Biochemical and Biophysical Research Communications xxx (2018) 1–6

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Applications of mass spectrometry-based targeted and non-targeted lipidomics

Hyeon-Cheol Lee*, Takehiko Yokomizo**

Department of Biochemistry, Juntendo University School of Medicine, Tokyo, Japan

ARTICLE INFO

Article history:
Received 9 March 2018
Accepted 10 March 2018
Available online xxx

Keywords:
Lipidomics
Targeted lipidomics
Non-targeted lipidomics
Mass spectrometry

ABSTRACT

Recent advances in mass spectrometry have expanded our knowledge of lipids and lipid metabolic pathways involved in many (patho)physiological events. Targeted and non-targeted lipidomics are powerful analytical strategies with distinct features, and a combination of these two approaches is often employed to maximize the coverage of lipid species detected and quantified in complex biological matrices. This review briefly summarizes the applications of targeted and non-targeted lipidomics, mainly focusing on electrospray ionization–liquid chromatography–tandem mass spectrometry (ESI–LC–MS/MS), along with recent technical advances in the field. Current limitations and challenges in lipidomics and possible solutions are also discussed.

1. Introduction

Lipids play fundamental and pivotal roles in a variety of (patho) physiological events by acting as constituents of biomembranes, sources of energy, and signaling molecules, which are often called lipid mediators. Lipids are classified into many classes (e.g., phospholipids, glycerolipids, sphingolipids, and sterols). Most lipid classes comprise a number of molecular species that vary in terms of their fatty acyl composition and stereochemical properties. These diversities in lipid molecular species and their homeostasis are implicated in a variety of pathological conditions [1–9]. Thus, analytical methods that enable lipid profiling of biological samples are indispensable for conducting lipid research.

Lipidomics, a subcategory of metabolomics, is such a strategy for profiling lipids and other lipophilic compounds present in biological samples. This field of study emerged as a result of technological advances in analytical tools such as mass spectrometry (MS), nuclear magnetic resonance (NMR), and high-performance liquid chromatography (HPLC). Targeted and non-targeted (also called untargeted) lipidomics are two approaches with distinct features, advantages, and disadvantages. One or both approaches are employed depending on the target lipids and experimental designs. This review briefly summarizes the technical and methodological features of these two approaches and their applications. Current technical limitations and challenges that researchers may face in lipidomics studies are also discussed. This review mainly focuses on lipidomics approaches that use electrospray ionization-liquid chromatography-tandem mass spectrometry (ESI-LC-MS/MS), one of the most commonly employed platforms for lipidomics analysis. Lipidomics using NMR has been reviewed elsewhere and is not a focus of the present review [10–12].

2. Targeted lipidomics

Targeted lipidomics is employed when researchers wish to study specific target lipids or lipid classes. Multiple reaction monitoring (MRM, also known as selected reaction monitoring (SRM)) employing a triple-quadrupole mass spectrometer is one of the most widely used platforms for targeted analysis because it provides high sensitivity, selectivity, and a wide dynamic range [13]. Ionized lipids with specific mass-to-charge ratios (m/z) (precursor ions) are isolated at the first quadrupole (Q1). Thereafter, ions that produce specific fragment ions (product ions) are re-selected at Q3 after fragmentation at Q2 by collision-induced dissociation (CID). Targeted lipidomics thus measures specific lipids of interest by selecting predefined pairs of precursor and product ions.

* Corresponding author.
** Corresponding author.
E-mail addresses: h-lee@juntendo.ac.jp (H.-C. Lee), yokomizo-tky@umin.ac.jp (T. Yokomizo).

https://doi.org/10.1016/j.bbrc.2018.03.081
0006-291X/

2.1. Lipid classes with characteristic fragmentation patterns

Lipid classes with characteristic fragmentation patterns are suitable for targeted analysis. For example, lipids containing phosphocholine (e.g., phosphatidylcholines and sphingomyelins) efficiently yield phosphocholine ions with *m/z* 184 as a major product ion in positive ion mode. Standard ceramides with a d18:1 long-chain base produce a characteristic ion with *m/z* 264. In negative ion mode, decomposition of phospholipids yields carboxylate ions of fatty acids liberated from the glycerol backbone, which can be used to determine the fatty acyl compositions of the precursor ions. Although free fatty acids (FFAs) generate product ions via neutral loss of H_2O and CO_2, the fragmentation efficiency seems to vary according to their fatty acyl chain length and degree of unsaturation [14]. Consequently, these product ions cannot be used to quantify some FFA species by MRM analysis. Instead, an excellent derivatization method for quantification of FFAs has been developed, as mentioned below [15]. Murphy and Axelsen wrote a detailed review on the mechanisms underlying the formation of product ions from major lipid classes, including those described above [14].

2.2. Low abundance lipid signaling molecules

The detection and quantification of low abundance lipids require high sensitivity, and consequently such lipids are usually investigated via targeted analysis. Polyunsaturated fatty acid (PUFA)-derived oxygenated metabolites, such as the bioactive lipid mediators eicosanoids (prostaglandins, leukotrienes, etc.), comprise hundreds or possibly thousands of molecular species that differ in terms of the numbers and positions of hydroxyl groups and double bonds [16–18]. These structural features result in an inherent fragmentation pattern that can be used to selectively measure individual lipid species [19–21]. Quantification of such low abundance lipids often requires the removal of abundant lipids by solid-phase extraction to avoid ion suppression. In most cases, other classes of lipid mediators, such as lysophospholipids, sphingosine 1-phosphate (S1P), and platelet-activating factor (PAF), are also measured in targeted analysis. A number of lipid mediators are implicated in diseases, and the levels of these mediators can be potentially used as biomarkers in clinical settings. Consequently, precise quantification of lipid mediators in biofluids and tissues by targeted lipidomics is an essential component of lipid research [22–26].

2.3. Derivatization of lipids

Derivatization methods are often employed for quantification of lipid classes that are difficult to ionize. In general, derivatization reagents enhance the ionization efficiency of the original lipids and form a good leaving group that is easily and sensitively detected by tandem mass spectrometry (MS/MS). Bollinger et al. developed a derivatization method for eicosanoids using *N*-(4-aminomethylphenyl)pyridinium (AMPP), which improved the sensitivity of detection by 10–20-fold compared with their original method [27]. The same derivatization method was also applied to quantify FFAs [15]. Although steroids can be quantified by ESI-LC-MS/MS without derivatization [28], derivatization reagents such as dansyl chloride and 2-hydrazino-4-(trifluoromethyl)-pyrimidine are frequently used [29,30]. Derivatization methods are also useful when the original lipids of interest are unstable. One such example is 4-hydroxyalkenal species, which are peroxidative products of PUFAs and sensitive oxidative stress markers [31,32]. It is challenging to quantify these aldehyde species because they are highly reactive and prone to form covalent adducts with other nucleophilic compounds. Consequently, a number of derivatization methods useful for MS analysis of 4-hydroxyalkenal species and other fatty aldehydes have been developed [33,34]. Phosphoinositides are low abundance membrane signaling lipids that play various roles in cells by recruiting their binding proteins to specific membrane compartments and organelle membranes where these phosphoinositides are synthesized [3–5,35]. Methylation of the phosphate groups of phosphoinositides by trimethylsilyl diazomethane enhances their stability and the sensitivity of their detection, which enables quantification of different fatty acyl species in this class of lipids [36–39]. Derivatization methods for shotgun lipidomics have been summarized elsewhere [40].

3. Non-targeted lipidomics

In contrast with targeted analysis, non-targeted (or untargeted) lipidomics analysis provides a plethora of metabolic features in a single run. A high-resolution MS (HRMS) platform is required to determine the exact mass and thereby discriminate each lipid species from its isobaric compounds. Quadrupole–time-of-flight (QTOF), Orbitrap, and Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometers are used in many cases. MS is often coupled with ultra-high-performance liquid chromatography (UHPLC) to separate isobaric and isomeric lipids present within complex biological matrices. The data-processing step includes noise filtering, peak picking and alignment, and normalization. A number of data-processing software packages with distinct features and algorithms have been developed and are available for MS users [12,41]. After data acquisition and processing, multivariate analysis methods, such as principal component analysis (PCA), partial least squares-discriminant analysis (PLS-DA), and orthogonal PLS-DA (OPLS-DA), are often employed to extract biologically relevant metabolic features [42].

Identification of metabolites is the most challenging step in non-targeted lipidomics. In general, information acquired from MS and/or MS/MS datasets (e.g., *m/z* values, fragmentation patterns, and retention times) is collated with internal or external databases to annotate individual metabolites [12,43]. A single or multiple candidate metabolite(s) is assigned to each metabolite when metabolites with the corresponding features are present in the databases.

3.1. Screening of biomarkers

Non-targeted lipidomics is a promising strategy to discover lipid biomarkers in biological samples. A study that discovered Fatty Acid Esters of Hydroxy Fatty Acids (FAHFA) is an excellent example of lipid biomarker discovery. FAHFA is a recently identified class of lipids that was discovered in a non-targeted lipidomics study aiming to screen metabolites with beneficial effects against type 2 diabetes [44]. The authors discovered four lipid molecules, which were later characterized as FAHFA, whose levels were strikingly elevated in the serum and tissues of mice overexpressing the Glut4 glucose transporter in adipocytes. Such non-targeted analysis is often accompanied by follow-up targeted analysis to validate the findings and to identify other cognate molecular species because only abundant species can generally be detected by non-targeted analysis. In the case of FAHFA, subsequent targeted analysis identified 16 FAHFA molecular species in mouse serum. Furthermore, FAHFA levels were reduced in insulin-resistant humans, and FAHFA species showed anti-diabetic effects *in vivo*. Non-targeted lipidomics is a powerful approach to discover not only lipids that can be used as diagnostic biomarkers, but also those that are therapeutically effective against diseases or are potential drug targets.

Please cite this article in press as: H.-C. Lee, T. Yokomizo, Applications of mass spectrometry-based targeted and non-targeted lipidomics, Biochemical and Biophysical Research Communications (2018), https://doi.org/10.1016/j.bbrc.2018.03.081