

Biological Effects of Naturally Occurring Sphingolipids, Uncommon Variants, and Their Analogs

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Abstract Sphingolipids (SPs) comprise a highly diverse class of lipids that serve biological roles both as structural components of cell membranes and as mediators of cell signaling. Pharmacologic and genetic manipulation of SPs and their signaling systems have underscored their importance in most biological processes, including central nervous system development and function. Likewise, perturbations of SP accumulation or signaling have been associated with a number of disease states, such as neural tube defects, neuroinflammation, stroke, and dementia. SPs can be endogenously synthesized *de novo*, and their metabolism is a well-regulated process, so their value as nutraceuticals has not been scrutinized. However, there is evidence that sphingolipid-rich diets can affect lipid homeostasis, and several mycotoxins are SP analogs that are known to cause profound derangement of SP metabolism or signaling. Furthermore, plants and invertebrates have SP species that are not present in mammals. Several of these have been shown to induce biological responses in mammalian cells. These findings suggest that dietary intake of SPs or SP analogs may have significant effects on

human health or disease outcome. This manuscript provides an overview of SP metabolism and signaling, their perturbations in neurological diseases, as well as potential impacts of modulating this system in the brain.

Keywords Sphingolipid · Fingolimod · Nutraceutical · Sphingosine 1-phosphate receptor · Ceramide · Review

Introduction to Sphingolipid Structure and Metabolism

Structural characteristics. SPs comprise one of the eight major, structurally distinct classes of lipids (Fahy et al. 2005). They are ubiquitous in all eukaryotic cells and in some prokaryotes such as anaerobic bacteria (Fyrst and Saba 2010; Olsen and Jantzen 2001). They are highly abundant in mammalian plasma membranes and make up approximately 20 % of the lipids in blood plasma (Shui et al. 2011). The defining characteristic of all SPs is the presence of a sphingoid backbone or “long-chain base” (LCB), which consists of a nitrogenous amine headgroup and an acyl chain (Fig. 1a). While there is some variability in the chain length (see below), the majority of sphingoid backbones in mammalian SPs have 18 carbons (Quehenberger et al. 2010). These are typically saturated or monounsaturated, but quantitatively minor species of LCBs have been identified that contain two double bonds at stereotyped positions (sphingadienes, see Renkonen and Hirvisalo 1969; Panganamala et al. 1969). The simplest members of the family (such as the canonical SP, sphingosine) consist only of an LCB, but modifications to this primary structure provide the material for highly diverse structural heterogeneity. Indeed, due to potential combinations of the known variations in the headgroup, the

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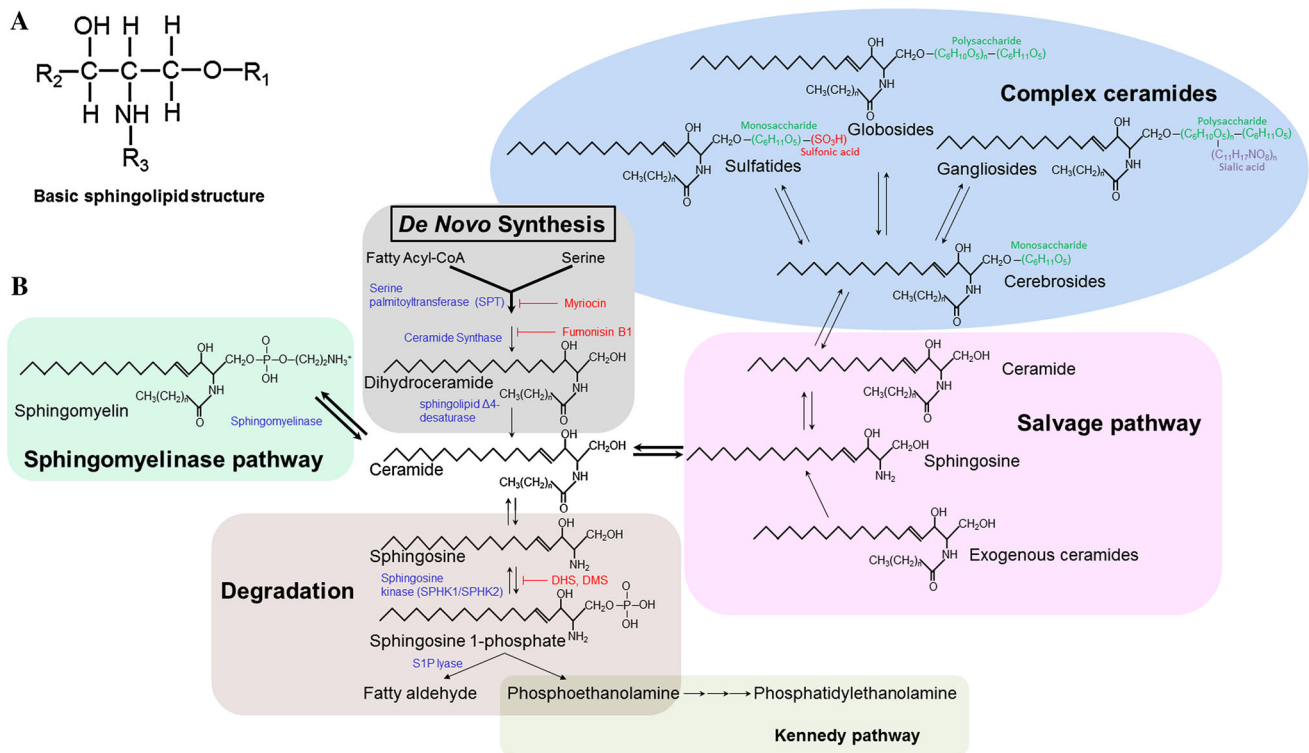


Fig. 1 Sphingolipid structure and metabolism. **a** Minimum structural requirement of typical SPs. $R_1 = \text{H}$, carbohydrate(s), sialic acid(s), sulfonic acid. $R_2 =$ saturated or mono unsaturated acyl chain. $R_3 = \text{H}$, or acyl chain. **b** Sphingolipid metabolic pathway. De novo synthesis is the major source of SPs in mammals, which begins with the condensation of serine and a fatty acyl-CoA, eventually leading to the formation of ceramide, a key metabolic intermediate which may

N-linked acyl chain, hydroxylation, and the length and degree of saturation of the LCB, theoretically, there are tens of thousands of unique SPs. However, of these possible combinations, 620 have been systematically classified as confirmed species or presumptive intermediates (Quehenberger et al. 2010; Merrill et al. 2005; Fahy et al. 2009).

General Overview of SP Metabolism

SPs can be obtained through the diet and incorporated into endogenous lipid metabolic pathways, but the majority of functionally active SPs are generated by de novo synthesis (Pyne and Pyne 2000). De novo synthesis begins with the condensation of a saturated fatty acyl-CoA and an amino acid, usually serine, via the action of serine palmitoyltransferase (SPT) as the first committed step in the generation of SPs (Fig. 1b). This results in saturated LCB intermediates (“sphinganine”) that are rapidly N-acetylated to form dihydroceramide, which is then acted upon by a sphingolipid Δ4-desaturase to introduce a double bond that generates ceramide. Much of the structural diversity of SPs results from head group modifications to ceramide. The addition of phosphorylcholine or phosphorylethanolamine results in the

be converted to sphingomyelins or complex ceramides. These “higher-order” SPs may be readily used to regenerate ceramide via the sphingomyelinase pathway or the salvage pathway, respectively. Irreversible degradation of all SPs is catalyzed by the activity of S1P lyase, resulting in the generation of a fatty aldehyde and phosphoethanolamine, which may serve as a substrate for the generation of phospholipids via the Kennedy pathway

formation of sphingomyelin, the most abundant SP making up to 20 % of the plasma membrane. Complex ceramides, or “higher-order sphingolipids,” can be generated by the addition of one or more carbohydrate groups. A single monosaccharide head group, typically glucose or galactose, results in the generation of a cerebroside. Increasing complexity occurs through elongation of this head group by further addition of monosaccharides (glucose, galactose, mannose, or fucose) or sialic acid, or by sulfonation. Complex ceramides are abundant in many tissues, particularly the brain, and can serve as a reservoir for the rapid generation of signaling pools of ceramide through the “salvage pathway” (Kitatani et al. 2008).

SP Degradation

Ceramide is deacylated to form sphingosine, the first described SP (Thudichum et al. 1884). By convention, the term “sphingosine” typically refers to 18-carbon, Δ4-monounsaturated, 1,3-dihydroxy sphingosine (D-erythro-sphingosine), which is the most abundant LCB in mammals. It is interesting to note that there is no known pathway by which sphingosine can be formed without an N-acetylated

intermediate. That is, non-acetylated LCBs cannot be directly desaturated (Kitatani et al. 2008). Once formed, sphingosine can then be phosphorylated to sphingosine 1-phosphate (S1P) via the action of two isoforms of sphingosine kinase: SPHK1 and SPHK2. S1P can then be irreversibly degraded by the action of S1P lyase (Saba et al. 1997), representing the only means by which SPs eliminated. While S1P was originally considered to be an inert intermediate in SP metabolism, it has become the most intensively studied SP after it was identified as a highly potent ligand for a family of cell surface receptors (Lee et al. 1998; Mutoh et al. 2012). Since SPHK1/SPHK2 activity controls S1P content in most tissues, this places these two enzymes as key regulators of many S1P signaling processes, as described below. S1P degradation via S1P lyase may contribute to the regulation of other phospholipid classes in that the breakdown of SPs results in the production of a fatty aldehyde and phosphoethanolamine, the latter of which is a substrate for phosphatidylethanolamine synthesis via the Kennedy pathway. Although SP metabolism is a relatively minor source of ethanolamine in mammals (Gibellini and Smith 2010), it has been shown that this may play a significant role in the regulation of the Kennedy pathway in some organisms (Zhang et al. 2007).

Phylogenic Variations in SP Structure

While SPs are ubiquitously found in eukaryotes, there are significant differences in the LCB structures that are utilized by different organisms. For example, insect SPs are typically shorter than their mammalian counterparts, with the most abundant LCBs containing 14 rather than 18 carbons (Fyrst et al. 2004). Yeast, on the other hand, contains LCBs with 16–20 carbons, but is typically saturated with backbones consisting of dihydrosphingosine or phytosphingosine (Cowart and Obeid 2007; Dickson 2008; Fig. 2). Increasingly divergent structures have been identified in plants, fungi, and marine invertebrates. LCBs with branched backbones (Pruett et al. 2008) or “bifunctional” SPs with multiple headgroups (Bensemhoun et al. 2008) have been isolated from phylogenetically distant organisms but appear to be rare or absent in mammals. Interestingly, however, some of these compounds have been shown to have potent biological effects on mammalian cells.

Effects of Bioactive Sphingolipids on Brain Development and Function

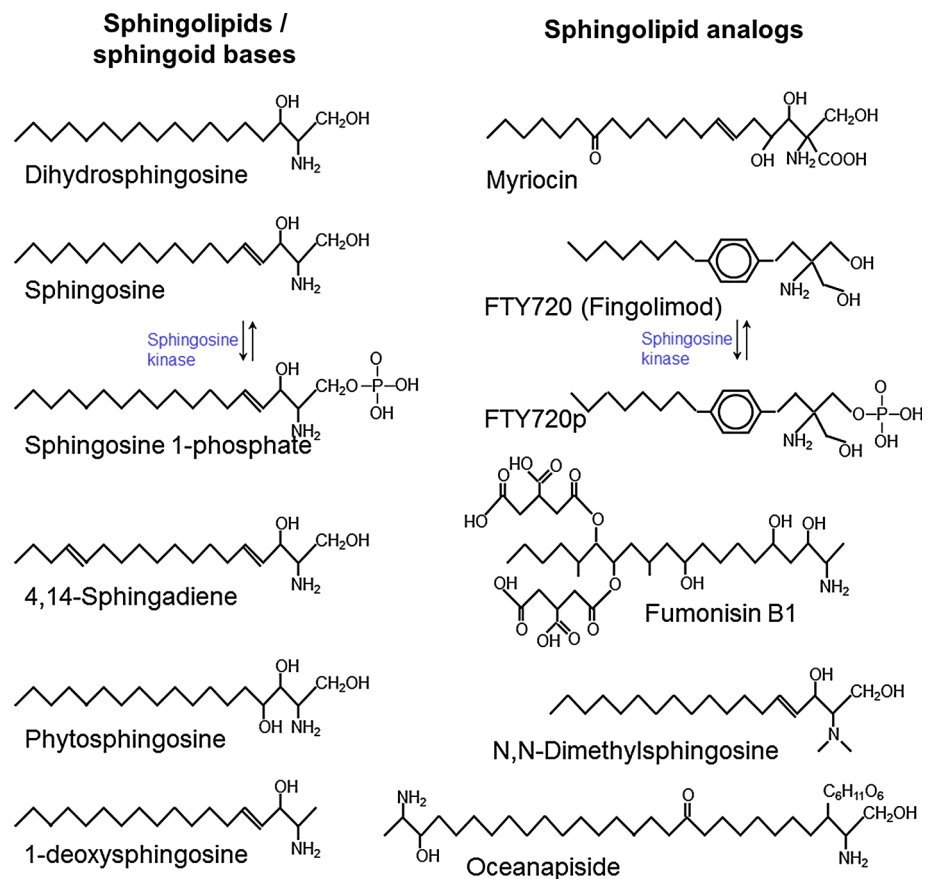
SPs are ubiquitous and, as such, regulate biological processes in many tissues. This review will emphasize the roles of SPs in the CNS since its biomedical relevance is underscored by the fact that the only FDA-approved drug

specifically acting on the SP system was approved for a neurological indication (see “Myriocin/Fingolimod” section below). However, other systems will be briefly discussed when necessary for context.

Taken as a whole, SPs are known to play critical roles in most aspects of normal brain function, such as neurogenesis and maintenance of neural stem cells (Kornhuber et al. 2014; Wang et al. 2014); integral structural components of both white and gray matter (O’Brien and Sampson 1965); formation and maintenance of lipid rafts which in turn regulate neuronal signaling (Aureli et al. 2015); and signaling molecules themselves modulating a wide range of responses, including neurotransmission, inflammation, cellular stress, autophagy, and apoptosis (Ghasemi et al. 2016; Ong et al. 2015; Pyne and Pyne 2000). Not surprisingly, perturbations of SP synthesis or regulation have been implicated in several neurological diseases, and specific phospholipids as well as associated enzymes and receptors have been investigated as potential therapeutic targets or biomarkers (Gomez-Munoz et al. 2016; Kornhuber et al. 2014; Mielke and Haughey 2012; Mutoh et al. 2012; Ong et al. 2015). In the following sections, we provide an overview of the biological functions of selected SPs, as well as their established or putative involvement in neurological diseases.

Ceramides

Ceramides are particularly well-studied SPs, primarily known for their pro-apoptotic effects, but also for regulating a number of important cellular processes (Fyrst and Saba 2010). Because of the remarkable structural diversity of ceramides, a comprehensive discussion of their roles is beyond the scope of this manuscript. Additional information may be found in more focused reviews (Reynolds et al. 2004; Ben-David and Futerman 2010). Ceramide can be rapidly generated in the CNS by tapping into the large reservoir of sphingomyelin in the plasma membrane. This occurs through the action of sphingomyelinase and can induce a number of effects such as neurotransmitter release, inflammation, and apoptosis of a number of cell types in the brain (Ong et al. 2015). Studies have also provided evidence for a possible bimodal role of ceramide on the effect of ethanol on the CNS. Excessive ceramide accumulation is thought to be generally neurodegenerative (Jana et al. 2009), and it has been suggested that this may mediate the harmful effect of alcohol consumption (de la Monte et al. 2009). Indeed, acute administration of ethanol in rats causes an increase in brain ceramide in adult and fetal brain (Saito et al. 2010). However, chronic *voluntary* consumption by ethanol-preferring rats resulted in a decrease in brain ceramides (Godfrey et al. 2015), suggesting that moderate ethanol intake may promote healthful bioactive lipid profiles. To further illustrate the

Fig. 2 Structures of key sphingolipids and sphingolipid analogs

conditional nature of ceramide function, a recent genetic analysis of ceramide metabolism identified a complicated relationship between ceramide and autophagy (Hebbar et al. 2015). When autophagic flux is inhibited in the brain, ceramide clearance is reduced and ceramides accumulate, accompanied by neurodegeneration (Hebbar et al. 2015; Finley et al. 2003). Unexpectedly, genetic reduction of ceramide (by reducing de novo synthesis) *exacerbates* the neurodegenerative phenotype, while increasing ceramide production through the salvage pathway *reduces* neurodegeneration. Apparently, although disruption of autophagy elevates ceramides, they remain sequestered in autophagosomes and are unable to engage their normal signaling functions. This provides evidence for a functional distinction among different metabolic sources of ceramide (Fig. 1b) and for the importance of subcellular localization in SP function.

Given the functional and pathogenic potential of ceramide perturbations, much research effort has understandably been focused on sphingomyelinase whose action is a main source of brain ceramides (see above). Altered regulation of sphingomyelinase activity has now been implicated in several neurological diseases or systemic diseases with CNS involvement, the best characterized of which are the Niemann–Pick disease types A and B arising

from a deficiency of the acid sphingomyelinase *SMPD1* gene. The resulting symptoms from the accumulation of ceramides in multiple organs are often systemic and may include cerebellar atrophy and dementia (Hulette et al. 1992; Obenberger et al. 1999; Macauley et al. 2008). Besides Niemann–Pick and related sphingolipidoses, altered sphingomyelinase function is thought to be involved in the progression of a number of diseases including Alzheimer’s disease, pain, and stroke, and sphingomyelinase may also be a target for novel antidepressant therapies (Gulbins et al. 2015; Ong et al. 2015).

Ceramides can be phosphorylated by the action of ceramide kinase (CERK) to form another bioactive lipid, ceramide 1-phosphate (C1P). C1P has a number of documented effects on mammalian cells that are generally proliferative or pro-inflammatory (reviewed in: Hoeflerlin et al. 2013; Gomez-Munoz et al. 2016). These effects are mediated by intracellular targets rather than cell surface receptors and involve the activation of phospholipase A₂, the MAPK/ERK pathway, and phosphoinositol 3-kinase (Lamour and Chalfant 2005; Arana et al. 2010). The role of C1P in the brain is largely unknown; however, several lines of evidence suggest biological significance in the CNS. For example, CERK activity was first identified in brain

synaptic vesicles (Bajjalieh et al. 1989), and C1P is particularly abundant in the brain tissue (Yamashita et al. 2016). Furthermore, it was shown that C1P can regulate calcium entry in pituitary cells, suggesting that it may modulate neuroendocrine secretion (Tornquist et al. 2004).

Sphingosine 1-Phosphate (S1P)

A paradigm shift in our understanding of the biological roles of sphingolipids occurred when it was discovered that some SP species could act as high-affinity, cognate ligands for G protein-coupled receptors (GPCRs; Lee et al. 1998). While a number of reports demonstrate that S1P may act as a second messenger on intracellular targets such as histone deacetylase and tumor necrosis factor-associated factor 2 (Kunkel et al. 2013), the majority of the biological effects of S1P are known to be mediated by cell surface receptors. Specifically, a family of 5 GPCRs has been identified as *bona fide* sphingosine 1-phosphate (S1P) receptors (Kihara et al. 2014), which has instigated a profound research effort into understanding the biological roles of S1P. These receptors (S1P₁–S1P₅) are expressed in distinct, but overlapping patterns in most mammalian cell types. As such, S1P can exert a wide spectrum of physiological responses depending on the cellular context, i.e., the complement of S1P receptors, and the presence of downstream effectors for receptor-specific signaling pathways. In the central nervous system, S1P receptors are expressed in neurons, glia, microglia, and endothelial cells and have been shown to participate in the regulation of multiple aspects of tissue development and cellular function (Herr and Chun 2007). The biological relevance of this signaling system has been established by *in vitro* and *in vivo* studies and by clinical evidence.

Numerous studies demonstrate that S1P induces responses in cultured astrocytes suggestive of an overall pro-inflammatory effect. Notably, S1P treatment stimulates growth and proliferation (Pebay et al. 2001) and induces the production of growth factors such as nerve growth factor (Furukawa et al. 2007), fibroblast growth factor-2 (Malchinkhuu et al. 2003; Sato et al. 1999), glial-derived neurotrophic factor (Yamagata et al. 2003), and arachidonic acid (Rao et al. 2003). This was shown to be functionally relevant in that S1P-primed astrocytes were able to promote the differentiation of neural progenitor cells (Spohr et al. 2012). These effects appear to be mediated by S1P₁, S1P₂, and S1P₃ (Rao et al. 2003). S1P receptors are also expressed in oligodendrocytes and mediate cell growth pathways (Yu et al. 2004). S1P₅ may be the most relevant mediator of S1P signaling for myelination since CNS expression of S1P₅ appears to be enriched in oligodendrocytes (Jaillard et al. 2005), although its function is not well characterized. While S1P₅ does induce process

retraction and cell survival in oligodendrocytes, there is no obvious myelination defect in S1P₅-null mice (Jaillard et al. 2005). In addition to S1P₅, there is evidence for the involvement of S1P₁ in regulation of myelination in that oligodendrocyte-specific conditional deletion of S1P₁ results in delayed differentiation of oligodendrocyte precursors (Dukala and Soliven 2016).

A number of mouse models have provided clear evidence for the involvement of S1P receptor-mediated signaling in CNS function *in vivo* (Yang et al. 2002). For example, genetic deletion of both isoforms of sphingosine kinase results in a severe depletion of S1P and embryonic lethality (Mizugishi et al. 2005). This is due to vascular defects and disruption of neurogenesis, demonstrating that S1P is essential for CNS development. On the other hand, increased S1P was found to accompany white matter lesions in an adult rodent bilateral carotid stenosis model of chronic hypoperfusion, both of which were ameliorated with sphingosine kinase inhibitor (SKI-II) treatment (Yang et al. 2016), although the effects of SKI-II may also involve its activity as an inhibitor of dihydroceramide desaturase (McNaughton et al. 2016; Cingolani et al. 2014). Taken together, these results suggest differential effects of S1P and sphingosine kinase alterations in different neurodevelopmental stages. Furthermore, S1P₂-specific knockout mice present with several rather pronounced neural phenotypes. Notably, young *S1pr2*^{-/-} mice display frequent seizures characterized by wild-running episodes followed by tonic-clonic episodes that are occasionally fatal (MacLennan et al. 2001; Akaoshi et al. 2011). This is associated with increased excitability of cortical neurons (MacLennan et al. 2001). Furthermore, surviving *S1pr2*^{-/-} mice uniformly display progressive hearing loss due to degeneration of hair cells and the afferent neurons of the spiral ganglia (Herr et al. 2007). This phenotype has recently been observed in humans who carry missense mutations of the S1P₂ gene (Santos-Cortez et al. 2016). Recently, we demonstrated that selective activation of S1P₂ is cytoprotective to neural cell lines by reduction of oxidative stress, suggesting that an S1P₂ agonist may be neuroprotective and/or otoprotective (Herr et al. 2016). Cumulatively, this demonstrates that S1P signaling plays an important role in the development and function of the nervous system.

It is likely that the effects of S1P in the CNS, particularly the glia, are relevant to neurological diseases. For example, it was shown that intracranial injection of S1P results in the robust activation of reactive astrogliosis (Sorensen et al. 2003), indicating that S1P is a regulator of neuroinflammation. This was validated in a mouse model for multiple sclerosis, whereby astrocyte-specific deletion of S1P₁ results in a significant reduction in inflammatory cytokine production due to autoimmune challenge (Choi

et al. 2011). Furthermore, S1P signaling has been shown to regulate neuronal loss in stroke models. Notably, transient middle cerebral artery occlusion/reperfusion results in an increase in S1P, S1P₃, and neuroinflammation, which can be attenuated by broad-spectrum antagonism of S1P receptors (Moon et al. 2015). In addition, S1P₂ may be an important regulator of vascular permeability and blood–brain barrier integrity during ischemia–reperfusion (Kim et al. 2015). These processes are likely to be clinically relevant since numerous studies collectively demonstrate that the S1P receptor modulator, fingolimod, reduces stroke damage in animal models (Liu et al. 2013).

Non-canonical Sphingolipids with Known Effects on Mammalian Cells

Mammalian SPs are generally characterized as structures containing the canonical sphingosine backbone. Variations of this typical structure are present, but at relatively low concentrations. For example, LCBs can contain 16–20 carbons, but more than 90 % are 18-carbon species (Quehenberger et al. 2010). Similarly, there are highly constrained variations in degree of saturation and hydroxylation. Recently, a number of studies have provided evidence that these non-canonical SPs have distinct biological effects, which suggests that they may be well-regulated mediators of cellular processes. This section describes variants that are emerging as potentially important, bioactive lipids.

Variations in Chain Length

The sphingoid base of SPs can vary in a number of ways, but much of the diversity in sphingoid bases is given by their different chain lengths. In mammals, the most abundant and ubiquitous long-chain base variant has 18 carbons (C18; Merrill 2011), but other forms, with shorter or longer chains, have been found in lower amounts. SPs containing a C16 sphingoid base have been detected in plasma (Quehenberger et al. 2010), heart tissue (Russo et al. 2013), and bovine milk (Byrdwell and Perry 2007), while a C20 sphingoid base has been found at high levels in brain SPs (especially gangliosides; Ikeda and Taguchi 2010), and in low levels in plasma (Narayanaswamy et al. 2014) and mucosal tissues (Keränen 1976). The presence of odd-chain sphingosines in low amounts has also been confirmed, and C17 or C19 (possibly also including branched chains) has been detected in human plasma (Quehenberger et al. 2010) and skin (Pruett et al. 2008). Of all tissues analyzed so far, skin has demonstrated the highest diversity in SPs, apparently due to their fundamental role in maintaining epidermal permeability barrier (t'Kindt et al. 2012;

Stahlberg et al. 2015). According to t'Kindt et al. (2012), the number of unique ceramide species present in human skin is around a thousand. The sphingoid base length distribution in skin SPs can vary between 16 and 26 carbons. Interestingly, phytosphingosine (with carbon numbers between 16 and 22), usually present in non-detectable amount in the majority of tissues, is the most abundant LCB in skin. A very unusual characteristic of skin ceramides is also the large content of C26 dihydrosphingosine, usually present with 18–20 carbon atoms in other tissues. It is still not clear whether the heterogeneous SP composition of the skin is only due to a different SP-related gene expression in epithelial cells, or whether it is also a result of a contribution of lipids derived from the microorganisms that colonize the skin surface (De Luca and Valacchi 2010).

In addition to the chain length diversity of the SP backbone, there is also remarkable variation in the chain length of the N-linked fatty acid (FA) of ceramides (Fig. 1b). The most structurally complex ceramides have been also observed in skin, containing extremely FAs, up to C36. Interestingly, skin ceramides have been reported that contain a unique modification where an ω -carbon group is hydroxylated and esterified to another fatty acid (typically linoleic acid), forming “ ω -O-acyl ceramide” (Novotny et al. 2010). This type of ceramide crucially contributes to the permeability properties of the skin lipid layers, due to its increased hydrophobicity, and its synthesis is often deregulated in skin-related pathologies like ichthyosis (Aldahmesh et al. 2011) or psoriasis (Motta et al. 1994). Ceramides with very short fatty acids, C2-ceramide, have also been found in mammals (Van Overloop et al. 2007). They are formed by the transfer of an acetyl group from platelet-activating factor (PAF) to sphinganine and have been detected in murine brain and liver (Lee et al. 1996), although they are present at a very low level when compared to more common molecular species. This C2-ceramide can also be converted to ceramide 1-phosphate, but its function is still unclear (Van Overloop et al. 2007).

At this point of time, not much is known about the biological effect of this chain length variability despite their recent detailed structural analyses. The reason behind this growing interest is that different structures present different biophysical properties, and this can influence membrane stability (Slotte 2016), binding with cognate receptors (Hanson et al. 2012), transporter function (Christoffersen et al. 2011), or enzymatic activity (Levy and Futerman 2010). S1P serves as an example of a specific function linked to structural requirements. S1P₁ is the best studied S1P receptor and preferentially binds C18 S1P (Hanson et al. 2012). The interactions between S1P and S1P₁ are both polar, with the zwitterionic headgroup of

S1P, and hydrophobic, with its acyl chain tail (Yuan et al. 2013). All five S1P receptors have conserved residues for the binding to the S1P headgroup, but they show an interesting diversity in the residues that line the hydrophobic cavity where the acyl chain of S1P is bound (Pulkoski-Gross et al. 2015). Ligands of S1P₁ can behave as agonists or antagonists depending on the length and position of their acyl chain in the binding pocket of the receptor (Hanson et al. 2012), where increasing chain lengths can shift the effect from antagonism to agonism. Since more molecular variants of S1P have been identified in mammals (Narayanaswamy et al. 2014)—variable in both chain length and degree of saturation—new developments in this field will likely identify how such variants affect the selectivity and signaling of their cognate receptors. This is of fundamental importance as structural analogues of S1P are used to treat multiple sclerosis (Willis and Cohen 2013) and rheumatoid arthritis (Fleischmann 2012).

The heterogeneity of the LCB chain length depends on the activity of the SPT enzyme, which is responsible for the first step in de novo synthesis (Fig. 1b). The combination of different catalytic subunits (SPTLC2 or 3; Hornemann et al. 2007, 2009) and other putative small subunits (ssSPTa, ssSPTb; Han et al. 2009) can shift the acyl-CoA substrate preference of the enzyme. The myocardium has highest expression of SPTLC3 of any tissue evaluated (Hornemann et al. 2009), and as a consequence its content in SPs containing C16 sphingoid bases is unusually high. This observation brought to the discovery that in mice, a diet rich in myristate enhances the expression of SPTLC3, promoting the synthesis of d16 SLs in the myocardium. To study the physiological effect of C16 sphinganine on mammalian cells, cultured myocytes were treated with C16 or C18 sphinganine. The treatment with C16 resulted in PARP-mediated apoptosis; however, C18 sphinganine did not show the same effect and instead induced autophagy, clearly suggesting that different chain length-containing SPs can have different functions (Russo et al. 2013).

Sphingadienes

The predominant LCB species in mammalian cells are dihydrosphingosine (also known as sphinganine) and sphingosine (Merrill et al. 1988; Valsecchi et al. 1996). Sphingosine contains an unsaturated hydrocarbon chain with one double bond at the Δ^4 position, which is absent in dihydrosphingosine (Fig. 2). Besides dihydrosphingosine and sphingosine, studies have also revealed the presence of other LCBs, which contribute to the formation of non-canonical sphingolipids (Fyrst et al. 2008; Renkonen and Hirvisalo 1969). These LCB species were shown to possess an additional double bond at various positions along the

hydrocarbon chain (T. U. Abeyunga 2015) and are commonly referred to as sphingadienes or sphingadienines (SDs; Renkonen and Hirvisalo 1969; Fyrst et al. 2008). The exact biosynthesis pathway(s) of SDs remain(s) elusive, but it has been suggested to involve desaturase enzymatic activity on sphingosine to introduce the second double bond into the hydrocarbon chain (Fyrst et al. 2008). These can then be readily incorporated into more complex SPs such as glucosylceramides (Abeyunga et al. 2008). Regulation of SDs was found to be highly dependent on S1P lyase (SPL) activity as shown by an increased accumulation of SDs in the *Drosophila* SPL mutant (*Sply*; Fyrst et al. 2008). SDs are most commonly found in plants such as soybean (Lynch and Dunn 2004), as well as being present across various organisms where the second double bond is located at different positions along the hydrocarbon chain (Table 1).

SPs obtained from the diet will undergo enzymatic lipolysis in the intestine to form free LCBs and ceramide (Imai et al. 1997; Ohlsson et al. 2010; Sugawara et al. 2003; Rozema et al. 2012b; Nyberg et al. 1997). This suggests that diets high in SD-rich food such as soy may result in significant exposure to SD isoforms that are not endogenously produced. While there is no consensus regarding the impact of this on human health, there are several lines of evidence that suggest that SDs exert potent effects on mammalian cells. For example, $\Delta^{4,8}$ -C₁₈-sphingadiene and 9-methyl- $\Delta^{4,8}$ -C₁₈-sphingadiene are the major constituents of plants cerebrosides, which were found to exhibit calcium ion ionophoretic properties and anti-ulcerogenic effects in addition to inhibiting replicative DNA polymerase activities (Okuyama and Yamazaki 1983; Shibuya et al. 1990; Mizushina et al. 1998). Moreover, dietary monoglucosylceramide from rice bran, which contains a $\Delta^{4,8}$ -C₁₈-sphingadiene backbone, was found to inhibit preneoplastic lesions in the colon of F344 rats (Inamine et al. 2005). Sphingoid bases from lactic yeast and maize were also shown to induce apoptosis in the Caco-2 human colon cancer cell line via caspase activation and reduction of intracellular β -catenin levels (Aida et al. 2004). In addition, sea cucumber sphingoid bases comprising sphingadienes were shown to dose-dependently inhibit the viability of three different human colon cancer cell lines (Sugawara et al. 2006). Soy glucosylceramide, which similarly contains a $\Delta^{4,8}$ -C₁₈-sphingadiene backbone, was found to inhibit colon tumorigenesis and reduce gene expression of transcription factors linked to cancer in two different mouse models (Symolon et al. 2004). The role of SDs as the active constituent of SPs in reducing colon tumorigenesis was further validated when both $\Delta^{4,6}$ -C₁₄-sphingadiene and $\Delta^{4,8}$ -C₁₈-sphingadiene reduced colon cancer cell viability in a time- and dose-dependent manner (Fyrst et al. 2009). SD-induced cell death was found to

Table 1 Molecular structure of SDs found across different organisms (Color table online)

Molecular structure	Sphingadiene type	Organism type
	$\Delta^{4,14}$ -C ₁₈ -sphingadiene	<i>Homo sapiens</i> (Renkonen and Hirvisalo 1969) <i>Homo sapiens</i> (Panganamala et al. 1969)
	$\Delta^{4,6}$ -C ₁₄ -sphingadiene	<i>Bombycis corpus</i> (Kwon et al. 2003) <i>Manduca sexta</i> (Abeytunga et al. 2004) <i>Drosophila</i> (Fyrst et al. 2008)
	$\Delta^{4,8}$ -C ₁₈ -sphingadiene	<i>Halichondria panicea</i> (Nagle et al. 1992) <i>Neanthes diversicolor</i> (Noda et al. 1993) <i>Glycine max</i> (Sullards et al. 2000) <i>Stichopus variegates</i> (Sugawara et al. 2006)
	9-methyl- $\Delta^{4,8}$ -C ₁₈ -sphingadiene	<i>Cortinarius tenuipes</i> (Tan et al. 2003)
	$\Delta^{4,8}$ -C ₁₆ -sphingadiene	<i>Euryale ferox</i> (Row et al. 2007)

The red arrows indicate the different positions of the second double bond

involve the PI3K/Akt pathway since translocation, activation, and cellular signaling of Akt in the colon cancer cells were repressed after SD treatment (Fyrst et al. 2009). Additionally, the chemopreventive effects of SDs were suggested to involve Wnt signaling inhibition via a protein phosphatase 2A/Akt/GSK3 β -dependent pathway (Kumar et al. 2012). Administration of $\Delta^{4,6}$ -C₁₄-sphingadiene also reduced intestinal tumorigenesis in vivo (Fyrst et al. 2009). Interestingly, both $\Delta^{4,6}$ -C₁₄-sphingadiene and $\Delta^{4,8}$ -C₁₈-sphingadiene had more pronounced cytotoxic effects on the colon cancer cells as compared to soy glucosylceramide, suggesting that enzymatic metabolism of glucosylceramide in the gut to release SDs is essential for their cytotoxic and tumor repression activities to occur (Fyrst et al. 2009).

The cytotoxicity of SDs is not limited to colon cancer cells. Novel ceramide analogs with the $\Delta^{4,6}$ -C₁₈-SD backbone induced apoptotic cell death in the MCF-7 and MDA-MB-231 breast cancer cell lines via mitochondrial activation (Struckhoff et al. 2004). Besides inducing cytotoxicity in different cancer cell lines such as HL-60 and RAW 264.7, $\Delta^{4,8}$ -C₁₈-sphingadiene also decreased cell viability and proliferation in non-cancerous cell lines such as HUVEC and HEK-293 (Rozema et al. 2012b). Nonetheless, ceramide analogs with the SD backbone exhibited reduced cytotoxicity toward normal breast epithelial cells, while non-cancerous human colon mucosal epithelial cells

also showed lower sensitivity to SD-induced cytotoxicity (Struckhoff et al. 2004; Fyrst et al. 2009).

The reason that this minor structural change results in significantly increased cytotoxicity is unclear; however, it may in part be explained by derangement of the sphingolipid rheostat. The oncogenic enzyme sphingosine kinase 1 (SPHK1), which is elevated in colon carcinogenesis, can phosphorylate sphingosine to form S1P. This shifts the rheostat toward phosphorylation, which is implicated in angiogenesis formation, Akt activation, and pro-tumorigenic inflammatory pathway signaling (Lee et al. 1999; Lee et al. 2010; Xia et al. 2000; Kawamori et al. 2006, 2009; Chumanovich et al. 2010; Kumar et al. 2012). The presence of the second double bond in sphingadienes was also suggested to contribute to their higher cytotoxicity in cancer cells as compared to ceramide or sphingosine (Struckhoff et al. 2004).

The suggested pharmacological actions of sphingadienes were not just limited to their cytotoxicity in cancer cell lines. $\Delta^{4,8}$ -C₁₈-sphingadiene was also shown to exert anti-inflammatory effects via inhibition of lipopolysaccharide (LPS) and tumor necrosis factor- α (TNF- α)-induced cytokine interleukin-8 (IL-8) and the inflammatory adhesion molecule E-selectin (Rozema et al. 2012a). Interestingly, lower concentrations of $\Delta^{4,8}$ -C₁₈-sphingadiene were required for its anti-inflammatory effects

compared to the concentrations where cytotoxic effects were observed (Rozema et al. 2012a). In addition, sphingolipids containing a $\Delta^{4,6}$ -C₁₄-sphingadiene backbone were found to induce neurotrophic effects and increased neurite outgrowth in PC12 cells similar to the effects of the nerve growth factor (NGF; Kwon et al. 2003).

Results from these studies suggest the possibility of utilizing SDs not only in colon cancer treatment but also in neurodegenerative diseases, particularly when inflammation and neuronal loss are involved. Added to the fact that they can be readily obtained from the diet, SDs represent an attractive nutraceutical compound for possible treatment of cancer and brain neurodegeneration. Nonetheless, there is still much work to be done regarding the role of SDs in human physiology and pathology, their direct targets and signaling mechanisms, and the metabolic pathways involved in SDs generation.

Deoxysphingolipids

Deoxysphingolipids are atypical constituents of the sphingolipidome (Merrill et al. 2009) that were originally identified in fungal species and marine sponges. They have only recently been shown to be present at low concentrations in mammalian tissues (Duan and Merrill 2015). Deoxysphingolipids such as 1-deoxysphinganine differ from the typical sphingoid base (e.g., sphingosine) due to the absence of a hydroxyl group at C1 position (Bode et al. 2015), but they are structurally diverse and can range from simple to complex. 1-deoxy- and 1-deoxymethyl-sphingoid bases are structurally simple deoxysphingolipids that are generated by many organisms including mammals (Zitomer et al. 2009), while at the opposite end of the spectrum, the “two headed” structure of oceanapiside isolated from marine sponges represents one of the more unique and complex structures seen in SPs (Nicholas and Molinski 2000; Fig. 2).

Deoxysphingolipids were initially thought to be a class of mycotoxins that are particularly cytotoxic to plant and animal cells (Abbas et al. 1998). Recently, however, two groups made the surprising discovery that they are synthesized in mammalian cells in relatively low concentrations (Penno et al. 2010; Zitomer et al. 2009). SPT is mutated in hereditary sensory and autonomic neuropathy type 1 (HSAN1; Penno et al. 2010). These mutations have been shown to alter substrate specificity of SPT, from L-serine to L-alanine or glycine, which results in the synthesis of 1-deoxysphingolipid compounds such as 1-deoxysphinganine (Penno et al. 2010). In addition to synthesis of deoxysphingolipids by mutant SPT, the synthesis of 1-deoxysphingolipid from wild-type SPT was seen in cell lines incubated with fumonisins B1, which inhibits ceramide synthases and results in the accumulation of

deoxysphingolipids (Zitomer et al. 2009; Venkataraman et al. 2002). CHO-LyB cells, which carry an inactive SPT subunit (SPT1), failed to synthesize 1-deoxysphingolipids from L-alanine but was genetically rescued with wild-type SPT, confirming that generation of deoxysphingolipids is the result of normal mammalian SP metabolism (Zitomer et al. 2009).

Ongoing work aims to understand the effects of these atypical lipids on human cells to develop a better understanding of their biological roles in health and disease. 1-deoxysphinganine has been studied for its cellular effects, as it is among the more abundant 1-deoxysphingolipids present in humans. While it can enhance proliferation in Swiss 3T3 fibroblasts (Schroeder et al. 1994), it has been shown to inhibit cell growth as in Vero kidney epithelial cells (Zitomer et al. 2009) and is cytotoxic to LNCaP and PC-3 prostate cancer cells at low micromolar concentrations (Sanchez et al. 2008). The growth inhibition and cytotoxicity of deoxysphingolipids have been attributed to de novo synthesis of ceramide, phosphorylation of P53, and activation of apoptosis (Sanchez et al. 2008; Salcedo et al. 2007).

HSAN1 patients show elevated plasma concentration of 1-deoxysphingolipids, which has been attributed to the neurodegeneration seen in HSAN1 (Penno et al. 2010). Similarly, the occurrence of diabetic neuropathy, a common and debilitating sequela of diabetes, correlates with elevation of 1-deoxysphingolipids in plasma samples of type II diabetes patients (Bertea et al. 2010). This was corroborated by an in vivo model in which supplementation of L-serine in a diabetic rat results in decreased plasma deoxysphingolipids and decreased neuropathic behavior (Othman et al. 2015). These findings support a possible connection between deoxysphingolipids and neuroinflammation/neurodegeneration. Recent evidence also indicates that 1-deoxysphingolipids play a role in TNF-mediated neurotoxicity of dopaminergic neurons (Martinez et al. 2012). Cumulatively, these data provide compelling evidence that deoxysphingolipids, although a quantitatively minor class of SLs, are important regulators of neuronal pathology. It has been proposed, however, that this cytotoxicity could be exploited therapeutically. For example, Enigmol, which is a synthetic 1-deoxysphingoid base, has shown to suppress tumors in colon and prostate cancer mouse models (Symolon et al. 2011; Garnier-Amblard et al. 2011).

Naturally Occurring Sphingolipid Modulators

It is likely that the dietary intake of structurally diverse SPs exerts significant biological effects on mammals. In addition, it is known that naturally occurring compounds,

present in diet, have potent effects on SP metabolism and/or signaling. This can have a rather large impact on the composition of the sphingolipidome or may profoundly affect biological processes that are regulated by SP signal transduction. This section describes the most notable examples of SP analogs with known biological effects.

Myriocin/Fingolimod

Successful therapeutic relevance of sphingolipids has been realized through approval of a sphingosine analog known initially as FTY720 (Chiba et al. 1996) that has, through a tortuous process, become a drug known as Gilenya (generic name, fingolimod; Kappos et al. 2006; Brinkmann et al. 2010; Chun and Brinkmann 2011; Groves et al. 2013) that was FDA-approved in 2010 as a first oral treatment for relapsing forms of the autoimmune disease, multiple sclerosis (MS; Chun and Brinkmann 2011). The compound was a chemical derivative of a naturally occurring immunosuppressant molecule initially identified from the fungus *Myriococcum albomyces* and named “Myriocin” (St-Jacques 1973; Kluepfel et al. 1972). Years later, myriocin was identified from another fungus, the sac fungus Ascomycota (formerly known as Ascomycetes) and includes the entomopathogenic fungus *Isaria sinclairii* (Fig. 3) as “ISP-1” that is myriocin. Its antifungal properties were extended to mammalian systems where it was observed to have immunosuppressant properties in culture attributed to SPT inhibition (Miyake et al. 1995). These properties suggested utility in a variety of therapeutic applications like organ transplantation, and myriocin was studied for possible in vivo use; however, exposure in animals was associated with unacceptable toxicity (Napoli 2000), which led to a concerted effort from academia and pharmaceutical collaborations in Japan involving chemical derivatization of myriocin to produce a more desirable compound, which resulted in the identification and synthesis of FTY720 (Fig. 2; the names reflecting the contribution by Professor Tetsuro Fujita at Kyoto University; and the companies Taito and Yoshitomi; Chiba et al. 1996; Fujita et al. 1994).

FTY720 was tested in a wide range of animal models, in which it was thought to be acting as a strong immunosuppressant (Suzuki et al. 1996; Chueh et al. 1997; Mitsusada et al. 1997; Ueda et al. 2000). Its mechanism, however, was distinct from Myriocin that inhibits SPT, whereas FTY720 did not (Napoli 2000). Nevertheless, basic studies on Myriocin indicated that its roles in sphingosine metabolism (Miyake et al. 1995) might be relevant to derivatives like FTY720; however, its precise actions were unclear. Another fungal agent, cyclosporine, was isolated from Ascomycota fungus *Tolypocladium inflatum* that is the same fungal order (Hypocreales) as *Isaria sinclairii*, the source of myriocin/ISP-1 from which



Fig. 3 Source of myriocin. Myriocin was originally isolated from the fungus *Isaria sinclairii*, which is commonly used in traditional Chinese medicine. Like other Cordyceps species it parasitizes insects, such as cicada larvae under the soil. The structures of FTY720 (derived from myriocin) and cyclosporine (widely used immunosuppressant drug also of fungal origin) are shown for comparison. Reprinted with permission from An Illustrated Guide to New Zealand Soil Invertebrates, <http://soilbugs.massey.ac.nz>. © Massey University

FTY720 emerged. Discovered by Sandoz scientists as an important immunosuppressant (Heusler and Pletscher 2001), cyclosporine was observed in animals to synergize with FTY720 (Chiba et al. 1996; Enosawa et al. 1996; Hoshino et al. 1996; Kawaguchi et al. 1996; Masubuchi et al. 1996). However, studies at Novartis and in academia produced less clear activities for immunosuppression, culminating with a pivotal phase III clinical trial for renal

transplantation rejection that combined reduced-dose cyclosporine—a proven immunosuppressant—with high-dose FTY720 that failed to reach its desired clinical endpoints (Tedesco-Silva et al. 2006). However, other pre-clinical work supported activity of FTY720 in an animal model of multiple sclerosis (MS; Webb et al. 2004; Brinkmann et al. 2002; Fujino et al. 2003), experimental autoimmune encephalomyelitis (EAE), which led to human clinical trials for MS and ultimately resulted in regulatory approval of the compound that is known by its generic name “fingolimod” and commercial name Gilenya (Imusera in Japan; Kappos et al. 2006; Chun and Hartung 2010; Cohen and Chun 2011; Chun and Brinkmann 2011). In the USA, Gilenya received FDA approval in 2010 for treatment of relapsing forms of MS as well as regulatory approval globally where it has been used in the treatment of over 125,000 patients worldwide by 2015 (www.gilenya.com).

During the period of myriocin modification that produced FTY720, a completely separate research effort identified the first of a novel GPCR family (Hecht et al. 1996; Fukushima et al. 2001; Kihara et al. 2014) that mediates the effects of lysophospholipids (LPs) that included lysophosphatidic acid (LPA) and S1P. As a family, LP receptors currently represent over 40 % of lipid-interacting GPCRs. The discovery by scientists at Novartis (Brinkmann et al. 2002) and Merck (Mandala et al. 2002) that FTY720 phosphorylation (FTY-P) produced an S1P receptor non-selective agonist brought these two fields together and has allowed mechanistic understanding of important physiological activities. FTY-P interacts with 4 of 5 S1P receptors with high affinity (all except S1P₂). Notably, despite initial identification as an agonist, the prominent effects of FTY720 in disease models involve receptor loss through ubiquitin-mediated receptor degradation that produces functional antagonism (Welsch et al. 2003; Bohler et al. 2005; Oo et al. 2007), thereby explaining phenocopying of results between prolonged FTY720 exposure and mice deficient for S1P receptors (Matloubian et al. 2004; Pappu et al. 2007; Schwab and Cyster 2007; Choi et al. 2011). The primary effects of FTY720 in MS appear to involve disruption of normal lymphocyte egress from lymphoid organs produced by loss of S1P receptors (Matloubian et al. 2004). In addition, there are direct effects of FTY720 mediated by S1P signaling within the central nervous system (CNS) that involve astrocytes, whereby removal of S1P₁ receptors from astrocytes reduces disease and eliminates FTY720 efficacy in EAE (Choi et al. 2011; Groves et al. 2013). The direct CNS effect was further supported by the observation that FTY720 can promote the regeneration of sciatic nerve following crush injury (Szepanowski et al. 2016).

Beyond the proven efficacy that FTY720 has in improving MS outcomes, it has also been suggested that it may be useful in other neurological indications, such as stroke (Moon et al. 2015; Liu et al. 2013) and Alzheimer’s disease (Aytan et al. 2016). In addition, there is evidence for the potential efficacy of FTY720 in treating certain cancers (Patmanathan et al. 2015), and it was recently shown that it induces apoptosis and attenuates proliferative signaling pathways in oral squamous cell carcinoma cells (Patmanathan et al. 2016). It is notable that the possible anticancer effects of FTY720 may in part be independent of its direct effects on S1P receptors. It has been reported to affect S1P metabolism by inhibiting SPHK and S1P lyase (Tonelli et al. 2010; Bandhuvula et al. 2005) and to act directly on other intracellular targets, such as protein phosphatase 2A (Matsuoka et al. 2003) and the 14-3-3 adaptor protein (Woodcock et al. 2010). The clinical relevance of these reports is unclear.

These effects and the success of FTY720 have spurred research into other therapeutics based upon lysophospholipid signaling (Kihara et al. 2015), and it will not be surprising to encounter new biology and therapeutics in view of the vast range of phenotypes observed in deletion mutants of S1P receptors (Ishii et al. 2001, 2002; Yang et al. 2002; Baudhuin et al. 2004; Nofer et al. 2004; Herr et al. 2007, 2013; Keller et al. 2007; Means et al. 2007; Tolle et al. 2008; Choi et al. 2011; Hopson et al. 2011; Means et al. 2008), some of which may be accessed by modified fungal metabolites.

Fumonisin B1

Fumonisin B1 is one of a family of mycotoxins produced by filamentous fungi from the genus *Fusarium* that commonly infect corn (maize). It is a naturally occurring SP analog that contains tricarballic acid and methyl modifications to an LCB (Fig. 2). This compound is particularly notable in that it has been shown to inhibit the activity of ceramide synthase and cause the accumulation of dihydrosphingosine in vivo (E. Wang et al. 1991; Wang et al. 1999). Sub-toxic exposure of fumonisin B1 routinely occurs through normal dietary intake in many countries including the USA and is safely tolerated at levels below 2 µg/kg/day (Stockmann-Juvala and Savolainen 2008). However, high level of fumonisin B1 intake is characterized by an increased dihydrosphingosine/sphingosine ratio (Riley et al. 1993) and may lead to deleterious effect on the CNS. Notably, fumonisin B1 exposure and elevated dihydrosphingosine were associated with an outbreak of neural tube defects that occurred in a population with a corn-rich diet that lived along the US–Mexico border in 1990–1991 (Missmer et al. 2006). This was experimentally reproduced in mice, whereby exposure of embryos to as little as 2 µM

Fumonisin B1 caused neural tube defects (Sadler et al. 2002), confirming that regulation of SPs is essential for normal development of the CNS. Interestingly, the occurrence of neural tube defects could be attenuated by co-administration of folic acid, suggesting that derangement of SP metabolism may disrupt the uptake or bioavailability of folate.

D,L-Threo-dihydrosphingosine and N,N-Dimethyl sphingosine

Studies involving the use of sphingosine analogs have demonstrated that a number of structural variants are recognized by SP metabolic enzymes, but may act as inhibitors. Two notable compounds are D,L-threo-dihydrosphingosine (DHS) and N,N-dimethyl sphingosine (DMS; Fig. 2), which can inhibit sphingosine kinase activity. DHS, an enantiomer of naturally occurring D-erythro-dihydrosphingosine, was shown to inhibit SPHK1 (Buehrer and Bell 1992), but acts as a substrate for SPHK2 (Pyne and Pyne 2002). Similarly, L-threo-dihydrosphingosine (safingol) is known to inhibit SPHK1, but also acts on protein kinases, such as protein kinase C (Canals and Hannun 2013). Phase I clinical trials have been completed for the use of safingol for solid tumors in combination with doxorubicin (Schwartz et al. 1997) or cisplatin (Dickson et al. 2011). Both studies provided evidence for safety, but did not evaluate efficacy. DMS was found to be endogenously present at low levels in splenocytes (Felding-Habermann et al. 1990) and epidermoid carcinoma cells (Igarashi et al. 1990) and can inhibit both isoforms of sphingosine kinase (Yatomi et al. 1996; Pyne and Pyne 2002). Both of these compounds have served as useful tools for elucidating the biological roles of sphingosine kinase and further demonstrate that dietary SPs and SP analogs not only act as substrates for lipid metabolism, but also may have significant effects on endogenous metabolic pathways.

Conclusions and Implications

In conclusion, SPs are abundant and well-studied lipids that are critical mediators of many functions of the CNS and other tissues. The metabolic pathways that regulate SPs and the signaling pathways that they influence are abundantly characterized and are biomedically relevant. However, the importance of dietary SPs and the effects of naturally occurring SP analogs on endogenous SP metabolism are not fully realized. Atypical SPs can be absorbed from the diet and incorporated into the mammalian sphingolipidome, and several analogs found in normal diet or herbal supplements (myriocin, fumonisin B1) have been shown to exert potent biological effects. It is a near

certainty that novel, naturally occurring SPs will continue to be identified and that therapeutically useful effects will be discovered from these nutraceuticals.

Compliance with Ethical Standards

Conflict of interest D.R.H. and G.L.H. have received consulting fees or grant support from Expression Drug Designs, LLC and Bayer Healthcare. J.C. has received honoraria, consulting fees and/or grant support from: Abbott, Amira Pharmaceuticals, Biogen-Idec, Celgene, GlaxoSmithKline, Johnson and Johnson, Merck, Mitsubishi Tanabe Pharma Corporation, Novartis, Ono Pharmaceutical Co., Pfizer and Taisho Pharmaceutical Co. M.K.P.L., W.S.C., F.T., and A.R. have no financial relationships to declare.

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