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Review

Current state-of-the-art of nontargeted metabolomics based on liquid chromatography–mass spectrometry with special emphasis in clinical applications☆

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ABSTRACT

Metabolomics, as a part of systems biology, has been widely applied in different fields of life science by studying the endogenous metabolites. The development and applications of liquid chromatography (LC) coupled with high resolution mass spectrometry (MS) greatly improve the achievable data quality in non-targeted metabolic profiling. However, there are still some emerging challenges to be covered in LC–MS based metabolomics. Here, recent approaches about sample collection and preparation, instrumental analysis, and data handling of LC–MS based metabolomics are summarized, especially in the analysis of clinical samples. Emphasis is put on the improvement of analytical techniques including the combination of different LC columns, isotope coded derivatization methods, pseudo-targeted LC–MS method, new data analysis algorithms and structural identification of important metabolites.

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Contents

☆ Invited paper for the Honor Issue of Professor Peichang Lu's 90th birthday.

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1. Introduction

Metabolomics has been recognized as an important platform of systems biology in the post genomics era [1–3]. The integration of the data from metabolomics and other -omics such as genomics, transcriptomics and proteomics can be applied to the construction of molecular networks, which may help to understand the complex biochemical processes in a better way [4,5]. Non-targeted metabolic profiling is a commonly used strategy for holistic analysis of endogenous metabolites, and provides metabolic information for phenotyping, or the understanding of physiological or pathophysiological mechanisms [3,4,6–9]. Non-targeted metabolomics is commonly considered as a hypothesis free survey, which is an important and powerful tool for many biological related studies, e.g. on the exploration of functional compounds or discovery of biomarkers [10–13]. It attempts to measure all endogenous metabolites in the biological samples. However, this seems to be an impossible mission to complete due to the complexity of the metabolome. More than 40,000 metabolites which cover a broad diversity in physical and chemical properties can exist in biological samples according to the data from the human metabolome database [14]. Also the metabolite concentrations cover more than 12 orders of magnitudes. In contrast, the best linear range of current analytical instruments is only about four to five orders of magnitudes. Therefore, analytical platform is still a challenge for scientists in this field.

High throughput and sensitive instruments such as nuclear magnetic resonance (NMR) and mass spectrometry (MS) are commonly used analytical tools for metabolomics [15–18]. As can be seen from the Web of Science (Fig. 1), MS is the dominant analytical platform in metabolomics because of its high sensitivity and high resolution [19]. Separation techniques such as chromatography improve the analytical performance of MS by spreading the complexity of the sample over time and by reducing the matrix effects of biological samples. As can be observed from Fig. 1, liquid chromatography–MS (LC–MS) method has become the most applied chromatography–MS tool because it can be used to achieve rich metabolome information [20–22] with minimum sample pretreatment required.

A LC–MS based metabolomics study commonly consists of several steps including study design, sample collection and preparation, instrument analysis, data handling and biological explanation [23]. In this review we will focus on the latest progress and novel approaches in LC–MS based nontargeted metabolomics for clinical applications mainly in the last 4–5 years. Emphasis is set on techniques improving the achievable metabolite coverage and data quality as well as on novel algorithms for data analysis and metabolite identification.

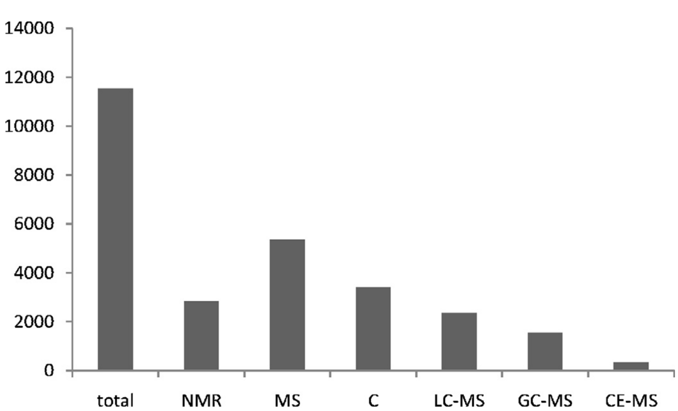

Fig. 1. The metabolomics paper number searched in the Web of Science™ on May 27, 2014. The used keywords are as follows: (1) Total: metabolomics OR metabonomics OR "metabolic profiling" OR lipidome OR "lipidomic profiling" OR metabolome OR metabonome; on the basis of search 1, searches 2–7 were carried out by using "AND" with following keywords. (2) NMR: NMR; (3) MS: MS OR "mass spectrometry"; (4) C: chromatography; (5) LC–MS: LC–MS OR UPLC–MS OR ("liquid chromatography" AND "mass spectrometry"); (6) GC–MS OR ("gas chromatography" AND "mass spectrometry"); and (7) CE–MS OR ("capillary electrophoresis" AND "mass spectrometry").

2. Samples preparation for LC–MS based metabolomics

The collection of biological samples is a critical step for the data quality of metabolic profiling. Standard operation procedures (SOP) are important to ensure the quality of samples, especially in the case of clinical samples [24]. According to metabolic profiling studies of the preanalytical steps on the collection of blood, it was not surprising to observe that many factors affect the quality of biological samples including the typical collection tubes used, the addition of anticoagulants, the occurrence of hemolysis and other operations during collection [24–26]. Moreover, the transportation and storage of samples could also influence the outcomes of metabolomics analysis [27]. The quality of biological samples is very critical in large scale metabolomics studies, especially when samples are collected from different clinical centers. Metabolite biomarkers can be used for checking the quality of samples, for instance, serotonin, sphingosine 1 phosphate and lysophosphatidylcholines [24,26,27] have been reported as significantly affected biomarkers for less standardized collection.

Generally, a practical protocol of metabolic profiling analysis includes minimal sample preparation to ensure 'unbiased' analysis and better repeatability [23]. Homogenization and quenching are necessary steps for the preparation of tissues and cell lines. For the extraction of metabolites, liquid–liquid extraction (LLE) and solid phase extraction (SPE) are commonly used methods in metabolomics. Organic solvent such as acetonitrile, methanol, isopropanol, chloroform or their mixture is the most commonly used for the sample preparation, including quenching, extraction of metabolites and protein precipitation [28].

Currently, there are different preparation protocols for the analysis of polar metabolites [29–32], lipids [33,34], and other metabolite species using different extraction solvent systems. 75–80% methanol (3:1–4:1, methanol/plasma or serum) is a classic protocol for the extraction of polar metabolites and protein precipitation [35–37]. Bruce et al. suggested two proportions of organic solvents for an optimal extraction of metabolites, and a ratio of 4:1 (solvent/plasma) for protein precipitation [38]. Lipids are commonly extracted by a solvent system of chloroform–methanol–aqueous [39–41]. However, the common approaches for sample preparation cannot extract all the metabolite species due to their polarity, hydrophobicity and so on. To achieve a wide coverage of the metabolome on multiple analytical platforms, several specific extraction procedures were executed [42,43], polar, apolar and hydrophilic metabolites could be extracted simultaneously and separated through different LC systems. Such strategies simplify the procedure of sample preparation and improve the utility of biological samples. To deactivate metabolism, quenching is particularly critical in the analysis of cells and tissues [34,35], cold solvent addition [44], freezing in liquid nitrogen, fast heating and addition of enzyme inhibitors are often utilized in quenching.

The challenges of sample preparation for metabolomics include efficiency, reproducibility, and coverage, etc. [44]. Meanwhile, clinical samples are not always sufficient or even limited, e.g. tissue samples collected from needle biopsy. Therefore, an effective preparation method using a small amount of sample is of great importance for the combined use of multiple analytical platforms in metabolomics. A new protocol to extract metabolome and lipids simultaneously in a small amount of tissue samples was developed

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