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Metabolome analysis for discovering biomarkers of gastroenterological cancer☆

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ABSTRACT

Improvements in analytical technologies have made it possible to rapidly determine the concentrations of thousands of metabolites in any biological sample, which has resulted in metabolome analysis being applied to various types of research, such as clinical, cell biology, and plant/food science studies. The metabolome represents all of the end products and by-products of the numerous complex metabolic pathways operating in a biological system. Thus, metabolome analysis allows one to survey the global changes in an organism's metabolic profile and gain a holistic understanding of the changes that occur in organisms during various biological processes, e.g., during disease development. In clinical metabolomic studies, there is a strong possibility that differences in the metabolic profiles of human specimens reflect disease-specific states. Recently, metabolome analysis of biofluids, e.g., blood, urine, or saliva, has been increasingly used for biomarker discovery and disease diagnosis. Mass spectrometry-based techniques have been extensively used for metabolome analysis because they exhibit high selectivity and sensitivity during the identification and quantification of metabolites. Here, we describe metabolome analysis using liquid chromatography-mass spectrometry, gas chromatography-mass spectrometry, and capillary electrophoresis-mass spectrometry. Furthermore, the findings of studies that attempted to discover biomarkers of gastroenterological cancer are also outlined. Finally, we discuss metabolome analysis-based disease diagnosis.

1. Introduction

Comprehensive investigations based on genomics, transcriptomics, proteomics, and metabolomics play an important role in life science. Metabolomics is the study of the final step in the "omics" cascade and is used to acquire comprehensive biological information about low molecular weight metabolites (<1,000 Da) [1,2]. The metabolome is the endpoint of the omics cascade, and hence, is the closest point in the cascade to the phenotype; therefore, studies of the metabolome can provide snapshots of the physiological state of an organism (Fig. 1). Over the last decade, metabolome analysis has developed markedly, as demonstrated by the increasing numbers of metabolome analysis-based publications in various research fields, such as clinical, cell biology, and plant/food science studies [3–6]. These developments have mainly been driven by improvements in analytical technologies, which have made it possible to rapidly analyze biological samples for thousands of metabolites. Metabolome analysis involves a qualitative and quantitative approach to analyzing all of the metabolites present in organisms. Currently, two complementary approaches are used for metabolome analysis:

Abbreviations: LC-MS, liquid chromatography-mass spectrometry; GC-MS, gas chromatography-mass spectrometry; CE-MS, capillary electrophoresis-mass spectrometry; NMR, nuclear magnetic resonance; FT-IR, Fourier transform-infrared; TCA, tricarboxylic acid; GLUT, glucose transporter; GLS2, glutaminase 2; *m/z*, mass-to-charge ratio; EI, electron-impact ionization; TMS, trimethylsilyl; Q, single quadrupole; IT, ion trap; MS/MS, tandem mass spectrometry; MRM, multiple reaction monitoring; QqQ, triple quadrupole; TOF, time-of-flight; FTICR, Fourier transform ion cyclotron resonance; HMDB, the Human Metabolome Database; KEGG, Kyoto Encyclopedia of Genes and Genomes; PCA, principal component analysis; OPLS-DA, orthogonal projection to latent structures-discriminant analysis; MLR, multiple logistic regression analysis; OSCC, oral squamous cell carcinoma; UPLC, ultra performance liquid chromatography; MALDI, matrix-assisted laser desorption/ionization; ROC, receiver operating characteristic; CEA, carcinoembryonic antigen; SFE, supercritical fluid extraction; VOCs, volatile organic compounds; HS-SPME, headspace-solid phase microextraction; ITEX, in-tube extraction; QC, quality control; QA, quality assurance.

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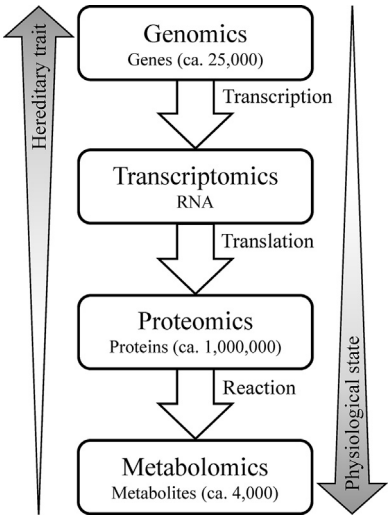

Fig. 1. The omics cascade: the pathway from the gene to metabolism.

metabolic profiling and metabolic fingerprinting. Metabolic profiling describes analyses that focus on a group of metabolites, e.g., a class of compounds, such as carbohydrates, amino acids, or the metabolites associated with a specific pathway [7]. Metabolic profiling requires unambiguous qualitative identification of the metabolites and correction for analytical inaccuracies [8]. Metabolic fingerprinting is a sample classification strategy based on the target samples' spectral patterns, which are considered to be derived from their biological state and/or origin [9]. Many analytical techniques based on liquid chromatography–mass spectrometry (LC–MS), gas chromatography–mass spectrometry (GC–MS), capillary electrophoresis–mass spectrometry (CE–MS), nuclear magnetic resonance (NMR) spectroscopy, or Fourier transform–infrared (FT–IR) spectroscopy have been developed for metabolome analysis [10–14]. Since MS-based techniques exhibit high selectivity and sensitivity during the identification and quantification of metabolites, they have been extensively used for metabolic profiling. On the other hand, NMR and FT–IR spectroscopy display low selectivity, but can be used to discriminate between biological samples on the basis of differences in their metabolite profiles. Therefore, these techniques are often used for metabolic fingerprinting.

Recently, metabolome analysis has been increasingly used to examine biofluids, e.g., blood, urine, or saliva, for biomarker discovery and disease diagnosis [15–18]. Most cancers are difficult to detect in their early stages because they exhibit a long asymptomatic period. Moreover, there is currently a lack of highly sensitive and specific cancer diagnostic tools. Since cancer cells are known to display highly unique metabolic phenotypes, metabolome analysis could be used to identify metabolic profiles, fingerprints, or signatures that would be useful for detecting cancer and assessing the pharmacodynamic effects of therapy. In clinical research, its utility has been demonstrated by the identification of new biomarkers for the diagnosis of gastroenterological diseases [19].

Most of the metabolic processes in the body, such as energy metabolism and amino acid catabolism, are common to all living cells. However, some metabolic pathways are up- or down-regulated in cancer cells. Metabolome analysis aims to determine the differences between biological samples based on their metabolic profiles or fingerprints. Cell growth requires a great deal of energy as well as the raw materials for membranes and other cellular components. Most cancer cells predominantly produce energy by glycolysis rather than oxidative phosphorylation via the tricarboxylic acid (TCA) cycle, even in the presence of an adequate oxygen supply: a phenomenon termed "the Warburg effect" [20]. In addition, it is likely that cancer cells exhibit profound changes in their metabolic pathways, such as those for amino acid metabolism and fatty acid oxidation. Such alterations in the metabolomes of cancer cells result in changes in the compositions of biofluids as well as tissues. Thus, metabolome analysis could become an ideal tool for cancer diagnosis.

In this review, we describe metabolome analysis using LC–MS, GC–MS, and CE–MS, as well as the findings of studies that attempted to discover biomarkers of gastroenterological cancer. Finally, we discuss metabolome analysis-based disease diagnosis.

2. The basis of carcinogenesis and metabolic alterations

Cancers are caused through the multistep accumulation of mutations in both oncogenes and tumor suppressor genes that control the growth, differentiation, and other behaviors of cells [21,22]. Evidence has accumulated that the mutations of oncogenes and tumor suppressor genes can stimulate the transcription of a number of genes that encode the proteins mediating the metabolic pathways [23]. Among them, *APC*, *KRAS*, and *p53* have been reported to play an important role in regulating several aspects of cellular metabolism.

The mutation of the *APC* gene occurs in the early stages of colorectal and gastric carcinogenesis [24,25]. APC proteins negatively regulate the Wnt pathway by aiding the degradation of β-catenin [26]. The Wnt pathway regulates stem cell pluripotency and cell fate decisions during development. The mutation of *APC* gene results in the stabilization of β-catenin and activation of the Wnt pathway, and then leads to aberrant cellular proliferation. Recently, it was revealed by proteome analysis that β-catenin activation in mouse liver might affect glutamine and glucose metabolism [27]. In addition, the metabolomic status of cells and tissues with *APC* gene mutation indicated that the *APC* gene might regulate amino acid related pathways and other energy related metabolic pathways [28].

The mutation of the *KRAS* gene is an early step in the sequence of pancreatic carcinogenesis. KRAS is a member of the RAS family of GTP-binding proteins that mediate a wide variety of cellular functions including proliferation, differentiation, and survival [29]. The *KRAS* gene can be activated by a point mutation that permits it to continue to stimulate cell proliferation even when the counter regulatory signals are trying to halt the process. Oncogenic KRAS signaling promotes glucose uptake by increasing glucose transporter (GLUT) 1 expression but decreases its utilization in the TCA cycle [30,31].

The *p53* tumor suppressor gene is the most commonly mutated gene in human cancer [32–36]. As a transcription factor, the p53 protein mainly exerts its function in tumor suppression through its transcriptional regulation of the target genes to initiate various cellular responses [37]. Recent studies have revealed a novel function of p53 in regulation of cellular metabolism. In cancer cells, mitochondrial oxidative phosphorylation activity is decreased in association with changes in the expression of synthesis of cytochrome c oxidase 2 that is regulated by p53 [38]. The expression of p53 protein also influences glycolysis by regulating the levels of a series of gene products. The mutation of *p53* gene abolishes the repressive effect on GLUT1 and GLUT4 that influence glucose metabolism and cell energy supply [39]. The p53 protein can induce the expression of TP53-induced glycolysis and apoptosis regulator, which lowers the intracellular concentration of fructose-2,6-bisphosphate that promotes glycolysis [40]. Additionally, the loss of p53 is associated with the increased level of phosphoglycerate