Mass Spectrometry-based Metabolomics in Translational Research

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Introduction

The metabolome represents the collection of all metabolites in a cell and includes all biomolecules, except for the genome, transcriptome, and proteome, and metals. Conventionally, low molecular weight biomolecules (<1500 Da) involved in endogenous metabolism are called metabolites. They play important roles as energy sources, signaling molecules, and metabolic intermediates in complex biological systems. Metabolite levels provide collective information on biomedical states and an instantaneous snapshot of biological responses caused by genetic and environmental perturbations. Metabolite signals result from the interplay of biochemical reactions across the genome, transcriptome, and proteome, and serve as biological modulators across multilayer omics to maintain cellular homeostasis (Yugi and Kuroda 2018). Thus, the metabolome is crucial to understand biological responses to diseases, and genetic and environmental changes.

Metabolites and their related features have been used as diagnostic markers since ancient times. Ancient people monitored the characteristic odor of an individual's breath in eastern traditional medicine for thousands of years. Recently, the sweet odor of a diabetic patient's breath was shown to be caused by the presence of acetone, which is related to high blood glucose level, the gold standard currently used in the diagnosis of diabetes (Wang and Wang 2013). In the Middle Ages, a urine chart was used to link the color, smell, and taste of urine to various medical conditions (Nicholson and Lindon 2008). Such features are based on the chemical patterns in response to biological conditions, and metabolites can represent these biochemical patterns with quantifiable data. Metabolites can define the molecular

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J. K. Kim et al. (eds.), *Advanced Imaging and Bio Techniques for Convergence Science*, Advances in Experimental Medicine and Biology,
https://doi.org/10.1007/978-981-33-6064-8_19

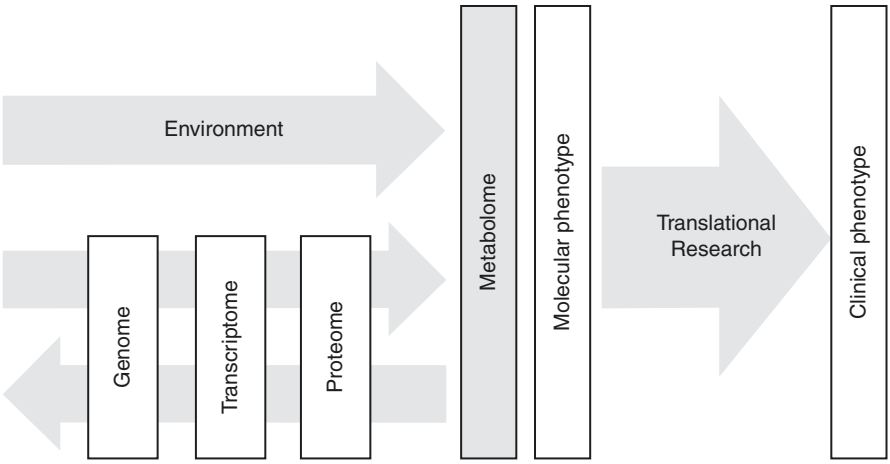

Fig. 1 Metabolomics provides the integrated information caused by complex biochemical reactions and environment, thus plays an important role in translational research

phenotypes of living organisms and be directly associated with the biological outcomes of diseases. In addition, the concentrations of metabolites are often highly correlated with biochemically related genetic variations (Gieger et al. 2008; Fendt et al. 2010; Bondia-Pons et al. 2011; Suhre et al. 2011a, b; Vander Heiden 2011; Jain et al. 2012). These characteristics allow metabolome alterations to be easily translated into disease states and can help us understand the pathophysiological mechanisms that contribute to various biomedical symptoms. Thus, translational research has included the metabolome and metabolomics (Fig. 1).

Metabolomics involves the systematic identification and quantification of metabolites. The physicochemical properties of metabolites vary widely, including in their polarity, acidity, and volatility. In addition, most metabolites consist of four to five atoms, including carbon, nitrogen, oxygen, hydrogen, and phosphorous, and many metabolites have the same elemental composition with slight variations in chemical bonds. Thus, the identification and quantification of metabolites are both difficult and challenging. Metabolomics has been implemented with various analytical platforms to identify and quantify diverse intra- and extracellular metabolites.

Methods for metabolite analysis include nuclear magnetic resonance (NMR) spectroscopy, mass spectrometry (MS), Fourier transform-infrared (FT-IR) spectroscopy, high performance liquid chromatography (HPLC), gas chromatography (GC), Raman spectroscopy, and other analytical platforms (Fig. 2) (Bogdanov and Smith 2005; Want et al. 2005; Villas-Boas et al. 2005; Boskey and Mendelsohn 2005; Defernez and Colquhoun 2003; Deleris and Petibois 2003). FT-IR and Raman spectroscopies are nondestructive and rapid techniques that can analyze various types of biological samples. Absorption spectra at specific wavelengths provide important clues crucial for determining the structure of unknown metabolic features, whereas the peak area under the curve of absorption spectra is used for quantitation. The sensitivity and selectivity of FT-IR and Raman spectroscopy, however,

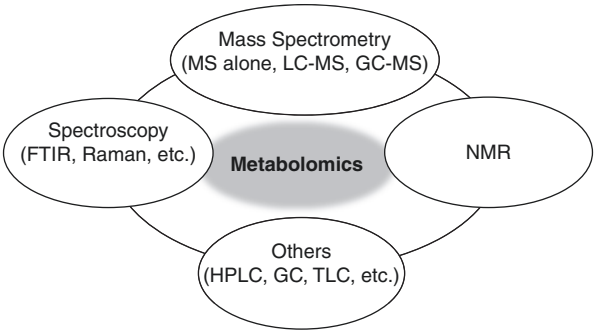

Fig. 2 Analytical instrumentations for metabolomics should be able to detect various kinds of metabolites present in biological systems

are not as high as those of other methods (Boskey and Mendelsohn 2005; Deleris and Petibois 2003). The detection methods used in HPLC and GC, such as UV absorption, electrochemical detection, and flame ionization, are non-selective, thus complete separation of metabolites is crucial in accurate quantitation of metabolites, especially from complex biological samples (Gathungu et al. 2014; Keyfi and Varasteh 2016; Richins et al. 2018). NMR spectroscopy, another rapid and nondestructive analytical method, requires negligible sample preparation. Chemical shifts dependent on the nucleus' chemical environment can be used to identify metabolites. NMR spectroscopy, however, is relatively insensitive, and millimolar to high micromolar concentrations are often required. Thus, only the most abundant metabolites are detected (Emwas 2015). Because of its high sensitivity and broad dynamic range, MS has become the most suitable analytical tool in metabolomics research (Johnson et al. 2016). MS can also detect various types of metabolites because mass is a universal property. Selectivity among isobaric (identical masses) metabolites can be ensured by various MS/MS techniques and by combination with separation modules such as GC or LC (Metz et al. 2007; Lei et al. 2011; Xiao et al. 2012; Fiehn 2016). MS-based metabolomics platforms have become more popular than NMR-based platforms in recent research (Fig. 3). Thus, this chapter will emphasize MS-based metabolomics.

Analytical Platforms for Mass Spectrometry-based Metabolomics

MS is an analytical method that measures charged molecules based on their mass-to-charge ratios. The signal intensities of charged metabolites reflect the amounts of them present in biological samples. The mass analyzer, a primary component of the MS system, separates molecules based on their mass to charge ratios. Several types of mass analyzers are currently available, including quadrupole, time of flight (TOF), ion-trap, orbitrap, and Fourier transform-ion cyclotron resonance (FT-ICR). High resolution mass spectrometry (HRMS) methods, such as TOF, orbitrap, and

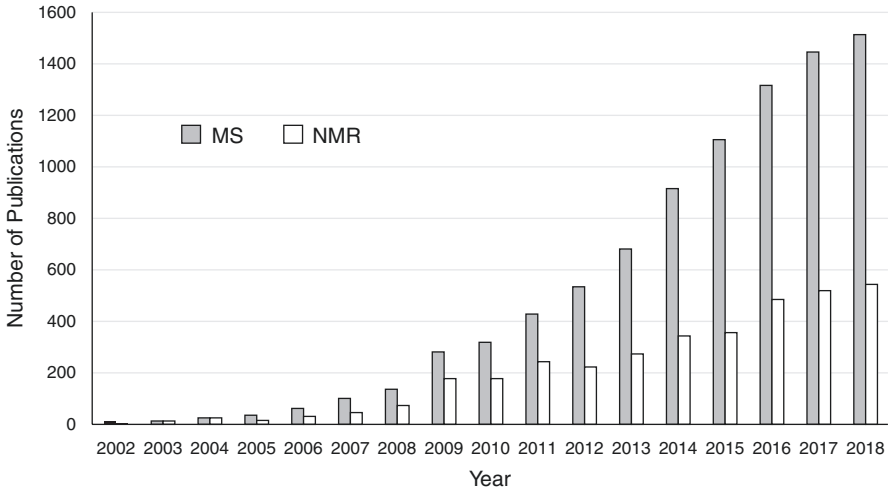

Fig. 3 Publications related to MS- or NMR-based metabolomics. PubMed search was performed with keywords of (MS and metabolomics) or (NMR and metabolomics)

FT-ICR, measure the exact masses of charged molecules. The exact mass is the theoretical mass of specific isotopic composition of a charged molecule (Murray et al. 2013). By contrast, low resolution mass spectrometry methods, such as quadrupole and ion-trap, measure the nominal mass, defined by the integer mass of the most abundant stable isotope of a molecular ion (Murray et al. 2013).

Various tandem mass spectrometric techniques (MS/MS) have been used to generate unique fragmentation patterns from different metabolites, which enables the specific characterization of unknown metabolites or differentiation among isobaric metabolites (Ceglarek et al. 2009). Upon collision with a neutral gas or interaction with activated electrons, any specific metabolite isolated from a mass analyzer produces characteristic fragment ions which are measured by a second mass analyzer. Sequential fragmentation (MS^n) can be performed as needed. Several MS/MS methods are available for fragmenting molecular ions for tandem mass spectrometry (Fig. 4). Collision induced dissociation (CID) is the most common MS/MS technique, and fragment ions are generated from collision with neutral gases (Xiao et al. 2012; Wang et al. 2008). By contrast, electron capture dissociation (ECD), electron-induced dissociation (EID), and electron transfer dissociation (ETD) are electron-based MS/MS methods, and their fragmentation patterns are normally different from those generated by CID (Johnson et al. 2016; Ongay et al. 2013; Yoo et al. 2007, 2011). The structural information collected from different MS/MS methods is often complementary and crucial in revealing the identities of unknown metabolites (Yoo et al. 2007; Liang et al. 2007; Liu et al. 2008).

Chromatography-based separation modules are often combined with MS systems in MS-based metabolomics. Liquid chromatography-mass spectrometry (LC-MS) and gas chromatography-mass spectrometry (GC-MS) have several advantages over MS alone. The matrix effect and ion suppression, caused by salts, ion pairing

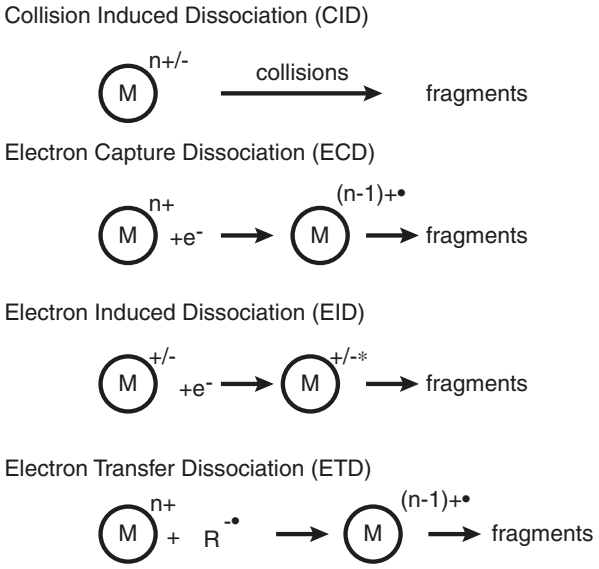

Fig. 4 Various tandem mass spectrometric techniques used for the structural characterization of metabolites

Table 1 Comparison of GC-MS and LC-MS based metabolomics

	GC-MS based metabolomics	LC-MS based metabolomics
Metabolites	Relatively nonpolar, small metabolites	Wide range of metabolites
Chemical derivatization	Generally necessary	Generally not necessary
Total run time	Longer run time (generally >1 h)	Shorter run time (generally 10–40 min)
Retention time drift	Rarely observed	Often observed
Ion source	EI (Electron impact)	ESI (Electrospray ionization)
Metabolite identification	Mass fingerprint generated from one metabolite is used	Molecular ion and/or its specific fragment ions during MS/MS (CID) are used
Dynamic range for quantitation	Shorter dynamic range (~10^2)	Wide dynamic range (~$10^{3–4}$)

agents, and any other interfering endogenous compounds in a complex biological matrix, can be alleviated by appropriate chromatographic separation among analytes and interfering compounds. In addition, metabolites in biological samples can be quantitated by measuring their chromatographic peak areas. GC-MS has been utilized to profile relatively more volatile and lower molecular weight metabolites, and chemical derivatization of metabolites is commonly required (Fiehn 2016; Papadimitropoulos et al. 2018; Garcia and Barbas 2011). By contrast, metabolites being analyzed by LC-MS are generally not subjected to chemical derivatization, and LC-MS has been applied to various types of metabolites with broad ranges of physicochemical properties and molecular weights (Table 1) (Zhou et al. 2012;

Lu et al. 2008; Theodoridis and Wilson 2008). Capillary electrophoresis (CE) can be used with MS, especially for highly polar metabolites (Ramautar et al. 2009, 2017; Zhang et al. 2017).

An ion source is a device that produces molecular ions in a mass spectrometer. Commonly used ion sources in metabolomics are electrospray ionization (ESI), electron impact ionization (EI), and desorption electrospray ionization (DESI). ESI, which links LC to MS, uses high temperature to evaporate solvents and high voltage to generate charged molecular ions from metabolites. ESI is a soft ionization technique and produces molecular ions with very little fragmentation, which is advantageous for database searches to identify unknown metabolites (Ho et al. 2003; Fenn et al. 1989; Yamashita and John 1984). EI is a harsh ionization technique in which high energy electrons (typically 70 eV) interact with molecules in the gas phase, and extensive fragmentation specific to each metabolite is generated. EI is used as an ion source in GC-MS, and is generally useful for small organic molecules (<600 Da) (Mark and Dunn 2013). DESI is a recently developed ionization technique, in which a fast-moving charged solvent stream extracts metabolites from the surfaces of samples (Takats et al. 2004). Like matrix-assisted laser desorption ionization (MALDI) and secondary ion mass spectrometry (SIMS), the DESI source is useful in revealing the location of selected metabolites on tissue samples (Petras et al. 2017). These techniques have allowed spatial localization and visualization of molecular distribution of target molecules (Fessenden 2016; Claude et al. 2017; Sun et al. 2019).

Metabolomics can be performed using two different approaches (Table 2). Nontargeted metabolomics can be performed to identify as many metabolites as possible in a biological sample, with the results generating novel hypotheses and/or metabolite targets. By contrast, targeted metabolomics can provide quantitative information for target metabolites involved in specific metabolic pathways. Targeted metabolomics is used to answer specific biochemical questions and hypotheses. These two approaches will be discussed in greater detail below.

Table 2 Nontargeted metabolomics and targeted metabolomics

	Nontargeted metabolomics	Targeted metabolomics
Purpose	To generate hypothesis	To prove hypothesis
	To find metabolic features related to disease pathophysiology	To confirm metabolite levels in any disease states
Targets	Not necessary	Necessary
Analytical platform	Universal methods are preferable to observe as many metabolic features as possible	Specific quantification methods are needed to measure target metabolites
Outcomes	Statistically meaningful changes in any metabolic features or metabolic pathways related to disease states or perturbations	Quantitative result of target metabolites for the specific disease states or perturbations
Validation	Necessary	Not necessary, but related biological experiments may be useful to interpret the result

Nontargeted Metabolomics

The purpose of nontargeted metabolomics is to measure the entire metabolome in a biological sample, and to generate a hypothesis. However, it is not possible to measure all constituents of an entire metabolome from a biological sample, because their amounts and physicochemical properties are quite diverse. Thus, the number of metabolites identified in a sample is dependent on sample preparation and analytical instrumentation. Because accurate measurement of mass is critical in identifying unknown metabolites, high resolution MS, such as TOF, orbitrap, or FT-ICR, should be utilized in nontargeted metabolomics. Advanced instrumentation and bioinformatics tools are important in nontargeted metabolomics. The general workflow of global metabolome profiling involves sample preparation, instrumental analysis, data analysis using various bioinformatics tools, and verification including biological interpretation (Fig. 5) (Want et al. 2013; Dunn et al. 2011; Osborn et al. 2013).

Sample preparation is designed to reduce the metabolome pool or sample matrices, based on the physicochemical properties of metabolites. Metabolites from a complex biological sample can be separated into several fractions with similar physicochemical properties. Initially, a cold organic solvent is added to a biological fluid to precipitate large biomolecules, such as the genome and proteome. This is followed by liquid–liquid extraction, which separates a sample solution into two aliquots, one containing hydrophobic and the other containing hydrophilic metabolites. When necessary, solid particulates from biological samples can be eliminated using a membrane filter.

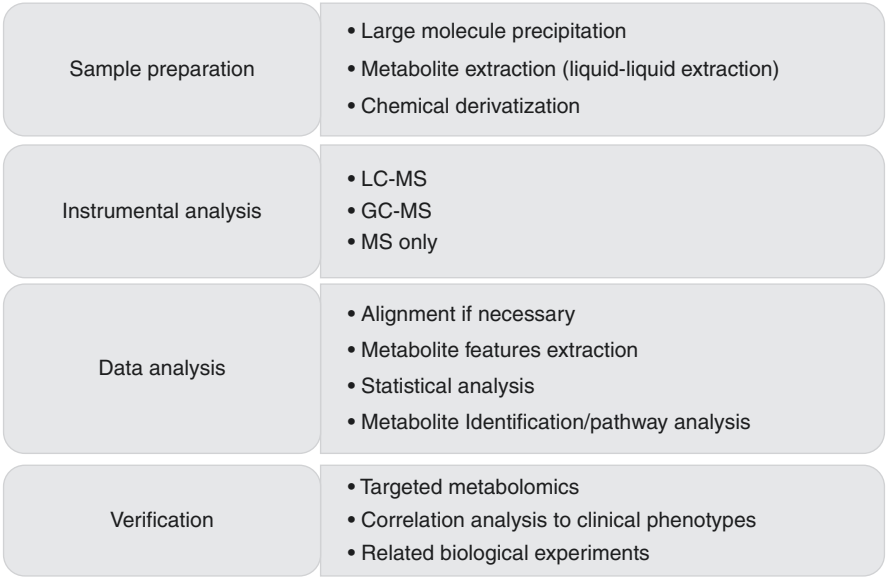

Fig. 5 Workflow of nontargeted metabolomics

Although nontargeted metabolomics using multiple metabolomics platforms may maximize the number of metabolites detected, access to multiple analytical platforms is often difficult. The choice of analytical platforms for nontargeted metabolomics depends on their availability and applications. If LC-MS or CE-MS is available for global metabolome profiling, metabolome profiles can be obtained from both positive and negative ion modes to maximize the number of metabolites observed. Basic and acidic metabolites can be detected better using positive and negative ion mode, respectively.

The data quality of global metabolome profiling is dependent on the types of sample, sample preparation, chromatographic separation, and types of mass analyzer. The data acquired from nontargeted metabolomics include a list of the mass-to-charge ratio (*m/z*) with/without its retention time and signal intensity of each metabolic feature. If GC-MS is used for global metabolome profiling, extensive fragment ion peaks are obtained, rather than a single molecular ion peak of an intact metabolite. A list of metabolite features, consisting of *m/z*, retention time, and peak area or *m/z* and ion intensity, should be generated from the intensive processing of all detected peaks in global metabolome profiling.

The workflow of data processing for global metabolome profiling begins with peak alignment (Fig. 6). Inevitable retention time shifts in chromatograms over runs are often observed and need to be corrected. Two types of peak alignment can be performed, profile-based and feature-based peak alignment (Vandenbogaert et al. 2008; Smith et al. 2015; Katajamaa and Oresic 2007). Profile-based methods align peaks using raw total ion chromatograms (Vahamaa et al. 2011; Tsai et al. 2013a), whereas feature-based methods align peaks after peak detection. Most feature-based alignment methods involve reference peaks as standard features for further retention time correction (Tsai et al. 2013b; Watrous et al. 2017). A typical LC-MS metabolomics analysis can involve spiking a limited number of internal standards (stable isotope-labeled exogenous chemical compounds) to samples (Dunn et al. 2011), and the peaks corresponding to these internal standards are used to align retention times (Li et al. 2016; Ren et al. 2016). In some cases, endogenous metabolites are preferred as reference standards.

To generate the list of metabolite features, consisting of *m/z*, retention time, and peak area or *m/z* and ion intensity, it is necessary to match peaks and group them by their *m/z* and/or retention times across samples. In some cases, data may be missing because low signal intensities are below the limit of detection. Most bioinformatics

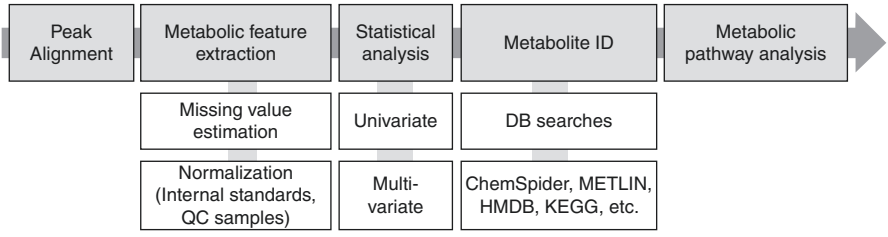

Fig. 6 Workflow of data processing in nontargeted metabolomics

tools used for global metabolomics provide several options to deal with missing values (Katajamaa et al. 2006; Smith et al. 2006; Xia and Wishart 2011; Chong et al. 2018; Forsberg et al. 2018). For example, missing values can be replaced by a very small value, generally half of the minimum value found in the data set, or by estimated values, such as the mean or median. If too many values are missing, the missing values can be automatically excluded. Data filtering can be performed to remove background noises and non-informative features in a data set, improving the statistical power of subsequent data analysis. Non-informative features, consisting of features with constant intensities throughout the experiments, can be identified by comparing statistical analyses, such as standard deviation or the coefficient of variance.

Data are subsequently normalized to reduce any systematic error or bias over analytical experiments, and to allow meaningful biological comparisons. Data normalization can be combined with data transformation and/or data scaling. Data can be normalized using several characteristics, including sum, median, reference sample, or a reference feature (Chong et al. 2018; Forsberg et al. 2018). If all samples are spiked with internal standards, then the internal standards can be used for data normalization as reference features. Data transformation, e.g., log transformation or cube-root transformation, is a method to alter features so that they exhibit a normal or Gaussian distribution. Data scaling is useful when features have very different orders of magnitude. Various scaling methods can be applied, such as auto-scaling, Pareto scaling, and range scaling. There is no optimal strategy for all types of metabolomics data. Users should perform trial-and-error testing by combining various options for data normalization, transformation, and scaling to obtain a Gaussian-shaped data distribution. Overall data quality should be assessed and apparent outliers should be checked prior to statistical analysis of metabolomics data.

Statistical analysis of metabolomics data can be univariate or multivariate, depending on the number of metabolic features. Univariate analysis evaluates differences among groups on a parameter-by-parameter basis, with statistical comparisons including t-tests and analysis of variance (ANOVA). By contrast, multivariate analysis assesses not only differences in single metabolite among groups of metabolites, but any relevant structures among individual metabolites. Commonly used multivariate analysis techniques are principal component analysis (PCA), cluster analysis, and partial least squares (PLS) regression. PCA and cluster analysis are unsupervised techniques, whereas PLS regression is a supervised technique. Multivariate analysis is able to visualize samples according to their groups or intrinsic similarities. The PCA and PLS techniques use a data matrix to evaluate the response vector using a linear regression model. The variables and metabolic features that maximize the discrimination among sample groups can be determined, and these variables should be confirmed further as significant metabolic features to discriminate among sample groups using targeted metabolomics or other biochemical approaches (Worley 2013; Smkmrmstt 2012).

Metabolite identification is challenging in global metabolomics (Matsuda 2016). Successful metabolite identification requires accurate measurement of the masses of the metabolic features observed, and database searches to find accurate matches.

If available, MS/MS spectral libraries can be used for metabolite identification (Bowen and Northen 2010; Vaniya and Fiehn 2015). However, this approach does not result in a definitive identification of compounds, because a single chemical formula may have many valid chemical structures. In addition, the metabolite databases (e.g., KEGG, HMDB, METLIN, and ChemSpider) are not complete, and many of the observed metabolic features remain unidentified (Sas et al. 2015; Sreekumar et al. 2009). Thus, a list of potential candidates would be obtained, and the identification should be confirmed using authentic standards or additional analysis. The process for the identification of all observed metabolic features requires considerable time and effort, suggesting that metabolite identification should be performed on the metabolic features that show statistical significance (Dunn et al. 2011; Evans et al. 2009; Want et al. 2010; Theodoridis et al. 2012).

Another challenge in global metabolome profiling is the changes in signal intensities or retention times over time. Various approaches have been applied to ensure that the results of metabolic profiling studies are valid. These include spiking of internal standards into all samples, use of QC samples, and randomized order of analysis. QC samples can be either pooled samples containing equal aliquots of all samples, or test mixtures containing a limited number of commercially available standards in solutions of a matrix similar to a real sample. QC samples should be used to assess experimental performance such as mass accuracy, signal response, retention time stability, and peak shape. Translational research would include at least tens of clinical samples for global metabolome profiling and may require long-term experimental periods. In that case, QC samples should be analyzed at regular intervals, e.g., every five to ten samples, throughout the analysis, and the reproducibility of the data should be evaluated to ensure that metabolome profiling is reliable (Want et al. 2010; Broadhurst et al. 2018).

Targeted Metabolomics

Targeted metabolomics can be used to obtain quantitative information on target metabolites involved in specific metabolic pathways. Targeted metabolomics is used to answer any specific biochemical questions and hypotheses (Yuan et al. 2012; Quehenberger et al. 2010; Quehenberger and Dennis 2011; Roberts et al. 2012). Sample preparation, chromatographic conditions, and specific mass fragmentation methods can be established or optimized to identify any specific metabolites in an analytical platform of targeted metabolomics. Low-level metabolites or a specific metabolite among several isobaric metabolites can be quantified selectively in complex biological samples. Greater effort is required to develop targeted than nontargeted metabolomics platforms, but, once targeted metabolomics platforms have been established, metabolites in specific metabolic pathways can be quantitated relatively easily. As a result, targeted metabolomics can provide a better understanding and interpretation of a specific hypothesis.

The metabolome from human serum contains about 20 biochemical classes, including amino acids, carnitines, carbohydrates, fatty acids, and bile acids

(Psychogios et al. 2011; Boudah et al. 2014). Many metabolomics platforms should be needed to quantify various metabolites, and novel targeted metabolomics platforms have been developed (Yoo et al. 2007; Yoo and Hakansson 2011; Strassburg et al. 2012; Han et al. 2013; Kim et al. 2017, 2012). Specific MS/MS and chromatographic separation can be useful to discriminate metabolites from isobaric metabolites or compounds having very similar chemical structures (Kim et al. 2014).

To outline the practical procedures encountered in targeted metabolomics, targeted metabolomics platforms focusing on fatty acids and their oxidized derivatives are highlighted. Fatty acids and their oxidized derivatives include short-chain fatty acids, medium and long-chain fatty acids, and eicosanoids. Short-chain fatty acids have different physicochemical and biological characteristics comparing to other fatty acids (Zeng and Cao 2018; Gao et al. 2009a; Cooper et al. 1995; Fushimi et al. 2006). Because short-chain fatty acids are more volatile and more water-soluble than medium and long-chain fatty acids, short-chain fatty acids are usually prepared from biological samples by aqueous extraction (Huda-Faujan et al. 2010). Organic phase extraction can be used to prepare longer chain fatty acids, with >5 carbons, from biological samples. Methyl esterification or trimethylsilylation is a commonly used chemical derivatization method for GC-MS analysis of medium- and long-chain fatty acids. These chemical derivatives of medium- and long-chain fatty acids are generally quantified by GC-MS. Intact molecular ions are rarely observed due to the harsh ionization conditions used by the electron impact ion source of GC-MS. Thus, the specific fragmentation pattern of each metabolite is used for identification and quantification (Antolin et al. 2008; Jayasinghe and Dias 2013). By contrast, short-chain fatty acids can be chemically derivatized using alanine, O-benzylhydroxylamine, methyl-/ethyl-/propyl-chloroformate, or a trimethylsilylation agent, prior to GC-MS or LC-MS analysis (Zeng and Cao 2018; Tao et al. 2008; Gao et al. 2009b; Zheng et al. 2013; Kvitvang et al. 2011; Qiu et al. 2007; Perez et al. 2016; Han et al. 2015; Chan et al. 2017).

Eicosanoids are oxidized derivatives of polyunsaturated fatty acids (PUFAs), and play a key role in human diseases related to inflammation and immune responses. The amounts of eicosanoids and their imbalances affect various biomedical processes and the pathophysiology of human diseases (Wenzel et al. 2007; Sanak et al. 2011; Pavord et al. 1999; Higashi et al. 2002; Dennis and Norris 2015; Huang and Peters-Golden 2008). In general, fatty acids are present at micromolar concentrations in human blood, whereas many eicosanoids are present with less than nanomolar concentrations (Psychogios et al. 2011). Thus, these lipid mediators from biological samples should be specifically extracted and concentrated (Yang et al. 2009, 2011). Eicosanoids share very similar chemical structures and some of them have the same molecular weight (isobaric). Thus, LC separation is crucial to discriminate against these isobaric eicosanoids (Yang et al. 2009). For example, prostaglandin D_2 (PGD_2) and prostaglandin E_2 (PGE_2) have the same elemental compositions, differing only in their stereochemistry. Elaborate optimization of chromatographic separation conditions allows discrimination between PGD_2 and PGE_2. LC-MS/MS has been applied to specifically profile about 40 eicosanoids (Yang et al. 2009).

Targeted metabolomics without authentic standard metabolites can also be useful in exploring human diseases and their pathophysiological mechanisms, despite obtaining relative quantitative results. Quantitative profiling of phospholipids or sphingomyelins is a good example. In CID, fragment ions corresponding to the headgroups of phospholipids or sphingomyelins are commonly obtained in positive ion mode, whereas fragment ions corresponding to fatty acyl chains are obtained in negative ion mode. Thus, LC-MS/MS in both positive and negative ion modes can provide valuable structural information about phospholipids and sphingomyelins (Li et al. 2016; Knittelfelder et al. 2014; Anand et al. 2016). In addition, MS/MS strategies such as neutral loss or precursor ion scan can be useful in profiling different types of phospholipids (Kim et al. 2017, 2012, 2014).

Metabolomics Applications in Translational Research

Metabolites are intermediates involved in various biochemical processes of biological systems. Metabolite levels change rapidly, depending on genomic or environmental perturbations, and can provide instant snapshots of changes throughout the human body. Metabolomics can be used to investigate metabolite–biological interactions and elucidate their roles in biomedical and clinical environments. The metabolome plays a crucial role in clinical applications, with about 95% of clinical assays based on metabolites and their related features (Wu 2006). One representative application is the blood glucose level in diabetic patients and sweetness in their urine samples (Karamanou et al. 2016). Human diseases are associated with adverse interactions of the genome or proteome with the metabolome. For example, many inherited metabolic disorders were found to be related to a specific organic acid or amino acid deficiency (Pitt et al. 2002). Every molecule in a human body may respond to a perturbation, and a system-wide approach may be needed to explain the biological complexity. Recent tangible advances in analytical platforms have resulted in metabolomics becoming the optimal strategy for translational research. Like other omics, metabolomics has a great impact on biomedical and translational research, especially in the areas of biomarker discovery and drug development. Metabolomics analyses can enhance understanding of the mechanisms underlying diseases or the adverse influence of exposure to toxic substances, and provide crucial clues to identify novel targets in drug development or treatment strategies. Thus, metabolomics should be a valuable tool in translational studies of novel drugs and treatments, of safety assessments, and in identifying clinical biomarkers for monitoring or diagnosing disease.

Diabetes

One of the earliest metabolomics studies in diabetes was the comparison of phospholipids in the plasma of patients with diabetes mellitus type 2 and normal controls (Wang et al. 2005). The study reported that metabolites could distinguish between

patients and normal controls using multivariate statistical analysis such as PLS and PCA. Moreover, the study identified several metabolites as potential biomarkers for group discrimination. A metabolomics analysis using LC-MS/MS and H-NMR to investigate metabolic pathways changed by insulin deficiency found that metabolic pathways related to amino acid oxidation, mitochondrial bioenergetics, and gluconeogenesis were altered due to insulin deficiency (Lanza et al. 2010). In another study, metabolome profiling revealed that metabolic signatures related to branched chain amino acids were changed due to obesity and contributed to insulin resistance (Newgard et al. 2009). Furthermore, monitoring of metabolome profiles in control and prediabetic groups for 12 years identified five branched chain and aromatic amino acids as possible prognostic biomarkers for an earlier risk of developing diabetes (Wang et al. 2011). The significance of these metabolites was confirmed using independent groups of patients. An evaluation of the predictive performance of these amino acids as prognostic biomarkers for the risk of diabetes involved the quantification of amino acids, nucleotide metabolites, and urea cycle metabolites using targeted metabolomics platforms. Use of nontargeted metabolomics to explore metabolome changes in the urine and plasma of prediabetic subjects showed that tryptophan, uric acid, fatty acids, bile acids, and lysophospholipids were major metabolites related to the prediabetes-associated alterations (Zhao et al. 2010). Analysis of volatile organic compounds (VOCs) from human exhaled breath has been also investigated in the diagnosis of diabetes. Acetone is produced in mammals by lipolysis or amino acid degradation, and elevated levels of acetone have been reported in the exhaled breath of diabetes mellitus patients (Das et al. 2016; Lebovitz 1995).

Brain

The high-throughput quantitative nature of metabolomics studies can lead to a comprehensive mapping of mammalian brain function. These results are expected to lead to the development of sensitive and accurate diagnostic tools and the design of personalized therapeutic treatments. Metabolomics has been applied to the study of central nervous system (CNS) physiology and pathophysiology to better understand the metabolic complexity of the CNS and the onset, progression, and treatment of multifactorial neurodegenerative diseases (Vasilopoulou et al. 2016). The blood–brain barrier (BBB) is a contraindication to the analysis of blood in brain biology. Rather, cerebrospinal fluid (CSF) may better reflect brain physiology. Metabolomics studies and genetic validation using CSF and serum samples from hundreds of patients and healthy controls found that cerebral metabolism of tryptophan was closely related to outcomes in patients with tuberculous meningitis (van Laarhoven et al. 2018). The finding suggested that the tryptophan metabolic pathway may be a novel drug target in patients with tuberculous meningitis, and interventions targeting tryptophan metabolism may benefit these patients. Metabolome changes in multiple sclerosis have also been explored. Global metabolome profiling identified that metabolomics signatures, including hormones, lipids, and amino acids, were

associated with the severity of multiple sclerosis (Villoslada et al. 2017). Vitamin D supplementation had different effects on metabolomes induced by oxidative stress and xenobiotic metabolism in multiple sclerosis patients and healthy controls (Bhargava et al. 2017). Metabolomics also revealed that chronic inflammation activated the kynurenine pathway, exacerbating the progression of multiple sclerosis. These results were validated in an independent cohort, suggesting that kynurenine metabolites in patient serum were biomarkers of multiple sclerosis (Lim et al. 2017). Glycerosphingolipids, including cerebrosides, which are important myelin lipids in the brain, were assessed to determine whether myelin membrane lipids interacted with infection and immune responses in demyelinating neurological diseases (Yang et al. 2011; Bergholt et al. 2018).

Fibrosis

Fibrosis results in the excess formation of fibrous connective tissues and interferes with the normal architecture and/or function of affected organs and tissues (Birbrair et al. 2014; Neary et al. 2015). Fibrosis develops differently in different individuals and is often related to wound healing response or disease severity (Vilar-Gomez et al. 2018; Wynn 2008; Leask and Abraham 2004). However, reliable molecular biomarkers to predict or diagnose fibrosis susceptibility are still lacking. Disturbance in mitochondrial homeostasis was reported in radiation-induced fibrosis, suggesting that mitochondrial dysfunction was closely related to fibrosis through metabolic perturbations such as lipid accumulation due to reduced fatty acid oxidation (Maeda 1982). Fatty acids and bile acids in fibrotic plasma were changed in cystic fibrosis, suggesting abnormal lipid metabolism in patients with cystic fibrosis (Guilbault et al. 2009; Teichgraber et al. 2008). In addition, the lipid mediator sphingosine-1-phosphate was found to be elevated in the human fibrotic liver, due to upregulation of sphingosine kinase (Li et al. 2011). Disturbed lipid metabolism in the liver was found to result in inflammation and fibrosis (Moustafa et al. 2012). Moreover, a high-fat diet resulted in cardiac fibrosis even before the development of obesity and hyperlipidemia (Aubin et al. 2008). In addition, eicosanoids were reported to be involved in cardiac fibrosis (Levick et al. 2007). Changes in phosphatidic acid and lysophosphatidic acid were observed in the bronchoalveolar lavage fluids of patients with idiopathic pulmonary fibrosis (Crow and Wakeland 2012). Branched chain amino acids such as leucine and valine were reduced in liver fibrosis via TGF_{β} inhibition (Cha et al. 2013). Exhaled ethane and pentane of systemic origin are produced by lipid peroxidation, a chain reaction induced by reactive oxygen species (ROS) (Miekisch et al. 2004; Larstad et al. 2007; Sarbach et al. 2013). Destructive oxidative stress was found to damage cells (Ross et al. 2011). Elevated levels of exhaled ethane in human breath have been reported in patients with asthma, chronic obstructive pulmonary diseases (COPDs), and cystic fibrosis (Paredi et al. 2000a, b, c). In addition, other VOCs, such as saturated hydrocarbons, were found in exhaled breath condensates of patients with COPD and idiopathic pulmonary fibrosis (Psathakis et al. 2006; Cazzola et al. 2015). These studies indicate that

metabolomics is likely a promising tool in identifying biomarkers of various lung diseases and in elucidating disease mechanisms.

Cancer

Most cancer cells utilize highly activated glycolysis to produce energy or substrates for rapid cell proliferation. This pathway is characterized by lactic acid accumulation, also called the Warburg effect (Liberti and Locasale 2016), which is responsible for adaptation to low oxygen and damage to mitochondria in a cancer environment (Warburg 1956). Metabolic modifications have been observed in tumors (Kim et al. 2009; Griffin and Shockcor 2004), and metabolic changes in a cancer environment may involve energy metabolism, lipid metabolism, and nucleotide metabolism (Kim et al. 2015). In addition, investigation of eicosanoids would be very useful in understanding tumor development, progression, and metastasis (Wang and Dubois 2010). However, the roles of lipid mediators in tumors remain incompletely understood. Lipidome profiling showed that fatty acid synthesis was activated in breast cancer, possibly for the synthesis of membrane phospholipids (Hilvo et al. 2011). Exhaled breath was explored as a noninvasive tool for early detection of lung cancer and characterization of suspicious lung nodules (Nardi-Agmon and Peled 2017; Phillips et al. 2015; Capuano et al. 2015; Peled et al. 2012; Broza et al. 2013; Shehada et al. 2016). In one study, metabolome profiles were assessed in 968 breath samples from 484 patients with gastric cancer to identify diagnostic markers for reduced cancer incidence and mortality. Eight significant VOCs in breath were found to correlate with cancer risk, suggesting the use of breath tests for follow-up surveillance of high-risk patients (Amal et al. 2015).

Conclusion

Metabolomics is a relatively new omics strategy, and is increasingly explored in translational research. Genomics can predict overall disease risk and potential drug responses, but has limitations in assessing alterations due to changes in diet and environmental factors. By contrast, metabolomics can quickly detect biochemical changes associated with specific disease states or detrimental environments, and provide valuable clues for novel drug targets or therapeutic interventions.

Significant time and effort are needed to identify metabolite biomarkers for diagnosis and surveillance that can replace traditional methods. These metabolite biomarkers may constitute a powerful tool to aid or enhance the early detection of primary or recurrent disease as well as for the reliable characterization of suspected disease signatures. In addition, an in-depth understanding of metabolism related to disease progression may help identify novel targets for treatment. Thus, metabolomics is expected to flourish as a valuable research platform in translational research.

Acknowledgments This study was supported by a grant (2018IL0540, 2019IL0540) from the Asan Institute of Life Sciences, Asan Medical Center, Seoul and by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (2019R1F1A1056286).

Conflicts of Interest The authors declare no competing interests relevant to this chapter.

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