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Sphingolipidomics analysis of large clinical cohorts. Part 2: Potential impact and applications

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ABSTRACT

It has been known for decades that the regulation of sphingolipids (SLs) is essential for the proper function of many cellular processes. However, a complete understanding of these processes has been complicated by the structural diversity of these lipids. A well-characterized metabolic pathway is responsible for homeostatic maintenance of hundreds of distinct SL species. This pathway is perturbed in a number of pathological processes, resulting in derangement of the "sphingolipidome." Recently, advances in mass spectrometry (MS) techniques have made it possible to characterize the sphingolipidome in large-scale clinical studies, allowing for the identification of specific SL molecules that mediate pathological processes and/or may serve as biomarkers. This manuscript provides an overview of the functions of SLs, and reviews previous studies that have used MS techniques to identify changes to the sphingolipidome in non-metabolic diseases.

1. Applications of sphingolipidomics

Sphingolipids (SLs) are ubiquitous macromolecules that participate in the regulation of structure, barrier function, metabolism, and signaling in every known eukaryotic cell type. The structural diversity of this class of lipids is the key feature underlying their pleotropic effects. As mass spectrometry-based analytical techniques are becoming increasingly sensitive and increasingly accessible, the field of SL biology is now entering a new level of sophistication. Part 1 of this two-part review series describes SL structure and provides an overview of the techniques and challenges involved in large-scale lipidomics projects. Part 2, presented here, addresses the "why" questions by discussing the pathological, therapeutic, and diagnostic implications of dysregulation of the sphingolipidome.

One particularly notable characteristic of the profile of SL composition is that it is not homogenous. That is, although SLs are abundant in all mammalian cells, the relative abundance of individual SL species varies significantly among tissue and cell types. This may be best exemplified by skin, which contains a highly unusual complement of ceramides relative to other organs, notably including an enrichment in omega-hydroxyceramides [1]. Furthermore, there is evidence that cells may undergo significant alterations in SL content due to pathological activation or transformation. For example, ovarian cancer cells show marked increases in sulfatide content compared to surrounding stroma [2], and macrophages have organelle-specific increases in some ceramide species upon activation [3]. Since SL content has the potential to provide "signatures" of cell types and cell behavior, this has a number of implications for the translational value of sphingolipidomics analyses. This review addresses these implications by

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providing examples of known derangements in SL content in disease states.

2. Biological roles of sphingolipids

The remarkable structural heterogeneity of SLs is highly conserved, as evidenced by the fact that the SL profiles of yeast, plants, and many bacteria and fungi, though different in composition, bear a similar complexity to that of mammalian cells [4–6]. This conservation presumably underlies a fundamental need for molecularly distinct SLs in basic cellular processes such as membrane structure and cellular signaling.

2.1. Membrane structure and integrity

SLs are major components of the plasma membrane of all eukaryotic cells. In fact, sphingomyelins alone represent up to 30% of the membrane lipids of many cell types, with a particular enrichment in the outer leaflet [7]. Since their aliphatic chains are almost entirely saturated or *trans*-monounsaturated, their biophysical properties contrast with that of the other major classes of membrane phospholipids, such as phosphatidylserine, phosphatidylcholine, and phosphatidylethanolamine, which typically incorporate unsaturated or polyunsaturated fatty acids. Thus, control of SL content endows cells with a mechanism to regulate the overall rigidity and shape of plasma membranes. In addition, the cylindrical shape of SLs allows them to pack tightly into the membrane to form solid-like gel phases which can be “fluidized” by the incorporation of cholesterol [7]. Cholesterol preferentially interacts with saturated acyl chains by stable stacking of linear acyl chains and flat sterol rings, or by energetically favorable condensation [8]. These interactions allow for the spontaneous assembly of SM/cholesterol aggregates resulting in lateral heterogeneity of the plasma membrane. Sometimes referred to as “lipid rafts” these membrane microdomains have been proposed to have unique scaffolding properties that can influence cell signaling [9]. In addition, raft structures are likely to play a role in pathological processes such as viral entry, atherosclerosis, and oncogenic signal transduction.

2.2. Signal transduction

While the quantitatively abundant SL species affect the biophysical properties of membranes which indirectly affects cell signaling, several quantitatively minor species are known to directly affect signal transduction as first or second messengers [10]. Specifically, ceramide, ceramide 1-phosphate (C1P), sphingosine, and sphingosine 1-phosphate (S1P) are the best-studied signaling SLs. While these mediators are known to elicit a wide variety of responses in many different cell types, the non-phosphorylated forms, ceramide and sphingosine, are generally associated with anti-proliferative or apoptotic effects, whereas S1P and C1P have more often been shown to induce proliferative or inflammatory responses [11]. All four of these lipids have been shown to act intracellularly as second messengers, but only S1P is a *bona fide* first messenger, acting as a high-affinity, cognate ligand for five G protein-coupled receptors: $S1P_1$-$S1P_5$ [12,13]. Nearly all mammalian cells that have been evaluated have been shown to express one or more S1P receptors. These receptors can act redundantly [14], antagonistically [15,16], or mediate distinct signaling pathways to induce different responses [17]. Consequently, the biological roles of S1P are complex and highly pleotropic, many of which have significant biomedical implications. Notably, the S1P/$S1P_1$ signaling axis mediates inflammatory processes in the central nervous system [18] and is required for egress of lymphocytes from the secondary lymphoid tissues [19]. In addition, S1P signaling has been shown to be associated with cardiovascular disease [20], hearing loss [21], cancer progression [22], and other pathological processes in humans [23].

3. Alterations in sphingolipid content associated with disease

Considering the important and pleotropic biological roles of SLs, it is not surprising that the sphingolipidome is exquisitely choreographed in most tissues, and that pathological states have characteristic disruptions of this regulation resulting from cellular dysfunction or contributing to disease sequelae. This is well-documented for metabolic diseases such as obesity and type 2 diabetes mellitus (for specialized reviews, see: [24–26]). Here, we summarize the evidence that many other diseases, not necessarily involving metabolic dysfunction, are also associated with signature alterations to the sphingolipidome (Table 1).

3.1. Neurodegeneration and dementia

Sphingolipids have been recognized as important signaling molecules in the brain, modulating a wide range of processes including neuroinflammation, oxidative stress, autophagy and apoptosis [27]. A recent study suggested that ceramide, through diverse mechanisms such as enhanced production of reactive oxygen species, decreased anti-apoptotic (Bcl-2) and increased pro-apoptotic (Bax, Hrk) mRNA/protein levels, may trigger neuronal death [28]. In contrast, it was shown that S1P exerts an inhibitory effect on apoptosis through members of the Bcl-2 protein family and the reduction of oxidative stress [28]. Given the importance of sphingolipids in regulating neuronal survival, perturbations in sphingolipids levels have been implicated in several neurological diseases, including Alzheimer's disease (AD), the commonest cause of neurodegenerative dementia in old age characterized by the neuropathological hallmarks of intercellular amyloid plaques consisting of aggregated β-amyloid (Aβ) peptides and abnormally hyperphosphorylated τ-proteins forming intracellular neurofibrillary tangles [27]. The formation of amyloid plaques, in particular, is considered a central pathogenic mechanism of AD which arises from the dysregulated cleavage of amyloid precursor protein (APP) resulting in the over-production of pro-aggregatory forms of Aβ [29,30]. Human studies examining post-mortem cortical tissues and peripheral sphingolipid levels have revealed aberrant lipid profiles in AD. A post-mortem LC-MS/MS study quantifying S1P and ceramide levels in multiple brain regions that are differentially affected by AD pathology revealed decreases in S1P which were most pronounced in brain regions affected early in the AD process, such as the hippocampal CA1 region and inferior temporal cortex [31]. Furthermore, the observed decline in S1P may be associated with reduced sphingosine kinase activity [31]. Overall, the study suggested that putatively neuroprotective S1P signaling declined in a region-specific manner which correlated well with neuropathological progression in the AD cortex [31]. However, only one S1P species (d18:1) was measured, and the status of other S1P species, as well as their relative contribution to potential S1P signaling dysregulation, are unclear. An earlier study by He et al. also demonstrated a decrease in S1P in the frontotemporal area of the AD brains compared to controls [32]. However, in contrast to Couttas et al. [31], He et al. observed elevated levels of ceramide in AD [32]. This finding is in agreement with other studies which showed increases in ceramide species such as C16, C18, C20 and C24, in brain tissues of AD patients (as well as in patients with non-AD neurodegenerative dementias such as frontotemporal lobar degeneration, tauopathy, and diffuse Lewy body disease) compared to controls [33,34]. In cellular models of AD process using either Aβ

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