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REVIEW

Nuclear Magnetic Resonance Spectroscopy and Mass Spectrometry-Based Metabolomics for Cancer Diagnosis

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Abstract: It is highly desirable to develop and validate novel methods for detecting cancer with higher sensitivity and better specificity. The aim of this review is to introduce a relatively new approach, metabolomics, and explore its potential for cancer diagnosis. We briefly introduced the concept of metabolomics and its relationship with other omics studies in systems biology for cancer detection. The field of metabolomics focuses on the parallel measurement of hundreds of small molecule metabolites in biological samples such as blood, urine, and biopsied tissue. Since metabolite levels are sensitive to subtle changes in the pathological status, metabolomics promises novel avenues for early cancer detection and a better understanding of cancer processes. In fact, many previous metabolomics studies have demonstrated the promises of metabolomics not only for the diagnosis of various kinds of cancer, but also for therapeutic monitoring as well as for drug development. In addition, in this review we discussed the challenges and future directions for developing metabolomics methods towards clinical applications for cancer diagnosis.

Key Words: Nuclear magnetic resonance spectroscopy; Mass spectrometry; Metabolic profiling; Metabolomics; Cancer; Oncology; Diagnosis; Review

1 Introduction

Cancer is a major public health problem around the world, *e.g.*, one in 4 deaths in the United States is due to cancer[1]. In China, cancer is also a major killer, responsible for 25% of all deaths in urban areas and 21% in rural areas[2], and more than 1.5 million people die due to cancer every year. The most common cancers include prostate cancer, breast cancer, lung cancer, and colon cancer. Currently, many cancers are eventually diagnosed by biopsy. Biopsy could be a simple procedure or a serious operation, depending on the location of the tumor. Many cancer patients need X-ray, computed tomography scan (CT) and/or magnetic resonance imaging (MRI) to determine the exact location and size of the tumors. In the past decade or so, the analysis of biomarkers at the molecular level attracted increased attention for cancer diagnosis, monitoring, and treatment[3]; for example, blood tests were used for examining a number of proteomic and genomic tumor markers.

It is well known that early and accurate diagnosis of cancer will not only improve survival but also help clinicians determine the best therapeutic strategies for patients by avoiding under- or over-treatment. For example, based on 13 studies including 2,263 patients, Lu *et al.* concluded that 5–8 lives could be saved in every 1,000 patients if breast cancer recurrence could be detected earlier, leading to a 17%–28% reduction in mortality[4]. However, the diagnosis performance of traditional methods is limited, for instance, mammography could produce 20%–40% misdiagnosis for breast cancer cases especially for those younger women with small or early tumor growth. In most cases, gene and protein markers are not sensitive or specific enough to detect cancer at an early stage.

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Therefore, alternative approaches are strongly desired for the early and accurate diagnosis of cancer with higher sensitivity and better specificity.

It is of increasing interest to develop novel diagnostic tests for earlier detection of cancer with improved accuracy. Metabolomics, also referred as metabolic profiling, is a fast growing field which focuses on investigating metabolites to directly observe the physiological status of biological systems, and thus allows a broad and highly efficient evaluation of altered metabolism[5–9]. Because of their sensitivity to biological status, metabolite markers may provide better diagnostic performance and earlier detection, which should result in improved therapy outcomes. Metabolomics can be regarded as a downstream omics field in systems biology, because it could be used to study the low molecular weight metabolites that are the end-products of genes and proteins. Meanwhile, metabolomics can be integrated with other omics studies to achieve a comprehensive understanding of complicated biological systems. For cancer diagnosis, in fact, the well known Warburg effect discovered in 1920s points out that cancer cells have altered metabolism[10–12]. Although genomic mutations initiate cancer, it is also important to study metabolic alterations during DNA damage and link them with tumorigenesis to understand the fundamental balances of bioenergetics and growth in cancer progression, which is essentially valuable for discovering novel targets for drug development and biomarkers for disease prognosis, diagnosis, and treatment. Therefore, metabolomics may promise novel avenues for early cancer detection as well as a better understanding of cancer processes.

Figure 1 shows the flowchart of a typical metabolomics study. Nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS) are the two most commonly analytical techniques in metabolomics. NMR is very reproducible and has strong ability for structure elucidation. Coupled to liquid chromatography (LC) or gas chromatography (GC), MS is a premium tool for both qualitative and quantitative measurements in metabolomics. A new advance in MS is the invention of ambient ionization techniques which include desorption electrospray ionization (DESI)-MS, extractive electrospray ionization (EESI)-MS, direct analysis in real time (DART)-MS, and so on[13–17]. Ambient MS requires minimal sample pretreatment and thus enables high-throughput analysis. In terms of MS analyzers, single quadrupole, tri-quadrupole (QQQ), time of flight (TOF), Q-TOF, and orbitrap are frequently used. In addition, because the data in metabolomics studies are generally complex, multivariate statistical analysis is used to extract useful information related to the research topics under investigation. Principle component analysis (PCA), a representative unsupervised method, can provide the information for the visual inspection of sample groups and for the identification of potential biomarkers as well. Based on the training sample set, the supervised methods could be used to build predictive models for the unknown samples. To avoid too optimistic clusters, it is important to apply rigid cross validation for the supervised methods such as partial least squares-discriminant analysis (PLS-DA), logistic regression, and support vector machines (SVMs)[18].

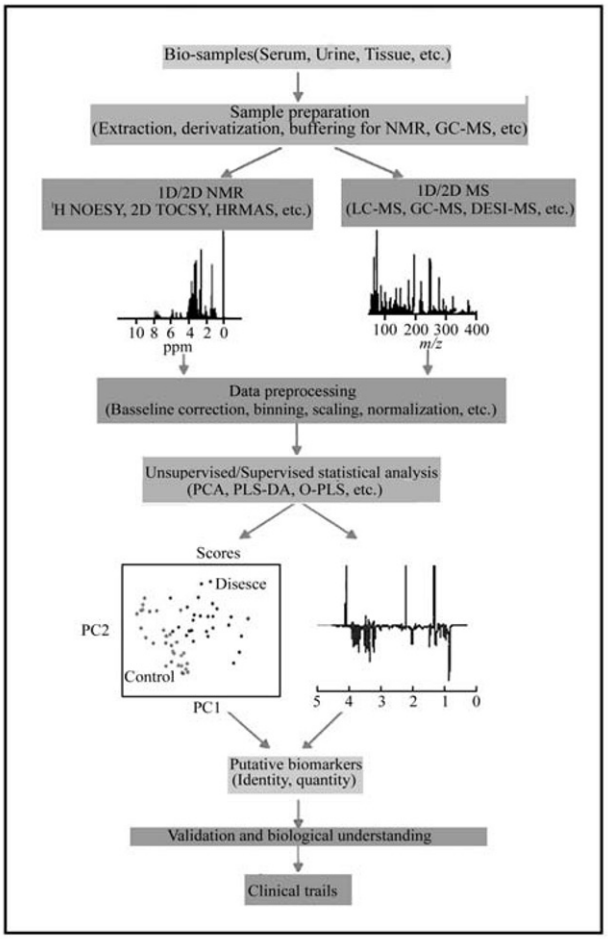

Fig.1 Schematic illustration of NMR- and MS-based metabolomics

2 Typical studies and challenges

Since 1990s, metabolomics has demonstrated promising applications in many different areas, including early disease detection, investigation of metabolic pathways, pharmaceutical development, toxicology, and nutritional studies[5–7,19]. For cancer diagnosis, many studies have shown that metabolomics methods potentially can be used for better diagnostic tests for various kinds of cancers. Bathe *et al.* used ^{1}H NMR to measure 58 unique metabolites in serum samples from patients with benign hepatobiliary disease (n = 43) and those with pancreatic cancer (n = 56)[20]. The metabolic profiles of patients with pancreatic cancer were significantly different from those of patients with benign pancreatic lesions (AUROC, the area under the receiver operating characteristic curve equals 0.8308). Odunsi *et al*[21] applied NMR-based metabolomics to detect epithelial ovarian cancer (EOC) using

preoperative serum specimens from 38 patients with EOC, 12 patients with benign ovarian cysts, and 53 healthy women. Statistical analysis was able to distinguish samples of different groups with 97%–100% accuracy. Rantalainen *et al.* used a combined metabolic and proteomic approach to study a mouse model of prostate cancer[22]. The correlations between a serotransferrin precursor and both tyrosine and 3-D-hydroxybutyrate, and between a decreased concentration of tyrosine and an increased presence of gelsolin were observed. Metabolite profiles in urine from lung cancer mice models were explored using NMR and DESI-MS[23]. Statistical analysis of both the NMR and MS data identified a large number of differentiating metabolites, many of which were localized to the purine metabolism pathway. Fan *et al.* used NMR and GC-MS in the stable isotope resolved metabolomics analysis (SIRM) to investigate metabolic changes due to lung cancer[24]. The ^{13}C-enrichment in lactate, Ala, succinate, Glu, Asp and citrate in lung cancer tumors suggested that glycolysis and Krebs cycle were more active in the tumor tissues. LC-MS was utilized to measure urinary metabolites from liver cancer patients and healthy volunteers[25]. PCA and PLS-DA models identified 21 metabolites as the potential biomarkers of liver cancer which were related to arginine and proline metabolism, alanine and aspartate metabolism, lysine degradation, nicotinate and nicotinamide metabolism, and fatty acids oxidation.

A number of investigations were also carried out to establish the breast cancer biomarkers using the approach of metabolic profiling. Based on the multivariate statistical analysis of NMR data, tumors and non-involved tissues could be classified with a high specificity (100%) and sensitivity (82%)[26]. Asiago *et al.* recently developed a metabolite profile (BCR model) using blood samples for the early detection of breast cancer recurrence[27]. As shown in Fig.2, the BCR model was a more accurate approach for early recurrent breast cancer detection, compared with that by CA 27.29 (a FDA-approved blood immunoassay for breast cancer treatment monitoring). More importantly, 55% of the patients could be correctly predicted to have recurrence 13 months (on average) before the clinical diagnosis, representing a large improvement over CA 27.29 in terms of early detection. Furthermore, one trend in metabolomics is to combine the advantages of both NMR and MS by using statistical analysis, also provide a high-throughput and reliable tool to detect the deviations of metabolites in biofluid samples or tissues[28]. Because NMR and MS generate unique metabolic profiles, the combination of these two analytical tools in various ways potentially can provide new avenues for the development of metabolomics for cancer diagnosis. For example, Gu *et al*[29] developed a principal component directed partial least squares (PC-PLS) approach to improve the detection of breast cancer when two datasets resulted from NMR and MS were available. This approach resulted in a significant improvement in the separation between the breast cancer samples and the samples from healthy subjects, which would be potentially useful to achieve more accurate disease detection and gain more insights into cancer mechanism. These studies and many others not mentioned herein have greatly stimulated the interest in the use of metabolomics to detect cancer by means of the measurements of altered metabolism.

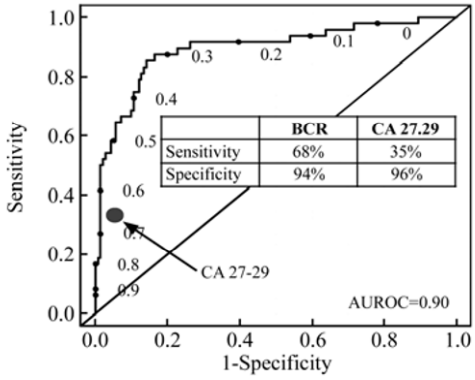

Fig.2 ROC curve of BCR model and the performance of CA 27.29[27]

Although metabolomics is evidently promising, currently a number of challenges persist in the field of metabolic profiling to diagnose cancer for earlier disease detection as well as for better accuracy. First, metabolic profiles of biological specimens can easily be affected by many factors that are of secondary importance such as diet, age, gender, ethnicity, drugs, lifestyle, environment, and confounding factors from other diseases. These factors need to be carefully controlled or deconvoluted to obtain information specific to the cancer under investigation. Second, further improvement in metabolomics for cancer diagnosis requires better access to advanced instrument platforms with wider coverage of metabolic profiles, better quantitative capacity for metabolites, and a stronger ability to identify unknowns. Human body approximately has 5000 to 7000 detectable metabolites, many of which are very different in structure, polarity, and biological concentration. Therefore, the analytical and statistical methods are in place for significant advances to provide better qualitative and quantitative measurements of metabolites that are significant to cancers. Third, the development of metabolic assay for cancer diagnosis and its clinical validation are time-consuming and expensive. To develop a sensitive and specific metabolic diagnostic test, the markers need to be identified and quantified, and the resulting test most likely needs regulatory approval (*e.g.*, FDA approval) according to a variety of related guidance. Last but not the least, cancer mechanism and cancer biology are still poorly understood. For example, the well-known Warburg effect indicates that cancer cells have increased aerobic glycolysis producing more lactate[10,12]. However, the energy mechanism related to cancer is still not fully understood. Additionally,

many studies of cancer mechanism were carried out using cells[30,31], instead of the connection and validation of these important markers in blood, which potentially can be developed as a routine and convenient diagnostic test, is rarely seen.

3 Summary and prospect

In summary, metabolic profiling is very promising to develop better diagnostic tests for cancer. The metabolomics approach for cancer diagnosis potentially has high sensitivity because metabolites are sensitive to subtle stimuli such as the early onset of tumor growth. Metabolomics is advantageous also because the major metabolic pathways are well-known and characterized, and a number of databases of human metabolites and metabolic information are available[32–34]. Moreover, the ability to link metabolome to genotype and phenotype can provide a better understanding of complex biological status, which promises routes to new pathological understanding, therapeutic treatment, and drug development.

Future directions in metabolomics for cancer diagnosis may focus on developing advanced analytical platforms with user-friendly software packages of statistical analysis and identifying robust metabolic biomarkers which are more specific to different kinds of cancers. Metabolic profiling and clinical validation are challenging, but not overwhelming barriers. We believe that it is more efficient and cost-effective to first examine biomarkers discovered in cell models and then to validate their diagnostic performance for cancers in animals and human beings. It can be anticipated with high confidence that new discoveries in cancer metabolism will continue to surprise us and perhaps lead to better prospects for diagnostics.

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