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Lipidomics Techniques and its Applications in Medical Research

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Abstract

Lipids are diverse families of biomolecules that are involved in essential structural as well as signalling roles in biology. The analytical measurement of lipids and their identification & quantitation has become a major research area, in particular in biomedical science as many human pathologies are associated with lipid metabolism disorders. This review provides a brief overview over experimental workflows of lipid isolation and mass spectrometry based detection methods robust enough to study lipid profiles in a clinical context.

Keyword: Lipidomics; Chromatography; Mass spectrometry; Lipidome profile; Targeted lipidome

Introduction

Lipidomics represents the comprehensive and systematic study of pathways and networks involving lipid molecules in a given cell, tissue or organism. Lipids can be classified into eight internationally accepted classes according to the LIPID Metabolites and Pathways Strategy (LIPID MAP) nomenclature. These include fatty acids, glycerolipids, glycerophospholipids, sterol lipids, sphingolipids, prenolipids, saccharolipids and polyketides [1]. It is estimated that about 10,000-100,000 lipid entities result from anabolic and catabolic reactions in biological systems across both the plant and animal kingdom [2]. The complexity and structural diversity of the "lipidome" makes lipidomics a unique subset in the research arena of metabolomics.

Lipids are Critically Important in Many Biological Functions

Lipids have largely been considered as membrane components and as molecules for energy storage. Studies using model organisms have expanded our understanding of lipid biology, in particular with regards to their function in cellular signalling. Sphingolipids (SLs) are one of the major lipids, members include ceramide (Cer), sphingosine (Sph), Sph-1-phosphate (S1P) and Cer-1-phosphate. SLs can be induced by various stimuli such as tumor necrosis factor-α (TNFα), interleukin-1 (IL-1), Fas ligand (FasL) and oxidative stress. Once activated, SLs can regulate several signal transduction pathways. For instance, numerous studies had shown that Cer are involved in the regulation of cell growth, differentiation, senescence and apoptosis by activating the protein phosphatases PP1A, PP2A, protein kinase C, raf-1 and kinase-suppressor of Ras [3]. Another example is S1P that can function as a tumor-promoting lipid by binding to and influence the function of G-protein-coupled receptors [4]. A different signalling lipid, phosphatidylinositol (PtdIns), is a member of the phospholipid family. PtdIns can be phosphorylated and dephosphorylated at the 3-,4- and 5 positions of the inositol moiety by lipid kinases and phosphatases to form seven phsophoinositide forms, termed PtdIns3P, PtdIns4P, PtdIns5P, PtdIns(3,4)P2, PtdIns(4,5)P2, PtdIns(3,5)P2 and PtdIns(3,4,5)P3, respectively. PtdIns(4,5)P2 can be converted into second-messenger molecules triacylglycerol and inositol-1,4,5-trisphosphate (Ins(1,4,5)P3) by phosphoinositide-specific phospholipase C. PtdIns play a regulatory role by interacting with specific target proteins. For instance, the PtdIns (4,5) P2 head group binds to the FERM protein domain (PDB ID: 1GC6) and Cofilin (PDB ID: ITVJ) [5]. The second-messenger Ins (1,4,5)P3 head group binds to the PH domain of Akt kinase [6]. Taken together, through PtdIns-protein interactions, PtdIns control a wide range of cellular processes including cell proliferation, differentiation, intracellular trafficking and glucose transport [7,8].

Lipidomics is Largely Driven by Advances in Analytical Techniques

Due to the complexity and diversity of the physic-chemical properties of lipid molecules, it is very challenging to cover the whole spectrum of the "Lipidome" with a single analytical approach, and often complementary analytical techniques are required. However, with the development of technology, particularly chromatography-based separation and mass spectrometry (MS) based detection, lipidomics progressed rapidly in last two to three decades. The sections below will review the most common approaches used for lipidomics studies, from lipid extraction to lipidome profiling and targeted lipidome analysis, focusing on frontiers of technologies and their applications in medical research.

Lipids Extraction From Tissue

Sample preparation is one of the most important steps for carrying out a successful experiment, and various protocols have been developed to extract different lipid classes. Generally, lipidome studies in tissue are more challenging than using cells or body fluids. In the latter case, lipids are ready to be extracted from fluid directly omitting the problem of solubilisation. Homogenization allows lipid contents to be released from tissue material in a more regular fashion. Incomplete tissue homogenization will not reveal a full picture of the lipidome, and can lead to sample variability that can complicate the interpretation of results. Bead-beater based extraction methods for homogenisation allow the reproducible and efficient homogenisation of tissues (Figure 1). Lipids can then be extracted by using organic solvents, for example by using a mixture of methyl tert-butyl ether/methanol (MTBE/MeOH (3:1)), which is easier to handle as compared to chloroform/MeOH (2:1) (Bligh and Dyer protocol) [9]. At the same time, aqueous fractions can be used to isolate more polar metabolites. Generally, the extraction protocol may be adapted to the type of metabolites under study.

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Chromatography-Based Separation of Lipids

To address the complexity of the "lipidome" from an analytical point of view, diverse chromatography-based techniques have helped to separate and thereby simplify the discovery and quantitation of lipids in biological samples. Solid phase extraction (SPE) is a technique to simplify lipid complexity by enriching for particular lipid sub-classes. For instance, Griffiths and colleagues showed that cholesterol and non-polar lipids could be separated from oxysterol and bile using C18 cartridges as stationary phase and 70% ethanol as the mobile phase [10]. SPE offers a rapid and preparative separation of lipids. Thin layer chromatography (TLC) is also commonly applied to separate lipid mixtures using a thin silica gel as stationary phase, and a variety of mobile phases can be selected dependent on the polarity of the lipid content. The limitation of TLC is the low sensitivity of TLC detection and the short length of the stationary phase which limits separation power. To overcome this, gas chromatography (GC) and liquid chromatography (LC) have been widely applied. GC is used to analyse low molecular weight (<500Da) molecules of all different classes in the gas phase. Helium is normally used as mobile phase in combination with fused silica glass capillary as a stationary phase. For lipids which are not volatile or unstable at high temperature, the samples need derivatization to increase stability and volatility [11]. Silylation is the most common derivatization technique used in GC-MS based analyses. Recently, 2-dimensional GC chromatography (GCxGC) (coupled by a modulator) was developed to increase separation power [12]. In such experiments, total tissue extracts can be separated on the first GC according to velocity, and further separated on the second GC based on polarity. The limitation of GC related techniques is that only small molecular weight lipids (predominantly<500 Da) can be resolved and separated. For high molecular weight lipids (>500 Da), LC has been proven to be suitable. Lipids can be separated either based on normal phase or reversed phase chromatography and even using hydrophilic interaction chromatography (HILIC). Ultra performance liquid chromatography (UPLC) was applied in lipid studies and achieved higher peak resolution, sensitivity and greater throughput compare to HPLC [13].

Different MS Ionization Techniques for Lipid Measurement

Mass spectrometry (MS) is a driving force of the analytical development in lipidomics. After chromatography separation, lipids are ionized and delivered to the mass spectrometer for detection. Electron impact ionization (EI, coupled to GC) at high energy (70-eV) generates stable and reproducible spectra with high sensitivity [14]. Fragmentation patterns produced by known or unknown lipid compounds can be used to search of a series of standard (70-eV) spectral libraries (NIST / METLIN / LIPID-MAPS) for lipid identification. Electrospray ionization (ESI) is a soft ionization technique, and it is most commonly used in MS and particularly effective for polar lipids (phospholipids) with great sensitivity. ESI-MS/MS based fragmentation can also be used for metabolite database search algorithms, but appear to be less reproducible as compared to EI spectra. The low energy employed by ESI keeps lipids in their intact form, so each spectrum represents essentially one lipid mass, which allows for a global lipidome profiling approach. The first biological lipid mixture was analysed by Han and Gross in 1994 using low resolution MS at that time. Low resolution MS could not distinguish a larger number of isobaric compounds exist in complex biological samples, making such top-down lipidomics approaches extremely challenging [15]. However, the development of high resolution mass spectrometer in early 2000s, in particular new time-of-flight (TOF) along with Orbitrap instruments, make the isobaric distinguishing practicable due to sub-isotope resolution. As an example, phosphatidylcholine (PC) 36:1 and phosphatidylserine (PS) 36:2 are isobaric, but the exact masses differ by 0.0726 Da, which can be distinguished by LTQ Orbitrap MS at the resolution of 100,000 [16]. Electron capture atmospheric pressure chemical ionization (APCI) increases MS detection sensitivity even further as compared to conventional ESI [17]. Matrix-assisted laser desorption/ionization (MALDI) is a conventional source for intact glycerophospholipid and sphingolipid detection, but the lack of quantitation limits MALDI-TOF applications in lipidomics. However, its ability to use tissue sections directly and its MALDI image function still attracts attention, as this provides spatial-temporal information in addition to mass.

Analytical Approaches to Address the Lipidome

Two approaches can be applied to lipidomics studies, referred to as global lipid analysis profiling and a targeted approach (the study of a discrete set of biologically relevant lipids). For global profiling, matrix assisted laser desorption ionisation time-of-flight (MALDI-TOF), quadrupole time-of-flight (Q-TOF) and ion trap (Orbitrap) mass spectrometry are commonly used. Mass (m/z) of lipid species in a given sample can be used for profiling analysis even without lipid species identification (top-down lipidomics). Moreover, with the development of tandem MS (MSn), such as Q-TOF and Orbitrap LC-MS/MS, selected precursor ions can be fragmented in second MS (MS/MS) which can aid with lipid identification and quantitation. Indeed, many lipidome profile analyses have used this approach successfully to profile and identify lipids (bottom-up lipidomics) [18].

For a targeted lipidome analysis approach, a triple quadrupole (QqQ) TOF MS is often employed. The first quadrupole is used for precursor ion selection, which is then fragmented in the second quadrupole, and the third quadrupole is used to monitor selected fragment ions. Neutral loss (NL) is a fragmentation behaviour of ionized lipids, which allows the set up specific precursor ions and neutral loss scans using QqQ MS [19]. NL scan significantly reduced chemical noise, decrease the number of scans required for structure characterization and hence increase detection sensitivity. Multiple reaction monitoring (MRM) has also been developed to measure lipids with known precursor ion and product ions. MRM MS increases detection sensitivity (even better than NL) and allows detection and quantitation of lipids at very low levels in biological samples, as has been demonstrated for eicosanoids [20]. Selected ion monitoring (SIM) scans unique fragment ions for particular lipid (e.g. m/z 87 for a saturated fatty acid), and the SIM method can be applied in both GC/EI-MS and ESI-QTOF MS with a 10-fold increase in sensitivity as compared to a full scan mode setting on the same instrument [21] (Figure 1).

Application of Lipidomics in Medical Research

MS based techniques and approaches allowed researchers to assess lipids functions in medical research. A crucial role of lipids and their associated metabolism has been demonstrated in many human diseases including diabetes and metabolic syndrome, neurodegenerative diseases and cancer. Metabolic syndrome is associated with pathologic metabolic states such as obesity, diabetes and hyperlipidaemia, and it increases the risk of developing atherosclerosis, inflammation and hypertension. Using an MS approach, Gross et al., evidenced that plasmalogens were major ethanolamine glycerophospholipids presented in the myocardium. End-stage human myocardium contained abundant amounts of triglycerides as well [22]. Lipidomics studies have demonstrated that there were major glycerophospholipids

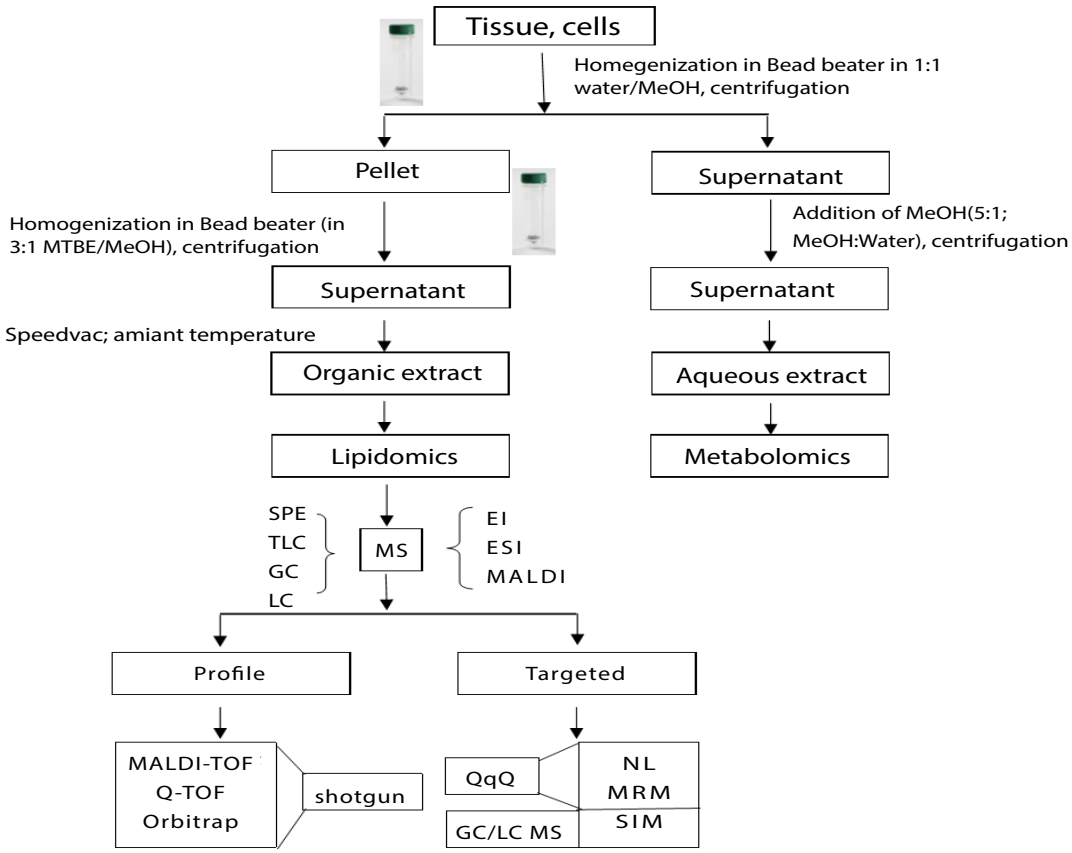

Figure 1: A scheme of an experimental and analytical workflow in lipidomics studies from tissue or cells extractions. Samples are first homogenized in a bead beater to release lipids from tissue completely. After centrifugation, homogenates are separated into an aqueous supernatant for sugars, amino acids, nucleotides studies, and a pellet fraction for further organic phase lipid extraction. Lipid mixtures can be chromatographically separated by TLC, SPE, LC or GC and subsequently analysed by ESI, EI or MALSI MS for lipid detection either on line or off line. Two approaches, a global profiling and a targeted approach can be applied to elucidate comprehensive pictures of the "lipidome". MALDI-TOF MS, Q-TOF LC MS/MS or Orbitrap LC-MS/MS platforms can be used for profiling. NL, MRM or SIM based scanning methods can now be used in GC-MS or LC-MS based workflows.

and sphingolipid deficits in the brain structure in neurodegenerative Alzheimer's disease (AD). Peroxisomal dysfunction in AD leads to glycerophospholipid deficits [23]. Numerous studies have demonstrated that elevations of plasma fatty acids (myristic acid, α-linoleic acid and eicosapentaenoic acids) were associated with the risk of prostate cancer. For instance, Zhou et al., demonstrated that phosphatidylethanolamine, ether-linked phosphatidylethanolamine and ether-linked phosphatidylcholine could be considered as biomarkers for the diagnosis of prostate cancer [24].

Conclusions

In summary, the study of lipidomics will help to understand fundamental biological questions as well to uncover their relevance and contributions to human diseases. Considering the diversity and structural complexity of the "lipidome", it is still challenging to disclose a full picture of the "lipidome". However, with techniques advancing, particularly chromatography separation methods and MS development, the combination of both techniques has been successfully applied in lipidome global profiling or targeted analysis studies, and these approaches have now reached a level to be applied to study clinical sample cohorts on a more robust level to understand lipid based molecular signatures in human diseases.

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