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Critical path

Metabolomic biomarkers: their role in the critical path☆

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Global metabolic profiling is being applied to identify biomarkers of health. Some small molecules are exquisitely sensitive indicators of health status. Metabolic profiling analyses are being used to determine biomarkers of drug safety and effectiveness as well as disease diagnosis and prognosis including organ transplant rejection. To understand the mechanism(s) of drug toxicity and disease, a *systems biology approach* that considers the information generated from metabolic profiling, genetics, transcriptomics and proteomics research paradigms is necessary. This will allow for a better understanding of the mechanism(s) of drug interactions and disease while possibly identifying susceptible populations, an important goal in the move toward personalized medicine.

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Introduction

Global metabolic profiling has long been used to determine biomarkers to aid in assessment of the pathophysiological health status of patients. The emergence of genomics and proteomics technologies has generated plausible mechanisms that correlate with the diagnostic and prognostic metabolic biomarkers in the health to disease continuum [1,2]. Global metabolic profiling has been referred to as either metabolomics [3] or metabonomics [4], though both identify metabolic alterations under varying conditions. In this review, metabolomics and metabonomics will be referred collectively to as global metabolic profiling or simply, metabolic profiling. Together, genetics, transcriptomics, proteomics and global metabolic profiling comprise the basis of the systems biology approach.

Systems biology has been described as the computational integration of data generated by a suite of 'omic' platforms to understand function across different levels of biomolecular organization [4–6]. These new research paradigms are likely to lead to new opportunities for personalized health because they add more detail to our current knowledge of the health and disease continuum. A systems biology approach provides a better understanding of the mechanisms and progression of disease as well as the ability to identify early and sensitive biomarkers of drug efficacy and toxicity. The ability to link changes in the metabolite profile to altered genes and proteins will help to elucidate the source of metabolite biomarkers in many cases. As part of a systems biology approach, global metabolic profiling will also help the medical community understand the complex interaction between metabolites, genes and proteins to overall health status.

Metabolomic analytical platforms

Metabolic profiling studies have been performed with a wide range of biofluids and tissues [7]. The large concentration range, over eight orders of magnitude and chemical diversity of metabolites found in cells, tissues and biofluids, requires multiple analytical methods to detect as many metabolites as possible. In general, the most common metabolic profiling

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technologies consist of high-resolution NMR and hyphenated mass spectrometric methods with an initial chromatographic separation step (e.g. LC–MS). The advantages and disadvantages of NMR and MS platforms have been thoroughly reviewed [8]. Initially, NMR was used primarily for the investigation of metabolite changes as it is highly reproducible and quantitative in nature and it detects each proton in the same manner. Mass spectrometry is a technology that is more frequently being applied in metabolic profiling studies due in part to its high sensitivity. The combination of NMR and MS platforms in metabolic profiling research will permit a more broad coverage of the metabolome [9].

Metabolomics in drug discovery

One of the major areas of research in pharmaceutical drug discovery is directed toward the identification of biomarkers of drug toxicity that can be used in preclinical and clinical studies of drugs [10–14]. There is a strong need to develop new biomarkers that can accurately predict toxicity in the preclinical development of new chemical entities (NCEs) early in the drug development process. Metabolic profiling has the potential to impact pharmaceutical drug discovery by lowering both the cost and time associated with the development and marketing of a NCE. The current estimate of the cost for developing a NCE is approximately one billion dollars [15]. Additionally, there is a high attrition rate of NCEs with only one in five actually making it to market. Of those NCEs that fail, approximately half of them fail in phase III clinical testing. Failed NCEs increase drug discovery costs and therefore, ultimately increase cost to the consumers. Time is an additional consideration with it taking an average of eight to ten years for a NCE to make it through the developmental and FDA approval processes. Therefore, a technique such as metabolic profiling that has the potential to identify toxicity early in the drug discovery process will save time and money for pharmaceutical companies. For example, metabonomics methods were used in a preclinical study at Merck on a compound known to cause hepatotoxicity in several species [16]. Multivariate statistics of the NMR spectra of urine showed the dosed group separated from the control group with the depletion of tricarboxylic acid cycle intermediates and the appearance of medium-chain carboxylic acid. *In vitro* experiments with this compound showed that it causes defective metabolism of fatty acids. This is a case where metabonomics was able to provide mechanistic insight to the hepatotoxicity of a drug.

In general, the most widely used biofluid for toxicity studies has been urine, which is easily obtained from a subject and provides information about the whole system following a toxic insult [17–19]. One advantage of using urine or plasma in drug toxicology experiments is that the sample is collected noninvasively so that it can be applied in clinical studies. Another advantage is that multiple biofluid samples from a single subject can be collected over a time course, which allows the determination of a metabolic trajectory that describes the toxic response and recovery period. The analyses of metabolites in biofluids permit a toxicological evaluation of the 'health of many different organs' within the same animal over time and may permit the simultaneous evaluation of drug efficacy from the same biofluid sample [19]. Further, since the same animal can be used over many time points, the number of animals needed for a toxicological study is greatly reduced. This makes the metabolic profiling method much more cost effective than many other biomarker detection methods. The potential to determine biomarkers from easily obtained, noninvasive samples also makes preclinical findings accessible in the clinical setting.

Another benefit of using a temporal study with the same animal is that it is not necessary to know the pharmacodynamics and pharmacokinetics of the drug before the biomarker investigation. This removes the need for a preparatory pharmacokinetic research, which saves time and financial resources. This is especially important in the early ADME-Tox (absorption, distribution, metabolism and excretion-toxicology) stage of drug discovery. Temporal metabolic profiling studies may permit a quick determination as to whether the toxic insult to the animal causes temporary metabolite concentration changes that return to normal after a period of time or results in metabolite concentrations that stay in a perturbed toxic state. This information can be evaluated to determine whether and when the animal recovers from the toxic distress [20].

Metabolomics in personalized health

NMR and GC–MS methods have long been applied to detect inborn errors of metabolism following birth [21]. Thus, some of the first attempts to determine biomarkers of disease by global metabolic profiling was applied to the study of inborn errors of metabolism [22,23]. Genetic alterations in DNA sequence by nucleotide deletion, insertion or single nucleotide polymorphism can alter the enzymatic activity of a protein that is responsible for converting one metabolite to another. If no other enzymes are able to interact with a particular metabolite, the concentration of the metabolite can build up. In an effort to return to a homeostatic state, the metabolite is exported from the cell to biofluids like serum and urine. In cases of inborn errors of metabolism, the metabolite biomarkers are diagnostic for a particular inborn disease and knowledge of genetics can be used to understand the link between altered metabolic pathway and disease state.

Another success for metabolic profiling has been its ability to diagnose renal, liver and heart organ transplant rejections better than measurements of standard clinical chemistry parameters [24–26]. Recent metabolic profiling evaluations of kidney transplants have revealed biomarkers that include altered levels of trimethylamine-*N*-oxide (TMAO), dimethy-

lamine, lactate, acetate and alanine. In many of these investigations, TMAO was increased by a factor of 3–4 compared to healthy controls. The increase in TMAO is believed to stabilize proteins when there is an increased concentration of protein denaturants such as urea and guanidine derivatives following a toxic insult to the kidney [27].

Metabolic profiling has been applied to diagnose and predict the outcome of diabetes [28], cirrhosis [29] and cancer [30,31] in preclinical and clinical studies. Many metabolites are species-independent and could form the basis of translational biomarkers that are determined in preclinical studies and applied during clinical studies. Metabolic profiling of urine, plasma, serum and cerebral spinal fluid has been applied effectively in a clinical research environment for the assessment of a range of health issues. Urine samples have been evaluated to investigate the efficacy of immunosuppressants in renal transplant [32] and to detect inborn metabolic diseases [22,23]. Plasma or serum samples have been used to evaluate differences in fat metabolism in lean and obese patients [28], to detect motor neuron diseases like amyotrophic lateral sclerosis [33], to detect pancreatic cancer [31], to detect coronary heart disease [34] and to detect ovarian cancer [30]. Finally, cerebral spinal fluid has been used for the detection of meningitis and ventriculitis [35]. Each of these metabolic profiling studies was able to develop a predictive relationship between the biofluid spectral patterns and a health disease state in humans. Most diseases occur later in life and have not only a genetic component but also environmental contributions. The later the onset of a disease, the more probably it is that contributions from the environment played a larger and significant role. Once a disease can be defined as a pattern of metabolites in tissue or biofluids, global metabolic profiling can be used as a diagnostic tool.

Metabolomics and the critical path

In March 2006, FDA published the *Critical Path Opportunities Report and List* as a follow up to FDA's initial Critical Path Challenges and Opportunities Report that was released in 2004. The *Opportunities Report and List* presented 6 major topic areas and 76 specific scientific opportunities. Metabolomics can play a significant role in the major topics for developing biomarkers, streamlining clinical trials and defining at-risk populations. The ability of metabolomics to provide translational safety biomarkers related to kidney, liver, heart and vascular damage should allow it to play a major role in many opportunities presented in Topic 1: better evaluation tools – developing new biomarkers and disease models. In Critical Path Topic 2: streamlining clinical trials – the ability of metabolomics to give noninvasive biomarkers of efficacy and toxicity will facilitate the advancement of clinical trial designs. By evaluating patient responses through pharmacometabonomic phenotyping techniques, pharmaceutical companies may be able to adjust which patients are used in each stage of the trial through accurate prediction of nonresponders and responders in terms of toxicity [36]. Finally, linking genetics, transcriptomics, proteomics, metabolic profiling, nutrition and gut microflora in relation to patient health to disease status is an essential component of the FDA's critical path to personalized medicine. Metabolic profiling has a potential to play a vital role in many facets of the FDA's Critical Path.

Conclusions

Metabolic profiling is an essential component that along with genetics, transcriptomics and proteomics data will permit a detailed description of the interactions between metabolites, proteins, transcripts and genes in the health and disease continuum. In many errors of inborn metabolism, the relationship between disease state, metabolic biomarker and genetics is easily understood. However, in many diseases, the relationship between health status, genetics and metabolic state is highly complex and not easily determined. The ability of metabolic profiling to provide noninvasive translational biomarkers makes it an integral part of a systems biology approach as well as in the move toward personalized medicine and within the FDA Critical Path opportunities.

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