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Lipidomics: When apocrypha becomes canonical

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Abstract

Lipidomics is a branch of the field of metabolomics. Although only about a decade since its inception, lipidomics has already had a major influence on the way in which questions about lipid metabolism and signaling are posed. The field is intertwined in the culture and rich history of mass spectrometry. Early work emphasized analytical issues such as limits of detection and numbers of molecular species quantitated in single injections. Increased sophistication in applications of lipidomic analysis and emerging technologies, such as imaging mass spectrometry, are facilitating the study of lipid metabolism and signaling species in cellular functions and human diseases. In the coming years we anticipate a richer understanding of how specific lipid species mediate complex biological processes and interconnections between cellular pathways that were thought to be disparate.

Background and History

Lipidomics is the systematic identification of the lipid molecular species of a cell, organelle, globule, or whole organism with emphasis on the quantitative determination of composition changes in response to a perturbation, such as stimulation of a receptor-mediated signaling pathway or alteration in metabolism. Since the first use of the term over a decade ago there has been controversy as to what truly constitutes “lipidomic” analysis, but most of the early contributors agree that the origins emerged from instrumentation advances in mass spectrometry, and computational biology. The ability to identify lipid molecular species and track changes in the composition of a cell membrane or biopsied tissue are rooted in the technological revolution of mass spectrometry instrumentation, particularly electrospray ionization mass spectrometry. Development of next generation instruments that could be used beyond the confines of advanced analytical chemistry laboratories, allowed the technology to be increasingly used by researchers in the biological sciences. Several investigators made major contributions to the methodology that has led to mass spectrometry as the preferred method of lipid identification (see reviews [1,2]) but in the late 1990s a transition began that engaged the power of systems biology in the quantitative analysis of lipids. As advances in the sensitivity and resolving power of mass spectrometry progressed, investigators pursued systematic identification of the lipomes of various cell types. An influential paper by McLafferty and colleagues [3] demonstrated the use of positive and negative mode Fourier-transform ion cyclotron resonance mass spectrometry (FTICR-MS) to identify the glycerophospholipid composition of a mucosal mast cell line. Subsequently, Ivanova et al [4] expanded this capability by tracking a greater number of species and measuring changes in multiple species during the process of regulated

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exocytosis. In 2001 the National Institute of General Medicine (NIGMS) formally sponsored the creation of a Lipidomics Core as part of the large scale collaborative initiative program, The Alliance for Cellular Signaling. This program was designed to define the components of signaling systems and describe the complex overlapping physical and regulatory interactions between these components in a quantitative manner [5] and quantitation of lipid species was acknowledged as critical element necessary for a comprehensive systems analysis of cellular signaling networks.

The early goals in lipidomics were oriented on technology development and building the infrastructure for the analytical and computational challenges [6,7]. The field has rapidly progressed and increasingly is being used to address questions in diverse biological systems. From its somewhat esoteric origins lipidomics has gained acceptance as previously unappreciated roles of lipid species in cellular processes are being discovered and molecular mechanism described. Among the contributions of the NIGMS-supported Lipid Metabolites and Pathways Strategy (LIPID MAPS) project has been the reorganization of lipid classification to facilitate bioinformatic organization and making databases more compatible with search functions [8]. A review on the structural organization of the Lipidomics database and online tools provided in the LIPID MAPS database (<http://www.lipidmaps.org/>) was recently contributed by Subramaniam and colleagues [9]. This includes an in-depth discussion of the various issues related to classification, ontology, nomenclature, and structural representation of lipid molecules that were considered in the creation of the database.

Current state-of-the-art Lipidomics

Lipid molecular species are a heterogeneous category of cellular metabolites. Historically, lipids were functionally defined as molecules that were soluble in certain kinds of organic solvents (*e.g.*, chloroform) or extracted from aqueous solutions. We have come to appreciate that all lipids found in nature derive from one of two different biosynthetic pathways involving either the condensation of acyl carrier protein intermediates derived from malonyl-CoA and acyl-CoA esters and the intermediacy of a carbanion structure. This mechanism leads to lipids that include fatty acids, phospholipids, and glycerolipids. The second route is the carbocation pathway that involves condensation of branched chain 5-carbon pyrophosphate intermediates, including steroids and polyisoprenoids [10]. Owing to this chemical diversity of species, it has been pragmatic to divide lipid species further into subcategories based on metabolic pathways. The six functional divisions of lipid categories are shown in TABLE 1. References provided cite the best current practices for methodologies on the extraction and analysis of species in these respective lipid categories.

Recently, a thematic issue of *Chemical Reviews* (2011) [11] provided a timely set of contributions on various topics in lipid biochemistry, metabolism, and signaling. The reader is directed to this issue for a more comprehensive overview of this topic. A central theme running through these scholarly reviews is the way that lipidomic analysis is shaping the direction of the field. The sequencing of genomes, proteomics, and metabolomics are increasingly integrating once seemingly disparate areas of research. In the first ten years of lipidomics, we have met several challenges that were technical in nature, including development of the proper workflow for the handling, extraction, separation, and analysis of lipid species. As noted, the methodologies differ in part due to the chemical heterogeneity of lipid species. However, successful workflows are described for each of the lipid classes in cited special issues [12, 13]. The workflow for glycerophospholipid analysis is illustrated in FIGURE 1. Several important features are shown including the choice of a profiling approach (*i.e.*, shotgun lipidomics) or a prior separation of classes using liquid chromatography (LC) followed by mass spectrometry detection and analysis. There has been

considerable debate over the relative merits of these two approaches. Our experience has been that shotgun protocols are particularly useful for initial identification of differences between two conditions (*i.e.*, profiling). Stimulation of a cell by an extracellular ligand or knockdown of a gene product are common challenges well suited to initial characterization using a direct infusion or shotgun approach. By contrast, LC-MS approaches are preferred when absolute quantitation is needed. The lack of linearity in ionization of hydrocarbon chains of different lengths has been well documented [14–17]. This lack of uniformity in ionization between extracted cellular metabolites can satisfactorily be addressed by strategic inclusion of chemically relevant internal standards and the rigorous generation of standard curves using defined amounts of the chemical constituents. Thus, as illustrated in FIGURE 1, we frequently use a shotgun approach to profile changes between two or more conditions subsequently followed by a rigorous, quantitative analysis using LC-MS/MS approaches with standard curves for absolute quantitation. The initial profiling helps to identify the types and amounts of chemically defined internal standards that are needed for absolute quantitation. The details of the computational analysis and data handling approaches are discussed in reviews elsewhere [18,19].

The identification of novel enzymes involved in lipid metabolism and signaling offers a new set of challenges for the second decade of lipidomics/metabolomics. Some exciting examples of identification of novel enzymes that modulate known lipid species [20] have recently been described. This serves to highlight where the field is heading. Four major challenges are outlined below that although vexing, clearly represent the maturation of the field as we move beyond technical issues. The first challenge is better establishment of substrate-product relationships in lipid metabolic and signaling pathways. Classical approaches to measure flux have typically labelled cells with a stable isotope containing lipid precursor and followed the generation of lipid products through identification of species containing the isotopic label. This classic approach has elucidated many biochemical pathways and continues to be widely utilized. However, we are to some extent victims of our own success. As the number of molecular species has multiplied the spectra of some regions have grown dense and a slight shift in a labelled species may cause it to overlap the mass to charge (m/z) atop another lipid species. This was the motivation for creating a type of “lipid affinity chromatography,” where species could be followed and resolved from the bulk of native species. Milne et al., (2010) [21] described the capture and release of alkyne-derivatized glycerophospholipids using cobalt chemistry. This technique allows alkyne labelled precursor lipids (*e.g.*, fatty acyls or glycerophospholipids) to be incorporated into cells and metabolism is followed by capture of the alkyne-labelled products. Unlike other types of click-chemistry the covalent attachment is reversible and generated probe lipids can be released. This technique offers a unique series of probes for defining the biotransformations of relatively low abundance signaling lipids.

A second major challenge is to go beyond the identification of lipid biomarkers in human diseases and to discern whether any of the changes described represent actual mechanistic causes of pathophysiology. Several reports have demonstrated that lipid molecular species are altered in human diseases. For example, lipid changes have been examined in clinical biopsies and animal models of various diseases, including Alzheimer’s disease [22, 23], Parkinson’s disease [24], hepatic liver diseases [25], and diabetes [26]. Moreover, the lipomes of pathogenic organisms have been analyzed to attempt to identify novel targets, including *Mycobacterium tuberculosis* [27,28] and human cytomegalovirus virions [29]. While these and other exciting discoveries are emerging, the field has yet to reach its full potential by identification of novel mechanisms that truly underlie human diseases. Indeed, we have made great strides from the early days where studies were more descriptive and observational. Recent studies are increasingly pursuing the identification of specific enzymes with altered activities and how these aberrations in metabolite concentrations affect

cellular processes. This leads to the third major challenge, namely translating these discoveries into new small molecule therapeutics. Progress in the use of mass spectrometry-based OMICS to discover targets and accelerate development of novel chemical modulators has been long anticipated. Recently, isoenzymes-selective inhibitors of phospholipase D [30,31] have been developed using mass spectrometry-based assays to screen large numbers of compounds generated by technology-enabled synthesis approaches. Increasingly discoveries made through the integration of genomics, proteomics, and lipidomics/metabolomics will accelerate the discovery of new therapeutic targets.

The final challenge looks ahead to subsequent technology development. The advances in mass spectrometry instrumentation over the last twenty years have roots in the genesis of lipidomics and metabolomics, but it was the emergence of the interdisciplinary field of chemical biology that has empowered chemists to use analytical tools to answer fundamental questions in biology. The biomedical sciences have been a primary beneficiary of this renaissance in lipidology [32]. Lipidomics/Metabolomics has largely been used to described temporal changes in lipid species content, but new approaches will provide spatial information on lipid localization within tissues and cells [33,34]. Advances will largely depend on the next generation of instrumentation being built [35,36] to resolve subcellular details of lipid composition.

Further improvements in the resolution and identification of lipid molecular species are emerging from other approaches as well. Blanksby and colleagues [37,38] have used ozone-induced dissociation to identify the positions of double bonds in unsaturated fatty acids. This advancement has the potential to vastly expand key molecular level detail between species that differ by only in the position of a double bond. Another technology that appears to hold great promise in advancing lipidomic capabilities is ion-mobility mass spectrometry [39]. The inclusion of cross-sectional areas of lipid molecules will potentially add yet another dimension of information that will facilitate identification of molecular species. More systematic analysis of a broader range reference standards need to be reported, but the approach appears well positioned to contribute to more facile identification of complex molecular species. These and other advances will undoubtedly lead to new discoveries about the roles of lipid species in cell biology and pathophysiology as the OMICS revolution continues to transition from the obscure to a mainstream research tool.

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Figure 1. Lipidomics Overview

The workflow begins with the extraction of lipids from a biological source. Tissues, cells, subcellular organelles or globules, bacteria, purified virions (among others) have been used in the determinations of lipid compositions. Depending on the specific class of lipid being analyzed different extraction and analytical separation procedures are used. Two citations provide comprehensive descriptions of these procedures (12,13). In the case of glycerophospholipids, the species may be profiled using a direct injection or "shotgun" protocol (upper). This is particularly effective at the initial identification of changes in lipid species between two or more conditions (*e.g.*, challenge with extracellular ligand or gene knockout). Subsequently, absolute quantitation of lipid species is obtained by a more detailed analysis using LC-MS/MS in combination with appropriate selection of chemically defined lipid standards. This approach gives more reliable absolute quantitation by taking into account the nonlinear ionization of lipid species with differences in hydrocarbon chain lengths and other chemical dissimilarities. Lastly, the tracking of low abundance lipid metabolites and flux analysis is facilitated by use of an alkyne-labeled lipid precursor. This allows the separation of alkyne-cobalt complexes from native lipid pools using a modification of click chemistry.

Table 1
Major Lipid Classes

Major lipid classes (with representative species shown) as organized by the LIPID Metabolites And Pathways Strategy (LIPID MAPS) consortium. This consortium has defined the mouse macrophage lipidome (an inventory of thousands of lipid molecular species) and worked to develop state-of-the-art lipid analysis protocols, developed website based tools (www.lipidmaps.org), and increase access of the broader scientific community to lipidomics [see further details in Ivanova et al., 2007 *Methods in Enzymology*; Myers et al., 2011 *Biochim Biophys Acta* (17,18)].

LIPID CLASSIFICATION	REPRESENTATIVE SPECIES
Glycerolipids	<p>[1] Diacylglycerol (DAG) and [2] Triacylglycerol (TAG)</p>
Glycerophospholipids	<p>[3] Phosphatidic Acid (PA) and [4] Phosphatidylcholine (PC)</p>
Sphingolipids	<p>[5] Ceramide (Cer) and [6] Sphingosine-1-Phosphate (S1P)</p>
Sterols	<p>[7] 25-Hydroxycholesterol and [8] Zymosterol</p>

LIPID CLASSIFICATION	REPRESENTATIVE SPECIES
Fatty Acids / Eicosanoids	<p>9 </p> <p>10 </p> <p>[9] Arachidonic Acid (20:4) and [10] Oleic Acid (18:1)</p>
Prenols	<p>11 </p> <p>12 </p> <p>[11] Dolichol and [12] Vitamin E</p>