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Review Article

METABOLOMICS: CURRENT TECHNOLOGIES AND FUTURE TRENDS

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ABSTRACT

The emerging field of metabolomics had profound importance in recent years as its wide applications in the field of drug discovery and drug development. With recent advances in the field of metabolomics it can be proved as a major tool in the process of drug discovery and development. In the agricultural/chemical industry, metabolomics may be used to develop herbicides and pesticides With increasing importance being placed on health and safety related aspects of our food, metabolomics can potentially be a valuable tool to monitor and improve the quality of what we eat; for example, in food processing and quality control, or in plant breeding for improved crop varieties and in the development of novel foodstuffs. The present review reveals the importance of metabolomics in drug discovery along with recent advances in the techniques of metabolomics along with its wide applications in various fields like drug discovery, drug toxicity profiling, food industries especially in food and allied beverages testing.

Keywords: Metabolome, metabolite, metabolomics.

INTRODUCTION

Drug development from the early stage of target identification validation through clinical trials to clinical practice is a long, tortuous, and extremely costly process. The net yield of such process has also been poor and often comes from incremental advances on existing therapeutic agents. The current paradigm for drug development calls for uncovering specific molecular targets, against which highly selective and potent inhibitors can be developed, with minimal off-target effects. Such agents can be synthetic small molecules that may require optimization by medicinal chemistry, or natural products and their synthetic derivatives. From target discovery, through target validation, to clinical testing and eventual clinical adoption, the whole process would logically require a systems biochemical understanding of the disease itself, pharmacological properties (i.e. absorption, distribution, metabolism, excretion, and toxicity or ADMET) of the therapeutic agents, and their functional impact on the human body both on-target and off-target. Systems biochemistry can be viewed as "global biochemical networks and molecular regulations". As with all systems approaches, this represents a tall order for drug discovery, development, and deployment using conventional

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approaches. The lack of systems biochemical approaches and thus functional understanding in the past presents a fundamental barrier to efficient and successful commercialization of potential therapeutic agents. Beginning with the development of genomics, followed by functional genomics, proteomics, and now metabolomics, it is for the first time that a systems biochemical understanding of the human body may be envisioned. Once developed, this will accelerate the understanding of disease mechanisms and therapeutic development at an unprecedented pace. [1]

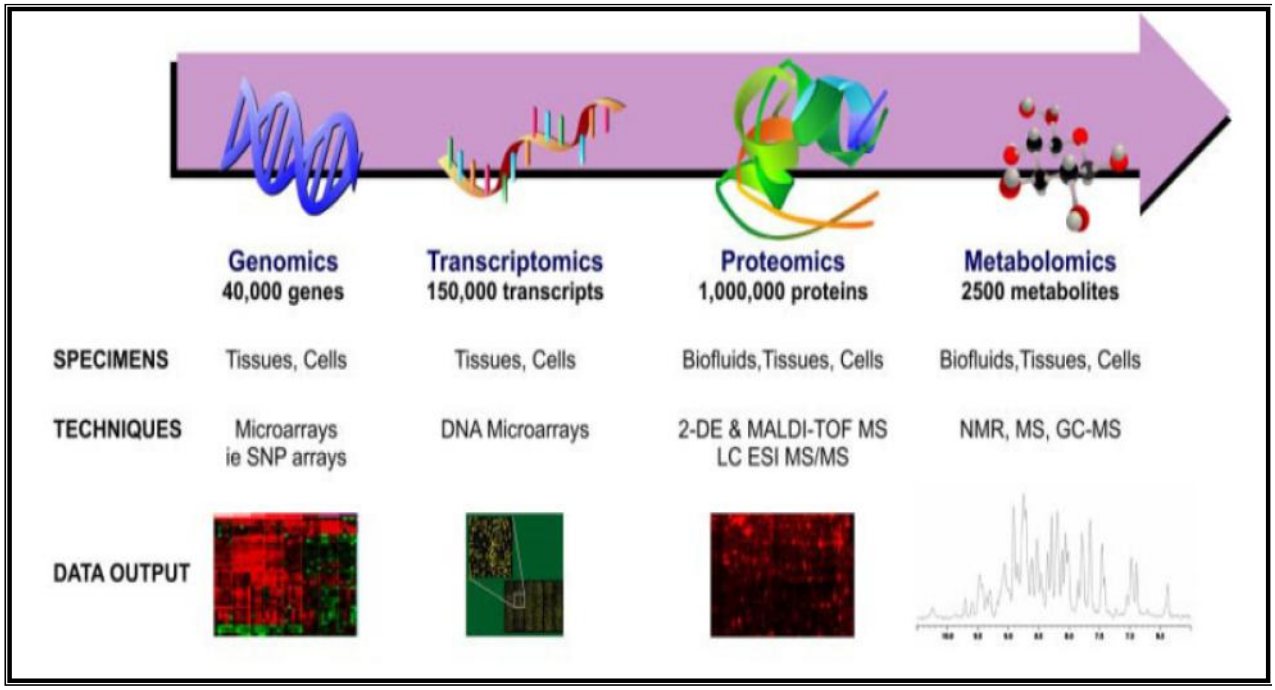

Fig.1 Introduction to metabolomics

The rapidly emerging field of metabolomics combines strategies to identify and quantify cellular metabolites using sophisticated analytical technologies with the application of statistical and multi-variant methods for information extraction and data interpretation. In the last two decades huge progress was made in the sequencing of a number of different organisms. Simultaneously, large investments were made to develop analytical approaches to analyze the different cell products, such as those from gene expression (transcripts), proteins, and metabolites. All of these so-called 'omics approaches, including genomics, transcriptomics, proteomics, and metabolomics, are considered important tools to be applied and utilized to understand the biology of an organism and its response to environmental stimuli or genetic perturbation. Metabolites are considered to "act as spoken language, broadcasting signals from the genetic architecture and the environment", and therefore, metabolomics is considered to provide a direct "functional readout of the physiological state" of an organism. A range of analytical technologies has been employed to analyze metabolites in different organisms, tissues, or fluids. Mass spectrometry coupled to different chromatographic separation techniques, such as liquid or gas chromatography or NMR, are the major tools to analyze a large number of metabolites simultaneously. Although the technology is highly sophisticated and sensitive, there are still a few bottlenecks in metabolomics. Due to the huge diversity of chemical structures and the large differences in abundance, there is no single technology available 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. Another challenge in metabolomics is to extract the information and interpret it in a biological context from the vast amount of data produced by high-throughput analyzers. The application of sophisticate statistical and multi-variant data analysis tools, including cluster analysis, pathway

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mapping, comparative overlays and heat maps has not only been an exciting and steep learning process for biochemists, but has also demonstrated that current thinking needs to change to deal with large data sets and distinguish between noise and real sample-related information.[2]

Metabolomics in drug discovery

It has been suggested that metabolomics greatest potential lies in disease marker discovery and detection. For instance, many in-born errors of metabolism (IEMs) and numerous acquired metabolic disorders (obesity, diabetes, cachexia and hyper-cholesterolemia) are characterized by unusually high (or low) levels of certain metabolites. For example, phenylketonuria is characterized by low levels of tyrosine, Tay Sachs disease by high levels of GM2 gangliosides, cystinuria by high levels of lysine and cystine and cachexia by high levels of methyl-histidine and glutamine. These metabolites are not only biomarkers for the disease, but also serve as 'biomarkers of efficacy'. This means they allow the utility of new drug leads to be rapidly assessed in cell-based or enzymatic assays. Furthermore, some metabolites may serve as potential targets for new drug therapies or as potential drugs, in and of themselves. Many genetic disorders arise from multiple gene defects, and being able to distinguish between these disorders and to identify their root causes is critical to finding appropriate drug targets, appropriate drugs (i.e. enzymes or enzyme replacements) or appropriate combinations of dietary supplements to treat them. In the past, where metabolite identification was typically limited to a few abundant and easily detectable compounds, the etiology of many metabolic diseases was not well understood. Indeed, until recently it was not widely appreciated that some disorders that presented with similar clinical symptoms had fundamentally different metabolic (i.e. causal) profiles. A greater understanding of many metabolic disorders – and the ways to treat them – is being achieved with the advent of metabolomics, the growing use of broad spectrum metabolite profiles and the continued improvements in sensitivity. [3] Metabolomics is a powerful and new scientific approach for the discovery and development of drugs and the early diagnosis of disease states. Metabolome has developed a patent pending technology that is poised to impact drug discovery and development processes dramatically by measuring accurately the spectrum of biochemical changes and mapping these changes to metabolic pathways. This technology provides data that is less complex, more precise, more relevant and more quantitative than genomics, transcriptomics or proteomics. With this methodology, it is now possible to develop an understanding of disease states and new treatment modalities much faster and more accurately than ever before. Metabolomics has broad applications across the drug discovery and development processes. Metabolon's proprietary technology platform in metabolomics will enable faster and more cost-effective processes in the following areas:

• Target identification and validation
• lead prioritization and optimization
• Preclinical studies
• Clinical trials
• Marketing studies and
• Diagnostics. [4]

Metabolic profiling

Quantitative analysis of set of metabolites in a selected biochemical pathway or a specific class of compounds. This includes target analysis, the analysis of a very limited number of metabolites, e.g. single analytes as precursors or products of biochemical reactions.

Metabolic fingerprinting

Unbiased, global screening approach to classify samples based on metabolite patterns or "fingerprints" that change in response to disease, environmental or genetic perturbations with the ultimate goal to identify discriminating metabolites.

Metabolic foot printing

Fingerprinting analysis of extra-cellular metabolites in cell culture medium as a reflection of metabolite excretion or uptake by cells. [5]

The metabolome, the intersecting systems chemistry of life processes, is the functional outcome of the activity of the genome, functional genome, and the proteome. Metabolic processes are the ultimate expression of gene and protein activities to meet the physiological demands for growth and survival, including responses to environmental factors such as nutrient availability, xenobiotics, and therapeutic agents. Metabolic products are often indispensible players in maintaining metabolic homeostasis via regulating enzyme activities, as well as protein and gene expression events in a

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feedback loop. This is simply illustrated by the well-known insulin production in response to blood.

Hippocrates with the Pythagorean theory. This theory that was formed by Galen was unchallenged and remained standard until the 17th century.

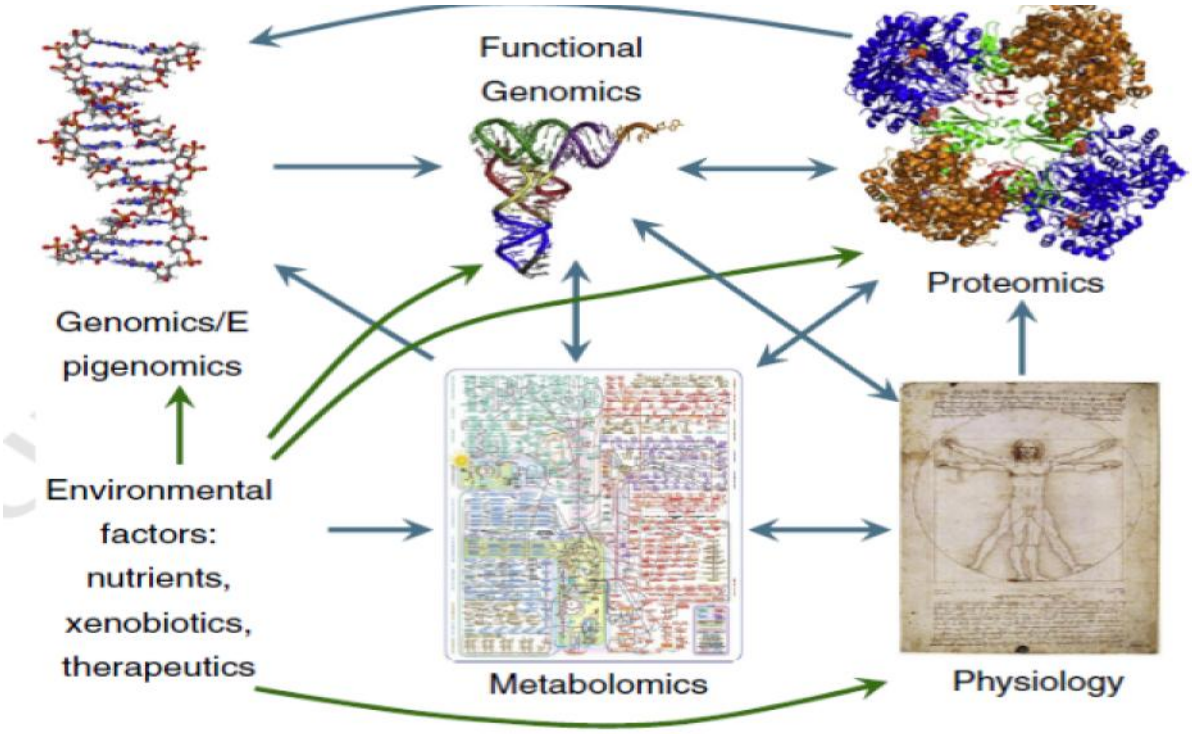

Fig.2 Integration of 'Omics approaches for system biology

History Of Metabolomics

The beginning of metabolomics traces back all the way to 2000-1500 B.C. when traditional Chinese doctors began using ants in order to evaluate the urine of patients to determine if the urine contained the high glucose of diabetics. At this time, others tasted the urine for sweetness in order to check for the same thing. Urine was also a factor in determining diabetes in Ancient Egypt where it was determined by frequent urination. This earliest use of body fluids to determine a biological condition can be considered the first early uses of metabolomics.

Galen and Metabolomics

More early steps towards metabolomics came in 300 B.C. when the ancient Greeks first recognized that it was essential to examine body fluids (called humor at the time) in order to predict diseases. From here the next step in the path of Metabolomics was in 131 A.D. when Galen created a system of pathology that combined the humoral theories of

Metabolomics after the Scientific Revolution

As the 17th century began Santorio Sanctorius became the man who is considered to be the founding father of metabolic studies. In 1614 he published work that he had done on "insensible perspiration" in De Statica Medicina and he determined that the total excrete (urine, feces, sweat) was less than the amount of fluid ingested. His work was the first to obtain physical data and provide quantitative basis of pathology based upon precise studies and instrumentations. The next step in the evolution of metabolomics came in 1674 when Thomas Willis, a physician from England, performed the first analysis of urine and he found that people with diabetes mellitus and diabetes insipidus could be distinguished based solely upon the sweetness of their urine. His research was taken one step further by Matthew Dobson in 1776 by evaluating the urine from diabetics and identifying that there was sugar in the urine of individuals with diabetes.

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The 20th Century

By 1905 J.J. Thomson of the University of Cambridge developed the first mass spectrometer. Also in this year there was more work in determining what other things were in urine and Otto Knut Olof Folin reported that methods for analysis of urine for urea, ammonia, creatine, uric acid. His findings were all published in one issue of Physical Review. The next step in the path to modern Metabolomics came by 1946 when Felix Botch of Stanford and Edward Purcell of Harvard simultaneously published the first NMR in the same issue of Physical Review. The separation of metabolites through chromatography also made the study of metabolomics possible. As chromatographic separations were discovered and made possible in the 1960's the ability to study individual metabolites was made possible and the technical aspects of the field were made possible.

Robinson and Pauling

With the necessary instruments in place there was a small gap of time until 1971 when Mamer and Horning performed the first mass-based metabolomics experiments. Shortly after they began their work Modern Metabolomics began to form when Arthur B Robinson and Linus Pauling investigated biological variability being explained by ranges of nutritional requirements. By studying early chromatographic separations in urine he found that the chemical constituents of the urine were loaded with useful information. The first paper on Metabolomics, though not called metabolomics at the time, was by Robinson and Pauling in 1971. It was titled "Quantitative Analysis of Urine Vapor and Breath by Gas-Liquid Partition Chromatography" and was published in Proceedings of the National Academy of Sciences.

The Human Metabolome Project

Less than one per cent of metabolites are measured in clinical tests such as blood and urine analyses, leaving medical professionals without a comprehensive picture of patients' health. The Human Metabolome Project led by Dr. David Wishart of the University of Alberta, Canada completed a first draft of his research on the human metabolome, which consists of 2500 metabolites, 1200 drugs and 3500 food components. The project had started in 2004 with $7.5 million in funding and involved 53 scientists. The first draft was finished on January 23, 2007. The findings have been archived on a freely accessible web resource called the Human Metabolome Database (HMDB). In addition to this work on endogenous metabolites, the group has identified and cataloged nearly 1200 drugs (now archived in Drug Bank) and is working to complete a similar database on food additives. The group is using advanced methods in NMR spectroscopy, mass spectrometry, multi-dimensional chromatography and machine learning to facilitate this work. There are two common components of the present research in metabolomics:

(1) Metabolites are profiled without any bias to any specific group of metabolites

(2) Relationships between metabolites are characterized; currently this is done through multivariate methods.

METABOLOMICS TECHNIQUES

Metabolomics is a multi-disciplinary science that includes aspects of biology, chemistry, and mathematics. It requires analytical techniques such as chromatography, molecular spectroscopy and mass spectrometry, coupled with multivariate data analysis methods. For target compound analysis and metabolic profiling, main techniques are gas chromatography (GC), high performance liquid chromatography (HPLC) and nuclear magnetic resonance (NMR). These approaches rely on chromatographic separations, often coupled with well-developed calibrations for specific analytes. In metabolic fingerprinting, samples are analyzed as crude extracts without any separation step, using NMR, direct injection mass spectrometry (MS), or Fourier transform infrared (FT-IR) spectroscopy. These fingerprinting approaches are often combined with multivariate analysis, to get the most out of the data. The aim of metabolomics is to obtain the widest possible coverage, in terms of the type and number of compounds analyzed. This is achieved by making use of several, complementary analytical methods. In particular, the 'hyphenated' techniques of LC/MS, LC/MS/MS and LC/NMR are likely to make increased impact in the future.

An overview of each of the approaches is given below.

- Gas chromatography (GC),
- High performance liquid chromatography (HPLC),
- Nuclear magnetic resonance (NMR),
- Mass spectrometry (MS),
- Fourier transform infrared (FT-IR) spectroscopy,

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- LC/MS, LC/MS/MS and LC/NMR,
- 'Fingerprinting' Methods,
- Multivariate Analysis.

Gas Chromatography

Developments involving gas chromatography have been responsible for the recent upsurge of interest in plant metabolomics. GC provides high-resolution compound separations, and can be used in conjunction with a flame ionization detector (GC/FID) or a mass spectrometer (GC/MS). Both detection methods are highly sensitive and universal, able to detect almost any organic compound, regardless of its class or structure. However, most of the metabolites found in plant extracts are too non volatile to be analyzed directly by GC methods. The compounds have to be converted to less polar, more volatile derivatives before they are applied to the GC column. Efficient derivatization methods are available, but relatively low sample throughput is a drawback of the GC method, particularly when there are many samples to be examined.

High Performance Liquid Chromatography (HPLC)

HPLC, with UV detection, is probably the most common method used for targeted analysis of plant materials, and for metabolic profiling of individual classes. A derivatization step is not essential (unless needed for detection), since non-volatile and volatile substances may be measured equally well. Selection of compounds arises initially from the type of solvent used for extraction (as with all methods that use an extraction step), and then from the type of column and detector. For example HPLC/UV will only detect compounds with a suitable chromophore; a column selected for its ability to separate one class of compounds will not generally be useful for other types. HPLC profiling methods all rely to a great extent on comparisons with reference compounds. The full UV spectrum gives some useful information on the nature of compounds in complex profiles, but often indicates the class of the compound rather than its exact identity.

'Fingerprinting' Methods

- Nuclear Magnetic Resonance (NMR)
- Fourier Transform Infrared (FT-IR) spectroscopy
- Direct Injection Mass Spectrometry

'Fingerprinting' techniques can be used for rapid profiling of large numbers of samples, whilst still being able to provide, to different extents, specific chemical information. Samples can be examined after solvent extraction (no derivatization required), or as intact tissues (magic angle spinning NMR), liquids or semi-solids (NMR and FT-IR), or dried materials (FT-IR).

NMR

In principle, proton (^{1}H) NMR can detect any metabolites containing hydrogen. Signals can be assigned by comparison with libraries of reference compounds, or by two-dimensional NMR. ^{1}H NMR spectra of plant extracts are inevitably crowded not only because there is a large number of contributing compounds, but also because of the low overall chemical shift dispersion. ^{1}H spectra are also complicated by spin-spin couplings which add to signal multiplicity, although they are an important source of structural information. In ^{13}C NMR, the chemical shift dispersion is twenty times greater and spin-spin interactions are removed by decoupling. Despite these advantages, the low sensitivity of ^{13}C NMR prevents its routine use with complex extracts. Sensitivity can be enhanced when seedlings are grown in the presence of ^{13}C enriched carbon dioxide, but this is obviously only an option for laboratory based studies.

Direct Injection MS

It is also possible to obtain metabolite 'mass profiles' without any chromatographic separation. Such profiles are obtained by injecting crude extracts into the electro spray ionization source of a high-resolution mass spectrometer. ESI generates mainly protonated, deprotonated or adduct molecules, such as $[M+H]^+$, $[M+cation]^+$ or $[M-H]^-$ for each species present in the mixture, with little or no fragmentation. Thus a fingerprint spectrum is obtained with a single peak for each metabolite, separated from other metabolites according to (accurate) molecular mass. The fingerprint can be used as a classification tool, for example in taxonomy. Some mass analyzers are capable of ultra-high resolution and permit the mass to be determined to four or five decimal places. This allows unique formulae to be assigned to peaks with masses of a few hundred or so. The coupling of high sensitivity with high resolution provides a method of determining a rough estimate of the number of metabolites present and a valuable first indication, from the formulae, of their identities. Its main weakness is the inability to separate isomers of the same molecular mass.

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FTIR spectroscopy

The attraction of FTIR spectroscopy as a fingerprinting method is the ease of sample preparation, the speed with which data can be acquired, and the high degree of reproducibility attainable. Samples that can be poured or spread to make good contact with a flat surface can be measured by the attenuated total reflectance (ATR) method, whereas powdered or dried samples are measured by diffuse reflectance. The spectra are less easily interpreted than with the other methods, but extremely subtle differences may be picked out using chemo metrics, providing a powerful classification tool.

LC/MS, LC/MS/MS and LC/NMR

LC/MS, LC/MS/MS and LC/NMR are potentially powerful solutions to the problems of detector generality and structure determination. LC/MS can be used to detect compounds that are not well characterized by other methods. The electro spray ionization (ESI) technique has made polar molecules accessible to direct analysis by MS, as well as being compatible with HPLC separations. Quantification of multiple compounds in crude extracts can, in principle, be achieved in the same way as described for GC/MS, although automation of the procedure presents greater practical difficulties. LC/MS/MS provides additional structural information that can be a very useful aid in the identification of new or unusual metabolites or in the characterization of known metabolites in cases where ambiguity exists. The lower sensitivity of LC/NMR means that at present it is most often used for structural characterization of unknowns, rather than for comparative analysis of numerous samples. However, NMR is a very general detection method, and can provide unique structural information, so with improvements in sensitivity, the use of LC/NMR is likely to grow.

Multivariate Analysis

Plant extracts are very complex in composition and, if many samples are examined, it is difficult to make meaningful comparisons of large numbers of spectra or chromatograms 'by eye'. Multivariate statistical methods can be extremely useful, as they are able to compress data into a more easily managed form. This can assist in visualizing, for example, how a given sample relates to other samples - a central issue in metabolomics. Multivariate analysis is practically essential in the fingerprinting approaches, but is also helpful in techniques where individual metabolites are explicitly quantified (e.g. GC/MS).

Principle component analysis (PCA) is a well-known and effective method of data compression. PCA transforms the original data (e.g. intensity values in a spectrum) into a set of 'scores' for each sample, measured with respect to the principal component axes ('loadings'). Due to these properties, a small number of PCs can replace the many original varieties without much loss of information. Scatter plots of the scores on the first few PC loadings provide an excellent means of visualizing and summarizing the data and often reveal patterns that cannot be discerned in the original data. The scores plots may show clustering of similar samples, separation of different sample types, or the presence of outliers. Plots of the loadings themselves may be used to explore which compounds are most responsible for, say, separating samples into groups: the most important compounds (peaks) tend to correspond to high absolute loading values. [6]

APLICATIONS OF METABOLOMICS

Application of NMR to stable isotope tracer studies

Target identification and verification by NMR profiling

There have been numerous SIRM-based studies that make use of NMR (and often MS) for drug target identification and validation in cultured cells and animal models. Based on the SIRM analysis of an experiment designed to inhibit LDH-A in breast cancer cells (which is known to be required for tumor genesis, Chesney's group showed that the LDHA inhibitor oxamate also impacted aspartate metabolism via the OAA/Glu transaminase, suggesting that such amino transferase might be legitimate targets in some cancers. These authors have also used SIRM to evaluate potential targets including choline kinase and phosphofructokinase 2, uncovered by both genetic and metabolic analyses with small molecule inhibitors specifically designed from in silica modeling. In addition to target validation, the SIRM approach is excellently suited for assessing the off-target effects and their origin, because of the breadth in metabolic coverage with this approach. [7]

In vitro NMR approach

NMR has a long history in analytical and natural products research, and along with MS and single crystal X-ray diffraction it has become one of the main analytical tools for

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structure analysis. The general principles and approaches have been described in great detail in many excellent texts. Although most such texts discuss analysis of pure compounds, NMR in fact can easily be applied to mixtures, which has a number of advantages for biological studies, particularly for the stable isotope tracer approach, as described in the majority of stable isotope tracer applications in metabolism have used 1D methods, especially direct observation of ^{13}C and making use of the ^{13}C–^{13}C coupling for isotopomer analysis. Such approaches have been successfully applied to many different disease states. However, the sensitivity and information content is significantly less than using 2D methods with proton detection. [8]

Metabolomics in ADMET

The absorption, distribution, metabolism, excretion and toxicology of drugs (ADMET), is one of the most critical areas for drug testing and drug development. It is also one of the most times consuming. While the drug discovery process is concerned almost exclusively with identifying active lead molecules, ADMET is concerned with identifying which of the leads is potentially hazardous, thereby preventing them from progressing too far down the drug development pipeline. ADMET is carried out both in preclinical and clinical trial phases of drug development. In pre-clinical studies, ADMET normally requires testing on large numbers of animals and performing detailed histological and pathological analyses. These are supplemented with clinical chemistry studies of blood, cerebrospinal fluid (CSF), urine and faeces. The invasive, manually intensive nature of most ADMET studies makes them expensive, prone to error and time consuming. Faster, simpler, less invasive methods are desirable – especially for human studies. Metabolomics has applied to ADMET appears to meet these desired criteria. In fact, the field of metabolomics largely began with ADMET studies. [9]

Metabolic fingerprinting

Metabolic fingerprinting describes the unbiased analysis of the metabolome by examination of metabolite patterns in different experimental groups and subsequent classification of the patterns. Samples can be classified if the metabolite fingerprints change between groups, resulting in sample clustering. In mass spectrometry-based investigation, metabolite fingerprints are described by m/z values and corresponding intensities of detected ions. If a separation step is performed, retention times are also used to index metabolites. Thus, m/z values, retention times, and intensities represent the metabolic fingerprint of the analyzed sample and are exported for sample classification using multivariate data analysis. The chemical structure of the detected metabolites typically remains unknown. The term metabolic fingerprinting refers not to investigations that aim at the analysis of a pre-defined set of metabolites but rather to the unbiased analysis of the entire detectable metabolome, even if semi-quantitative data are generated, which could justify using the term metabolomics instead of metabolic fingerprinting. [10]

Key applications:

- **Toxicity assessment / toxicology**. Metabolic profiling (especially of urine or blood plasma samples) can be used to detect the physiological changes caused by toxic insult of a chemical (or mixture of chemicals). In many cases, the observed changes can be related to specific syndromes, e.g. a specific lesion in liver or kidney. This is of particular relevance to pharmaceutical companies wanting to test the toxicity of potential drug candidates: if a compound can be eliminated before it reaches clinical trials on the grounds of adverse toxicity, it saves the enormous expense of the trials.
- **Functional genomics**. Metabolomics can be an excellent tool for determining the phenotype caused by a genetic manipulation, such as gene deletion or insertion. Sometimes this can be a sufficient goal in itself—for instance, to detect any phenotypic changes in a genetically-modified plant intended for human or animal consumption. More exciting is the prospect of predicting the function of unknown genes by comparison with the metabolic perturbations caused by deletion/insertion of known genes. Such advances are most likely to come from model organisms such as Saccharomyces cerevisiae and Arabidopsis thaliana.
- **Nutrigenomics** is a generalised term which links genomics, transcriptomics, proteomics and metabolomics to human nutrition. In general a metabolome in a given body fluid is influenced by endogenous factors such as age, sex, body composition and genetics as well as underlying pathologies. The large bowel microflora are also a very significant potential confounder of metabolic profiles and could be classified as either an endogenous or exogenous

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factor. The main exogenous factors are diet and drugs. Diet can then be broken down to nutrients and non- nutrients. Metabolomics is one means to determine a biological endpoint, or metabolic fingerprint, which reflects the balance of all these forces on an individual's metabolism.

- **Environmental Metabolomics** is the application of metabolomics to characterize the interactions of organisms with their environment. This approach has many advantages for studying organism–environment interactions and for assessing organism function and health at the molecular level. As such, metabolomics is finding an increasing number of applications in the environmental sciences, ranging from understanding organism responses to abiotic pressures, to investigating the responses of organisms to other biota. These interactions can be studied from individuals to populations, which can be related to the traditional fields of ecophysiology and ecology.[11]

Metabolomics in Organ Transplantation

Metabolite measurements have been part of organ transplant monitoring for more than 40 years While most metabolite measurements have been restricted to just a few well-known compounds (creatinine, glucose), there is a surprisingly large body of literature describing injury dependent changes for a large number of lesser-known metabolites. Until recently, most of these measurements have been done using 'classical' clinical chemistry methods such as GC-MS, but since 1999 a growing number of reports have described the use of true metabolomics methods (NMR, LC-MS, spectral pattern analysis, etc.) to monitor organ function. Regardless of the technique or speed by which these compounds were measured, it is still quite instructive to explore what these metabolite measurements have revealed and what compounds are proving to be particularly good diagnostic or prognostic biomarkers. In general, metabolite measurements have been performed to monitor two key aspects of organ physiology:

(i) Organ reperfusion injury and

(ii) Organ function (or dysfunction).

Both types of measurements have been performed and reported on almost all solid organs that can be transplanted including kidneys, liver, lung and heart. The frequency of these reports for specific organs closely reflects the reported frequency of the corresponding organ transplant, with kidney (60%) leading the way, followed by liver (21%), heart (10%), pancreas (5%) and lung (4%). Most metabolite measurements associated with organ transplant analysis have been performed *ex vivo*, using biofluids such as urine, serum or bile. More recently, a few measurements have been performed *in vivo* using NMR chemical shift imaging techniques. These primarily measure inorganic phosphate or phosphorylated metabolites (ATP, ADP and phosphocreatine). [12]

Metabolomics approaches for discovering biomarkers of drug-induced hepato toxicity and nephrotoxicity

Hepato toxicity and nephrotoxicity are two major reasons that drugs are withdrawn post-market, and hence it is of major concern to both the FDA and pharmaceutical companies. The number of cases of serious adverse effects (SAEs) in marketed drugs has climbed faster than the number of total drug prescriptions issued. In some cases, preclinical animal studies fail to identify the potential toxicity of a new chemical entity (NCE) under development. The current clinical chemistry biomarkers of liver and kidney injury are inadequate in terms of sensitivity and/or specificity, prompting the need to discover new translational specific biomarkers of organ injury. Metabolomics along with genomics and proteomics technologies have the capability of providing translational diagnostic and prognostic biomarkers specific for early stages of liver and kidney injury. Metabolomics has several advantages over the other omics platforms such as ease of sample preparation, data acquisition and use of biofluids collected through minimally invasive procedures in preclinical and clinical studies. The metabolomics platform is reviewed with auricular emphasis on applications involving drug-induced hepato toxicity and nephrotoxicity. Analytical platforms for metabolomics, chemo metrics for mining metabolomics data and the applications of the metabolomics technologies are covered in detail with emphasis on recent work in the field. [13]

Use of metabolomics to discover metabolic patterns associated with human diseases

Metabolomics techniques aim at detecting unexpected effects comparing stressed/unstressed or mutant/wild type experiments. This chapter asks the question if metabolomics could also go one step further for analyzing changes in metabolic patterns that are associated with the time course

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of nutritional-dependent human diseases. Such diseases are typically hard to predict, and existing biological markers such as for type 2 diabetes mellitus have limited value for the assessment of individual risks. Many factors may be involved in disease progression such as genetics, nutritional habits, age, or sex, resulting in the need to study large cohorts in order to draw statistically sound conclusions. Due to this inherent biological variability, severe constraints are posed on the validation of the analytical methods used. Using diabetes as an example, the economic and scientific needs for accurate diagnostic tools are discussed with respect to the available analytical and computational approaches for cost-effective high throughput methods.

Metabolomics in Alcohol Research and Drug Development

Metabolomics—a systems biology approach to characterizing metabolites produced in biochemical pathways—is contributing to many studies of disease progression and treatment, although it has not yet been extensively applied in research on metabolic perturbations associated with alcohol abuse. However, numerous metabolomics approaches may contribute to alcohol-related research, as illustrated by studies on alcohol-related metabolic dysfunctions such as alterations in fat metabolism and thiamine deficiency. By further increasing the number and types of metabolites that can be measured in a given biological sample, metabolomic approaches may be able to help define the role of the many different metabolic pathways affected by alcohol abuse and support discovery and development of novel medications for the treatment of alcoholism and related conditions. [14]

Metabolomics in cancer research

Recent developments in cancer research have led to reconsiderations regarding metabolic dysfunctions in cancer cell proliferation and differentiation. The original concept stemmed from the observation that, even in presence of oxygen, highly proliferating cells tend to generate energy strictly from the glycolytic pathway, through a process called aerobic glycolysis, also known as the Warburg effect. More recently, advances in the field of metabolomics applied to cancer research enabled the documenting of the generality of the Warburg effect in a broad variety of tumors. Through metabolomics, cancer cells told us that oxidative stress, while representing one leading cause of genetic instability underpinning carcinogenesis, could also deliver a window of probable therapeutic opportunities that is worth opening. [15]

Metabolomics study on the anti-depression effect.

The emerging field of metabolomics provides a promising opportunity to generate novel approaches for addressing the therapeutic effect of drugs, molecular mechanisms, and ultimately towards exploiting new ideal antidepressants. It has been increasingly used as a versatile tool for the discovery of molecular biomarkers in many areas such as diagnosing or prognosing clinical diseases, exploring the potential mechanism of diverse diseases, and assessing therapeutic effects of drugs. Recent metabolomics technology has successfully applied high through put analytical tools to analyze various biological samples and utilized multivariate statistics to extract meaningful biological information from the resultant complex and huge data sets. Urine has been heavily used in metabolomics studies because it is minimally invasive to the animals or human and primarily reveals an overall metabolic state of the given organism. [16]

The use of metabolomics for the discovery of new biomarkers of effect

Classical metabonomics approaches investigate the levels of these metabolites in urine and plasma of mostly laboratory animals, e.g., after exposition to toxic compounds. Increasing the sensitivity of the analytical methods offers the possibility to detect a far broader range of metabolites and thus increasing the chance of finding relevant biomarkers or patterns of change. [17]

Application of Metabolomics to Cardiovascular Biomarker and Pathway Discovery

Metabolites change rapidly in response to physiologic perturbations; they represent proximal reporters of disease phenotypes. The profiling of low molecular weight bio chemicals, including lipids, sugars, nucleotides, organic acids, and amino acids, that serve as substrates and products in metabolic pathways is particularly relevant to cardiovascular diseases. In addition to serving as disease biomarkers, circulating metabolites may participate in previously unanticipated roles as regulatory signals with hormone-like functions. Cellular metabolic pathways are highly conserved among species, facilitating complementary functional studies in model organisms to provide insight into metabolic changes

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identified in humans. Although metabolic profiling technologies and methods of pattern recognition and data reduction remain under development, the coupling of metabolomics with other functional genomics approaches promises to extend our ability to elucidate biological pathways and discover biomarkers of human disease. [18]

Application of Metabolomics to Unique Human Cardiovascular Disease Models

Novel metabolomics techniques still suffer from signal-to noise issues, however, and applications to humans may be limited by interindividual variability. Although recent studies have evaluated the diurnal and even seasonal variation of hemostatic and inflammatory proteins (e.g., fibrinogen, D-dimer, and C-reactive protein), systematic studies have yet to be performed for metabolites in humans. Studies to identify novel disease-related pathways are also restricted by the inherent unpredictability of the onset of pathological states. As noted previously, human metabolomics studies are also at high risk for potential clinical confounders, such as diet or drug effects, as well as age, gender, and comorbidities. It has been advocated that the analysis of samples from large patient cohorts, stratified by known risk factors or exposures, may minimize the impact of clinical confounding variables. However, the throughput of most current metabolomics technologies, particularly those that are MS based precludes the analysis of large patient cohorts. [19]

CONCLUSION

The concept of research is directed towards the development of newer chemical entities for the treatment of various life threatening diseases and disorders. The fields of drug discovery and drug development mostly rely on systems biochemistry as it offers the global biochemical networks and molecular regulations. The field of metabolomics gaining its importance in relation to the study of these global biochemical networks and their regulations which will be proved as base for future drug discovery and development.

The Future Directions of Metabolomics

In the future metabolomics will most likely be based on finding biomarkers in order to determine when disease is present in an individual biological system. Since there is already a use of biological keys to determine disease, such as glucose in urine means diabetes, or high cholesterol being more susceptible to heart disease, so it is clear metabolomics can take advantage of biochemical pathway knowledge. Currently metabolomics is focusing on specializing on 20-100 different metabolites, and although this is just a small portion it is making strides in discovering biomarkers. There is presently a speculation that metabolomics is the key to finding universal biomarkers for diagnosing disease. This has already been begun in experimentation as in the case for biomarkers for reversible myocardial ischemia which can be found through metabolomics, rather than through genomics or proteomics. This is because there is a sign of 60% to 70% rise in citric acid cycle components when there is a restriction of cardiac flow to the heart. These metabolomics changed can be found in the plasma of the blood and that this is a good new biomarker to find signs of somebody suffering from this disease. With some diseases already being diagnosed by these metabolomics biomarkers they could easily become the future of medical detection to diseases.

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