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Review

Metabolomics for laboratory diagnostics

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ABSTRACT

Metabolomics is an emerging approach in a systems biology field. Due to continuous development in advanced analytical techniques and in bioinformatics, metabolomics has been extensively applied as a novel, holistic diagnostic tool in clinical and biomedical studies. Metabolome's measurement, as a chemical reflection of a current phenotype of a particular biological system, is nowadays frequently implemented to understand pathophysiological processes involved in disease progression as well as to search for new diagnostic or prognostic biomarkers of various organism's disorders. In this review, we discussed the research strategies and analytical platforms commonly applied in the metabolomics studies. The applications of the metabolomics in laboratory diagnostics in the last 5 years were also reviewed according to the type of biological sample used in the metabolome's analysis. We also discussed some limitations and further improvements which should be considered taking in mind potential applications of metabolomic research and practice.

Contents

1. Introduction

Dynamic homeostasis is a common feature of each living system. It means that particular biological organism is changing in time, when is exposed to various exogenous stimuli, such as pharmacotherapy, diet or environmental factors. Disease initiation and progression can also lead to disturbances of internal balance of biological system and its components, such as cell, tissue, organ or whole organism. Nowadays, to understand complex, dynamic living systems, an integral, holistic approach, called systems biology (systeomics), is commonly applied [1]. Systeomics focuses on the structure and dynamics of the particular biological organization levels in order to predict behavior of the living system (cell, tissue, organ, organism) based on a set of biological components and interactions between them [2]. Systeomics approach includes few crucial – "*omics*" sciences: genomics, transcriptomics, proteomics and metabolomics, which aim to determine genome, transcriptome, proteome and metabolome, respectively. Firstly, development in genomics area led to genome sequencing of

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0731-7085/

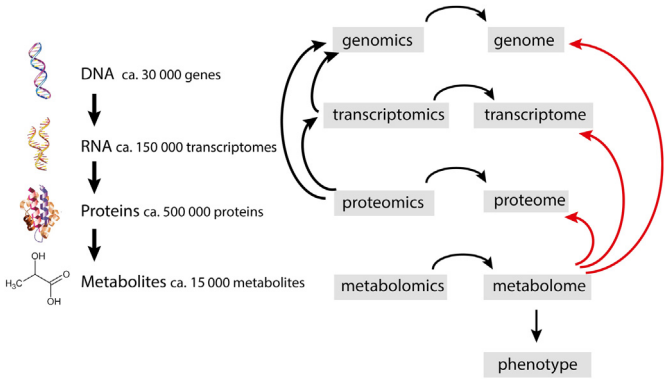

Fig. 1. The "*omics*" cascade in systems biology approach.

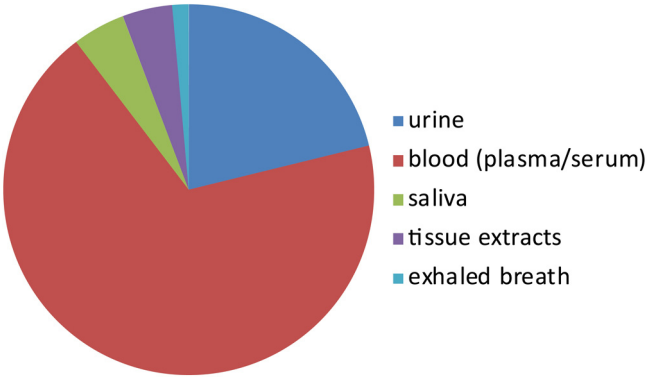

Fig. 2. The proportional contribution of biological materials in metabolomics research. The chart is based on searching of the following terms: metabolomics together with plasma, serum, urine, saliva, tissue extracts and exhaled breath in *ScienceDirect* and *PubMed* databases and considering the studies reported in the last decade.

different organisms and stimulated progress of other systems biology disciplines, as transcriptomics and proteomics. These – "*omics*" sciences are focused on determination of mRNA transcription level (transcriptome) or proteins abundance (proteome), respectively. Consequently, development in proteome research initiated progress of metabolomics approach, which aims to measure the products of individual proteins expression, i.e. the low-molecular-weight compounds, named metabolites. Thus, the biological information in the specific living system goes from genes to transcripts through proteins and finally to metabolites. The typical "*omics*"-cascade was displayed in Fig. 1. It should be underlined here that there are numerous network and feedback interactions between metabolites, proteins, transcripts and genes (Fig. 1) [3].

The changes at the genome or proteome level predispose to the specific behaviors of the particular biological system. The alterations in metabolome composition reflect the current status of the organism. Moreover, changes observed in the metabolome represent the dynamic perturbations of genome, transcriptome and proteome of the specific biological system. Therefore the metabolome is considered to be a chemical reflection of a molecular phenotype. Metabolome is also thought to be a promising link between the genotype-phenotype gap. Therefore, metabolomics is becoming dominant approach in systems biology research and is extensively used in biomedical, pharmaceutical and toxicological studies. Additionally, continuous development in sensitive analytical techniques and advanced biostatistics provides feasible metabolites' determination and identification in complex biological samples, for instance in blood, urine or in tissue extracts. In case of diseases which are developing asymptotically, metabolite changes might occur much earlier that any specific symptoms. For that reason, metabolomics is frequently applied to get a deeper understanding of pathomechanisms of complex diseases, like cancer, diabetes, cardiovascular or pulmonary disorders, as well as in searching for new diagnostic and prognostic disease biomarkers [4,5]. Therefore, the main aims of this review are focused on current applications of the metabolomics in the biomedical research, mainly in the disease diagnosis and progression. Metabolomics is considered to be promising and potentially useful tool for laboratory diagnostics. However, there are still some limitations and improvements that should be taken into account, what will subsequently be discussed in the present review. Current examples of application of metabolomics in diagnosis of various diseases will be provided in Section 4 and discussed according to the type of biological sample used in metabolomics experiment.

A type of biofluid is a crucial factor, which determines the proper design of a whole metabolomic study. Fig. 2 represents the chart with biological samples which have the most frequently been applied for metabolomics research in the last decade.

2. Metabolomics: aims and research strategies

The beginning of metabolomics research can be dated back to ancient Greece, where urine colors, tastes or smells, which are metabolic in origin, were tested to diagnose diabetes [6]. However, systematic studies in the 1970s by Horning and Horning [7,8] as well as by Pauling et al. [9], initiated a new age in metabolomics research, which was focused rather on analysis of the comprehensive state-specific set of metabolites in biological fluids instead of a determination of a single metabolite. Modern approach to metabonomics and metabolomics was developed during the last two decades. Nicholson et al. [10] defined metabonomics, as "the quantitative measurement of the dynamic multiparametric metabolic response of living systems to pathophysiological stimuli or genetic modification". Then, Fiehn [11] evaluated metabolomics as a "comprehensive and quantitative analysis of all metabolites in a system". It should be noticed here that the difference between metabonomics and metabolomics is subtle and rather linguistic than technical. Therefore, in practice, these terms are commonly used interchangeably and utilize the same analytical and modeling methods [6]. In general, modern metabolomics is a comprehensive and systematic identification and quantification of small molecule metabolites (<1500 Da) in biological samples at a given point of time. Similar to genome or proteome, metabolome is defined as a set of all metabolites present in cell, tissue, organ or organism at a given state. However, contrary to genome or proteome, human metabolome's composition is still not fully defined. Few publicly available databases, such as *Human Metabolome Database*-HMDB (http://.hmdb.ca), METLIN (http://metlin.scripps.edu) or KEGG (http://www.genome.jp/kegg), provide information on metabolites present in human biofluids. For instance, HMBD comprises around 40,000 human metabolites and is constantly updated and expanded. However, it should be noticed that human metabolome's size, proposed in HMDB, can be overestimated due to the presence of various exogenous metabolites originating from diet, pharmacotherapy as well as compounds produced by endogenous gut microflora [12].

There are a few research approaches, which have emerged in metabolome's analysis, like metabolic profiling, metabolic fingerprinting and metabolic footprinting [13]. Metabolic profiling is an example of targeted approach, which focuses on the identification and quantification of predetermined groups of metabolites with similar physicochemical properties (e.g., carbohydrates, amino acids, organic acids, nucleosides) or taking part in the same biochemical pathway (e.g., glycolysis, gluconeogenesis, β-oxidation or citric acid cycle) [14]. In this strategy the hypothesis about