liver nuclei from 500 nmoles/gm dry tissue to 1600 nmoles/gm dry tissue (~3 fold change) and a significant increase in saturated fatty acid species (C14:0, C16:0 and C18:0) (Chocian et al., 2010). Although Cer has been implicated in apoptosis, no specific nuclear target of Cer has been identified. Cer localized in NLM may be involved in 1,25-dihydroxy vitamin D_3 mediated differentiation of embryonic hippocampal cells (Marini et al., 2010), as exposure of the cells to exogenous SMase or serum deprivation increased SMase activity, changed the lipid composition of NLM and impaired the localization of vitamin D_3 receptor in the NLM. It is unclear if Cer traffics out from the nucleus into the cytoplasm or vice-versa via lipid protein(s) such as Cer transport protein CERT, and FAPP2 that have been identified (Schroeder et al., 2008; Yamaji et al., 2008). However, there is evidence in support of the blood-borne fatty acids to rapidly enter nuclear lipid pools in hepatocytes (Bucki et al.,

1997). Conversion of Cer to C1P catalyzed by Cer kinase (CERK) takes place at the Golgi and C1P-transfer protein (CPTP) transfers C1P to the plasma membrane and other organelles including the nucleus (Simanshu et al., 2013). In addition to CERK's primary localization in Golgi, it is also associated with the nucleus through the nuclear import signal at the N-terminal and exported to the cytosol with its nuclear export signal at the C-terminal region (Rovina et al., 2009). The C1P generated in the nucleus may have specific signaling function(s) that are yet to be described. C1P is an activator of group IVA cytosolic phospholipase A2, the rate-limiting step in eicosanoid generation (Lamour and Chalfant, 2005). Most of the cellular effects of C1P have been described to take place in intracellular membrane compartments; however, C1P also stimulated cell migration that was blocked by pertussis toxin suggesting signaling of C1P via PTx-sensitive G-protein coupled receptor(s) on the plasma membrane of the cell (Arana et al., 2013). Further studies are necessary to determine the role of nuclear C1P in nuclear eicosanoid synthesis and cellular functions such as proliferation, apoptosis and survival.

Nuclear Sphingosine Signaling and Regulation of Gene Transcription

Total cellular and nuclear free sphingosine (Sph) levels are much lower compared to Cer and nuclear Sph levels are regulated by ceramidases that hydrolyze ceramide to Sph and ceramide synthase, a reversible enzyme that can convert Sph to Cer (Tsugane et al., 1999; Lucki and Sewer, 2012). Sph is a ligand for steroidogenic factor 1 (SF-1) and regulates the transcription of CYP17 (Urs et al., 2006). Under basal conditions, Sph is bound to SF-1 in the nucleus with Sin3A, HDAC and other co-repressor proteins and stimulation with adrenocorticotropin hormone (ACTH) activates protein kinase A that facilitates release of Sph from SF-1 receptor's ligand binding pocket and stimulates transcription of genes involved in steroid hormones synthesis from cholesterol via binding of PA to SF-1 (Lucki and Sewer, 2012). This study implicates an important role for nuclear Sph in regulating gene transcription. Sphingolipids play an important role in host-pathogen interaction and detection of host-derived Sph by *Pseudomonas aeruginosa* (*PA*) is essential for survival of the Gram-positive bacterium in the murine lung. Sph, present in the pulmonary surfactant is generated via metabolism of SM ▶ ceramide ▶ S1P, induced a transcript *sphA* (*PA5325)* that was regulated by an AraC-family transcription factor, PA5324 (SphR) (LaBauve and Wargo, 2014). Deletion of *sphR* resulted in reduced bacterial survival during *P. aeruginosa* infection of mouse lung. Sph is bactericidal and deletion of *sphR* increased sensitivity of the antimicrobial effects of Sph suggesting that Sph detection by the AraC-family transcription factor SphR may be more prevalent in other bacteria and not limited to *P. aeruginosa*.

P. aeruginosa infection of mouse lung significantly reduced free Sph levels in BAL fluids from 40 pmoles/ml to 10 pmoles/ml 6–48 h post-infection; however, the Sph levels in ling tissue was elevated at 48 h post-infection (Ebenezer, Fu and Natarajan, unpublished data) indicating modulations in Sph levels in BALF and lung tissue after bacterial infection. The pathophysiological significance of Sph reduction in BAL fluids is yet to be correlated to *P. aeruginosa* infection of the lung and resolution but there is evidence for sphingoid long chain bases to prevent lung infection by *P. aeruginosa* Jung and Tabazavareh, 2014). The contradictory role of Sph in *P. aeruginosa* survival and infection of mouse lung needs further interrogation.

Nuclear Sphingosine-1-Phosphate Metabolism and Signaling

S1P, the simplest bioactive sphingophospholipid of the human sphingolipidome, is one of most extensively investigated lipid that is known to play a key role in various cellular functions. S1P is enriched in plasma (0.1–1.0 μM) compared to tissues and interstitial fluids(Olivera et al., 2013). Cellular sources of plasma S1P are unclear; however recent studies suggest that platelets (Yatomi et al., 2004), red blood cells (Ito et al., 2007) and non-hematopoietic sources such as liver sinusoidal endothelial cells (Venkataraman et al., 2008) may be the predominant sources (Książek et al., 2015). S1P is biosynthesized in mammalian cells by phosphorylation of Sph, catalyzed by sphingosine kinases (SphKs) 1 & 2 (Neubauer and Pitson, 2013; Gault et al., 2010). Although both the isoforms of SphK catalyze S1P formation from Sph, the sub-cellular localization and expression vary in different cells that dictate spatio-temporal S1P production and function. SphK1 expression is higher in the lung and heart, and SphK2 in the liver and spleen (Melendez et al., 2000; Siow et al., 2011). Two functional nuclear export signal sequences (NES) direct SphK1 localization to the cytosol (Inagaki et al., 2003); in contrast, SphK2 has nuclear import and export sequences, and is found predominantly in the nucleus in many cell types (Igarashi et al., 2003). Both SphK1 and SphK2, when activated by growth and survival factors, undergo translocation, post-translational modifications, protein-lipid, and protein-protein interactions that ultimately result in elevated S1P levels in the cell (Alemany et al., 2007), which regulates various biological responses. Cytosolic S1P produced by SphK1 enhances cell growth, whereas Sphk2 generated S1P in ER and/or membranes promote apoptosis, as evident from studies using different model systems (Liu et al., 2003; Hait et al., 2006). Despite their differences, *in vivo* studies have shown that SphK1 and SphK2 can compensate for each other, as neither SphK1 nor SphK2 knockout mice models reveal any ostensible phenotype in adulthood; however the double knockouts are embryonically lethal. Additionally, S1P could be also be generated from C1P by the action of an amidase to cleave the long chain fatty acid or sphingosylphosphorylcholine by autotaxin (ATX)/ lysophospholipase D (Lyso PLD) (Aoki et al., 2008). These pathways are yet to be established and may not represent the major pathway of S1P formation in the cell or the nucleus.

S1P signals via G-protein coupled $S1P_{1–5}$ receptors present on the cell surface (“inside-out” signaling) and initiate downstream cellular responses (Pyne and Pyne, 2010; ; Natarajan et al., 2013; Ebenezer et al., 2016a). However, the possibility of intracellular action of S1P via intracellularly localized S1PRs is understudied and cannot be ruled out. Recent evidences suggest localization and differential expression of S1PRs in normal and malignant human tissues. All the five S1PRs are expressed in both the cytoplasm and nucleus of benign and malignant tissues from multiple human organs as evidenced by IHC and ICC (Wang et al., 2014). Additionally, in estrogen receptor-positive breast cancer, high cytoplasmic $S1P_1$ and nuclear $S1P_2$ and $S1P_3$ expression and association of these receptors with signaling proteins such as ERK1/2, AKT or SphK1 was reported to be associated with survival or recurrence of estrogen receptor-positive breast cancer (Ohotski et al., 2013). S1P, generated within cells is catabolized to sphingosine by S1P phosphatases (SPPs) 1 & 2 (Pyne et al., 2009; Lépine et al., 2011). Additionally, S1P is also terminally hydrolyzed by S1P lyase (S1PL) to ethanolamine phosphate and hexadecenal in cells (Van Veldhoven et al., 2000; Saba and Hla,

2004). Thus, S1P accumulation is the result of a balance between synthesis, catalyzed by SphKs, and degradation mediated by LPPs, SPPs, and S1PL. In addition to inside-out signaling, S1P can also signal inside the cell by increasing free Ca^{2+} levels (Birchwood et al., 2001; Itagaki and Hauser, 2003); however, the intracellular target(s) is yet to be identified. Recent studies suggest binding of S1P to human telomerase (Panneer Selvam et al., 2015) and HDACs 1& 2 in normal and cancerous epithelial cells (Hait et al., 2009; Ebenezer et al., 2016b), suggesting a role for nuclear S1P and signaling in normal physiology and pathophysiology (Fig. 2).

Nuclear S1P or S1P analog(s) Generation Requires SphK2 Phosphorylation and Activation in the Nucleus

SphK2/S1P signaling axis in the nucleus and its role in gene regulation via epigenetic mechanism(s) is an emerging paradigm. In many cells, SphK2 is localized in the nucleus; however, its role in S1P production and nuclear signaling is yet to be fully defined. S1P levels in the nucleus of epithelial (<10 pmoles/mg protein) (Fu et al., 2014; Ebenezer et al., 2017a) and breast cancer cell line MCF-7 (~2 pmol) (Hait et al., 2009) is low as compared to Sph and ceramide. However, infection of epithelial cells with *P. aeruginosa* or overexpression of SphK2 in MCF-7 cells resulted in enhanced nuclear S1P levels by several fold (Ebenezer et al., 2016; Hait et al., 2009). Similarly, treatment of mouse embryonic fibroblasts with FTY720, an analog of Sph, resulted in significant accumulation of FTY720-P in both the cytoplasm and nuclear fractions (Gardner et al., 2016a) and blocking SphK2 attenuated nuclear S1P levels in cells (Hait et al., 2009; Ebenezer et al., 2017a) as well as FTY720-P (Gardner et al., 2016a). Administration of FTY720 in wild type mice resulted in accumulation of FTY720-P in the hippocampal region and mice with genetic deletion of SphK2 showed reduced FTY720-P (Hait et al., 2014). A critical step in nuclear S1P or FTY720-P production is phosphorylation of SphK2 and its nuclear localization in response to stimuli. Stimulation of cells with phorbol myristoyl acetate (PMA) (Hait et al., 2009) or infection of mouse lung or lung epithelial cells with *P. aeruginosa* induced SphK2 phosphorylation at ser/thr 614 and nuclear localization (Ebenezer et al., 2017). Inhibition of SphK2 activity with ABC294640, a specific inhibitor of SphK2, reduced the amount of S1P (Ebenezer et al., 2017a) accumulation in the nucleus suggesting a role for nuclear SphK2 in cell functions. Similarly, SH-SY5Y neuroblastoma cells treated with FTY720 exhibited elevated levels of FTY720-P in the nucleus compared to the cytoplasm, which required SphK2 activity as transfection of the catalytically inactive $SphK2^{G212E}$ plasmid attenuated FTY720-P accumulation (Hait et al., 2014). As SphK2 is localized in the cytoplasm as well as nucleus of many cell types including lung epithelial cells (Ebenezer et al., unpublished data), it will be important to determine if SphK2 is phosphorylated in the cytoplasm and translocated to the nucleus or nuclear SphK2 is phosphorylated preferentially compared to cytoplasmic SphK2 or if both the steps are operative in response to an exogenous stimulus. It appears that both the cytoplasmic and nuclear SphK2 are phosphorylated in lung alveolar and bronchial epithelial cells after infection with heat-inactivated *P. aeruginosa* and further characterization of the phosphorylation status is warranted (Ebenezer et al., 2017a; Ebenezer et al., 2017b). Both SphK1 and SphK2 are phosphorylated at ser/thr residues by ERK1/2 (Pitson et al., 2003; Hait et al., 2007), and other protein kinases such as PKC. *P. aeruginosa* infection of alveolar and bronchial epithelial cells stimulated phosphorylation of PKC δ, ε,

and ζ and blocking PKC δ, but not PKC ε or ζ, attenuated SphK2 phosphorylation in the nucleus and S1P production suggesting an important role for this PKC isoform in nuclear S1P accumulation after bacterial infection of epithelial cells (Fu et al., 2014; Ebenezer et al., 2017a). Further, the phosphorylated PKC δ is co-localized with SphK2/p-SphK2 in the nucleus of the epithelial cells after *P. aeruginosa* infection. The role of ERK1/2 or other protein kinases in phosphorylation of SphK2 needs to be evaluated. Additionally, total cellular and nuclear S1P, dihydro S1P or FTY720-P levels could be elevated by inhibiting S1P lyase, dihydroceramide synthase and ceramide synthase or overexpression of either SphK1 or SphK2 *in vivo* or *in vitro*. Genetic deletion of *sgpl1* in mouse embryonic fibroblasts (Ihlefeld et al., 2012) or inhibition of S1PL by chemical agent such as 2-acetyl-5-tetrahydroxybutl imidazole (THI) increased total lung (Zhao et al., 2011) and nuclear S1P (Nguyen-Tran et al., 2014) levels. Further, treatment of the mouse embryonic fibroblast with fumonisin B1 (an inhibitor of both dihydroceramide synthase and ceramide synthase) resulted in elevated levels of cytoplasmic and nuclear dihydro S1P that was significantly reduced by Sphk1 inhibitor PF-543 and SphK2 inhibitor, ABC294640 (Gardner et al., 2016b) however, the underlying mechanism of enhanced accumulation of S1P or dihydro S1P is not known. Recent evidences suggest that elevated nuclear S1P and S1P analogs are linked to increased risk for neural tube defects (Gardner et al., 2016a; Gardner et al., 2016b) neurodegeneration in neuron models of Huntington disease (Moruno-Manchon et al., 2017) and bacterial lung inflammation (Fu et al., 2014; Ebenezer et al., 2016a; Ebenezer et al., 2017a; Ebenezer et al., 2017b). Further studies are necessary to define the role of elevated nuclear S1P or S1P analogs in vivo and in vitro to understand the role of nuclear S1P signaling pathway(s) in the development human pathologies for potential therapeutic intervention.

Nuclear S1P and FTY720-P are Co-Epigenetic Regulator of Chromatin Remodeling, Gene Transcription and Inflammatory responses via association with HDACs

The role of nuclear S1P and S1P analogs mediated regulation of chromatin remodeling and transcriptional regulation of pro-inflammatory and cell cycle genes is a new and novel emerging area. Nuclear SphK2/p-Sphk2 and S1P co-immunoprecipitated with HDAC1/2; S1P and FTY720-P inhibited HDAC activity and increased histone acetylation at H3K9, H4K8, H3K18 and H3K23 in MCF-7, MLE-12 and MEFs (Hait et al., 2009; Ebenezer et al., 2016a; 2017a; Hait et al., 2014; 2015; Gardner et al., 2016a). Blocking SphK2 activity with ABC294640 or down-regulation of SphK2 siRNA reduced PMA or *P aeruginosa* mediated H3 and H4 histone acetylation in MCF-7 (Hait et al., 2009) and MLE-12 cells (Ebenezer et al., 2017a), respectively. Transcription regulation through chromatin compaction and decompaction is regulated through two major co-repressor complexes in mammalian cells, namely Sin3a, and NuRD/Mi2 (Vermeulen et al., 2006; Baymaz et al., 2015). Both the Sin3 and NuRD co-repressor complexes are large multi-protein complexes in which all the components and their functions have not been identified; however, two of the key components include HDAC1 and 2, and methyltransferases (including methyl-CpG-binding proteins) (Delcuve et al., 2012; Chen et al., 2015; Li and Seto, 2016). SphK2/p-SphK2 was associated with HDAC1/HDAC2 as part of the Sin3 and NuRD co-repressor complexes and was selectively enriched at the promoters of genes encoding pro-inflammatory cytokines such as IL-6 and TNF-α (Ebenezer et al., 2017a), and cyclin-dependent kinase inhibitor p21

or transcriptional regulator of c-fos (Hait et al., 2009). More recently, down-regulation of SphK2 with siRNA or inhibiting SphK2 activity with a selective inhibitor K145 reduced hypoxia-mediated nuclear S1P, histone acetylation and nuclear HIF-1α synthesis in breast cancer cell lines (Hait and Maiti, 2017). Thus, HDAC1/2 are direct nuclear targets of S1P and link nuclear S1P production to epigenetic regulation of pro-inflammatory, transcriptional factor and cell cycle genes. The role of SphK2/S1P signaling in histone acetylation was not restricted to cells in culture but was observed *in vivo* in animal models of inflammation and injury. Genetic deletion of SphK2, but not SphK1, protected mouse against *P. aeruginosa* mediated lung inflammation and injury and SphK2 knockdown resulted in reduced H3 and H4 histone acetylation and secretion of pro-inflammatory IL-6 and TNF-αsecretion in bronchoalveolar lavage (BAL) fluids (Fu et al., 2014; Ebenezer et al., 2015; Ebenezer et al., 2016b). Administration of SphK2 inhibitor, ABC294640 to mice reduced lung inflammation, pro-inflammatory cytokines in BAL fluids, and histone acetylation both pre- and post-challenge of *P aeruginosa* implicating a therapeutic role of blocking SphK2 in reducing bacterial lung inflammation (Ebenezer et al., 2016b) (Fig. 3). In the dystrophic mouse (*mdx*), increasing nuclear S1P levels by inhibiting S1PL with THI decreased HDAC activity, and increased histone acetylation that resulted in up-regulation of muscle metabolic genes and key micro RNAs miR-29 and miR-1 (Nguyen-Tran et al., 2014). These effects observed in *mdx* mouse muscle after blocking S1PL lyase seem to be beneficial for the maintenance of energy metabolism in mice while S1P generated by SphK2 activation in epithelial cells seem to regulate the pro-inflammatory cytokine secretion via modulation of HDAC1/2. Partial deletion of *sgpl1* in mice (*sgpl1*$^{+/-}$ heterozygous) offered protection against *P.aeruginosa* mediated lung inflammation and injury; however, the involvement of cytosolic and/or nuclear S1P in chromatin remodeling and mechanism of this protective response to bacterial inflammation needs to be established (Ebenezer, Fu, and Natarajan unpublished data). Recent studies with the Sph analog, FTY720 showed that FTY720-P generated in the nucleus by SphK2 mediated phosphorylation of FTY720 reactivated expression of silenced estrogen receptor-α (ERα) and sensitized the breast cancer cells to tamoxifen that required inhibition of HDAC activity (Hait et al., 2015) suggesting an important role for SphK2/FTY720-P nuclear signaling for effective treatment of therapy-resistant breast cancer. The SphK2/FTY720-P signaling axis also seems to play a key role in ameliorating lipid accumulation and trafficking defects in Niemann-Pick type C (NPC) syndrome, a fatal neurodegenerative disorder caused by mutations in the *NPC1* and *NPC2* genes leading to cholesterol and sphingolipids in late endosomes and lysosomes (Loftus et al., 1997) (Vanier, 2013). FTY720 administration in mice increased hippocampal and cerebellar NPC1 and NPC2 expression that required SphK2 (Newton:2017d) Further, FTY720 mediated expression of NPC1 and NPC2 in *NPC1* mutant fibroblasts correlated with generation of FTY720-P and decreased accumulation of cholesterol and glycosphingolipids in *NCP1* mutant fibroblasts (Newton et al., 2017). FTY720-P akin to S1P is a potent HDAC inhibitor and hence the FDA approved drug, Fingolimod (FTY720), for treatment of multiple sclerosis may be useful for the treatment of lipid storage disorders such as NPC in humans.

Mechanisms of Nuclear S1P Mediated Regulation of HDAC1/2 and Chromatin Remodeling

(i) S1P binding to HDAC1/2 and modulation of activity—Based on the ability of S1P to bind to HDAC1/2 and inhibit its activity *in vitro*, modeling of S1P into the active site of HDAC1 homologue from *Aquifex aeolicus* indicated that it docked well in the pocket with the highly conserved Arg27 with a predicted binding energy of −6.85 kcal/mol comparable to −7.09 kcal/mol of the HDAC1/2 inhibitor suberoylanilide hydroxamic acid (SAHA) (Hait et al., 2009). Further, the bound [^{32}P] S1P was totally displaced by inhibitors of HDAC1/2, SAHA and trichostatin A, and unlabeled S1P suggesting competitive binding. While these studies support that S1P is an endogenous ligand of HDAC1/2, the mechanism(s) of S1P mediated inhibition of HDAC1/2 activity remains to be investigated.

(ii) ROS-mediated oxidative modification of HDAC1/2—Inhibition of HDAC1/2 and enhanced histone acetylation by SphK2/S1P signaling axis in the nucleus may involve S1P mediated reactive oxygen species (ROS) generation in the nucleus that modulates HDAC1/2 oxidation of lysine residues as well as oxidation of SH groups to sulfenic acid and formation of disulfide linkage in HDAC1/2. *In vitro,* S1P stimulated p47^{phox} dependent ROS generation in human lung endothelial cells and genetic deletion of SphK1 in mice attenuated hyperoxia-induced ROS generation in BAL fluids (Harijith et al., 2016). *P. aeruginosa* infection of mouse lung increased S1P and hydrogen peroxide levels in lungs and BAL fluids while genetic deletion of SphK2, but not SphK1, in mice also attenuated accumulation of hydrogen peroxide after bacterial infection (Fu et al., 2014). Blocking SphK2 activity with ABC294640 or knockdown of PKC δ with siRNA reduced nuclear ROS generation, H3/H4 histone acetylation and IL-6 secretion. Inhibition of SphK2 with ABC294640 had no effect on cytoplasmic or mitochondrial ROS production as determined by p-Hyper redox sensor measurement (Ebenezer et al., 2017a; Ebenezer et al., 2017b). ROS production in mammalian cells is regulated by NOX1–5 and DUOX1 &2 (Altenhofer et al., 2012; Brandes et al., 2014). *P. aeruginosa* activation of mouse and human lung epithelial cells stimulated ROS production via NOX2 and NOX4 (Fu et al., 2013) and NOX 4 was predominantly localized in the nucleus compared to NOX2 (Fu P et al., 2014; Ebenezer et al., 2016a &b). The *P. aeruginosa* mediated H3 and H4 histone acetylation and IL-6 production was reduced by NOX4 siRNA and blocking SphK2 with ABC294640 attenuated NOX4 mediated hydrogen peroxide production (Fu et al., 2014; Ebenezer et al., 2017a & b). Thus, *P. aeruginosa* infection of lung epithelial cells drives SphK2-dependent S1P generation that stimulates nuclear hydrogen peroxide via NOX4 modulating HDAC activity, and histone acetylation pattern. Increased NOX4 dependent oxidative stress in the nucleus of cardiac myocytes induced NOX4 dependent cysteine oxidation of HDAC4 and nuclear exit of HDAC4, resulting in cardiac hypertrophy response to phenylephrine and pressure overload (Matsushima et al., 2012). These studies suggest a role for oxidative stress in modulation of chromatin remodeling. The mechanism of regulation of HDAC1/2 activity by *P. aeruginosa* mediated nuclear hydrogen peroxide seems to involve oxidation of HDAC1/2. Further, *P. aeruginosa* induced oxidation of HDACs was attenuated by PKC δ, SphK2 and Nox4 siRNAs (Ebenezer et al., 2017a) (Fig. 4).

(iii) Modulation of HDAC1/2 by Aldehydes Generated in the Nucleus—Short-chain and long-chain fatty aldehydes released in cell nucleus could interact with ε-amino

groups of lysine residues or SH groups of HDAC1/2 to generate adduct resulting in modulation of HDAC activity and histone acetylation/methylation patterns, and cellular functions. Δ2-hexadecenal is one of the products generated by S1PL action on S1P in cells (Van Veldhoven, 2000; Saba and Hla, 2004; Natarajan et al., 2013). Although S1PL is predominantly localized in the endoplasmic reticulum, nuclear fractions isolated from human bronchial and mouse lung epithelial cells exhibited immunostaining for S1PL and activity as measured by ability to generate Δ2-hexadecenal by mass spectrometry (Ebenezer et al., 2015; Ebenezer et al., 2016a). The nuclear fractions isolated from mouse lung epithelial MLE-12 cells were enriched in lamin B (marker for nuclear membrane) with least contamination of cytosol (LDH marker); however, showed positive staining for ER markers suggesting potential ER contamination (Ebenezer et al., 2015). It is noteworthy to point out that nuclear outer membrane is contiguous to ER and use of mild detergents to release the ER and nuclear outer membrane may be necessary to further characterize S1PL localization in the nucleus. Inhibition of S1PL by 4-deoxypyridoxine in mouse lung epithelial cells not only elevated *P. aeruginosa* mediated S1P levels but also reduced H3/H4 histone acetylation in the nuclear fraction suggesting a potential role for long-chain fatty aldehyde(s) released from S1P to modulate HDAC activity and histone acetylation (Ebenezer et al., 2015; Ebenezer et al., 2017a; Ebenezer et al., 2017b). Further, exogenous addition of Δ2-hexadecenal to nuclear preparations from MLE-12 cells stimulated H3K9 histone acetylation in a dose- and time-dependent manner (Ebenezer et al., 2015) suggesting a role for S1PL released fatty aldehyde in chromatin remodeling. Trapping of the aldehydes released by agents such as phloretin, a polyphenol, also reduced *P. aeruginosa* stimulated histone acetylation. These studies suggest a potential role for reactive aldehydes such as 4-hydroxynonenal and Δ2-hexadecenal generated by lipid peroxidation and S1P catabolism, respectively as potential modulators of HDAC1/2 and could serve as epigenetic co-regulators of pro-inflammatory gene expression. In the nucleus, long chain fatty aldehydes such as hexadecanal (C16:0 fatty aldehyde) could also be generated from ethanolamine or choline plasmalogens through plasmalogenase activity (Ansell and Spanner, 1968; McMaster et al., 1992). Further studies are necessary to quantify hexadecenal and hexadecanal generated in the nucleus and delineate molecular mechanism(s) of short and long-chain fatty aldehyde mediated modulation of nuclear HDAC1/2 in lung pathologies (Fig. 5).

Nuclear Glycosphingolipids

Complex glycosphingolipids, comprised of oligosaccharide chain containing hexose and N-acetylneuraminic acid (sialic acid) residues, are intrinsic components of cell organelles including the nucleus. Gangliosides are sialic acid containing glycosphingolipids that have been identified to occur both in the outer and inner membranes of the nucleus (Keenan et al., 1972; Matyas and Morré, 1987; Ledeen and Wu, 2006; Lucki and Sewer, 2012). Two major species of Gangliosides, GM1, GD1a, were found to be present in both membranes of NE (Ledeen and Wu, 2008; Ledeen and Wu, 2011) along with two isoforms of neuraminidase (Neu), which convert GD1a to GM1 (Wang et al., 2009). GM1 in the inner membrane interacts with Na^+/Ca^{2+} exchanger (NCX) (Xie et al., 2002) and conversion of GD1a to GM1 by Neu1 or Neu3 potentiates the NCX activity. The nuclear NCX/GM1 complex situated in the inner membrane of NE mediates Ca^{2+} transfer from cytosol to endoplasmic

reticulum (Gerasimenko et al., 1995; Wu et al., 2009). Furthermore, nuclear NCX/GM1 complex may provide cytoprotection as GM1 knockout mice exhibited temporal lobe seizures of greater severity and duration to kainic acid compared to normal mice, and administration of LIGA-20, a membrane permeable analog of GM1 reduced the duration of kainic acid induced seizures by enhanced penetration to the nucleus of hippocampal neurons and activate the NCX of the NE (Wu et al., 2005). Thus increasing GM1 and other gangliosides in the nucleus may offer a therapeutic protective application in neurodegenerative disorders such as Parkinson's disease.

CONCLUSION AND FUTURE PRESPECTIVES

This perspective highlights the role of nuclear sphingolipids signaling in cellular responses in normal and pathological conditions. Nuclear sphingolipids is an area that has been actively investigated for more than a decade; however, only in the past 5 years considerable progress has been made in delineating the link between sphingolipid metabolism and epigenetic regulation of cellular functions such as gene expression, cell proliferation, and inflammation. Among the nuclear sphingolipids, S1P generation in the nucleus and signaling have been implicated in several human pathologies including cancer, pulmonary inflammation, and neurodegenerative disorders. There is strong evidence for S1P generation in the nucleus mediated by activation/phosphorylation of SphK2 catalyzed by PKC δ or ERK 1/2 and modulation of H3 and H4 histone acetylation *in vivo* and *in vitro*. In addition to S1P, the S1P analog FTY720-P is also emerging as a modulator of nuclear HDAC activity and gene transcription. SphK2 or p-SphK2 in the nucleus is associated with HDACs 1 & 2 as part of the co-repressor complex of Sin3, NuRD or CoREST and S1P generated by SphK2 activation affects HDAC 1/2 activity, and H3 and H4 histone acetylation pattern in the chromatin. At least three potential mechanisms have been identified for S1P dependent regulation of HDAC1/2 activity. The first proposed mechanism involves docking of S1P in the pocket of HDAC1/2 inhibitor SAHA binding site while the second mechanism points out to the role of nuclear S1P signaling in nuclear ROS generation and oxidation of HDACs1/2 leading to inhibition of HDAC activity, increased acetylation of H3 and H4 histones, chromatin remodeling and altered gene expression. A third potential pathway might involve short and long fatty aldehydes such as 4-hydroxynonenal and Δ2-hexadecenal released in the nucleus by lipid peroxidation and S1P degradation, respectively to modulate HDAC activity. Nuclear S1P levels are regulated by synthesis and catabolism. Very little is known regarding breakdown of S1P in the nucleus either by S1P phosphatases or S1P lyase, which require further investigation. Similarly, histone acetylation is a balance between HDACs and histone acetylases (HATs); however, S1P modulation of HATs is unclear. Limited information is available on the role of Sph and ceramides in the nucleus. Further interrogation is necessary to identify the role of these two sphingoid bases in nuclear signaling and function. To-date only a few genes have been identified to be epigenetically regulated by nuclear S1P and use of ChIP-Sequencing (ChIP-Seq) will provide a power tool to identify genome-wide DNA binding sites for co-repressor complexes such as Sin3, NuRD and CoREST that contain HDACs, SphK2 and other target proteins. Finally, detailed lipidomics analysis of nuclear sphingolipids in different cells types will provide a better understanding of these lipids in nuclear signaling involved in various human pathologies.

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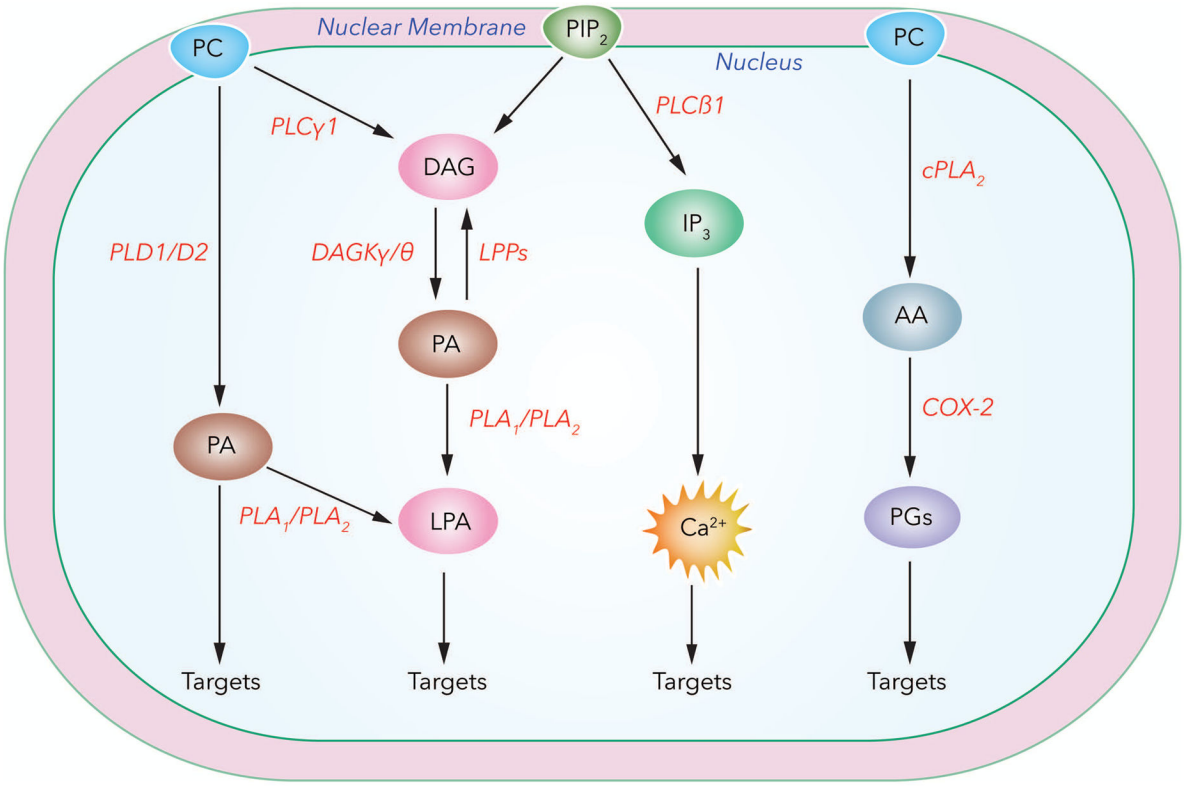
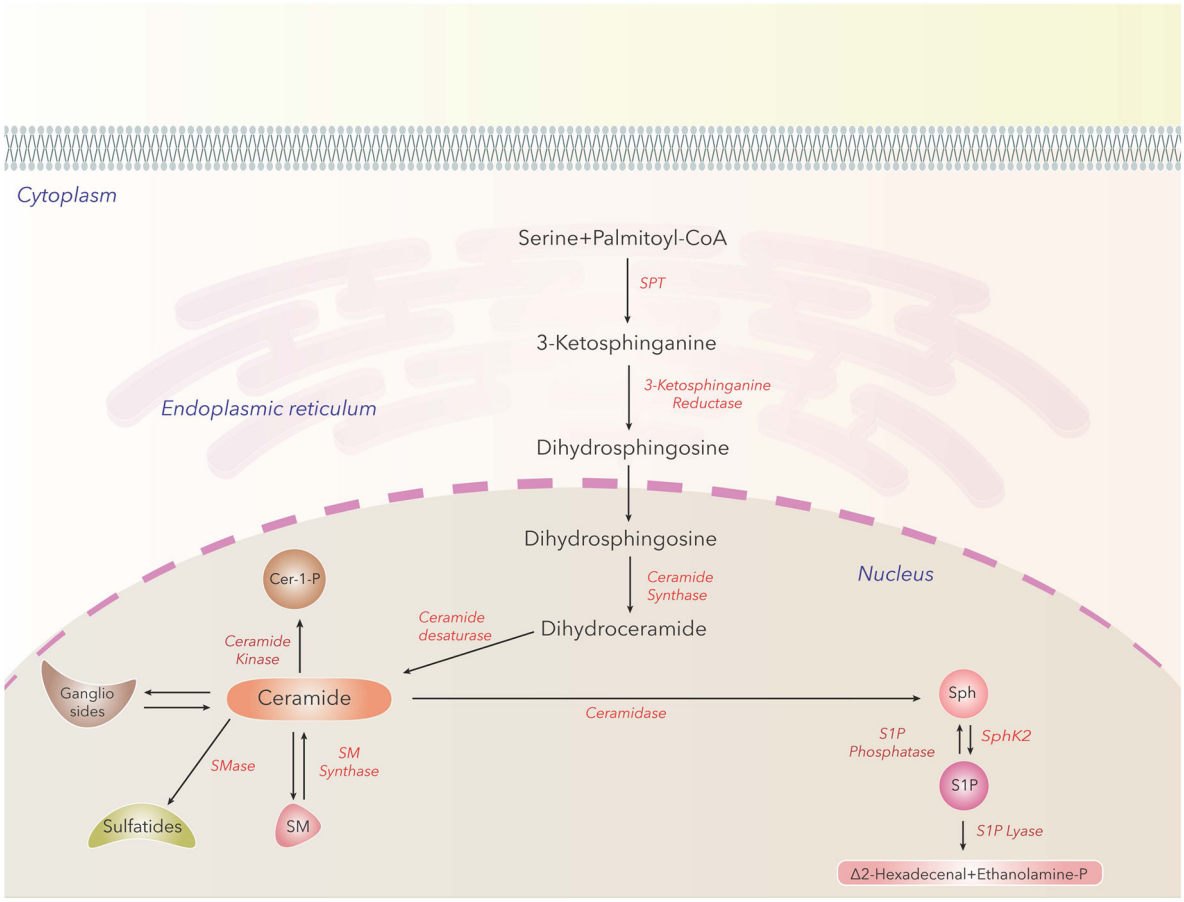


Fig. 1.



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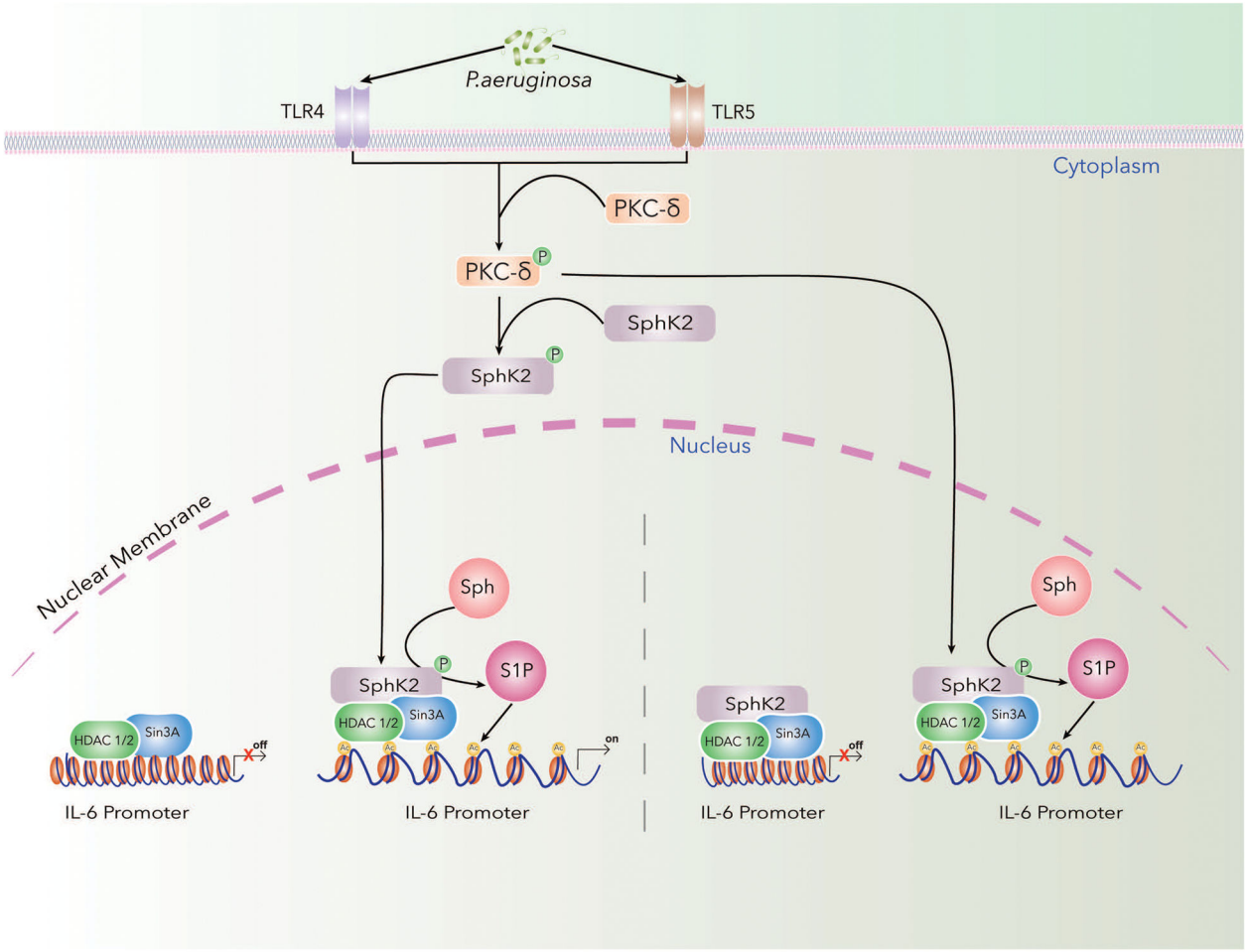

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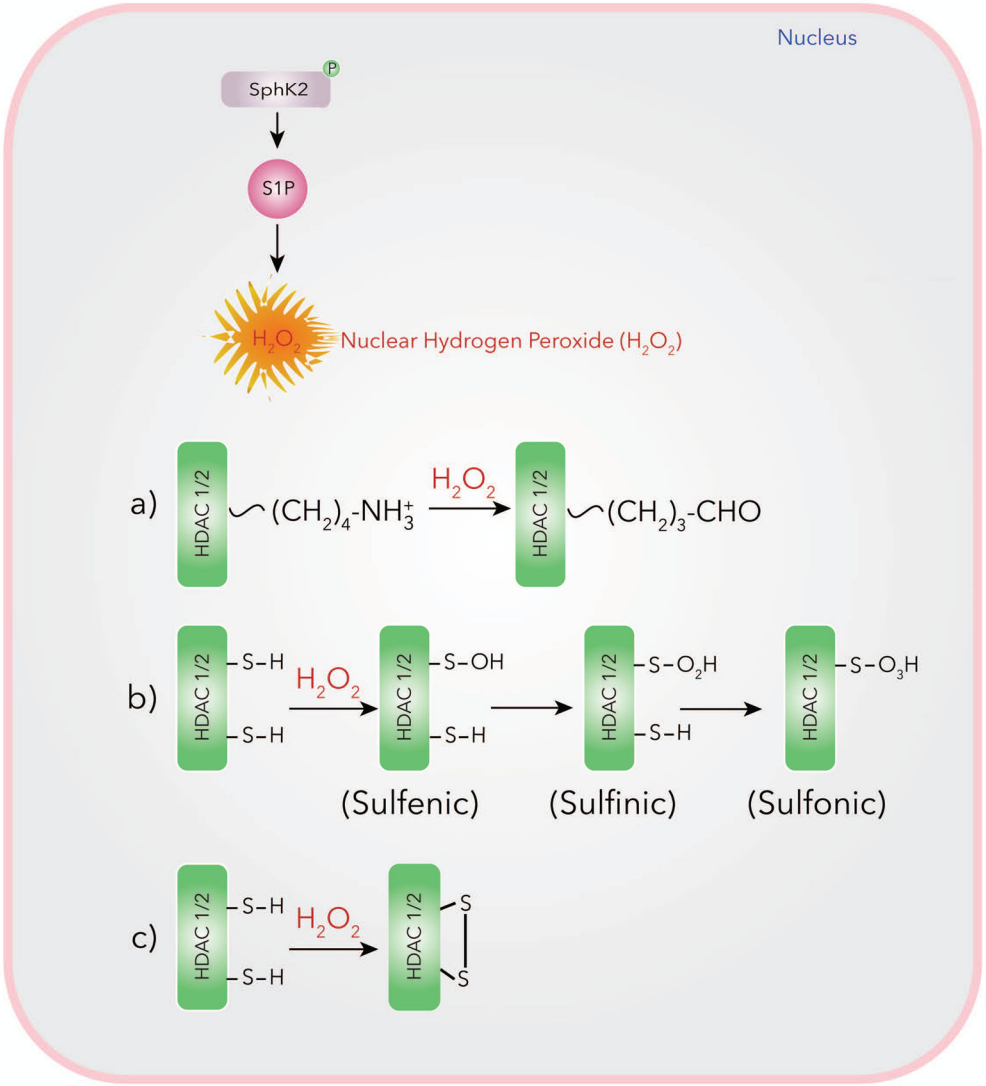


Fig. 4.

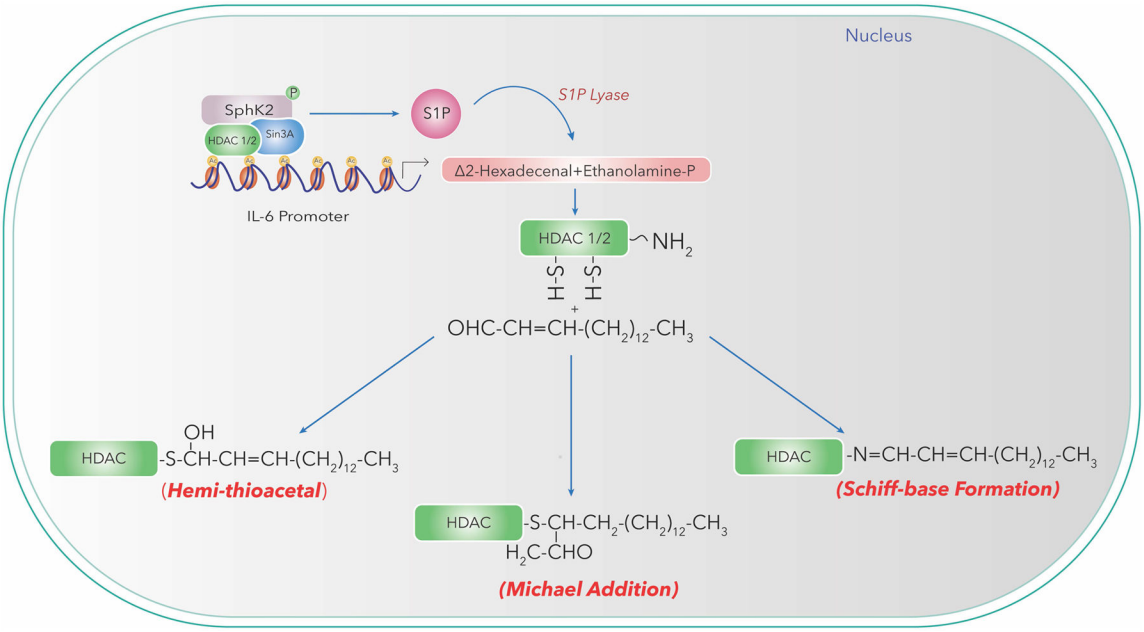
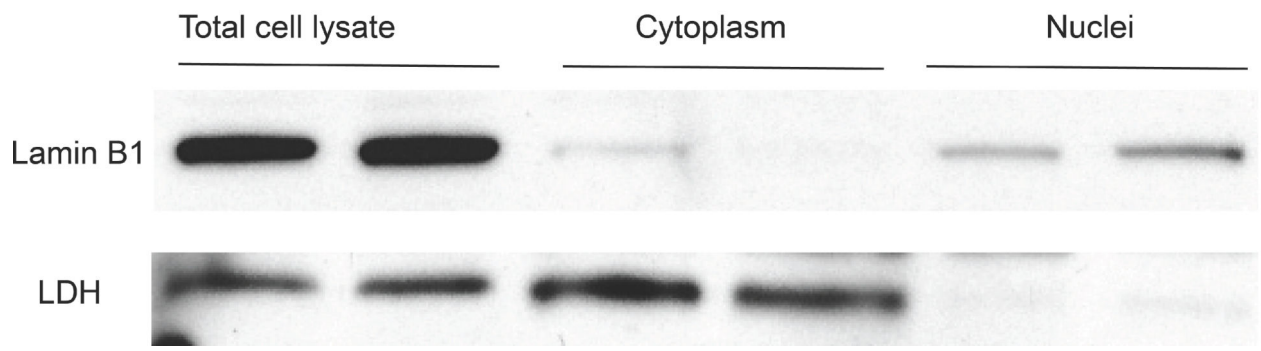


Fig. 5.

Table 1

Sphingoid bases Levels in Epithelial Cell Nuclear Fraction

Sphingoid base	**Cytosolic Fraction** (*pmols/mg Protein*)	**Nuclear Fraction** (*pmols/mg Protein*)
Sphingosine (Sph)	42	77
Dihydro Sphingosine (DH-Sph)	8	15
Sphingosine-1-Phosphate (S1P)	6	6
Dihydro Sphingosine-1-Phosphate (S1P)	2	2
Ceramide (Cer)	233	560
Dihydro Ceramide (DH-Cer)	13	24



Cytosol and nuclear fractions were isolated from mouse lung alveolar epithelial cell (MLE-12) lysate using differential sucrose density gradient-centrifugation. Lipids were isolated by Bligh and Dyer extraction and sphingoid based were quantified by LC/MS/MS and normalized to mg protein in the nuclear fraction. The purity of nuclear fraction was verified by western blotting for Lamin B1 and Lactose dehydrogenase.

Table 2

Nuclear localization and function of Sphingolipids

Sphingolipid	Generated From	Nuclear Localization	Function	References
Sphingomyelin	Ceramide + Phosphatidylcholine	Nuclear Envelope	DNA synthesis, Chromatin Assembly, RNA Stability	Ledeen & Wu., 2011 Lucki & Sewer., 2012 Albi et al., 2003b Chocian et al., 2010
Ceramide	Dihydro Ceramide	Chromatin/Nucleosome	Apoptosis Protein degradation	Albi et al., 2003a Albi & Magni. 2004 Tsugane et al., 1999
Ceramide-1-P	Ceramide	Perinuclear	$cPLA_2$ activation	Simanshu et al., 2013 Lamour & Chalfant, 2005
Sphingosine	Ceramide	Chromatin/Nucleosome	SF-1 gene transcript, Host-pathogen Interaction	Tsugane et al., 1999 Lucki & Sewer., 2012 LaBauve & Wargo., 2014
S1P	Sphingosine	Chromatin/Nucleosome	Histone Acetylation, ROS Generation	Hait et al., 2009 Gardner et al., 2016a Ebenezer at al., 2016a, 2017a,b Fu et al., 2014 Matsushima et al., 2012
Dihydro S1P	Dihydro-Sphingosine	Chromatin/Nucleosome	Hisone Acetylation, ROS Generation	Gardener et al., 2016b
Ganglioside M1	Ceramide + Hexoses + Mono-Sialic acid	Nuclear Envelope	Nuclear Ca^{2+} Homeostasis	Keenan et al., 1972 Matyas & Moore., 1987 Ledeen & Wu., 2006, 2008, 2011 Lucki & Sewer, 2012 Xie stal., 2002 Gerasimenko et al., 1995 Wu et al., 2009
Ganglioside D1a	Ceramide + Hexoses + Di-Sialic acid	Nuclear Envelope	Storage Precursor of GM1	Ledeen & Wu., 2008, 2011 Wang et al., 2009 Xie et al., 2002
▵2-Hexadecenal	S1P	?	Modification of HDACs	Ebenezer at al., 2015., 2016a, 2017a,b

Table 3

Summary of Sphingolipid Metabolizing enzymes in Cell Nucleus

Metabolizing Enzyme	Sphingolipid Subtrate ▶ Sphingolipid Product	References
Sphingomyelin Synthase	Ceramide + Phosphatidylcholine▶ Sphingomyelin	*Albi et al., 2003a* *Farooqui, 2009* *Lucki & Sewer, 2012*
Reverse Sphingomyelin Synthase	Sphingomyelin + Diacylglycerol ▶ Ceramide	*Albi et al., 2003a* *Albi & Magni, 2004*
Ceramidase	Ceramide ▶ Sphingosine	Shiraishi et al., 2003
Sphingomyelinase	Sphingomyelin ▶Ceramide	Alessenko & Chatterjee, 1995 Tsugane et al., 1999 Albi et al., 2006a
Sphingosine Kinase 1	Sphingosine ▶ Sphingosine-1-Phosphate Dihydro Sphingosine ▶ Dihydro Sphingosine-1-Phosphate	Ohotski et al., 2013
Sphingosine Kinase 2	Sphingosine ▶ Sphingosine-1-Phosphate FTY720 ▶ FTY720-Phosphate	Hait et al., 2009 Fu et al; 2014 Ebenezer et al., 2016b Gardner et a.l., 2016a
Sphingosine-1-Phosphatases 1 & 2	Sphingosine-1-Phosphate ▶ Sphingosine	*Not shown*
Ceramide Kinase	Ceramide ▶ Ceramide-1-Phosphate	Rovina et al., 2009
Sphingosine-1-Phosphate Lyase	Sphingosine-1-Phosphate ▶ Δ2-Hexadecenal Dihydro Sphingosine-1-Phosphate ▶ Hexadecanal + ethanolamine-P	Ebenezer et al., 2015, 2016a Ebenezer et al., 2017b
Neuraminidase	Ganglioside GD1a ▶ Ganglioside GM1	Wang et al., 2009 Ledeen & Wu., 2011