Review Article:

Metabolomics - the new "omics" of health care

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ABSTRACT

Metabolic diseases are caused by chronic dysregulations of metabolism that differ among individuals due to interactions of the genetic and environmental influences manifesting as a particular phenotype. These are usually diagnosed with the help of individual metabolites which serve as disease biomarkers. However, single biomarker based diagnostics for metabolism-based diseases fails to identify the cause of the alteration of these surrogate markers. This is where metabolomics comes into the clinical picture as it not only helps in assessing the risk of an individual for developing a particular disease but also helps in individualizing therapy based on the metabolic defect. Metabolomics is named in line with other recently developed approaches of diagnostics, i.e., genomics and proteomics. This review provides an overview of the fundamental basic principles, the techniques involved and the applications of this new -omics of healthcare.

Keywords: *Metabolic disorders, Metabolomics, Health care*

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Knowledge of biochemistry serves to deal with the ever increasing challenges faced by today's healthcare system. Several diseases are known to occur as a result of deranged metabolism and it is well known that human metabolism is an integration of several biochemical pathways. These biochemical pathways do not exist as isolated systems; rather they are interrelated. Though metabolic diseases are identified by their respective metabolites [e.g., diabetes mellitus and glucose, cardiovascular disease (CVD) and cholesterol], individual metabolite measurement does not help in understanding the metabolic basis of the disease completely. The changes in these metabolites need to be understood in the context of their biochemical or metabolic pathways which helps in better appreciation of pathophysiology of disease[1] logically leading to more effective management. This approach, known as metabolomics, helps to coalesce the knowledge of biochemistry to address the health care challenges of the modern world.[2]

The idea of metabolomics was conceived by Arthur Robinson and Linus Pauling in 1971[3] and the term was first used in 1998.[4] Metabolomics has a potential role in functional genomics, toxicology, drug discovery, nutrition, cancer and diabetes.[5] Metabolic diseases are known to be caused by chronic dysregulations of metabolism that differ among individuals, yet giving rise to a single disease state[2] (e.g., insulin resistance in diabetes mellitus, hypercholesterolaemia in cardiovascular disease etc.). Understanding of these different metabolic dysregulations helps in a more personalized management of the disease. Individual metabolites are being used as biomarkers of diseases since a long time. For example elevated glucose levels are indicative of diabetes and cholesterol is associated with CVD. However, single biomarker based diagnostics for metabolism-based diseases fails to identify the cause of the alteration of the surrogate markers. In this regard, metabolomics provides for a personalized metabolic assessment since it allows assessment of a large number of metabolites which are substrates or products in metabolic pathways involved in the disease concerned. In addition to serving as potential biomarkers of disease, study of metabolomics of a disease includes metabolites that serve as

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regulatory signals with hormone like functions or effectors of the disease process itself.[6] It can thus help in better understanding of the disease process so that management can be targeted to improve the efficacy of treatment and possibly prevention strategies.[2] Metabolomics provides sufficient information not only on the presence of the metabolites that predict pathology but also helps in identifying the molecular mechanisms responsible for the dysregulation and a logical strategy for intervening in the process. By accurately diagnosing the metabolic defect underlying a phenotype, the most appropriate drug treatment can be chosen thus personalizing the intervention.[2]

Metabolomics: the next step after genomics and proteomics

According to the central dogma of molecular biology, DNA is transcribed into RNA, which is then translated into proteins. While there may be about 37,000 to 40, 000 genes encoded in the human genome and 125,000 to 250,000 unique transcripts represented in the transcriptome, there could be as many as 1,000,000 unique proteins represented in the proteome. Metabolites are the intermediates and products of metabolism and metabolomics is the study of the repertoire of these non proteineceous, endogenously synthesized small molecules or metabolites present in an organism (Figure 1).[7] A metabolome represents the

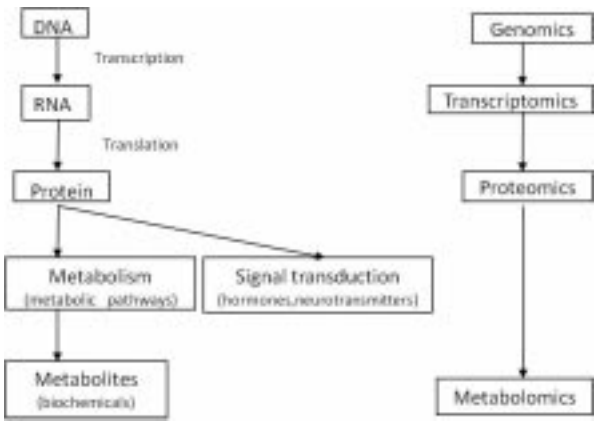

Figure 1: Central dogma of molecular biology and the -omic sciences used in diagnostics
DNA= Deoxyribonucleic acid; RNA= Ribonucleic acid

complete set of small molecule metabolites in a specific organism e.g. human metabolome. As per the human metabolome data base (HMDB) version 2.5, the number of metabolites that were identified includes about 7,982.[8]

The core benefit of metabolomics

It is known that metabolites are an end result of all regulatory complexity present in the cell, tissue or organism. Thus, metabolic changes represent the most proximal reporters of alterations in the body in response to a drug therapy or a disease process.[9] It can be considered as an emerging omics science that allows for a global assessment of the cellular state within the context of the immediate environment, taking into account genetic regulation, altered kinetic activity of enzymes and changes in metabolic reactions.[10-12] In this respect it goes hand in hand with the other omic sciences in that the upstream changes in genes and proteins are measured downstream as changes in cellular metabolism.[10,13]

Human metabolome

The global collection of metabolites in a cell or organism is known as metabolome and includes all small molecules other than nucleic acids and proteins. Estimates of metabolome are likely to be revised as technologies to detect metabolites become more sensitive and comprehensive. The metabolome spans a variety of chemical compound classes, including anionic and cationic; hydrophilic and lipophilic compounds. Some of the endogenous compounds profiled using metabolomic technologies include alcohols, aldehydes and ketones, amino acids, bile acids, carbohydrates, fatty acids, prostaglandins etc.[9]

Techniques of metabolic profiling

Metabolomic assessment can be done using cells, fluids or tissues. Biofluids are the easiest samples to work with and include serum, plasma, urine, ascitic fluid, saliva, bronchial washes, prostatic secretions or fecal water.[14] Metabolomic techniques are usually broadly

gouped into the targeted and non-targeted methods.[15]

Non-targeted analysis

This technique is used for simultaneous measurement of as many metabolites as possible in a biological sample irrespective of its chemical class. These methods are based on comparision of two biological states that statistically differ on qualitative analysis as against the quantitative nature of targeted analysis. This is the method of choice in some situations but usually is succeed by development of targeted analysis.

Targeted analysis

This is the most developed approach in metabolomics that is used to measure the concentration of a limited number of chemically related metabolites like amino acids or hormones. These are quantified in an absolute manner using calibration curves and/or stable isotope labeled internal standards. It is a well established approach in the diagnosis of inherited metabolic disorders.[16]

This can be explained from the following study done to assess early markers of insulin resistance (IR). Insulin resistance is a risk factor for type 2 diabetes mellitus and cardiovascular disease progression. Current diagnostic tests, such as glycemic indicators, have limitations in the early detection of insulin resistant individuals. The Relationship of Insulin Sensitivity to Cardiovascular Risk (RISC) study,[17]comprising a nondiabetic cohort, was initiated to address how IR may contribute to type 2 diabetes mellitus and CVD progression. The study reported a biochemical profiling technology for the discovery of new biochemical biomarkers in a subset of the RISC cohort, in which insulin sensitivity was measured directly by the hyperinsulinemic euglycaemic (HI) clamp. Initial nontargeted analysis revealed four classes of metabolites [organic acids α-ketobutyrate (α-KB), α-hydroxybutyrate (α-HB), lipid species such as acylcarnitines and lysoglycerophospholipids, fatty acids and creatine] that differentiate normal glucose tolerant-insulin sensitive (NGT-IS) from normal glucose tolerant-insulin resistant (NGT-IR) and/or NGT-IS from dysglycaemia [impaired fasting glucose (IFG) or impaired glucose tolerance(IGT)].

Further targeted analysis revealed only α-HB to be the most significant metabolite associated with insulin sensitivity and an early marker for dysglycaemia.

Analytical methods

Metabolites in tissues or body fluids are present in a broad range of concentration. Hence, no single analytical method is capable of analyzing all the metabolites.[9] Several analytical methods are used to measure the metabolites.[18-20] Methods such as chromatographic procedures gas chromatography (GC), high performance liquid chromatography (HPLC) and capillary electrophoresis (CE) have been used to identify and quantify metabolites. Mass spectrometry (MS) and nuclear magnetic resonance (NMR) spectroscopy are the other commonly used techniques in metabolomic measurement.[9] MS identifies metabolites on the basis of difference in the mass/charge (M/Z) ratio. NMR spectroscopy uses magnetic properties of nuclei to determine the number and type of chemicals in a molecule. Proton (1 H) NMR spectroscopy can detect soluble proton containing molecules with a molecular weight of 20 kDa or less.[9] MS has higher sensitivity[18] and covers a wider range of metabolites. However, each technique has both advantages and limitations[21] and hence, a combination of different analytical techniques is commonly used. For example, GC-MS[22], LC-MS[23-25], CE-MS[26-28] have been used for analyzing a majority of metabolites.

Bioinformatics and metabolomics

Large amounts of data are generated in metabolomics. Handling, processing and analyzing the data requires specialized mathematical, statistical and bioinformatical tools.[29] Bioinformatics is particularly useful for data

and information management, raw analytical data processing, statistical analysis and data mining, data integration and mathematical modeling of metabolic networks within the frame work of systems biology.[5]

Applications of metabolomics

Metabolomics was originally proposed as a method of functional genomics,[4] but now its applications extend well beyond that. It is used for comparing mutants, assessing responses to environmental stress, studying global effects of genetic manipulation, comparing different growth stages, toxicology, drug discovery, nutrition, cancer, Diabetes and natural product discovery.[5]

The beneficial role of metabolomics in the management of metabolic and chronic diseases is well appreciated. As has been mentioned earlier, metabolomics provides sufficient information that helps in identifying the molecular mechanisms responsible for the metabolic dysregulation and a logical strategy for intervening in the process. The advantage of metabolic profiling strategy over a biomarker approach can be observed in the example of cholesterol. Serum cholesterol can be high in an individual due to several mechanisms, but three are illustrative: (i) the individual can absorb cholesterol inordinately well through the intestine, or (ii) the individual can produce too much cholesterol through endogenous biosynthesis; or (iii) the individual can convert cholesterol to bile acids very slowly. Total cholesterol measurement does not distinguish between these three mechanisms, but if the analytical measurement of cholesterol is extended to include sterols and their metabolites, all the required information can be obtained.[2] Individuals who absorb excessive cholesterol hyper absorb both cholesterol and phyto sterols[30] and the concentration of phyto sterols in plasma reflect the higher absorption from the intestine. For these individuals, treatment which is targeted at intestinal absorption of cholesterol is recommended.[31] Those individuals who produce excess cholesterol show increased concentration of mevolanate,[32] an intermediate in cholesterol biosynthesis. For these individuals, treatment with inhibitors of cholesterol biosynthesis remains most appropriate. Individuals with decreased bile acid conversion of cholesterol can be identified by the measurement of 7-α hydroxy 4- cholesten -3- one in plasma.[33] Metabolomic diagnostics thus helps to provide a personalized and effective approach in the selection of drug treatment thereby increasing the efficacy and safety of therapy.

Most of the drugs act at the level of metabolites and metabolomics is a more direct measure of the action of a drug. Thus, measuring the biochemical status using metabolomics helps in understanding how the disease is manifest, how drugs work and also in identifying responders and non responders to treatment.[7] The use of metabolomics for assessment of treatment efficacy of both traditional chemotherapy and hormonal agents has been shown through in-vitro experiments.[14] Intermediates of choline phospholipid metabolism have been found to be potential biomarkers for monitoring treatment efficacy in a variety of human cancers.[34] In general, when using standard metabolomic methods, tumours display elevated phospholipid levels characterized by an elevation of total choline containing compounds (tCho) and phospho choline. Thus, a decrease in tCho signal on proton NMR may indicate response to chemotherapy or radiation and may be used as an early marker of effect in malignancies of breast, prostate and brain.[14] Positron emission tomography (PET), a form of in vivo metabolomics with the use of radioactive glucose, choline or thymidine as metabolic end points has been evaluated as a predictor of drug efficacy in certain tumour types.[14] In recurrent gastrointestinal stromal tumours (GIST), [18F] fluor-deoxy glucose (FDG) PET was found to be superior to the standard com-

puted tomography scanning in predicting early response to treatment with imatinib.[35,36]

Another application of metabolomics is in the characterization of toxic effects of drugs. It can be used as a biomarker of hepatic, renal and lung toxicity by analyzing various metabolites such as glucose, lactate, lipoproteins and amino acids which may either increase or decrease thus providing for a recognizable pattern associated with an organ dysfunction.[37-44] Though much of the data have not been validated and some overlap exists between various toxins, the pattern, temporal rate of change and extent of change in metabolites can still provide toxicity assessments[45] which can be used for preclinical drug screening and for following a patient clinically to monitor target organ effects.

In conclusion, metabolomics is a novel discipline in which biochemical profiling of hundreds of metabolites in complex mixtures such as plasma is done. It helps in deducing the metabolic status of a cell, tissue or organ. It has been found to have a great potential not only as a diagnostic tool but also helps in achieving a fully personalized and preventive health care. Comprehensive measurement of metabolites helps in targeting the treatment at the molecular basis of the disease processes and not simply at the end point symptoms. It has a broad potential not only by itself but also when used in conjunction with other -omic sciences such as proteomics and transcriptomics. However, to meet the growing challenges in metabolomics, further developments are required in analytical science and bioinformatics as well as standards of data management, analysis and reporting.

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