Redox Biology 12 (2017) 946–955

Contents lists available at ScienceDirect

Redox Biology

journal homepage: www.elsevier.com/locate/redox

Review article

Shotgun lipidomics in substantiating lipid peroxidation in redox biology: Methods and applications

Changfeng Hu[a], Miao Wang[b], Xianlin Han[a,b,*]

[a] College of Basic Medical Sciences, Zhejiang Chinese Medical University, Hangzhou, Zhejiang 310053, China
[b] Center for Metabolic Origins of Disease, Sanford Burnham Prebys Medical Discovery Institute, Orlando, FL 32827, USA

ARTICLE INFO

Keywords:
Shotgun lipidomics
Oxidative stress
Lipid peroxidation
Oxidized fatty acids
4-hydroxyalkenal species
Plasmalogen

ABSTRACT

Multi-dimensional mass spectrometry-based shotgun lipidomics (MDMS-SL) has made profound advances for comprehensive analysis of cellular lipids. It represents one of the most powerful tools in analyzing lipids directly from lipid extracts of biological samples. It enables the analysis of nearly 50 lipid classes and thousands of individual lipid species with high accuracy/precision. The redox imbalance causes oxidative stress, resulting in lipid peroxidation, and alterations in lipid metabolism and homeostasis. Some lipid classes such as oxidized fatty acids, 4-hydroxyalkenal species, and plasmalogen are sensitive to oxidative stress or generated corresponding to redox imbalance. Therefore, accurate assessment of these lipid classes can provide not only the redox states, but also molecular insights into the pathogenesis of diseases. This review focuses on the advances of MDMS-SL in analysis of these lipid classes and molecular species, and summarizes their recent representative applications in biomedical/biological research. We believe that MDMS-SL can make great contributions to redox biology through substantiating the aberrant lipid metabolism, signaling, trafficking, and homeostasis under oxidative stress-related condition.

1. Introduction

1.1. Lipid, lipidomics, and multi-dimensional mass spectrometry-based shotgun lipidomics

Lipids involve numerous biological processes and play many crucial roles in cellular functions, including cellular barriers, signaling, energy storage, and growth and survival. Therefore, perturbations in lipid homeostasis are closely associated to diverse phenotypes and disease states, such as obesity, diabetes, cancer, neurodegenerative disorders, and autoimmune diseases [1–5]. It is clear that investigation of lipid alterations can make great contributions to elucidate disease pathogenesis and discover potential biomarkers for early diagnosis of diseases and drug efficacy.

However, cellular lipids are highly diverse and complex. These lipids consist of different polar head groups, backbones, and various aliphatic chains which connect to backbones in different linkages. The aliphatic chains are different in length (i.e., different numbers of carbon atoms), different degrees of unsaturation, different locations of double bonds, and potential branches, etc [6]. It is predicted that tens of thousands to hundreds of thousands possible lipid species exist in cellular lipidome at the levels of amol/mg to nmol/mg of protein [7,8]. Moreover, many new lipid species are continually being discovered [9].

In addition to their diversities in chemical structures, lipids are also highly dynamic. Lipid molecular species and compositions are varied from species, cell types, cellular organelles, and subcellular membrane, leaflets of membrane bilayers, and membrane microdomains (i.e., rafts) [10]. They are dynamically changing with life cycle, environmental conditions, or pathological perturbation [11–13]. Furthermore, their metabolism is interwoven via numerous pathways and networks [14].

After genomics and proteomics, lipidomics was also coined in early 2000 as a disciplinary field to investigate all lipids in a large scale and at the levels of intact molecular species [15,16]. It has been demonstrated that lipidomics analysis serves as a powerful tool for understanding the biochemical mechanisms underlying lipid-related disease processes through quantifying the changes of individual lipid classes,

Abbreviations: AMPP, N-(4-amiomethylphenyl)pyridinium; aPC, plasmanylcholine; aPE, plasmanylethanolamine; dPC, phosphatidylcholine; dPE, phosphatidylethanolamine; DEGs, differently expressed genes; EETs, epoxyeicosatrienoic acids; 4-HDDE, 4-hydroxy-2*E*,6*Z*-dodecadienal; HDL, high-density lipoprotein; 4-HDTE, 4-hydroxy-dodecatrienal; HETE, hydroxyeicosatetraenoic acid; 4-HHE, 4-hydroxy-2*E*-hexenal; 4-HNDE, 4-hydroxy-nondienal; 4-HNE, 4-hydroxy-2*E*-nonenal; HPLC, high performance liquid chromatography; MDMS-SL, multi-dimensional MS-based shotgun lipidomics; MS, mass spectrometry; Ox-HDL, oxidized HDL; PG, phosphatidylglycerol; PLA_2, phospholipase A_2; pPC, plasmenylcholine; pPE, plasmenylethanolamine; PS, phosphatidylserine; PUFAs, polyunsaturated fatty acids; ROS, reactive oxygen species; SLE, systemic lupus erythematosus; TAG, triglycerides

* Corresponding author at: College of Basic Medical Sciences, Zhejiang Chinese Medical University, Hangzhou, Zhejiang 310053, China.
E-mail address: xhan@sbpdiscovery.org (X. Han).

http://dx.doi.org/10.1016/j.redox.2017.04.030
Received 24 March 2017; Received in revised form 10 April 2017; Accepted 23 April 2017
Available online 24 April 2017

subclasses and molecular species and identifying the altered pathways and networks underlying changed lipid classes, subclasses, and molecular species [14]. In recent years, lipidomics has made great advances due to the rapid development in novel analysis strategies and approaches [17], and new instruments and techniques of MS [18].

Depending on whether LC separation is coupled to a mass spectrometer, MS-based lipidomics can be classified into two major categories, i.e., LC-MS-based and direct infusion-based lipidomics. The latter one is usually termed shotgun lipidomics. Based on the unique features and the mass spectrometers employed, at least three different approaches of shotgun lipidomics, including tandem MS-based, high mass accuracy MS-based, and multi-dimensional MS-based (MDMS-SL), have been developed and well documented in the literature [6,19].

MDMS-SL, which maximally exploits the unique chemical and physical properties inherent in discrete lipid classes enabling for analysis of very low-abundance levels, overcomes the majority of the limitations of other shotgun lipidomics approaches and has many significant advantages [6,19]. At its current development, this platform allows the researchers to analyze nearly 50 lipid classes and thousands of individual lipid species with > 90% accuracy [17], including identification of fatty acyl positional isomers [20] and fatty acid isomers [21] of the species. The typical workflow of MDMS-SL analysis of biological samples is schematically illustrated in Fig. 1.

1.2. Overview of conventional methodology for analysis of lipids indicative of oxidative stress

Oxidative stress arises due to redox imbalance between the oxidative and anti-oxidative systems of cells and/or tissues. It results in the over productions of oxidative free radical and reactive oxygen species (ROS), which could attack cellular proteins, lipids, and nucleic acids leading to cellular dysfunction [22]. A large number of studies have demonstrated that oxidative stress is tightly associated with many diseases [22–26]. The changed levels of a variety of lipids are indicative of oxidative stress. For example, 4-hydroxyalkenal species and eicosanoids are lipid peroxidation products generated through complex enzymatic and nonenzymatic reactions [27,28], and plasmalogens serve as one of endogenous antioxidants [29]. Many methods have been developed to measure the levels of these classes of lipids present in biological fluid and tissue samples, including immunoassay methods [30], separation-based MS methods (i.e., GC-MS, normal phase LC-MS, and reversed phase LC-MS) [31], and shotgun lipidomics [17]. All of these methods have advantages and limitations. Enzyme-linked immunosorbent assays are popular and simple to perform and accessible to most of laboratories, but the questionable specificity when so many isomers can cross-react with the various antibodies has limited their utilities [32]. HPLC methods coupled with UV detection are only useful for analysis of relatively high abundance metabolites [33]. More attention should be paid to uniform derivatization and interfering substances when using derivatization coupled with fluorescence detection [34]. Although MS-based approaches are sometimes complex and require sample preparation involving extraction and purification, they are the “gold standard” methods for allowing researchers to measure all of the different species [35–37]. Compared with shotgun lipidomics, separation-based MS methods need more internal standards [38]. Chromatographic separation leads to the differential elusion of internal standard and analytes, whereas co-elution of an analyte with its standard is very important to compensate for matrix effects and varying ionization efficiencies during gradient elution [38]. Both 4-hydroxyalkenal species and eicosanoids are instable and would decompose during the procedure of chromatographic separation. In addition, separation-based MS methods are generally time-consuming, which is not suitable for larger sample profiling [39].

In last few years, MDMS-SL for analysis of these oxidative stress-related lipid species has been developed rapidly. Although a serial of reviews on shotgun lipidomics have been published [6,14,17], they have different focuses. In this review, we first introduce the principles of MDMS-SL for 4-hydroxyalkenal species, eicosanoids, and plasmalogens, and then summarize their recent representative applications under different disease states. Finally, we discuss the advantages of MDMS-SL for analysis of these oxidative-stress-related lipids and how to further explore the methods in future work.

2. Quantitative analysis of lipid molecular species associated with redox biology by shotgun lipidomics

2.1. Shotgun lipidomics of 4-hydroxyalkenal species

Among the cellular components, phospholipids, which usually contain high levels of polyunsaturated fatty acids (PUFAs), are the most sensitive to be attacked by ROS induced by oxidative stress [40]. 4-Hydroxyalkenal species, a class of α, β-unsaturated aldehyde, are considered to be one of the most reactive electrophilic end-products of lipid peroxidation generated from PUFAs. Therefore, the levels of 4-hydroxyalkenal species are the indicator of the oxidative stress of a biological system. 4-Hydroxyalkenal species are highly reactive due to the presence of three reactive functional groups in the chemical structure: a carbonyl group on C1, a conjugated double bond (alkene) between C2 and C3, and a hydroxyl group on C4 (Fig. 2). These groups make the 4-hydroxyalkenal species highly reactive toward nucleophilic thiol and amino groups, and can readily form covalent adducts with various cellular (macro)molecules (e.g., lipids, proteins, and nucleic acids). The interactions could lead to inhibition of protein and DNA synthesis, dysregulation of enzyme activities, alteration in mitochon-

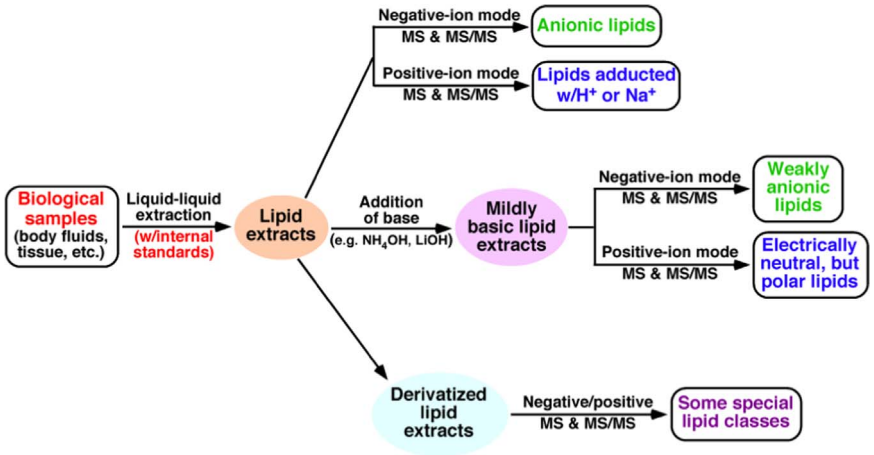

Fig. 1. Schematic illustration of the workflow of MDMS-SL for analysis of cellular lipidomes directly from crude extracts of biological samples [20,21].