



A Review on Metabolomics – The Harmonizing Field in Systems Biology

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ABSTRACT

Metabolomics is a promising technology that demonstrates promising opportunities to practice the precision medicine which may be defined as the inclusive analysis of metabolites in a biological specimen. Traditionally, metabolites were used to diagnose complex metabolic disorders and monogenic disorders. However, recent metabolomic technologies have leaped beyond the scope of standard clinical chemistry techniques. They are competent to analyze hundreds to thousands of metabolites precisely. Accordingly, metabolomics affords thorough characterization of metabolic phenotypes thus and facilitating precision medicine at a number of different levels, including the cataloging of metabolic derangements that trigger disease, novel therapeutic targets breakthrough, and detection of biomarkers that may be used for diagnosis or monitoring the activity of therapeutics. Thus, metabolomics corresponds to the edge between genetic pre-disposition and environmental persuades and this unique status in the systems biology hierarchy makes it possible for metabolomics to prove invaluable in the quest for understanding the function of genes, to be able to manage and/or devise novel organisms that may benefit the health or lifestyles of humankind, and to comprehend more entirely the molecular physiology of ourselves and that of other organisms.

Keywords: Metabolomic, Targeted metabolomics, Untargeted metabolomics, Metabolizer subpopulation.

INTRODUCTION

The objective of precision medicine is to propose disease prevention and clinical care strategies by considering individual variability in surrounding settings, lifestyle, genetics, and molecular phenotype. The clinical genomics in disease like cancer is to notify the selection of therapies and envisage upshots has been the front line of the field. Genomic tools comprises of a mightily informative objective lens to scrutinize individual variability using a microscope as an analogy. However, it does not offer a view to other biomolecules like metabolites, which also define molecular phenotypes. Preferably a molecular microscope would be outfitted with supplementary objectives to inspect biochemistry more largely for precision medicine. However, the development of blood glucose test strips in the 1950s for diabetes¹ or measuring phenylalanine in newborns for phenylketonuria screening proves that there has been decades of precedence for employing analyses of small numbers of metabolites to identify disease and impact clinical care.² Undeniably, both in complex disease and monogenic disorders measurable changes occur in metabolite levels and in disparity to the genome, these changes can display tissue specificity and temporal dynamics. Metabolomics defined as comprehensive measurement of metabolites and low-molecular-weight molecules in a biological specimen is a budding area. As metabolomics affords profiling of high numbers of metabolites than those roofed in standard clinical laboratory techniques presently, and thus complete coverage of biological processes and metabolic pathways, it clutches guarantee to serve as an crucial objective lens in the molecular microscope for precision medicine.³

1. METABOLOMICS

Metabolism driven approaches

Metabolites are small molecules chemically transformed during metabolism and provide a functional readout of cellular state unlike genes and proteins, the functions of which are subject to epigenetic regulation and posttranslational modifications. Metabolites act as direct signatures of biochemical activity and are easier to correlate with phenotype and have become a powerful approach that has been widely adopted for clinical diagnostics. The metabolome may be defined as the collection of small molecules produced by cells that offers a window for interrogating how mechanistic biochemistry relates to cellular phenotype. It is now possible to rapidly measure thousands of metabolites simultaneously from only minimal amounts of sample⁴ with developments in mass spectrometry. Recent innovations in instrumentation, bioinformatic tools and software enable the comprehensive analysis of cellular metabolites without biasness and in many instances; these metabolites can be spatially localized within biological specimens with imaging mass spectrometry.^{5,6}

System-wide alterations of unexpected metabolic pathways related to phenotypic perturbations have been revealed from the application of these technologies. However, many of the molecules detected are currently not included in databases and metabolite repositories which clearly indicate incomplete picture of cellular metabolism.^{7,8} Nevertheless, the field of metabolomics has made remarkable progress within the past decade and implementation of new tools has offered mechanistic



insights by allowing for the correlation of biochemical changes with phenotype.

Metabolomics combines strategies for identification and quantification of cellular metabolites using sophisticated analytical technologies with the application of statistical and multi-variant methods for information extraction and data interpretation. Huge progress has been made in the sequencing of a number of different organisms in the last two decades. Concurrently, large investments were made for development of analytical approaches to analyze the different cell products, such as those from gene expression (transcripts), proteins, and metabolites. All of these which are so-called 'omics approaches, including genomics, transcriptomics, proteomics, and metabolomics, are considered vital tools to be applied and utilized for understanding the biology of an organism and its response to environmental stimuli or genetic perturbation.⁹ Metabolomics is considered to provide a direct "functional readout of the physiological state" of an organism.¹⁰ An array of analytical technologies has been employed for analyzing metabolites in different organisms, tissues, or fluids. Mass spectrometry coupled to different chromatographic separation techniques, such as liquid or gas chromatography or NMR, are utilized to analyze a large number of metabolites simultaneously. There are still a few bottlenecks in metabolomics in spite of highly sophisticated and sensitive technology. Moreover, huge diversity of chemical structures and the large differences in abundance makes it more difficult for a single technology to analyze the entire metabolome. Therefore, a number of complementary approaches have to be established for extraction, detection, quantification, and identification of as many metabolites as possible.^{11, 12} Extraction of the information and interpretation in a biological context from the vast amount of data produced by high-throughput analyzers is the another challenge in metabolomics. However, application of sophisticated statistical and multi-variant data analysis tools, including cluster analysis, pathway mapping and comparative overlays has demonstrated that there is a need to change current thinking to deal with large data sets and distinguish between noise and real sample-related information. Moreover, we are only beginning to even assume where metabolomics, together with the other omics technologies, is going to lead us.

Potential and applications of metabolomics

The four conceptual approaches in metabolomics are: target analysis, metabolite profiling, metabolomics, and metabolic fingerprinting.¹³ Target analysis includes the determination and quantification of a small set of known metabolites (targets) using one particular analytical technique of best performance for the compounds of interest and has been applied for many decades. On the other hand, Metabolite profiling aims at the analysis of a larger set of compounds, both identified and unknown with respect to their chemical nature and has been applied for many different biological systems using GC-

MS, including plants¹⁴, microbes¹⁵, urine¹⁶, and plasma samples¹⁷. Metabolomics the third approach employs complementary analytical methodologies, for example, LC-MS/MS, GC-MS, and/or NMR, for determination and quantification of as many metabolites as possible, either identified or unknown compounds. The fourth conceptual approach is known as metabolic fingerprinting (or foot printing for external and/ or secreted metabolites) where a metabolic "signature" or mass profile of the sample of interest is generated and then compared in a large sample population to screen for differences between the samples, when signals that can significantly discriminate between samples are detected, the metabolites are identified and the biological relevance of that compound can be elucidated, greatly reducing the analysis time. Metabolomics can be used for a large range of applications, including phenotyping of genetically modified plants and substantial equivalence testing, determination of gene function, and monitoring responses to biotic and abiotic stress because metabolites are closely linked to the phenotype of an organism. Therefore, Metabolomics can be seen as bridging the gap between genotype and phenotype¹³, which provides a more comprehensive view of how cells function, as well as identifying novel or striking changes in specific metabolites. Hence analysis and data mining of metabolomic data sets and their metadata can lead to new hypotheses and new targets for biotechnology.

2. DESIGNING A METABOLOMIC EXPERIMENT

Determination of the number of metabolites to be measured is the first step in performing metabolomics. While in some case, examining a defined set of metabolites by using a targeted approach may be of significance in other an untargeted or global approach may be taken wherein as many metabolites as possible are measured and compared between samples without bias. Eventually, the number and chemical composition of metabolites to be studied is a defining trait of any metabolomic experiment and outlines experimental design with respect to sample preparation and choice of instrumentation.

Targeted metabolomics

This approach comprise of a method in which a specified list of metabolites is measured focusing on one or more related pathways of interest. A specific biochemical question or hypothesis that motivates the investigation of a particular pathway is generally the driving force for the targeted metabolomic approach (Figure 1(1)) which can be efficient for pharmacokinetic studies of drug metabolism as well as for measuring the influence of therapeutics or genetic modifications on a specific enzyme.¹⁸ Advancement in mass spectrometry and nuclear magnetic resonance (NMR) makes it possible for performing targeted metabolomic studies because of their specificity and quantitative reproducibility; however, there are several analytical tools available such as ultraviolet-visible spectroscopy and flame ionization.¹⁹



There is abundant literature investigating optimal protocols for the sample preparation and analysis of specific classes of metabolites that has been discussed extensively elsewhere making it significant in the development of the field of metabolomics.^{20, 21} Moreover, advances have been made in using triple quadrupole (QqQ) mass spectrometry for performing selected reaction monitoring experiments such that routine methods are now accessible for analysis of most metabolites in central carbon metabolism, as well as amino acids and nucleotides at their naturally occurring physiological concentrations.^{22,23} A highly sensitive and robust method for measuring a significant number of biologically important metabolites with relatively high throughput has been provided with these developments. Furthermore, as QqQ mass spectrometry methods are quantitatively reliable opportunities to achieve absolute quantification of low-concentration metabolites that are difficult to detect with less sensitive methods such as NMR is quite possible (Figure 1(1)). Targeted lists of metabolites can be screened by applying QqQ mass spectrometry-based methods to human plasma as potential metabolic signatures for disease. Targeted screening in recent time has revealed citric acid metabolites and a small group of essential amino acids as metabolic signatures of myocardial ischemia and diabetes, respectively.²⁴ Another diabetes-related study involved targeted metabolomic methods for investigating patient response to glucose challenge.

Untargeted metabolomics

Untargeted metabolomic methods can simultaneously measure as many metabolites as possible from biological samples without bias and are global in scope (Figure 1(2)). While untargeted metabolomics can be performed by

using either NMR or mass spectrometry technologies, liquid chromatography followed by mass spectrometry (LC/MS) absolutely enables the detection of the most metabolites and is considered the choice for global metabolite profiling efforts.^{25, 26} Thousands of peaks can be routinely detected from biological samples by using LC/MS-based metabolomic methods (Figure 1(2)) with each of the peaks referred to as a metabolite feature and corresponds to a detected ion with a unique mass to-charge ratio and a unique retention time (it should be noted that some metabolites may produce more than one peak). Untargeted metabolomic data sets are exceedingly complex, with file sizes on the order of gigabytes per sample for some new high-resolution mass spectrometry instruments in contrast to targeted metabolomic results. Moreover, manual inspection of the thousands of peaks detected is unfeasible and complicated due to experimental drifts in instrumentation. In LC/MS experiments, for example, there are deviations in retention time from sample to sample as a consequence of column degradation, sample carryover, small fluctuations in room temperature and mobile phase pH, as well as other variations. However, major progress has been made in the past decade such that the ability to measure dysregulated peaks in global metabolomic data sets has now become routine with the introduction of metabolomic software such as MathDAMP, MetAlign, MZMine and XCMS1,^{27,28} and has revealed that an astounding number of metabolites remain uncharacterized with respect to their structure and function and, furthermore, many of these uncharacterized metabolites change as a function of health and disease. Thus untargeted metabolomics has great potential to provide insights into fundamental biological processes.²⁹

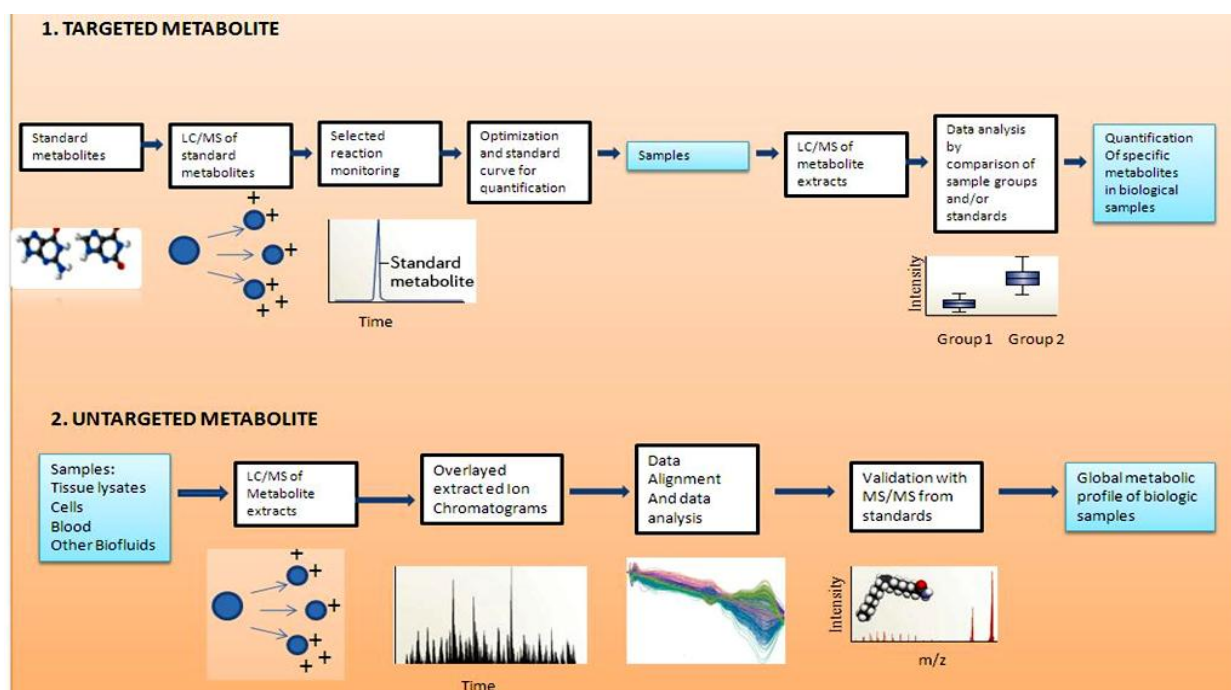


Figure 1: The targeted and untargeted workflow for LC/MS-based metabolomics.

IMPROVING METABOLITE DATABASES

The information catalogued in metabolite databases has evolved ahead of lists of one-dimensional data traditionally acquired by mass spectrometry- and NMR-based screens over the past decade e.g. Human Metabolome Database includes a 'MetaboCard' for each of its included metabolites (~8,550).³⁰ MetaboCards also list the information on each compound's biochemical pathway, concentration, anatomical location, metabolizing enzymes and related disorders along with molecular weights and experimental NMR spectra, when available. METLIN contains experimental data for a subset of the total number of compounds included (~45,000), having MS/MS data available for more than 10,000 metabolites which are experimentally generated from model compounds analyzed at four different collision energies in both positive and negative mode. The Human Metabolome and METLIN databases can facilitate both metabolite identification and data interpretation when used together with other publicly available tools. At present, the Human Metabolome Database and METLIN are the most widely used metabolite databases publicly available.

Meta-analysis: prioritizing unknowns

A cascade of metabolic perturbations that are functionally unrelated to the phenotype of interest can occur by alterations in a single enzyme. Untargeted metabolomic profiling of a particular disease or mutant thus reveals hundreds of alterations without having mechanistic implications. Strategies to reduce lists of potentially interesting features before committing to identifying them are of great utility provided that the resources needed to identify both known and unknown compounds are available. One such strategy is meta-analysis, where untargeted profiling data from multiple studies are compared. E.g. by comparing multiple models of a disease, features that are not similarly altered in each of the comparisons may be de-prioritized as being less likely to be related to the shared phenotypic pathology. For automation of the comparison of untargeted metabolomic data, freely available software called metaXCMS has been recently developed.³¹ MetaXCMS has been applied to investigate three pain models of different pathogenic etiologies: inflammation, acute heat and spontaneous arthritis. Only three were similarly dysregulated among all the groups in spite of the fact that hundreds of metabolite features were found to be altered in each model. Histamine, a well-characterized mediator of pain was one of the shared metabolites that were identified that works by several mechanisms. The application of similar data-reduction strategies to other biological systems may thus justify aggressive analytical investigations of unknown features which are likely to be physiologically relevant.

Imaging approaches for localizing metabolites

Metabolite isolation by sample homogenization is one of the first steps in the untargeted metabolomic workflow applied to biological tissue. High-resolution spatial localization of metabolites within samples is not permitted by standard metabolic profiling techniques. Investigations of heterogeneous tissues such as the brain are as a result complicated by the averaging of various cell types, each with a potentially unique metabolome. Thus correlating a dysregulated metabolite with a specific region of tissue or cell type can be challenging. Although NMR-based imaging technologies have been applied to spatially localize metabolites in intact samples, these methods have limited chemical specificity and sensitivity.³² In contrast to mass spectrometry based approaches relying on matrix-assisted laser desorption ionization (MALDI) which offers improved chemical specificity and sensitivity, but are limited in their application to metabolites owing to background interference caused by the matrix in the low-mass region that is characteristic of metabolites. A matrix-free technique called nanostructure-initiator mass spectrometry (NIMS) has been developed as an alternative for the analysis of metabolites with high sensitivity and spatial resolution. Analysis of 3 "m sections of brain tissue from mice with impaired cholesterol biosynthesis by using NIMS revealed localized metabolic precursors of cholesterol in the cerebellum and brainstem. Thus these types of NIMS imaging applications coupled with histology can allow metabolite localization patterns to be correlated with tissue pathology and drive developments in understanding of chemical physiology.

3. METABOLIZERS SUBPOPULATIONS

Physiological responses associated with a particular drug are linked to biochemical attributes in the body of the recipient. Several studies have attempted elucidation of the mechanisms/factors that modify the clinical response to a greater or lesser extent the result of which has demonstrated that variability in the function of drug-metabolizing enzymes (DME) is responsible for many differences in the disposition and clinical consequences of drugs. While it is a central issue to PGx, decisions about a medicine prescription in clinical practice are largely based on the classic factors responsible for drug variability, including co-existing disease (especially those that affect drug distribution, absorption or elimination), body mass, diet, alcohol intake, interaction with others drugs and mechanisms to improve patient compliance and all of these have been established to directly affect the indicated dose of the drug. Conversely, they only partially explain the reason behind major drugs being effective in only 25 to 60 percent of patients. Additionally, there are certain questions to be answered for better understanding like taking into consideration the patients with same physical and demographic characteristics, why does a standard dose toxic to some patient but not to others? Why not all patients demonstrate the expected



efficacy in drug treatment trials? These and many others questions have opened the door for a new era of the personalized medicine and treatment perspectives.

Drug levels can be raised by increasing the dose or frequency of administration in a non-responder patient. On the other hand, if a higher plasma drug level with a standard dose administration is expected (in a patient with cirrhosis or malnutrition, for example), a reasonable attitude may be either increasing the time of administration or suspending the dose. Even though advances in medical technology and potential predictive models have improved the choice of dose, they are not yet adequate for preventing high level of morbidity and mortality caused by adverse drug reactions.³³ Thus, genetic variation interface with drug metabolism study, especially in genes codifying DMEs, may lead to improve drug safety. Factors affecting the expression and activity of DMEs are classified into three major groups: genetic factors, non-genetic host factors (such as diseases, age, stress, obesity, physical exercise, etc.) and environmental factors (environmental pollutants, occupational chemicals, drugs, etc.). Recent studies indicate that the most important causes of drug response differences are inter individual variation in drug metabolism. In general, common pharmacokinetic profile is a lighthouse for most prescribers in clinical practice. Figure 2A demonstrate a simplified model of a drug biotransformation route. Most pharmaceuticals compounds or molecules (M1 in figure 2) administrated orally are enough lipid-soluble to be reabsorbed (in the kidneys) and are eliminated slowly in small amounts in an unchanged form in urine. Consequently, drug biotransformation by enzymes (represented by E1) has an important role in the control of plasma drug concentration. However, the metabolites (M2) might also exert pharmacological effect. In addition, low activity of the metabolic step might cause accumulation of the drug and/or its metabolites in the body if the drug is continuously taken (Figure 2B). Genetic mutations in coding and noncoding regions may be involved in inborn altered enzymatic activity examples of which are polymorphisms in CYPs (cytochrome P450)

genes, which may result in absence of protein synthesis (2A6*4, 2D6*5), no enzyme activity (2A6*2, 2C19*2, 2C19*3, 2D6*4), altered substrate specificity (2C9*3), reduced affinity for substrate (2D6*17, 3A4*2), decreased stability (2D6*10) or even increased enzyme activity (2D6*2xn).³⁴ Such genetically determined enzyme variation may directly interfere in the drug concentration at the target tissue, and although the pharmacological effect may be exerted, the risk of toxicity is higher in “poor metabolizers” because of drug accumulation to possibly harmful levels. Reduction in drug biotransformation, as is observed in drug-drug interactions, also results in altered expected values for the constant of elimination (K_e), half-life of the drug ($t_{1/2}$), volume of distribution (Vd), area under the curve (AUC) and others common useful pharmacokinetic parameters used in therapeutic drug monitoring and adjustment. Thus PGx approaches may contribute to the augmentation of clinical outcomes by providing a more effective match between patient and drug dose or type, and consequently reducing the probability of an adverse drug reaction.

Since changes in DNA sequence are responsible for the effect of inherited variation (genotype) on enzymatic activity, it is conceivable that there are diverse subgroups of subjects who have different metabolic capabilities (phenotype). Certainly, epidemiologic studies have revealed at least two sub-populations of individuals based on drug metabolizing profile, known as “rapid” or “slow” metabolizers. For example, administration of a prodrug have higher therapeutic efficacy in a rapid than in slow metabolizer phenotype because of the fact that metabolization of such drug is necessary to make it active. Moreover, drug biotransformation is fundamental to generate an active-molecule (M2) from a less (or not) active form (M1) (Figure 2C). Besides, it is evident that PGx approaches cited here are simplified assumptions of metabolism, many drugs are sequentially metabolized (Figure 2D) by parallel pathways or a broad range of enzymes to other intermediary metabolites.³⁵

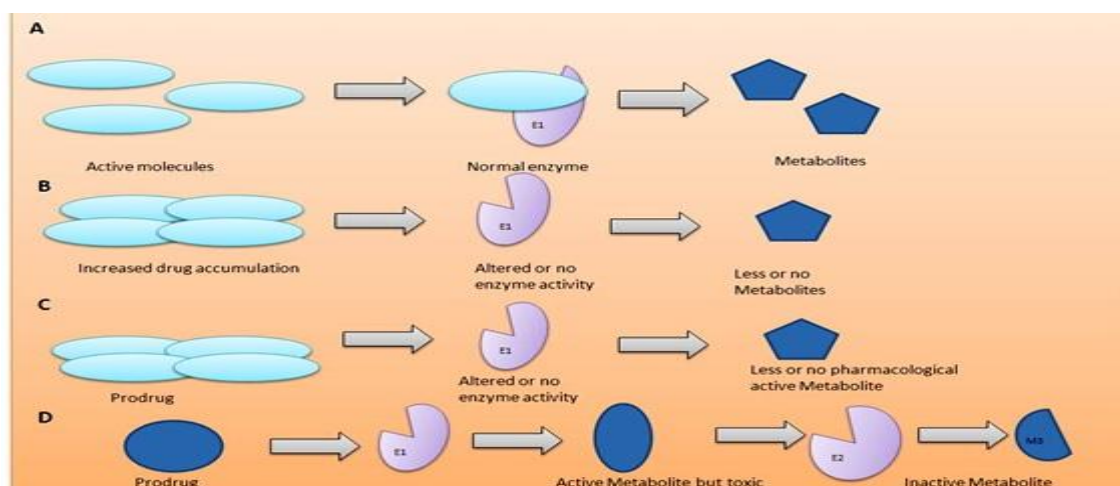


Figure 2: The expected clinical result and its relation with activity of drug metabolizing enzymes. M1: pharmaceuticals compounds; E1: phase I Biotransformation; M2 and M3: metabolites; E2: phase II biotransformation

CONCLUDING REMARKS

Technologies that enable the global analysis of metabolites at a systems level, comparable to its omic predecessors have recently emerged despite long-standing interest in metabolic profiling. Metabolomics provides a tool for measuring biochemical activity directly by monitoring the substrates and products transformed during cellular metabolism contrasting other techniques like genomics, transcriptomics and proteomics. Phenotypic readout that can be used effectively in diagnosing pathologies, identifying therapeutic targets of disease and investigating the mechanisms of fundamental biological processes can be served by untargeted profiling of these chemical transformations at a global level. Though untargeted metabolomics is still in its infancy, early studies have shown that the complexity of cellular metabolism exceeds that expected on the basis of classical biochemical pathways and thus the understanding of metabolism is evolving greatly. The potential of untargeted metabolomics to shape our understanding of global metabolism is yet to be fully realized as metabolomic technologies are continually advancing and facilitating the characterization of unknown pathways.

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