Front. Biol. 2011, 6(3): 183–189
DOI 10.1007/s11515-011-1147-1

MINI-REVIEW

Systems level analysis of lipidome

Guanghou SHUI

Life Science Institute, National University of Singapore, Singapore 117456, Singapore

Abstract **Lipids, once thought to be mainly for energy-storage and structural purpose, have now gained immense recognition as a class of critical metabolites with versatile functions. The diversity and complexity of the cellular lipids are the main challenge for the comprehensive analysis of a lipidome. Lipidomics, which aims at mapping all of the lipids in a cell, is expanded rapidly in recent years, mainly attributed to recent advances in mass spectrometry (MS). MS-based lipidomic approaches developed recently allow the quick profiling of hundreds of lipids in a crude lipid extract. With the aid of latest computational tools/software (chemometrics), aberrant lipid metabolites or important signaling lipid(s) could be easily identified using unbiased lipid profiling approaches. Further tandem MS (MS/MS)-based lipidomic approaches, known as targeted approaches and able to convey structural information, hold the promise for high-throughput lipidome analysis. In this review, I discussed the basic strategy for systems level analysis of lipidome in biomedical study.**

Keywords lipidomics, lipid, mass spectrometry, metabolite

Introduction

Lipids are essential components of cellular membranes and involved in a wide range of biological processes including membrane trafficking and signal transduction (Di Paolo et al., 2004; Wenk, 2005). Cholesterol and polar lipids including phospholipids and sphingolipids constitute major membrane lipids (Fig. 1), while triacylglyerols (TAGs) are major components of lipid droplets. Lipids also represent an important class of metabolites. It is becoming increasingly clear that deregulated lipid metabolism plays an important role in many human diseases (Fernandis and Wenk, 2009; Gangoiti et al., 2010). Despite the major revamping in the biological roles of lipids, many biological functions of lipids remain poorly understood, in particular at the molecular level. Traditional techniques, such as thin layer chromatography (TLC), gas chromatography (GC) and high performance liquid chromatography (HPLC) are proven insufficient to adequately address many important functional roles of diverse lipids (Wenk, 2005). A global snapshot of the cellular lipidome is a prerequisite to understanding the exquisite interactions and dynamics of lipids during cellular perturbations or in various physiological and pathological conditions. This demands methodological innovations to detect and monitor the fine changes in the diverse array of lipid species at the molecular level.

Lipidomics, which aims at systems scale detection, characterization and quantification of lipids, is developing rapidly as an independent discipline at the interface of lipid biology, technology and medicine, and has contributed tremendously to the advancement of our knowledge in the realm of lipid biology. Undoubtedly, systems scale research of lipids has benefited from a number of recent achievements and developments in analytical techniques. Technological advancements, most notably in MS, allow sensitive and highly selective analysis of lipids with diverse chemical composition and in complex mixtures. Various MS-based approaches have been successfully applied for systems scale analysis of lipids (Han and Gross, 2005; Merrill et al., 2005; Guan et al., 2006; Ejsing et al., 2009; Griffiths et al., 2006; Shui et al., 2010b; Shui et al., 2007; Taguchi and Ishikawa, 2010). Those approaches are developed in two directions, namely unbiased global (non-targeted) lipidomics and targeted lipidomics. In this article, we will briefly review the recent technical advances as well as the basic approaches used in analysis of lipidome.

Received March 1, 2011; accepted March 11, 2011
Correspondence: Guanghou SHUI
E-mail: lsisgh@nus.edu.sg, bchsgh@nus.edu.sg

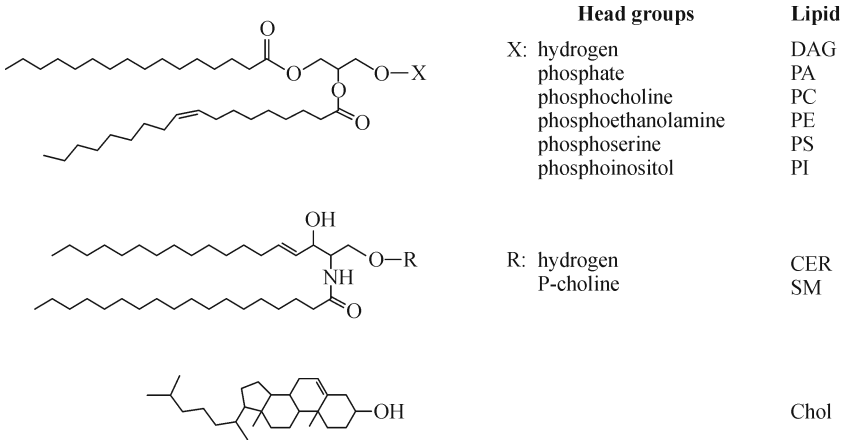

Figure 1 Typical structure of major membrane lipids. DAG, diacylglycerol; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; PG, phosphatidylglycerol; SM, sphingomyelins; Cer, ceramides; Chol, cholesterol.

Mass spectrometry of lipids

Lipids can be classified into the following eight categories: fatty acyls (FA, wax, etc.), glycerolipids (TAG, triacylglycerol; DAG, diacylglycerol; MGDG, monogalactosyldiacylglycerols; DGDG, digalactosyldiacylglycerols, etc), glycerophospholipids (PA, phosphatidic acid; PE, phosphatidylethanolamine; PC, phosphatidylcholine; PI, phosphatidylinositol; PG, phosphatidylglycerol; CL, cardiolipin, etc), sphingolipids (sphingosine, Sph; sphingosin-1-phosphate, S1P; ceramide, Cer; SM, sphingomyelin; GM3, Ganglioside mannoside 3, etc), sterols (Chol, cholesterol; CE, cholesterol ester; CS, cholesterol sulfate, etc), prenol lipids (isoprenoids, quinines, etc.), saccharolipids and polyketides (Fahy et al., 2005; Fahy et al., 2009). A wide range of building blocks from each category (of lipids) could give subsequently an amazing number of combinations. The complexity of chemistry, illustrated by the great differences in both the structures and biological levels among the lipids, remains a challenge for researchers to have a complete profile of a lipidome with a common method. Lipidomics, being a very promising and relatively new field, requires continuous development of more specific, sensitive and comprehensive analytical/biochemical approaches. Advances in mass spectrometry (MS) have paved a new and powerful path for lipid analysis, being able to separate and characterize ionized analytes according to their mass-to-charge ratios (m/z). Besides, it can also provide structural information by fragmenting the parent ions through tandem mass spectrometry techniques; quantitative analysis by comparing intensity of ion signals to that of corresponding internal standard is also achievable.

The procedure for analyzing major cellular lipids is illustrated in Fig. 2. First, analysis of specific lipids or the whole lipidome usually starts from extraction of lipids from biological samples such as tissue, cell or body fluids (Fig. 2), although recent imaging MS (IMS) could directly probe certain lipid species at the specific position of a tissue or intact cells (Fig. 2). The obtained crude lipid extract could be directly introduced to an MS machine with or without upfront chromatography separation. When passing through an ion source, lipid analytes are converted into charged ions in the gas phase, and a mass analyzer measures their m/z ratios. The intensity of a charged ion will be recorded by a detector and could reflect its corresponding concentration. With the aid of chemometrics, such as alignment of spectra using Correlation Optimized Warping (COW) (Guan et al., 2006), perturbation of lipids under different conditions could be rapidly identified from differential plotting of single-stage MS profiles using non-targeted approaches (Fig. 2). MS/MS approaches also serve as powerful tool for quantitative and/or comparative analysis of targeted lipid species (Taguchi et al., 2005). Statistical analyses are applied to identify the lipid species with significant changes (Fig. 2), which could be associated with biological pathways of interest (Fig. 2) (Niemelä et al., 2009).

Sample preparation

An ultimate protocol for extraction of all classes of lipids has never been realistic due to various reasons including stability and contamination issues, chemical diversity as well as extractability issue of lipids which are covalently-bound with protein, etc. Even though, most-widely used extraction protocols, which are based on or modified from Bligh and Dyer or Folch methods and able to extract cellular lipids quantitatively, are proven to be reliable for qualitative and comparative studies (Folch et al., 1957; Bligh and Dyer, 1959).

Ionization of lipids

A variety of ionization techniques can be used for MS, depending on the internal energy transferred during the

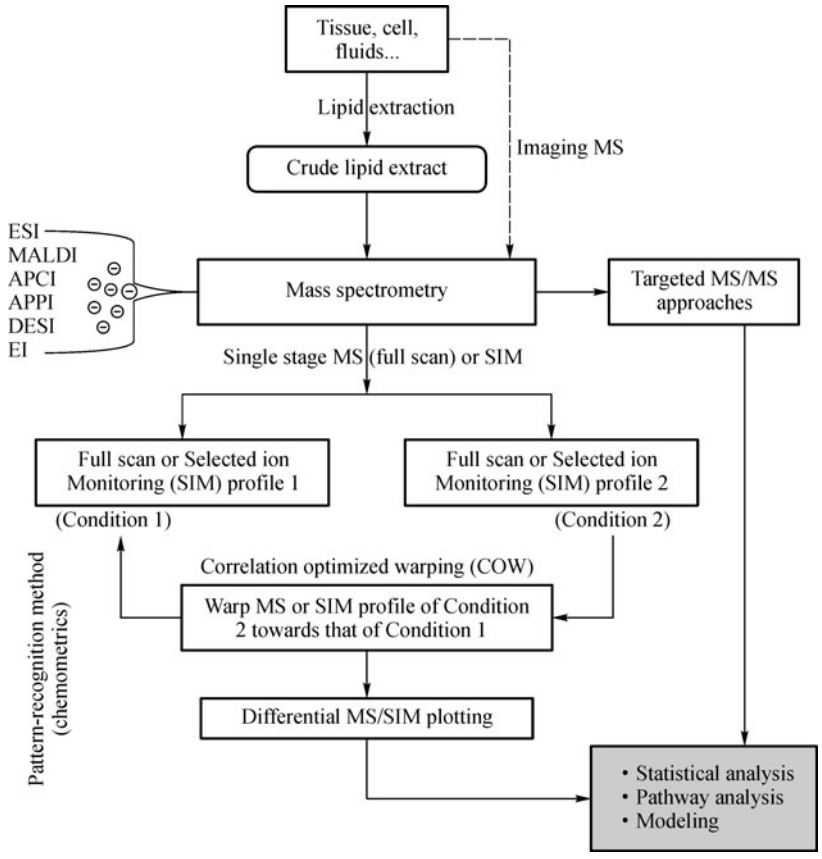

Figure 2 Mass spectrometry-based analysis of lipidome in biological samples. Flow chart illustrating quantitative and comparative lipidomic approaches.

ionization process and the nature of the lipids to be ionized. The commonly used ionization methods include electron ionization (EI), chemical ionization (CI), atmospheric pressure chemical ionization (APCI), electrospray ionization (ESI), matrix-assisted laser desorption/ionization (MALDI), fast atom bombardment (FAB) and desorption electrospray ionization (DESI). In the past, MS has been limited to analyzing fatty acids or other small volatile lipids using GCMS with EI, which has the drawback of lacking molecular ions due to high-energy-induced fragmentation. This was overcome by the technological advances like the development of soft ionization techniques such as ESI and MALDI, which have significantly expanded the range of lipids that can be qualitatively and quantitatively analyzed by MS (Karas and Hillenkamp, 1988; Fenn et al., 1989; Han and Gross, 1994; Pulfer and Murphy, 2003; Han and Gross, 2005; Jackson et al., 2005; Raith et al., 2005; Taguchi et al., 2005; Huang et al., 2006; Stubiger and Belgacem, 2007; Karu et al., 2007; Fuchs et al., 2010). ESI/MS has been successfully used to measure lipids in various cell types. Many cellular phospholipids and sphingolipids are effectively ionized and detected by ESI-MS in the negative ionization mode, with or without a chemical modifier or signal enhancer. For instance, phosphatidylcholine (PC) and sphingomyelin (SM), which are more sensitively detected in positive ionization mode, are readily detectible as their de-methylated or chloride-adduct (negative) ions. Negative ESI mass spectra of a crude cellular extract therefore cover most, if not all classes of polar phospholipids (Shui et al., 2007).

Non-targeted lipidomics

MS could be used to obtain the full scan MS spectra of the crude lipid extracts. For instance, the MS profiles of the yeast *Saccharomyces cerevisiae* and its *Slc1p* mutant were acquired in negative ESI mode (Fig. 3A-B). The MS scan range was between 400 and 1200 amu, which includes various polar lipids such as lysophospholipids, phospholipids (PC, PE, PI, PS, PG and PA) and sphingolipids (ceramides, inositol sphingolipids). The comparison of MS profiles can be achieved by aligning the spectra to give a differential profile (Fig. 3C, modified from Shui et al. (2010a)). The Δ*slc1* cells showed dramatic decreases in PI 30:0 (m/z 781), PI 28:0 (m/z 753), PI 26:0 (m/z 725), LysoPI 14:0 (m/z 545) and LysoPI 12:0 (m/z 517). As compensation, increases in PI 32:1 (m/z 807) and LPI 16:1 (m/z 569) were observed in Δ*slc1* cells (Fig. 3C).

The non-targeted lipidomic approaches offer the advantages of discovering unexpected lipid metabolites or novel lipids under different physiological conditions or in patients.

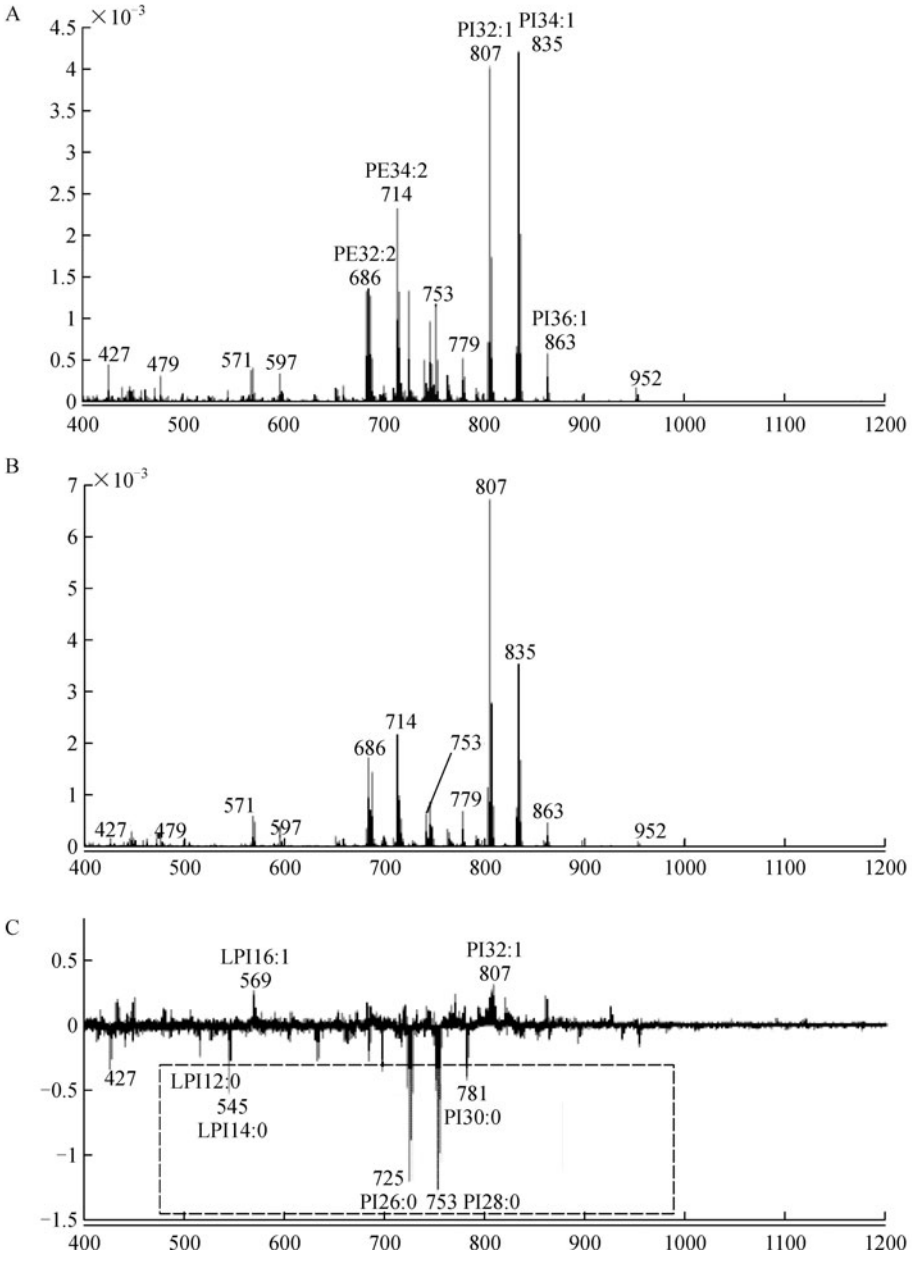

Figure 3 Non-targeted lipidomic analysis (modified from Shui et al. (2010a)). (A) Normalized lipid profile for lipid extract from the wild type cell of yeast *S. cerevisiae*; (B) normalized lipid profile for the *SCL1* mutant; (C) differential plot (log ratio) of the *SCL1* mutant compared to wild type.

However, poor ionization of certain lipid species under selected MS condition as well as sensitivity issues might lead to missing information on such lipid(s). In addition, further MS/MS-based techniques are usually required for structural elucidation.

Targeted analysis of lipids

Tandem MS serves as the basis for targeted lipidomics. The product ion scan (PIS) conveys structural information and can be used to characterize a specific ion of interest. When MS is operated in PIS mode, the first MS analyzer is set to transmit a selected ion for fragmentation inside the second MS analyzer (MS2); the fragmented ion species are resolved in the third MS analyzer (MS3) (Fig. 4A). When MS is operated in precursor ion scan (PreIS) mode, the first MS analyzer (MS1) is scanned in a selected mass range, while the third MS analyzer is set to transmit a specific ion of interest (Fig. 4A). In MRM experiments, both MS1 and MS3 analyzers are set to transmit a specific ion of interest, which require specific or

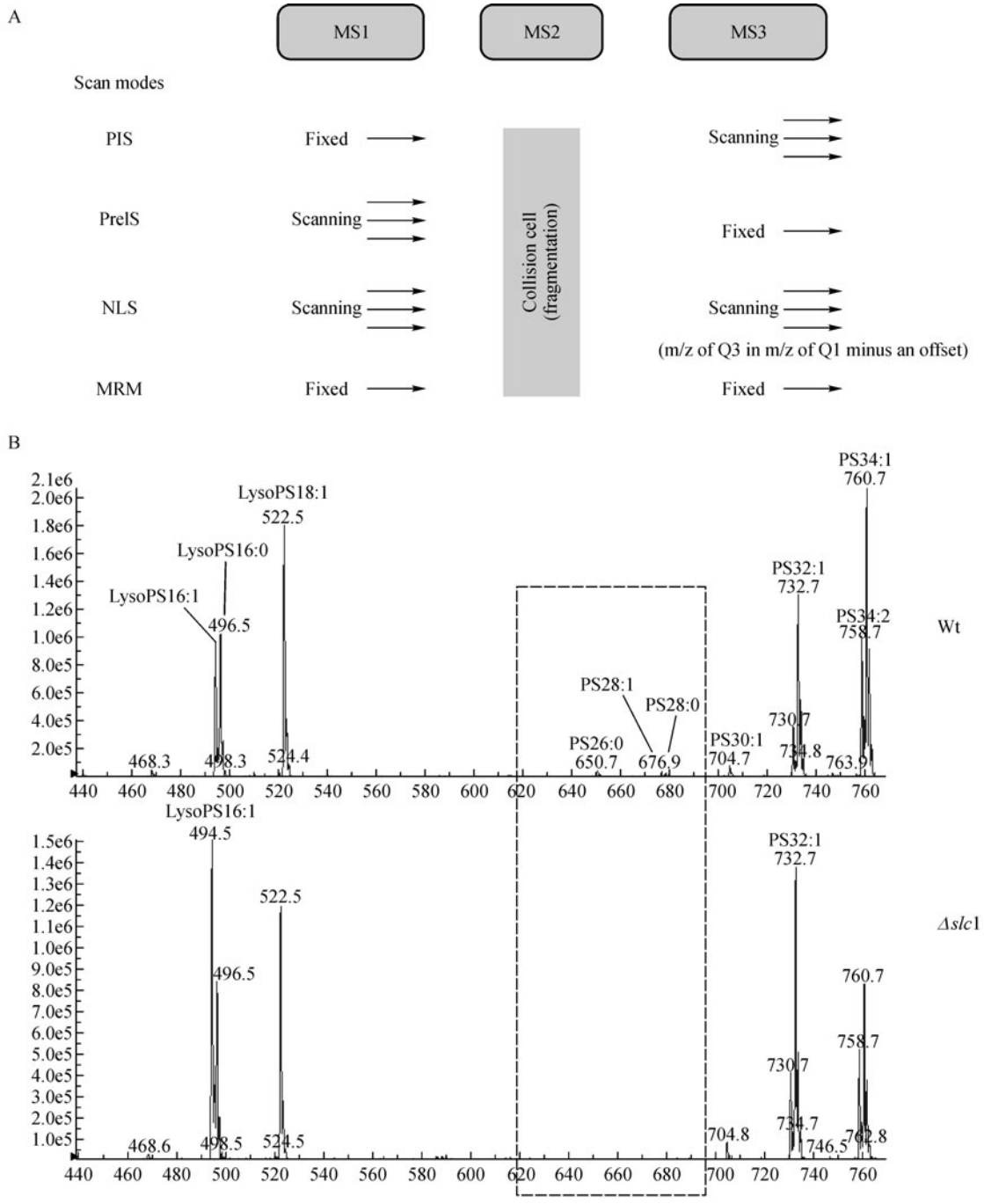

Figure 4 Tandem mass spectrometric (MS/MS) strategies for lipidomics. (A) various MS/MS techniques: PIS, product ion scan; PreIS, precursor ion scan; NLS, neutral loss scan; MRM, multiple reaction monitoring. (B) NLS scan of 87 to detect specific PS species in yeast *S. cerevisiae* Δ*slc1* mutant (unpublished data).

characteristic product ion to ensure the structural specificity of lipid of interest (Fig. 4A). In the neutral loss scan (NLS) mode, both the first MS analyzer and the third MS analyzer are scanning through a defined mass range, but MS3 is set to scan a fixed mass difference below MS1(Fig. 4A). For instance, NLS of 87 in the negative ESI mode results in detection PS species in the yeast *S. cerevisiae* and its *Slc1p* mutant (Fig. 4B). NLS scan spectra showed dramatic decreases in PS 28:0 (m/z 678), PS 28:1 (m/z 676) and PS 26:0 (m/z 650), while increases in PS 32:1 (m/z 732) and LPS16:1 (m/z 494) were observed in the Δ*slc1* cells (Fig. 3C). The advantages of MS/MS-based (targeted) lipidomic approaches lie in its high sensitivity because of the extremely-low noise signals thus high sensitivity and high specificity. In the differential plotting of Δ*slc1* over wild type using unbiased approaches, mainly changes of PI species,

which were the most prominent ions, could be clearly visualized (Fig. 3). Changes of minor species, such as short chain PS 28∶0, 28∶1 and 26∶0, were not reflected as obviously as PI species. However, NLS-based scan undoubtedly demonstrated that these species were dramatically reduced in the Δ*slc1* cells (Fig. 4B).

Dramatic decreases in short-chain PI species demonstrated by unbiased profiling and short-chain PS species by targeted approaches were attributed to the specific substrate preference of s*lc1p*, a known acyltranferase (Benghezal et al., 2007; Shui et al., 2010a). Using high-throughput lipidomic approaches, i.e. MRM-based screening, a few hundred phospholipid species were semi-quantitated in the Δ*slc1* cells, and the substrates of *slc1p* were readily assigned and further verified using biochemical approaches (Shui et al., 2010a).

The MS/MS-based targeted lipidomic approaches offers the advantages of high-throughput, high-sensitivity as well as easiness for data handling and processing. However, information on unexpected lipid metabolites or uncharacterized lipid species will not be achieved using targeted lipidomic approaches.

Summary

Lipidomics, the large-scale characterization and analysis of lipidome in biological samples, has recently emerged as a crucial component in biomedical studies. Both non-targeted (unbiased) and targeted approaches have proven powerful and efficient to investigate the potential roles of lipids in various biomedical researches including (1) lipid cell biology; (2) biomarkers in diseases including neurodegenerative diseases, cancers and mycobacterial infection diseases; and (3) lipid metabolism in organisms and animals.

References

Benghezal M, Roubaty C, Veepuri V, Knudsen J, Conzelmann A (2007). SLC1 and SLC4 encode partially redundant acyl-coenzyme A 1-acylglycerol-3-phosphate O-acyltransferases of budding yeast. J Biol Chem, 282(42): 30845–30855

Bligh E G, Dyer W J (1959). A rapid method of total lipid extraction and purification. Can J Biochem Physiol, 37(8): 911–917

Di Paolo G, Moskowitz H S, Gipson K, Wenk M R, Voronov S, Obayashi M, Flavell R, Fitzsimonds R M, Ryan T A, De Camilli P (2004). Impaired PtdIns(4,5)P2 synthesis in nerve terminals produces defects in synaptic vesicle trafficking. Nature, 431(7007): 415–422

Ejsing C S, Sampaio J L, Surendranath V, Duchoslav E, Ekroos K, Klemm R W, Simons K, Shevchenko A (2009). Global analysis of the yeast lipidome by quantitative shotgun mass spectrometry. Proc Natl Acad Sci USA, 106(7): 2136–2141

Fahy E, Subramaniam S, Brown H A, Glass C K, Merrill A H Jr, Murphy R C, Raetz C R, Russell D W, Seyama Y, Shaw W, Shimizu T, Spener F, van Meer G, VanNieuwenhze M S, White S H, Witztum J L, Dennis E A (2005). A comprehensive classification system for lipids. J Lipid Res, 46(5): 839–861

Fahy E, Subramaniam S, Murphy R C, Nishijima M, Raetz C R, Shimizu T, Spener F, van Meer G, Wakelam M J, Dennis E A (2009). Update of the LIPID MAPS comprehensive classification system for lipids. J Lipid Res, 50(Suppl): S9–S14

Fenn J B, Mann M, Meng C K, Wong S F, Whitehouse C M (1989). Electrospray ionization for mass spectrometry of large biomolecules. Science, 246(4926): 64–71

Fernandis A Z, Wenk M R (2009). Lipid-based biomarkers for cancer. J Chromatogr B Analyt Technol Biomed Life Sci, 877(26): 2830–2835

Folch J, Lees M, Sloane Stanley G H (1957). A simple method for the isolation and purification of total lipides from animal tissues. J Biol Chem, 226(1): 497–509

Fuchs B, Süss R, Schiller J (2010). An update of MALDI-TOF mass spectrometry in lipid research. Prog Lipid Res, 49(4): 450–475

Gangoiti P, Camacho L, Arana L, Ouro A, Granado M H, Brizuela L, Casas J, Fabriás G, Abad J L, Delgado A, Gómez-Muñoz A (2010). Control of metabolism and signaling of simple bioactive sphingolipids: Implications in disease. Prog Lipid Res, 49(4): 316–334

Griffiths W J, Wang Y, Alvelius G, Liu S, Bodin K, Sjövall J (2006). Analysis of oxysterols by electrospray tandem mass spectrometry. J Am Soc Mass Spectrom, 17(3): 341–362

Guan X L, He X, Ong W Y, Yeo W K, Shui G, Wenk M R (2006). Non-targeted profiling of lipids during kainate-induced neuronal injury. FASEB J, 20(8): 1152–1161

Han X, Gross R W (1994). Electrospray ionization mass spectroscopic analysis of human erythrocyte plasma membrane phospholipids. Proc Natl Acad Sci USA, 91(22): 10635–10639

Han X, Gross R W (2005). Shotgun lipidomics: electrospray ionization mass spectrometric analysis and quantitation of cellular lipidomes directly from crude extracts of biological samples. Mass Spectrom Rev, 24(3): 367–412

Huang Q, Shen H M, Shui G, Wenk M R, Ong C N (2006). Emodin inhibits tumor cell adhesion through disruption of the membrane lipid Raft-associated integrin signaling pathway. Cancer Res, 66(11): 5807–5815

Jackson S N, Wang H Y, Woods A S (2005). Direct profiling of lipid distribution in brain tissue using MALDI-TOFMS. Anal Chem, 77 (14): 4523–4527

Karas M, Hillenkamp F (1988). Laser desorption ionization of proteins with molecular masses exceeding 10,000 daltons. Anal Chem, 60 (20): 2299–2301

Karu K, Hornshaw M, Woffendin G, Bodin K, Hamberg M, Alvelius G, Sjövall J, Turton J, Wang Y, Griffiths W J (2007). Liquid chromatography-mass spectrometry utilizing multi-stage fragmentation for the identification of oxysterols. J Lipid Res, 48(4): 976–987

Merrill A H Jr, Sullards M C, Allegood J C, Kelly S, Wang E (2005). Sphingolipidomics: high-throughput, structure-specific, and quantitative analysis of sphingolipids by liquid chromatography tandem mass spectrometry. Methods, 36(2): 207–224

Niemelä P S, Castillo S, Sysi-Aho M, Oresic M (2009). Bioinformatics and computational methods for lipidomics. J Chromatogr B Analyt Technol Biomed Life Sci, 877(26): 2855–2862

Pulfer M, Murphy R C (2003). Electrospray mass spectrometry of phospholipids. Mass Spectrom Rev, 22(5): 332–364

Raith K, Brenner C, Farwanah H, Müller G, Eder K, Neubert R H (2005). A new LC/APCI-MS method for the determination of cholesterol oxidation products in food. J Chromatogr A, 1067(1–2): 207–211

Shui G, Bendt A K, Pethe K, Dick T, Wenk M R (2007). Sensitive profiling of chemically diverse bioactive lipids. J Lipid Res, 48(9): 1976–1984

Shui G, Guan X L, Gopalakrishnan P, Xue Y, Goh J S, Yang H, Wenk M R (2010a). Characterization of substrate preference for Slc1p and Cst26p in *Saccharomyces cerevisiae* using lipidomic approaches and an LPAAT activity assay. PLoS One, 5(8): e11956

Shui G, Guan X L, Low C P, Chua G H, Goh J S, Yang H, Wenk M R (2010b). Toward one step analysis of cellular lipidomes using liquid chromatography coupled with mass spectrometry: application to *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* lipidomics. Mol Biosyst, 6(6): 1008–1017

Stubiger G, Belgacem O (2007). Analysis of lipids using 2,4,6-trihydroxyacetophenone as a matrix for MALDI mass spectrometry. Anal Chem, 79(8): 3206–3213

Taguchi R, Houjou T, Nakanishi H, Yamazaki T, Ishida M, Imagawa M, Shimizu T (2005). Focused lipidomics by tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci, 823(1): 26–36

Taguchi R, Ishikawa M (2010). Precise and global identification of phospholipid molecular species by an Orbitrap mass spectrometer and automated search engine Lipid Search. J Chromatogr A, 1217 (25): 4229–4239

Wenk M R (2005). The emerging field of lipidomics. Nat Rev Drug Discov, 4(7): 594–610