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REVIEW ARTICLE

Nuclear Magnetic Resonance technique in tumor metabolism

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Abstract Cancer is one of the most serious diseases that cause an enormous number of deaths all over the world. Tumor metabolism has great discrimination from that of normal tissues. Exploring the tumor metabolism may be one of the best ways to find biomarkers for cancer detection, diagnosis and to provide novel insights into internal physiological state where subtle changes may happen in metabolite concentrations. Nuclear Magnetic Resonance (NMR) technique nowadays is a popular tool to analyze cell extracts, tissues and biological fluids, etc, since it is a relatively fast and an accurate technique to supply abundant biochemical information at molecular levels for tumor research. In this review, approaches in tumor metabolism are discussed, including sample collection, data profiling and multivariate data analysis methods etc. Some typical applications of NMR are also summarized in tumor metabolism.

Introduction

Cancer nowadays is a major public health problem in the world. The occurrence of cancer is increasing because of the growth and aging of the population, as well as increasing formed risk factors such as dirty air, polluted water, overweight, smoking, too much pressure in life.[1] Based on GLOBOCAN estimates, about 14.1 million new cancer cases and 8.2 million deaths occurred in 2012.[2] In China, it has been estimated that there are up to 3 million new cases and over 2 million deaths. More seriously, the cancer death rate will continue to increase without effective therapeutic options. Fortunately, some diagnostic methods and techniques are already widely used in clinic, such as chest radiograph,[3,4] fibreoptic bronchoscopy,[5] Computed Tomography (CT) scan,[6,7] Magnetic Resonance Imaging (MRI)[8,9] and positron-emission tomography (PET),[10] etc. Although they can provide helpful information about

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the size and location of the tumors, combining two or even more of these methods, they provide little information about metabolic profiling of the tumors.[11,12] It is vitally crucial for analyzing biomarkers at the molecular level for tumor diagnosis, monitoring and treatment.[13–15] Therefore, some effective complementary methods are especially valuable and challenging to investigate tumor metabolic profiling.

Metabolomics is a fast growing field of research downstream of transcriptomics, genomics, which mainly involves the multicomponent analysis of cell extracts, tissues and biological fluids.[16–20] It provides a snapshot of the metabolic dynamics that reflect the response of living systems to both pathophysiological stimuli and/or genetic alteration.[21–23] It has been reported that tumor metabolism differs from that of normal tissue.[24,25] Exploring the tumor metabolome may be the best way to reveal the phenotypic changes relative to biological function, especially where subtle changes in metabolite concentrations can be tractable.[26] In general, the primary analytical techniques used in metabolomics are Mass Spectrometry (MS)[27–29] and Nuclear Magnetic Resonance (NMR).[30–32] NMR is a non-destructive and non-invasive technique that can provide complete structural analysis of an extensive range of organic molecules in complex mixtures,[33,34] thus, can be used to differentiate metabolism between tumor and healthy tissues, aiming at finding possible biomarkers of presence and/or degree of different cancers such as brain,[35] prostate,[36] breast,[37] liver,[38] renal[39] and esophageal[40] cancer.

NMR technique analytical platform

NMR has been employed as an excellent tool in exploring tumor metabolism due to its advantages, such as non-invasive, non-destructive, highly reproducible, providing real-time detection for biological samples near the physiological environment,[41,42] offering both qualitative and quantitative information of compounds in complex mixtures precisely and exquisite spectral editing technique make the method flexible and efficient.[43,44] NMR technique can uses not only ^{1}H spectra but also other nuclei like ^{13}C, ^{15}N and ^{31}P etc. to obtain helpful information about the tumor metabolic pathways.[45–47] These spectroscopies have pretty wide spectral width, meaning low possibility of signal overlapping, but their low sensitivity also limit their application, although some metabolites contain phosphorus and nitrogen.[48] Because of high sensitivity and almost all compounds contain proton in biological tissues, ^{1}H-NMR is the most widely used method to differentiate metabolism between tumor and normal tissues.[32,49]

Fig. 1 shows a standard workflow of tumor metabolism studies by NMR, including sample collection, data profiling, pattern recognition and final validation.[50] NMR spectroscopy combined with pattern recognition technique is a vitally helpful tool in tumor metabolic profiling as it has the potential to obtain comprehensive characteristics and pathways between tumor and healthy tissues.[36,51–53] Usually, final results may supply abundant biochemical information at molecular levels for further tumor research, aiming at effectively promoting the process of early diagnosis and treatment of tumor control.

Sample preparation

NMR-based metabolomics has been performed to study a range of different biofluids types including plasma,[54] serum,[55] urine,[56] cerebrospinal fluid,[57] saliva,[58] and feces.[59] Intact tissues and cells samples can also be analyzed by NMR technique.[60–62] To validate those data, sample collection, storage and processing procedures are extremely critical.[63,64] For example, some deuterated buffer should be added to adjust the pH value to avoid the systematic bias by different pH values.[65,66] Typically, urine, serum and plasma samples should be stored in appropriate conditions after collection to reduce sample degradation from multiple freeze/thaw cycles.[67,68] Biofluids and tissue samples should be stored at or below −70 °C.[69] Blood plasma or serum samples have high protein content which will obscure the resonances of small molecule metabolites. Therefore, it is necessary to physically remove proteins prior to analysis, which is done by precipitation or extractant or to weaken the intensity of protein resonances in the ^{1}H-NMR spectrum due to their faster rates of T_2 relaxation.[70,71] In addition, samples collected from a group of volunteers should be careful to minimize and account for effects from factors such as gender, age, diet, fasting state, exercise, and physical activity.[72–74]

Data analysis

NMR and MS both have been employed as common analytical tools in tumor metabolism research. NMR data can be quantitatively analyzed more directly, while MS technique has higher sensitivity. One-dimensional NMR spectroscopy of biological samples usually has hundreds of peaks, so it is a big challenge to obtain all its useful information.[75–77] Therefore, it is vitally helpful to combine the pattern recognition. Several multivariate statistical analysis methods can be employed to discriminate metabolites between complex systems such as principle component analysis (PCA), hierarchical cluster analysis (HCA), partial least squares (PLS), discriminant function analysis (DFA) and artificial neural networks (ANN).[78–80]

As a kind of unsupervised methods, PCA is the most widely used statistical analysis methods in metabonomics which can reveal outliers, groups and trends in the original dataset effectively.[81,82] It reduces the multidimensional data space into a much lower dimensional model space while converting the dataset in two matrices, so called scores and loadings. The result shows how samples related to each other and performs the variable contribution to the classification results. PCA can identify the largest variation in the dataset, but the latent variables responsible for the class separation may not be in the direction of the largest variation.[83,84] Instead, orthogonal partial least squares discriminant analysis (OPLS-DA), a very powerful supervised method in metabolomics is a regression model that reflects the correlation between multivariate data and dependent variables with class information. In OPLS-DA, a single component is used as a predictor to describe the separation between classes, where the other components are orthogonal variations to reflect the separation within classes. OPLS-DA emphasizes separations between classes, reduces separation within class at the same time.[85–88] Therefore,

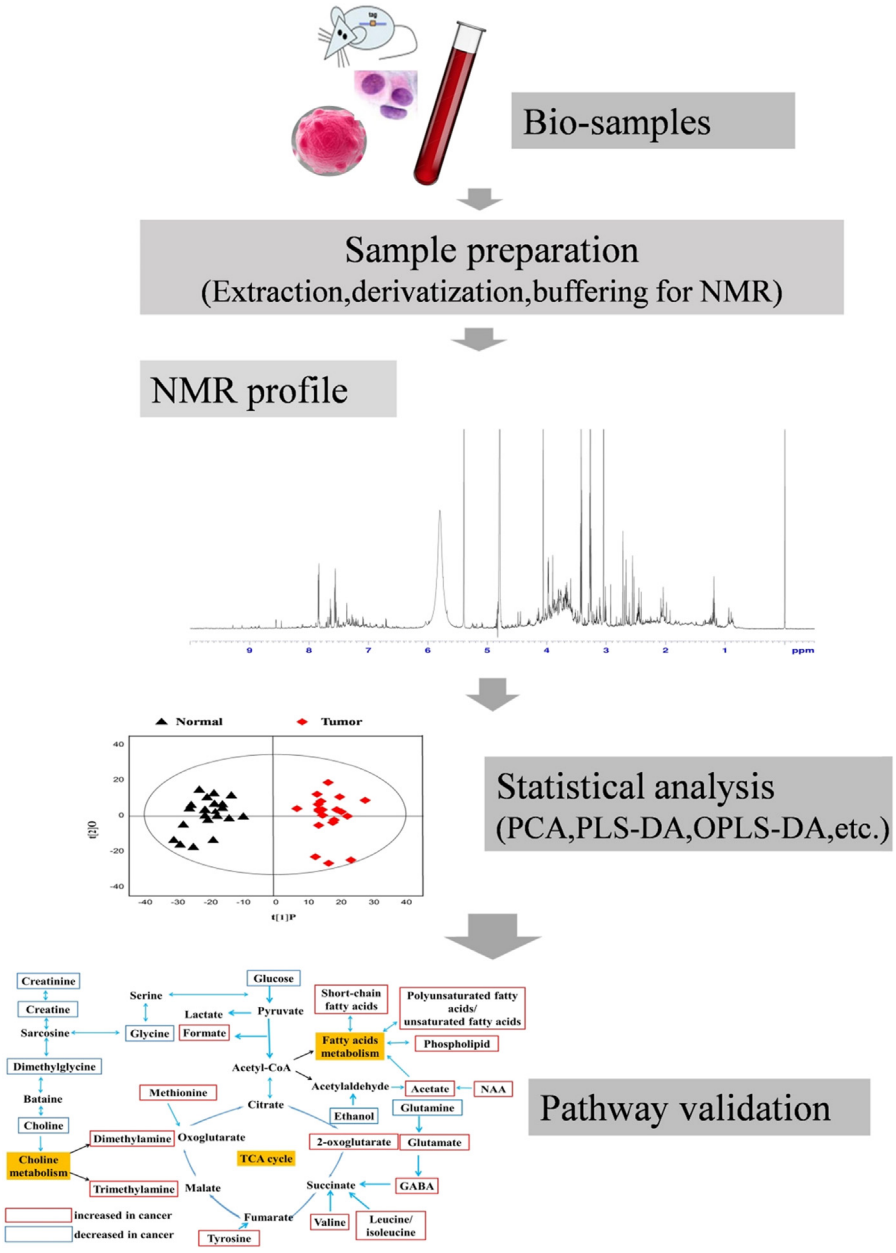

Fig. 1 Tumor metabolism studies by NMR.

an OPLS-DA scores plot will have tighter clusters with a larger separation between groups compared to PCA.[89] Usually, supervised and unsupervised multivariate statistical techniques are commonly used together to provide a deep explanation of the different metabolite concentrations between experimental groups.

NMR databases

The great challenge of NMR-based metabolomics is to recognize obtained peaks which can be assigned to different compounds.[15] Nowadays, there are a number of public databases available to promote the identification of metabolites including the Human Metabolome Database (HMDB),[90] Biological Magnetic Resonance Data Bank,[91] NMR database of Linkoeping, Magnetic Resonance Metabololomics Database and Madison-qingdao Metabolomics Consortium Database (MMCD).

These databases can be time-saving in metabolite identification and facilitate the validation of relevant biological pathways. HMDB, the most comprehensive curative human metabolite database in the world, provides a comprehensive compound description, names, structural information, reference NMR spectra, biofluids concentrations, pathway information and other public databases.[92]

Applications of NMR technique in tumor metabolism

In the following sections, we summarized some typical applications of NMR in various tumor metabolomics. To facilitate systematization and comparison of those studies,

Table 1 shows an overview of the main cancer-related metabolic findings in different cancer types.

Colorectal cancer

Colorectal cancer (CRC) is one of the most prevalent tumor types. Understanding the metabolic profile of colorectal tumor is important for therapeutic approaches and molecular diagnosis. Amiot et al[94] investigated the fecal metabolic phenotype of patients with advanced colorectal tumor and controls using ^{1}H-NMR and multivariate modeling. PCA results showed that advanced colorectal tumor demonstrated increased fecal concentrations of four short-chain fatty acids (valerate, acetate, propionate and butyrate) and decreased signals relating to β-glucose, Gln, and glutamate. The prediction accuracy of the method is higher than that of the guaiac-fecal occult blood test and the Wif-1 methylation test. Beatriz et al[102] performed high resolution Magic Angle Spinning (HR-MAS) NMR to analyze metabolites in intact tumor samples and samples of adjacent mucosa obtained from colorecal cancer patients. The results indicated marked biochemical differences between the two types of tissues by PCA and OPLS-DA. Moreover, metabolic profiles were able to differentiate tumors of different T-and N-stages (T stage has greater weight than the N stage and the former affects survival more significantly) on the basis of tumor node metastasis (TNM) classification. Bertini et al used NMR to profile the serum metabolome in patients with metastatic colorectal cancer (mCRC) and determine whether a disease marker may exist that is strong enough to predict overall survival (OS).[103] In the validation set, supervised predictive models allowed a separation of 96.7% of patients from the healthy controls. Wang et al[104] applied PCA, PLS-DA and OPLS-DA to analyze the ^{1}H-NMR profiling data to identify the distinguishing metabolites of rectal cancer. Results showed excellent separation among the different stages of rectal cancer tissues and normal controls. These modified metabolites revealed disturbance of energy, amino acids, ketone body and choline metabolism, which may be correlated with the progression of human rectal cancer. Piotto et al[105] applied HR-MAS NMR to characterize the metabolic fingerprint of both tumoral and normal tissue samples obtained from a cohort of patients affected by primary colorectal cancer. By analyzing the data using PLS-DA revealed that tumor tissue samples are richer in taurine, glutamate, aspartate and lactate whereas normal tissues contain a higher amount of myo-inositol and β-glucose. The statistical model was subsequently used to perform a blind test on tumor and healthy tissue.

Table 1 Main cancer-related metabolic findings in different cancer types unveiled by NMR technique.

Cancer type	Sample	Metabolic changes in cancer VS controls	Reference
Colorectal	Serum	(+) acetate, acetoacetate, 3-hydroxybutyrate, lactate,pyruvate (−) glucose, myo-inositol,taurine, dimethylglycine	Ludwig et al[93]
	Fecal extracts	(+)acetate,valerate, propionate, butyrate (−)β-glucose, Gln, glutamate	Amiot et al[94]
	Biopsies	(+)taurine, glutamate, aspartate, lactate (−)myo-inositol, β-glucose	Piotto et al[95]
Liver	Serum	(+) acetate, N-acetylglycoproteins,Gln, glycerol, α-ketoglutarate,1-methylhistidine, Phe, pyruvate, Tyr (−) acetoacetate, Cho, LDL, VLDL, Val	Gao et al[96]
	Urine	(+)carnitine, creatine (−) acetone, creatinine,glycine, hippurate, TMAO	Shariff et al[97]
Lung	Plasma	(+) lactate, VLDL, LDL, pyruvate (−) acetate, alanine, citrate, formate, Gln, HDL, His, methanol, Tyr, Val	Rocha et al[54]
	Urine	(+) N-acetylglutamine, citrate, creatinine, 3-hydroxyisobutyrate, 3-hydroxyisovalerate (−) hippurate, trigonellinamide, trigonelline	Carrola et al[98]
Breast	Tissues	Changes in Cho,creatine, β-glucose, GPC, glycine, myo-inositol, PCho,taurine	Bathe et al[99]
	Serum	Metastatic vs early disease: (+)acetoacetate, glycerol, pyruvate, mannose, glutamate, 3-hydroxybutyrate, N-acetylglycoproteins, (−)His, alanine,betaine	Elodie et al[100]
Pancreatic	Serum	(+)soleucine, triglyceride,leucine,creatinine (−)3-hydroxybutyrate, 3-hydroxyisovalerate, lactate, TMAO	Ouyang et al[55]
	Plasma	(+)N-acetyl glycoprotein, DMA, VLDL,acetone (−)lactate, 3-hydroxybutyrate, HDL, LDL, citrate, glutamate, alanine,Gln, His, isoleucine, lysine, Val	Lin et al[101]

Abbreviations: Cho, choline; Tyr, tyrosine; Val, valine; His, Histidine; Phe,phenylalanine; PCho, phosphocholine; GPC, glycerolphosphocholine; Gln,glutamine; DMA,dimethylamine; HDL, high density lipoprotein; LDL, low density lipoprotein; TMAO,trimethylamine N-oxide; VLDL, very low density lipoprotein; (+) increased in cancer, (−) decreased in cancer relatively to control.

Breast cancer

Breast cancer (BC) is the globally highest incidence and mortality form of all malignant diseases in women and its recurrence rate is also very high. More than three quarters of BC patients can achieve a high survival rate if the cancer is diagnosed at its early stage. It is imperative to identify markers for BC diagnosis and management including prediction, early diagnosis and individualized treatment. Li et al examined 31 breast tissue samples (13 cancer, 9 benign and 9 normal) obtained by core needle biopsy using multivariate modeling.[106] Although it was impossible to distinguish the benign tumors and normal tissues, cancer and non-cancer samples can be discriminated well with OPLS-DA on the NMR spectra. A subsequent blind test showed 69% sensitivity and 94% specificity in the prediction of the tumor status. Beathe et al compared the metabolic profiles of tissues collected from 85 breast cancer patients, where the concentration levels of GPC, PC and choline were monitored carefully.[99] The concentrations of choline and glycine were much higher in tumor samples larger than 2 cm compared with smaller tumors samples, indicating that HR-MAS NMR could be an efficient tool for BC diagnosis with the ability to identify cancer stages. Then they performed Electronic Reference to access In vivo Concentrations (ERETIC) applying with HR-MAS NMR to quantify metabolites in intact BC samples.[107] The nine significant discriminatory metabolites were b-glucose, lactate, glycine, myo-inositol, taurine, GPC, PC, choline and creatine. Elodie et al attempted to identify metabolic serum changes associated with advanced metastatic breast cancer (MBC) in comparison to the localized early disease (EBC).[100] The results clearly distinguished EBC and MBC samples with 89.8% sensitivity and 79.3% specificity, where higher levels of serum concentrations of acetoacetate, 3-hydroxybutyrate, glycerol, pyruvate, mannose, N-acetyl-glycoproteins, glutamate and lower concentration of histidine, alanine and betaine metabolites were observed in MBC tissues. Giskeodegard et al conducted biopsies which were excised during surgery and analyzed by HR-MAS NMR from BC patients.[108] The data were analyzed by PLS-DA, probabilistic neural networks (PNNs) and Bayesian belief networks (BBNs). It was also found that estrogen and progesterone receptor status can be successfully predicted to improve predictions of the hormonal therapy of breast carcinomas. Vincent et al employed a combination of NMR and MS methods to build and validate a model for early BC recurrence detection based on a set of 257 retrospective serial samples.[109] Utilizing the model, over 55% patients recurrence could be detected as early as 13 months before the recurrence was diagnosed.

Lung cancer

Lung cancer is one of the most serious health problems, also the most common cause of cancer death. There has been a large amount of research in the field. Claudia et al applied PCA and HCA followed by ^{1}H-NMR resulted in good discrimination between tumor and non-involved tissues, showing that inherently different metabolic profiling characterize the two tissue types.[54] In a similar study, NMR was utilized to measure metabolites in urine from lung cancer patients and control healthy group.[110] The multivariate modeling of urinary profiles discriminate the two groups, where the Monte Carlo Cross Validation of the classification model highlighted 93% sensitivity, 94% specificity and an overall classification rate of 93.5%. Liu et al conducted a new algorithm named PSO-SVWL-PLSDA to rectify the weakened efficacy by the high similarity of metabolic profiles combined with ^{1}H-NMR metabonomics.[111] Data clustering in discriminative metabolites for the lung cancer diagnosis were lactate, glucose, threonine, valine, taurine, trimethylamine, Gln, glycoprotein, proline and lipid. Chen et al performed the metabonomic characteristics of 51 lung tissues from 17 patients with lung cancer using the ^{1}H-NMR and the multivariate data analysis methods.[112] The findings clearly disclosed metabonomic characteristics of lung cancer tissues at various sites. Durate et al searched for the metabolic markers of lung cancer in urine by combining analysis of the NMR data including PCA, PLS-DA, and OPLS-DA.[113] The obtained results showed a high level of sensitivity and 100% specificity. Moreover, PLS-DA of a subset of tumour samples allowed adenocarcinomas to be discriminated from carcinoid tumors and epidermoid carcinomas, showing differences in metabolite levels between these histological types. Jordan et al investigated discrimination between tissue and serum metabolites for squamous cell carcinoma (SCC) and adenocarcinoma (AC) in the lung tumors by HR-MAS NMR.[114] The results disclosed the potential to differentiate between the tested lung cancer types and controls. Rocha et al investigated the variations in the metabolic profile of blood plasma from lung cancer patients and control group by NMR-based metabonomics.[115] PLS-DA modeling of CPMG spectra from plasma, subjected to Monte Carlo Cross Validation provided the potential of this approach to screen and diagnose for lung cancer. The distinguishing metabolites between the patients and the control group were lower levels of HDL, higher levels of VLDL and LDL. The patients' plasma had significantly high level of lactate and pyruvate but significantly low levels of citrate, formate, acetate, glucose, Gln, alanine, tyrosine and valine.

Liver cancer

Hepatocellular carcinoma (HCC) is a leading cancer worldwide in terms of incidence and mortality, for the lack of surveillance of patients. Yang et al compared metabolite levels in sera of HCC tumor and non-involved adjacent liver tissues by HR-MAS NMR.[116] The discriminating candidate biomarkers were lactate, amino acids including glutamate, glutamine, Gly, leucine and alanine, Cho, PC, GPC, triglycerides, glucose, phosphorylethanolamine and glycogen. Gao et al monitored the changes in endogenous metabolites of liver cirrhosis (LC) and HCC using single blood samples.[96] Results indicated a higher level of acetate, pyruvate, Gln, a-ketoglutarate, glycerol, tyrosine, 1-methylhistidine and phenylalanine, together with lower level of LDL, isoleucine, Val, acetoacetate, creatine, Cho and unsaturated lipids in HCC patients. Moreover, pathway analysis suggested altered energy metabolism with changes in the tricarboxylic acid (TCA) cycle in LC and HCC patients. In a similar study, Nahon et al compared the metabolic profiles from

154 cirrhotic patients with and without HCC.[117] The result indicated that serum NMR spectra combined with the OPLS analysis model provided an evident discrimination between cirrhotic and large HCC tumors. Perturbations observed in the synthesis of glutaminolysis, citrate cycle, phospholipid and glycolysis metabolism have potential to assess pathological hepatic lesions. Debora et al investigated the metabolic profiles from primary HCC, cirrhotic tissues (CIR), hepatic metastases from colorectal carcinomas and non-cirrhotic normal tissues by ^{1}H-NMR combined with pattern-recognition and visualization techniques.[117] Results indicated clear metabolic differences in the studied grades and the tissue signals of lactate and the glucose were primarily discriminate the different histological samples, which can be of great benefit to liver tumor diagnosis. Antonio et al obtained NMR spectroscopy of 51 needle biopsies (14 primary nodules, 14 recurrent, and 23 paired cirrhotic specimens) to elucidate the metabolite changes associated with HCC.[118] The results clearly differentiated primary HCC from recurrences using PLS-DA models and disclosed alterations in choline metabolism.

Pancreatic cancer

Pancreatic cancer is a malignant tumor. Once diagnosed, surgical cure is no longer an efficient option for most patients, thus early detection of pancreatic cancer is vital for its treatment. OuYang et al studied the serum collected from pancreatic cancer patients with NMR.[55] Results showed good distinction between cancer and benign group. The analysis were revealed by higher level of soleucine, leucine and creatinine and lower values of 3-hydroxybutyrate, 3-hydroxyisovalerate, lactate, triglyceride and TMAO from pancreatic cancer patients compared to control group. These metabolite changes could be used as metabolic markers for the early detection of pancreatic cancer. Oliver et al analyzed the sera from pancreatic cancer patients and healthy volunteers by ^{1}H-NMR followed by statistical analyses.[119] The diagnostic model distinguished benign from malignant pancreatic lesions accurately via excluding patients with overt diabetes mellitus. Lin et al used ^{1}H-NMR spectra to profile the plasma metabolites obtained from 19 pancreatic cancer patients.[101] The levels of N-acetyl glycoprotein, DMA, VLDL and acetone in the PC group increased, whereas lactate, 3-hydroxybutyrate, HDL, LDL, alanine, glutamate, Gln, His, isoleucine, lysine and valine were found to be reduced.

Conclusions and future perspectives

NMR technique has become one of the most powerful tools in biology and medicine research. On the basis of the combination of ^{1}H-NMR spectroscopy with pattern recognition methods of multivariate data analysis, metabolomics can provide comprehensive characteristics of the major metabolic pathways to evaluate the status of the tumor more precisely. Therefore, NMR application in tumor metabolomics research could have a great potential to provide valuable information about early diagnosis, treatment options, processes and prognosis estimate of cancer and other serious diseases. Nowadays, NMR sensitivity has been greatly improved by higher magnetic field, cryoprobe, fast 2D NMR experiments, polarization transfer etc., so NMR technique can be more widely applied in tumor metabolomics studies.

Conflicts of interest

All authors have none to declare.

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