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A REVIEW ON METABOLOMICS

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Abstract
Recent genetic studies have made major contributions to understanding disease processes. However, biological processes operate through complex interactions between genes, RNA, proteins, and metabolites—the composite of this complex interaction network is defined as the interactome.Furthermore,environmental factors modify the interactome and consequently disease processes . Metabolomics aims at a comprehensive qualitative and quantitative analysis of all small molecules present in a cell a tissue or an organism to study the interplay in response to intrinsic and extrinsic factors. Metabolomics recently caught up with complementary efforts to understand the genomes, transcriptomes and proteomes to establish holistic pictures of biological systems.

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INTRODUCTION:

Recent genetic studies have made major contributions to understanding disease processes. However, biological processes operate through complex interactions between genes, RNA, proteins, and metabolites—the composite of this complex interaction network is defined as the interactome. Furthermore, environmental factors modify the interactome and consequently disease processes. These complex interactions define the phenotype of a disease. Hence, genetic research providesonly part of the picture and ultimately understanding the interactome in disease pathogenesis provides the optimal route to developing new treatments and monitoring their effects. The desire to understand the interactome in disease processes has driven the development of new research methods. One important example is metabolomics, which is the study of endogenous and exogenous metabolites in biological systems. It aims to provide semi-quantitative information on metabolite abundances in a biological system.

Metabolomics aims at a comprehensive qualitative and quantitative analysis of all small molecules present in a cell a tissue or an organism to study the interplay in response to intrinsic and extrinsic factors. Metabolomics recently caught up with complementary efforts to understand the genomes, transcriptomes and proteomes to establish holistic pictures of biological systems.

To determine functions of "unknown" genes in functional genomics studies, Metabolomics has gained increasing attention since changes in small molecule concentrations are closely related to the observed phenotype.

Also disease, drugs, or environmental variation can perturb concentration and fluxes in intermediary metabolic pathways. These responses involve adjustment of intracellular and extra cellular environments in order to maintain homeostasis. Both perturbation and adjustment are expressed as changes in the normal patterns in cells and biofluids that are characteristic (a 'fingerprint') of the nature of the site of the action.

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.

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 sophisticated statistical and multi-variant data analysis tools, including cluster analysis, pathway mapping, comparative overlays, and heatmaps, 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.

Sample collection and preparation:

Turnover of metabolites over time (metabolic flux) can occur in seconds to minutes, unlike changes in levels of proteins and transcripts, which typically occur over minutes or hours.1Therefore, sample collection and preparation are one of the most crucial steps in metabolomics experiments, as suboptimal handling can significantly reduce the accuracy and precision of the results.

Samples for analysis can be in the form of blood, serum, plasma, urine, cerebrospinal fluid (CSF), solid tissues, and cells. In the eye, commonly used tissues or fluids are cornea, lens, retina, vitreous, and aqueous. Typically,430 mg wet tissue is recommended for untargeted studies and this can be challenging particularly in small animal models. Different sample types require different collection processes and preparation although the principles remain similar. The aims of this stage are to quench metabolic activity in the samples and then to isolate or extract the metabolites in an appropriate solvent for the analytical instrument.

Quenching is performed to stop or slow down the metabolic activities so that metabolic flux is minimized or eliminated from the sample as soon as possible after collection. It can be performed by reducing the temperature to sub-zero immediately after collection and storing it at−80 °Cuntil the sample is ready for further processing.9Othermethods used in quenching include increasing the temperature

of the sample or introducing organic solvents with the aim of denaturing enzymes required formetabolism.1The biological samples obtained are highly complex and requires‘ extraction ’to separate the metabolites of interest from a complex sample of matrix, which may include proteins, carbohydrates, DNA, and inorganic salts.

DIRECTED AND UNDIRECTED METABOLOMICS:

Two major approaches are available, which are targeted (directed) and untargeted (undirected) metabolomics. Directed analysis is the approach in which a specific set of metabolites are measured, usually focusing on one or more related pathway(s)of interest. In directed analysis, the analytical method is optimized for the desired set of metabolites using authentic standards. This is followed by applying the method to biological samples, and reporting the results for this set of metabolites. On the other hand, undirected metabolomics is the approach which aims to analyze as many metabolites as possible. In undirected analysis, a global separation and detection method should be applied that can separate and detect a wide range of metabolites. It also requires a high resolution mass spectrometer.

ANALYTICAL DETECTION TECHNIQUES IN METABOLOMICS:

The rapid development of metabolomics in recent times is mainly attributed to the advances in the instrumentation used to detect metabolites. One of the advantages of metabolomic experiments over, for example, proteomics is that higher sample throughput is possible. Depending on the type of sample, the analytical throughput can betens of samples a day.2Automation of sample preparation and loading also means that the instruments can operate for 24 h a day. Traditionally, the study of metabolism focused on single or specific metabolic pathways. However, with advances in instrument sensitivity, development in mathematical data transformation and powerful statistical methods, large numbers of endogenous metabolites can now be analysed in a single sample.

NMR based metabolomics:

NMR is the most common spectroscopic analytical techniques. NMR can uniquely identify and simultaneously quantify a wide range of organic compounds in the micro-molar range. NMR has been introduced to the emerging field of metabolomics where it can provide unbiased information about metabolite profiles. NMR is straight- forward and largely automated and non-destructive, so samples can continue to further analysis. It has been extensively used for metabolite fingerprinting, profiling and metabolic flux analysis . NMR-based metabolomics is becoming a useful tool in the study of body fluids and has a strong potential to be particularly useful for the non- invasive diagnosis of diseases that are very common and significant public health problems. [11]

NMR has been the technique of choice, due to its ability to measure intact biomaterials nondestructively as well as the rich structural information that can be obtained. Hence, extensive research and significant improvements have been performed using NMR to measure populations of low-molecular-weight metabolites in biological samples [12].

The major limitation of NMR for comprehensive metabolite profiling is its relatively low sensitivity, making it inappropriate for the analysis of large numbers of low-abundance metabolites. NMR has been the technique of choice, due to its ability to measure intact biomaterials nondestructively as well as the rich structural information that can be obtained. Hence, extensive research and significant improvements have been performed using NMR to measure populations of low-molecular-weight metabolites in biological samples. High-resolution NMR could provide an ideal mechanism for the profiling of metabolites within biofluids or tissue extracts.

Although it has many advantages, the sensitivity of NMR is relatively poor compared with MS methods, and concentrations of potential biomarkers may be below the detection limit. A number of biofluids such as blood, urine, cerebrospinal fluid, cell culture media and many others can be obtained at a high sampling frequency with minimal invasion, permitting detailed characterisation of dynamic metabolic events. NMR can provide detailed information regarding the structural transformation of a compound as a consequence of metabolism in drug discovery and development.

A decrease of urine succinate, citric acid, and serum acetoacetate, together with an increase of serum lactate, suggests that chronic cysteamine supplementation results in perturbation of rat energy metabolism. Metabolomics is a powerful tool for investigating any disturbance in the normal homeostasis of biochemical processes. In particular, urine metabolomics provides information on the metabolite phenotype of the human being and therefore is appropriate to study the status of the global system. Jung *et al.* applied

an NMR metabolomics approach to investigate the altered metabolic pattern in plasma and urine from patients with cerebral infarctions and in order to identify metabolic biomarkers associated with stroke.

The plasma of stroke patients was characterized by an increase in lactate, pyruvate, glycolate, and formate, and by a decrease in glutamine and methanol; the urine of stroke patients was characterized by decreased levels of citrate, hippurate, and glycine. These detected biomarkers were associated with anaerobic glycolysis and folic acid deficiency. It indicated that magnetic resonance methodologies will be paramount in future disease management. However, because of their sensitivity and specificity, these techniques have been currently adequate for use as diagnostic tools in individual patients. Thus, NMR has been used for analysis of metabolites, including analysis of Alzheimer's disease, prostate cancer, amino acids, nucleotides and nucleoside ,vitamins, thiols, carbohydrate, peptides [13].

Advantages of NMR:

- NMR is the only detection techniques which does not relay on separation of the analytes.this mean the sample is not destroyed
- All kind of small molecules metabolite can be measured simultaneously
- Sample analysis is fast and robust, enabling high throughput

Disadvantages Of NMR:

- It is not as sensitive as MS techniques
- As a consequence's high-resolution NMR of intact biofluid doesnot yet identify all the metaboloites
- Cannot analyse the organic layer from cells/tissue.

MASS SPECTROSCOPY:

The field of metabolomics has witnessed an exponential growth in the last decade driven by important applications spanning a wide range of areas in the basic and life sciences and beyond. Mass spectrometry in combination with chromatography and nuclear magnetic resonance are the two major analytical avenues for the analysis of metabolic species in complex biological mixtures. Owing to its inherent significantly higher sensitivity and fast data acquisition, MS plays an increasingly dominant role in the metabolomics field. Mass spectrometry (MS) is an analytical technique that measures the mass-to-charge ratio (*m/z)* of charged particles (ions). Although there are many different kinds of mass spectrometers, all of them make use of electric or magnetic fields to manipulate the motion of ions produced from an analyte of interest and determine their *m/z.* The basic components of a mass spectrometer are the ion source, the mass analyzer, the detector, and the data and vacuum systems.

The ion source is where the components of a sample introduced in a MS system are ionized by means of electron beams, photon beams (UV lights), laser beams or corona discharge. In the case of electrospray ionization, the ion source moves ions that exist in liquid solution into the gas phase. The ion source converts and fragments the neutral sample molecules into gas-phase ions that are sent to the mass analyzer. While the mass analyzer applies the electric and magnetic fields to sort the ions by their masses, the detector measures and amplifies the ion current to calculate the abundances of each mass-resolved ion. In order to generate a mass spectrum that a human eye can easily recognize, the data system records, processes, stores, and displays data in a computer [15].

Advantages of MS:

- It is much more sensitive than NMR.more metabolite can be analysed.
- Not limited to sample type /potentially more global.
- Take up much space than any NMR machines and cheaper to buy.

Disadvantages of MS:

- Similarity of isomers can make identification difficult.For LC-MS libraries and not completed.
- Sample must be derivatised before analysis which increases the preparation time.
- Sample analysis time is more than NMR and less reproducible.
- Some large molecules cannot be measured by these techniques.

GAS CHROMATOGRAPHY _ MASS SPECTROSCOPY:

The goal of metabolomics analysis is systematic understanding of all metabolites in biological samples. Many useful platforms have been developed to achieve this goal. Currently, as a core analytical method for metabolomics, GC-MS has been used as a platform in non-targeted analysis, especially for hydrophilic metabolites. Generally, GC-MS-based metabolomics requires a high-throughput technology to handle a large

volume of samples and accurate peak identification through the standard retention times and mass spectra. GC-MS has been widely used for metabolomics and can provide efficient and reproducible analysis. For separation on the GC column, GC-MS requires a derivatization reaction to create volatile compounds. Non-volatile compounds are not derivatized and will not be detected in the GC-MS analysis. This limits the applicability to metabolomics.

CAPILLARYELECTROPHOROSIS-MASS SPECTROSCOPY:

CE-MS is a powerful and promising separation technique for charged metabolites, offering high-analyte resolution, providing information mainly on polar or ionic compounds in biological fluids. CE-MS, as an analytical platform, has made significant contributions in advancing metabolomic research. Capillary electrophoresis–mass spectrometry (CE-MS) is an analytical chemistry technique formed by the combination of the liquid separation process of capillary electrophoresis with mass spectrometry. CE-MS combines advantages of both CE and MS to provide high separation efficiency and molecular mass information in a single analysis. It has high resolving power and sensitivity, requires minimal volume and can analyze at high speed. Ions are typically formed by electrospray ionization, but they can also be formed by matrix-assisted laser desorption/ionization or other ionization techniques [20].

It has application in basic research in proteomics and quantitativeanalysis of biomolecul es as well as in clinical medicine. Since its introduction in 1987, new developments and application has made CE-MS powerful separation and identification technique. Use of CE-MS has increased for protein and peptides analysis and other biomolecules. However, the development of online CE-MS is not without challenges. Understanding of CE, the interface setup, ionization technique and mass detection system is important to tackle problems while coupling capillary electrophoresis to mass spectrometry.

LIQUID CHROMATOGRAPHY-MASS SPECTROSCOPY:

Liquid chromatography mass spectrometry (LC-MS) offers the broadest coverage of metabolites due to its ability to work with different column chemistries. Two column chemistry examples include reversed phase liquid chromatography (RPLC) for non-polar to moderately polar metabolites, and hydrophobic interaction liquid chromatography (HILIC) for ionic and polar compounds not retained by RPLC

The analysis of metabolites in human body fluids remains a challenge because of their chemical diversity and dynamic concentration range. Liquid chromatography (LC) in combination with tandem mass spectrometry (MS/MS) offers a robust, reliable, and economical methodology for quantitative single metabolite analysis and profiling of complete metabolite classes of a biological specimen over a broad dynamic concentration range.

Liquid chromatography is a method of physical separation in which the components of a liquid mixture are distributed between two immiscible phases, i.e., stationary and mobile.

In common applications, the mobile phase is a mixture of water and other polar solvents (e.g., methanol, isopropanol, and acetonitrile), and the stationary matrix is prepared by attaching long-chain alkyl groups (e.g., n-octadecyl or C_{18}) to the surface of irregularly or spherically shaped 5 μm diameter silica particles LC-MS based nontargeted metabolomics has been thoroughly tested, validated, and applied to screen/identify and validate novel metabolic biomarkers for epithelial ovarian cancer; six key-metabolites were considered as potential biomarker candidates, ready for early stage detection. In a study, an LC-MS method was successfully applied for metabolomic analysis of hydrophilic metabolites in a wide range of biological samples. Classification separation for metabolites from different tissues was globally analyzed by PCA, PLS-DA and HCA biostatistical methods.

There are numerous analytical platforms that have been used for metabolomic applications, such as NMR, Fourier transform- infrared spectroscopy (FT-IR) and MS coupled to separation techniques, including NMR, GC-MS, LC-MS, FT-MS and UPLC-MS. Whilst NMR spectroscopy is particularly appro- priate for the analysis of bulk metabolites and GC-MS to the analysis of volatile organic compounds and derivatised primary metabolites.

LC-MS is highly applicable to the analysis of a wide range of semi-polar compounds including many secondary metabolites of interest. Since LC-MS can avoid chemical deriv- atization, it is a widely used instrument. MS-based metabolomics offers high selectivity and sensitivity for the

identification and quantification of metabolites, and combination with advanced and high-throughput separation techniques can reduce the complexity of metabolite separation, while MS-based compound retention times and mass spectra.

ULTRA PERFORM LIQUID CHROMATOGRAPHY-MASS SPECTROSCOPY:

UPLC-MS technology is a powerful technique in biomolecular research and can also be used to quantify the activity of signal- ling and metabolic pathways in a multiplex and comprehensive manner. The recent introduction of UPLC, employing porous particles with internal diameters smaller than 2 mm, in conjuction with throughput capabilities compared to conventional HPLC columns, therefore making it even more suitable for a metabolomics approach. Because the optimum linear velocity has a broader range, UPLC also allows a more rapid analysis without loss of resolution. The combination of UPLC with MS detection covers a number of polar metabolites and thus enlarges the number of detected analytes. In view of the recent developments in the separation sciences, the advent of UPLC and MS based technology has shown ever improving resolution of metabolite species and precision of mass measurements [24]

Liquid chromatography–mass spectrometry (LC-MS) is an analytical chemistry technique that combines the physical separation capabilities of liquid chromatography (or HPLC) with the mass analysis capabilities of mass spectrometry (MS). Coupled chromatography - MS systems are popular in chemical analysis because the individual capabilities of each technique are enhanced synergistically. While liquid chromatography separates mixtures with multiple components, mass spectrometry provides structural identity of the individual components with high molecular specificity and detection sensitivity. This tandem technique can be used to analyze biochemical, organic, and inorganic compounds commonly found in complex samples of environmental and biological origin. Therefore, LC-MS may be applied in a wide range of sectors [25].

DATA ANALYSIS:

The data generated in metabolomics usually consist of measurements performed on subjects under various conditions. These measurements may be digitized spectra, or a list of metabolite features. In its simplest form this generates a matrix with rows corresponding to subjects and columns corresponding with metabolite features (or vice versa). Several statistical programs are currently available for analysis of both NMR and mass spectrometry data. A great number of free software are already available for the analysis of metabolomics data shown in the table. Some statistical tools listed in the table were designed for NMR data analyses were also useful for MS data. For mass spectrometry data, software is available that identifies molecules that vary in subject groups on the basis of mass-over-charge value and sometimes retention time depending on the experimental design.

Linear models are commonly used for metabolomics data, but are affected by multicollinearity. On the other hand, multivariate statistics are thriving methods for high-dimensional correlated metabolomics data, of which the most popular one is Projection to Latent Structures (PLS) regression and its classification version PLS-DA. Other data mining methods, such as random forest, support-vector machine, k-nearest neighbors etc. are received increasing attention for untargeted metabolomics data analysis. In the case of univariate methods, variables are analyzed one by one using classical statistics tools (such as Student's t-test, ANOVA or mixed models) and only these with sufficient small p-values are considered relevant. However, correction strategies should be used to reduce false discoveries when multiple comparisons are conducted. For multivariate analysis, models should always be validated to ensure the results can be generalized.

APPLICATIONS OF METABOLOMICS:

Toxicology:

Metabolic profiling of the urine or blood could be utilized for the assessment of toxicity. Several techniques are able to detect physiological changes in the physiological sample that result from the presence of a toxin or toxins.

The information that is revealed by way of metabolic profiling can also be related to a specific health condition or syndrome, such as a lesion in the liver or kidney. Pharmaceutical companies have expressed interest in this field because the ability to test the toxicity of potential drug candidates via its metabolic effects has the potential to make significant savings in the funding needed for clinical trials before a new drug is opened to the public.

Functional genomics:

Metabolomics could be very useful in researching the phenotypes that may result from a certain genetic manipulation in the field of functional genomics. For

example, sufficient knowledge about the behavior of the metabolome could help to predict the phenotypes that would present if a gene was deleted or inserted into the genome.

The detection of phenotypic changes can be applied in a number of practical settings. As a prime example, genetically modified plant material for human consumption can be examined for phenotypic changes. Any changes that have the potential to alter the metabolome of the organisms that consume the genetically modified material may be predicted before they are made available for public consumption.

Additionally, metabolomics could make it possible to predict the function of unknown genes, by way of comparing metabolic perturbations caused by the modifications of known genes. Model organisms of *Saccharomyces cerevisiae* and *Arabidopsis thaliana* are currently being undertaken and could lead to such advances in the future.

Nutrigenomics:

Nutrigenomics combines the knowledge obtained from genomics, transcriptomics, proteomics and metabolomics with nutritional principles for humans.

A metabolome of any body fluid depends on various endogenous factors including the individual's age, gender, body composition, genetic susceptibilities, and concurrent health conditions, as well as exogenous factors such as nutrients, other components of food and medications.

Metabolomics can be applied in nutrigenomics to determine the metabolic fingerprint of an individual, which portrays the effect of the endogenous and exogenous factors in the body on the metabolism of the individual.

Health and medical:

Metabolomics also promises to help advance the current understanding, diagnosis and treatment of several health conditions, such as endocrine diseases and cancer. The field can help to identify the pathophysiological processes of disease and mechanisms that can be targeted to manage the disease.

For example, metabolomics biomarkers in tissue samples or biopsies can be used to categorize and stage the progression of cancers. The information can then be used to guide the appropriate decisions for treatment.

CONCLUSION:

Metabolomics is a collection of powerful tools for the analysis of phenotype, both by hypothesis generation and by hypothesis testing. Building on the strengths of the "omics technologies that came before, metabolomics uniquely comprises analytical technologies that can provide diagnostic patterns via fingerprinting, absolute quantitation of targeted metabolites via pool analysis, relative quantitation of large portions of the metabolome using metabolite profiling, and tracing of the biochemical fate of individual metabolites through a metabolic system via flux analysis. Each of these technologies is supported by the two most commonly used and powerful techniques currently available for metabolomics: mass spectrometry and NMR.

Mass spectrometry-based metabolomics techniques are the most sensitive for simultaneous analysis of a large number of compounds. While limited in quantitation capabilities without appropriate labeled standards, the sheer quantity of information available in a single LC-MS or GC-MS experiment can provide detailed information on the patterns of metabolite change in an entire metabolic network.

Discovery and validation of biomarkers are exciting and promising opportunities offered by metabolic analysis applied to biological and biomedical experiments. This review highlights the importance and benefit of the role of integrated tools in metabolomic research. The recent rapid development of a range of integrated analytical platforms in metabolomics is an ideal strategy and will provide sensitive and reproducible detection of thousands of metabolites in a biofluid sample, accelerating integration of metabolomics into systems biology.

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