Clinica Chimica Acta 495 (2019) 436–445

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

Review

Metabolomics workflow for lung cancer: Discovery of biomarkers

Yuqing Tang, Zhou Li, Lissy Lazar, Zhiling Fang, Chunlan Tang*, Jinshun Zhao*

Department of Preventative Medicine, Zhejiang Key Laboratory of Pathophysiology, Medicine School of Ningbo University, 818 Fenghua Road, Zhejiang Province, Ningbo 315211, China

ARTICLE INFO

Keywords:
Lung cancer
Metabolomics
Workflow
Biomarker
Metabolic pathways

ABSTRACT

Lung cancer is one of the most common cancers in the world. Due to the limitations of current diagnostic techniques and methods, most lung cancers are diagnosed at the advanced stage, which is not conducive to early treatment. The rise of metabolomics has provided new ideas for the early diagnosis of lung cancer. As a method for the comprehensive analysis of endogenous metabolites of the biological system, metabolomics has shown significant application potential for the early diagnosis and individualized treatment of various cancers including lung cancers. Via advanced analytical techniques and bioinformatics tools, the metabolome was excavated to find biomarkers related to cancer and its prognosis. In this review, the research methods and workflow of metabolomics are summarized, with an emphasis on the recent discovery of biomarkers and major metabolic pathways for lung cancers.

1. Introduction

Cancer is a disease that threatens human life and health through the malignant proliferation of cells. The risk factors for cancer mainly include aging, exposure to harmful environmental factors, and adverse lifestyles [1]. Although researchers have studied cancer for decades, the early diagnosis and detection of cancer and improvements in the survival time and quality of prognosis remain a major challenge. Lung cancer is the most prevalent cancer in the world and is responsible for 1.3 million deaths annually. Lung cancer is also one of the deadliest cancers due to difficulties in early detection, with a 5-year survival rate of less than 15% [2]. Most patients in the early stages of lung cancer have no obvious symptoms and signs, which decreases the possibility of early diagnosis and treatment. The current diagnostic methods for lung cancer are mainly through physical, biochemical, and histopathological examinations. These traditional methods are used to determine the stage, location, and metastasis of lung cancers, and provide a reliable basis for further treatment [3]. However, they also have some limitations, such as difficulties in early diagnosis, high cost of examination, and unsuitability for screening in population [4]. Therefore, a new method is urgently needed for early detection and to improve the prognosis of lung cancer treatment.

Metabolomics is a new discipline following genomics and proteomics that is an important part of systems biology. Since the mid-1990s, metabolomics has developed and rapidly penetrated many fields, such as diagnosis, drug research and development, nutrition, food science, botany and toxicology, the environment, and human health, which are all closely related to health care [5]. Metabolomics is a relatively new member of the “omics” family, which aims to study global metabolic differences in biological systems by monitoring the levels of small molecular metabolites in biological fluids or tissues [6].

Metabolomics has recently been applied to the discovery of tumor biomarkers for the diagnosis, treatment, and prevention of lung [7], pancreatic [8], liver [9], breast [10], and prostate cancer [11]. Application of metabolomics in lung cancer started around 2000. The main research has included identifying the biomarkers for the early diagnosis of lung cancer, predicting prognosis by comparing the changes in metabolites before and after lung cancer surgery, discovering possible metabolic pathways of lung cancer [12], and determining lung cancer staging and chronic obstructive pulmonary disease [13]. These metabolomics studies have demonstrated that there are indeed differences in the metabolome between lung cancer patients and the control group, and some reliable biomarkers and possible biological metabolic pathways for lung cancer diagnosis have been gradually discovered. Some studies have shown that the lung cancer stage can also be determined by measuring the changes in the metabolic group at different stages

Abbreviations: EBC, exhaled breath condensate; BALF, bronchoalveolar lavage fluid; CSF, cerebrospinal fluid; NMR, nuclear magnetic resonance; GC–MS, gas chromatography–mass spectrometry; LC-MS, liquid chromatography-mass spectrometry; PLS-DA, partial least squares discriminant analysis; OPLS-DA, orthogonal projection-potential structure analysis; PLA, principal component analysis; BCAAs, branched-chain amino acids; TCA cycle, tricarboxylic acid cycle

* Corresponding authors.
E-mail addresses: tangchunlan@nbu.edu.cn (C. Tang), zhaojinshun@nbu.edu.cn (J. Zhao).

https://doi.org/10.1016/j.cca.2019.05.012
Received 9 March 2019; Received in revised form 13 May 2019; Accepted 13 May 2019
Available online 17 May 2019

over the course of lung cancer. However, these methods have not yet been applied in clinical practice, and more clinical studies are needed to prove the accuracy and reliability of these metabolomics methods.

Sample types and analytical technologies are also important in the study of the metabolomics for cancers. Current metabolomics samples mainly include plasma, serum, urine, and tissue samples. Studies have explored other biological samples such as sweat, exhaled breath condensate (EBC), bronchoalveolar lavage fluid (BALF), cerebrospinal fluid (CSF), sputum, and more [14,15]. Compared with commonly used samples, these non-invasive samples are not only less harmful to patients, but are also more accessible, which might represent the tendency in the sample selection. As for analytical methods, frequently employed nuclear magnetic resonance (NMR) [16], gas chromatography–mass spectrometry (GC–MS) [17], and liquid chromatography-mass spectrometry (LC-MS) [18] technologies have both advantages and disadvantages during their applications.

This paper reviewed the metabolomics studies of lung cancer over the past 10 years, mainly including the sample collection, storage and preparation, instrumental analysis and data processing, and the potential biomarker discovery and pathway analysis. Yearly published literatures on the application of metabolomics in lung cancer research over the past 10 years are summarized in Fig. 1. The distribution of often employed samples and methods are summarized in Fig. 2.

According to the previous studies [19–21], the metabolomics workflow for the study of lung cancer generally includes the following six steps: (1) study design, (2) sample collection and storage, (3) sample preparation, (4) instrumental analysis, (5) data processing and analysis, and (6) metabolite identification and pathway interpretation.

2. Study design

A good metabolomics study begins with a well-developed and reliable study design scheme to obtain a significant and credible result. First, targeted or non-targeted metabolomics study should be decided, different purposes present various methods. Targeted metabolomics aimed to quantitatively determine a specific set of metabolites (for example, amino acids, lipids, sugar, and/or fatty acids) and can analyze the specific metabolic pathways to verify the use of targeted biomarkers, which requires prior knowledge of the metabolites and known compounds of interest. In contrast, the non-targeted metabolomics method presented global analysis of metabolome, which was usually used for biomarker discovery [22]. Both methods have their pros and cons; non-targeted metabolomics generally provide more information, but targeted metabolomics are generally more quantitative.

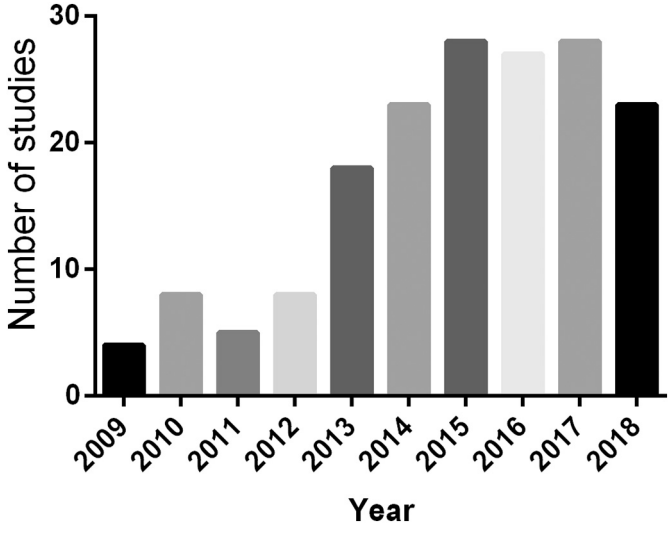

Fig. 1. The number of published studies on the application of metabolomics in lung cancer research over the past 10 years.

Basic information about the subjects is also an important factor in the study design, including data (age, gender, BMI, poor living habits, and physical labor) and medical history (current illness, past illness, and family genetic history). After information collection, the subjects can be further clinically diagnosed to determine the disease and stage. However, not all previous metabolomics studies of lung cancer provide detailed information. For example, Chen et al. provided information only on age, sex, and the stage of lung cancer without specific details. Wikoff et al. provided information on age, sex, and baseline smoking status, but was still not comprehensive enough. Lokhov et al. presented more comprehensive information, including age, BMI, smoking history, disease history, and the stage of lung cancer. Therefore, future studies with more detailed information on the subjects should be considered in the study design.

3. Sample collection and storage

Traditionally, plasma, serum, and urine were mainly the samples employed for metabolomics studies of lung cancer because they reflect an individual's global metabolic status and the collection processes are minimally invasive [23]. However, these complex samples are easily diluted by small metabolic changes from a specific part of the body. So in these cases, according to the characteristics of lung cancer, sweat, EBC, BALF, and sputum and saliva samples play important roles in the metabolomics of lung cancer. Based on the metabolic complexity, collecting multiple types of biological specimens at the same time is preferred, as they provide complementary metabolic information or cross-validation results. The following will highlight the collection methods for sweat and EBC samples. All of the other sample collection methods are summarized in Table 1.

Sweat samples: the sweat collection system consists of a sweat inducer and a sweat collector. The sweat inducer provides 1.5 mA of current strength for 5 min through two guide gel discs located on the forearm. The skin is then rinsed in the collector and distilled water to cover the skin and collect sweat for 15 min [14]. Approximately 50 μl of sweat is collected from each individual.

EBC samples: EBC sample collection employs a non-invasive method to obtain non-volatile substances from the lungs. The principal component is condensed through water evaporation, which can represent nearly 99% of EBC samples [17]. In Wang's study, gas samples from the ipsilateral lung and contralateral lung were collected before and after tumor resection. A specially designed sample collection tube was inserted through the tracheal catheter and placed in the tracheal bifurcation. Approximately 10 ml of the gas is collected and injected into the bottle to ensure that the gas collected is unilateral alveolar gas. All of the gas samples should be processed within 3 h after collection [24]. Peralbo-Molina et al. used the ECOScreen 2 device to collect EBC samples, which offers controlled collection of EBC into two separate bags for physical separation between the air exhaled from the upper breath and the lower breath and prevents saliva from contaminating the gas [25]. Following the collection of fresh samples, they should be immediately stored in a −80 °C or liquid nitrogen freezer.

4. Sample preparation

Sample preparation usually involves different methods according to the type of samples, the metabolites of interest, and the analysis platform used. Generally, for LC or GC analysis, the first step is to remove the high-molecular-weight species by adding an organic solvent or solvent mixture to precipitate these species followed by a centrifugation step to separate the precipitate and the metabolite of interest. In addition, the sample purity is paramount, and no large particles should be present in the samples. For NMR analysis, there is no such high requirement for the sample preparation, so only a simple centrifugation and separation operation is needed. Detailed sample preparation

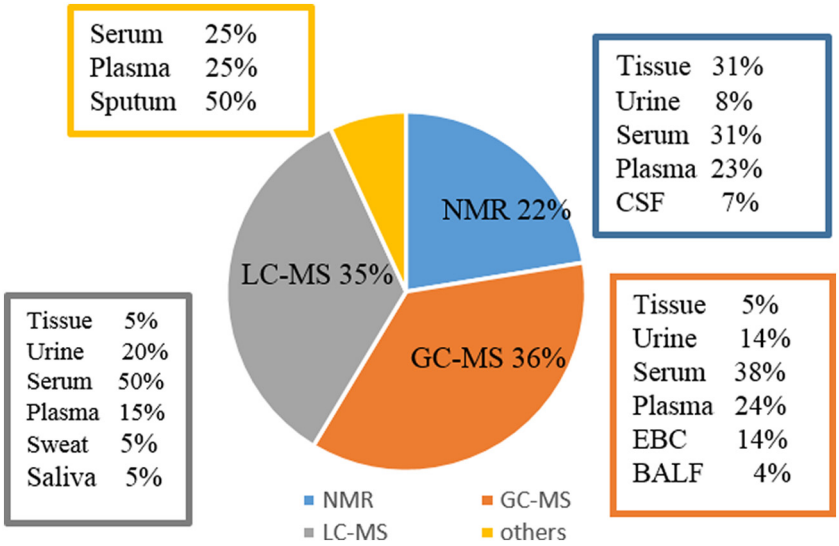

Fig. 2. The distribution of employed samples and methods in metabolomics study of lung cancer over the past 10 years.

methods are shown in Table 1.

5. Instrumental analysis

Metabolomics studies based on NMR spectrum and mass spectrometry are both increasingly used to screen biomarkers for cancer. Selecting a suitable platform largely depends on the specific samples to be analyzed. GC–MS is widely used to evaluate small molecules, but there is a lack of a widely adopted and validated method of metabolomics based on high-throughput discovery, and the detectable compounds are limited to the available compounds. In contrast, LC-MS based metabolic profiling experiments have proved to be comparable between different laboratories to reveal pathological metabolic differences in human samples, although these methods are currently undergoing verification [26]. Furthermore, LC-MS is very sensitive and allows for highly specific multi-metabolic recognition at low concentrations [27].

NMR spectroscopy provides a rapid, high-throughput, and repeatable method to measure metabolites with few sample preparation steps. Currently, there are many applications, especially in the study of metabolomics of lung cancer in plasma [28], serum [29], and urine [30]. Compared with MS, NMR is less sensitive and requires more expensive instrumentation but provides unbiased metabolite detection, quantitative properties, and repeatability [31].

Due to the different advantages and limitations of NMR and MS techniques, multiple complementary analysis platforms are often employed to maximize the use of samples to detect the relevant metabolic spectrum. In addition, multiple analysis platforms can also conduct metabolic elucidation and target quantification of the metabolites of interest.

6. Data processing

With the development of analytical techniques, advanced bioinformatics tools are needed to process, analyze, and interpret high-dimensional metabolomics data from complex biological samples. Based on the large and complicated data, many statistical methods are used, including partial least squares discriminant analysis (PLS-DA), orthogonal projection-potential structure analysis (OPLS-DA), principal component analysis (PLA), clustering analysis, linear discriminant analysis, and several other stoichiometric methods [32]. Different analytical instruments produce raw data files in different formats; however, the data are often processed with similar workflows, including noise filtering, peak feature detection, spectral deconvolution, and chromatographic alignment. Furthermore, before data analysis, data preprocessing is a first and important step. For example, standardization is used to eliminate

Table 1
Collection, storage, and preparation of samples.

Sample	Collection	Storage	Preparation
Blood/plasma/serum	- Collected in heparin tubes - Centrifuged	−80 °C	- Centrifuged after thawing at 4 °C - Add deuterium oxide (to lock) - Add acetonitrile (for protein precipitation)
Urine	- Collected in a sterile cup - Aliquots of approximately 1 ml were transferred into sterile cryovials	−80 °C	- Centrifuged after thawing at 4 °C - Remove cells and other precipitated material - Add deuterated buffer to urine
BALF	- Divided into 1 ml aliquots in Eppendorf tubes	−80 °C	- Add deuterium oxide to BALF - Add methanol/chloroform extraction
CSF	- Obtained during surgery - Snap-frozen in liquid nitrogen	−80 °C	- Add deuterium oxide to CSF
EBC	- The device used for sampling directly collects - Condenses the EBC in disposable polyethylene bags at −20 °C	−80 °C	- Add deuterium oxide to EBC
Tissue	- Retrieved from surgical specimens - Snap-frozen in liquid nitrogen	−80 °C	- Add a few drops of deuterium oxide - Add saline and thaw cold tissue - Add methanol/chloroform extraction to tissue
Sweat	- Use the sweat inducer to collect sweat - The Macroduct collector covers the skin to collect sweat - Transfer the sweat to micro tubes	−80 °C	- Add 0.1% formic acid to sweat - Add deuterium oxide to sweat

urine sample differences, and data transformation and scale transform are used to reduce the main metabolite bias at high concentrations. In actual operations, care should be taken in processing the data, because data preprocessing methods and execution sequences may significantly affect statistical results, and improper processing may lead to the loss of information. In addition, the screening and analysis of low concentrations of metabolites and the isolation of coalition compounds remain challenges of data processing. Therefore, robust and novel algorithms need further development.

After processing, associated changes in the spectral regions in the NMR spectrum or mass spectrum with specific metabolites should be investigated. To achieve this, database searches are often used, such as the Kyoto Encyclopedia of Genes and Genomes (KEGG) and the Human Metabolome databases, among others.

7. Metabolite identification and pathway interpretation

Differential metabolite identification is a complex process in metabolomics because the human metabolome has not been completely elucidated. Experimentally characterized database data applied for identification are not yet complete to reflect all known metabolites. Metabolites detected by GC–MS are generally identified using mass spectral libraries, such as the National Institute of Standards and Technology, Environmental Protection Agency, and National Institutes of Health, among others. For LC-MS, the accurate mass and molecular formula can be searched in the METLIN and KEGG databases, among others, to identify the metabolites. After a literature review, the potential biomarkers for lung cancers are summarized in Table S1 [14,15,17–19,21,22,24,25,29,30,33–66], which is available in the Supplementary Material. A total of 213 biomarkers were identified in the previous studies. All of these potential biomarkers were further analyzed by MetaboAnalyst for pathway interpretation to form the overview of the pathway result. MetaboAnalyst consists of two parts, enrichment analysis and extension analysis. The enrichment analysis results in the y-coordinate *P* value (take the negative common logarithm, that is, $-\log10\ P$); the impact value of the abscissa is obtained by the extension analysis. Each bubble in the bubble graph represents a metabolic pathway, and the vertical position and color of the bubbles represent the *P* value of the enrichment analysis. A darker color or smaller *P* value represents a more significant enrichment degree. The abscissa position and bubble size of the bubble represent the influence factor size of this pathway in the topological analysis. The more significant enrichment degree and the greater influence factor lead to the higher credibility of the pathway. Based on the analysis results of MetaboAnalyst (Fig. 3), the three bubbles are relatively darker in color, larger in size, smaller in *P* value, and greater in influence factor. Therefore, we chose these three pathways for further discussion. The three bubbles represent the valine, leucine, and isoleucine biosynthesis; alanine, asparate, and glutamate metabolism; and glycine, serine, and threonine metabolism pathways, respectively. We selected the most representative biomarkers for discussion and summarized these biomarkers in Table 2.

The first pathway is valine, leucine, and isoleucine biosynthesis as shown in Fig. 4. Qin et al. [36] and Liu et al. [57] reported the concentration of valine in lung cancer patients was higher than that in healthy controls. Valine is an essential amino acid involved in many metabolic processes and is also a glucogenic amino acid for biosynthesizing macromolecules such as proteins and lipids, which are essential for cancer cell growth. This may be why valine exhibited higher content in the case groups than in the controls. Zhang et al. reported that leucine and isoleucine concentrations in lung cancer patients were higher than in healthy controls. All three essential amino acids, known as branched-chain amino acids (BCAAs), are involved in stress, energy, and muscle metabolism with different metabolic pathways [67]. The BCAAs are involved in several cancers that regulate various signaling pathways such as protein synthesis, lipid synthesis, cell growth, and autophagy [68]. BCAAs are mainly decomposed in skeletal muscle and have high transaminase activity. In addition, the

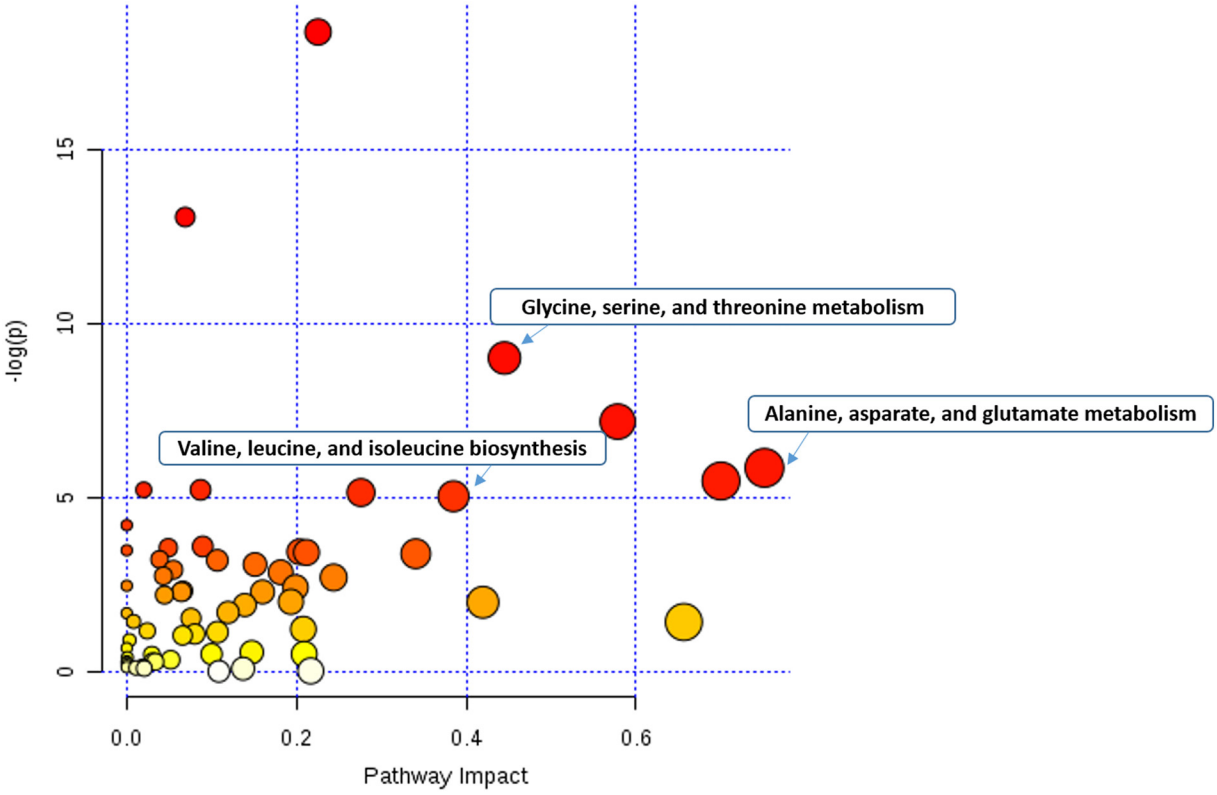

Fig. 3. Summary of pathway analysis.

Table 2
List of identified metabolite biomarkers for lung cancer diagnosis in pathways.

Name	Tissue		Urine			Blood/serum/plasma			CSF	Sweat	BALF	Sputum
	NMR	GC–MS	NMR	GC–MS	LC-MS	NMR	GC–MS	LC-MS	NMR	LC-MS	GC–MS	FIE-MS
4-Methyl-2-oxopentanonte				↑								
Betaine								↑				
Creatine									↓			
Choline			↑			↑						
Glycine	↓		↓			↓	↓					↓
Glyceric acid		↑										
L-Alanine								↑				
L-Aspartic acid											↑	
L-Glutamine											↑	
L-Leucine							↑					
L-Isoleucine								↑				
L-Threonine							↑					
L-Valine							↑	↑				
L-Cysteine		↑						↑				
L-Glutamic acid							↑					
L-Tryptophan					↑		↑	↑				
L-Serine							↑					
Pyruvic acid							↑	↑				

Note: The upward arrows in the table indicate that the number of metabolites is increased compared with the control group, while the downward arrows indicate that the number of metabolites is decreased compared with the control group.

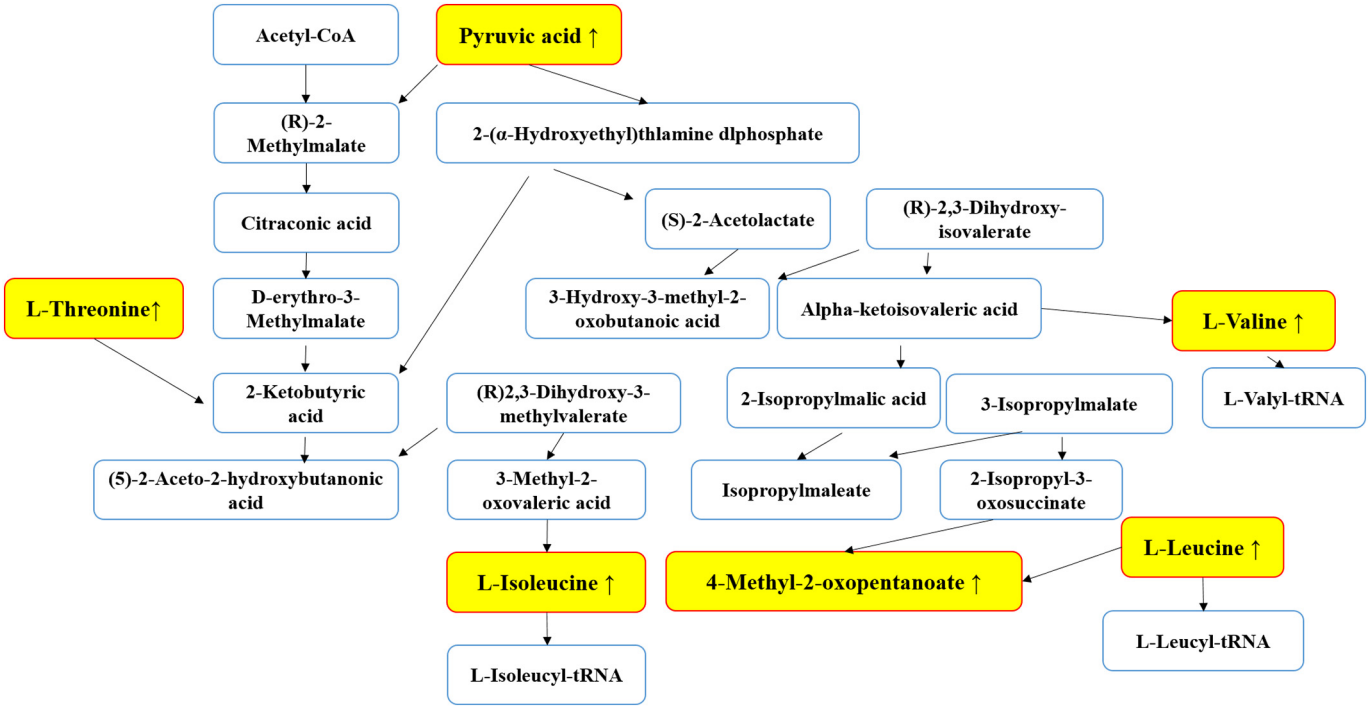

Fig. 4. Valine, leucine, and isoleucine biosynthesis.

catabolism of BCAAs plays an important role in amino acid synthesis (such as glutamine and alanine) [69]. Therefore, the upregulation of leucine and isoleucine in lung cancer patients can be explained by the energy and proliferation requirements of the host and tumor. But the maximum decrease in BCAA is often found in cancer cachexia [67]. In addition, the upregulation of 4-methyl-2-oxopentanoate in this pathway may be due to the upregulation of leucine. The increase in pyruvic acid may be due to the decrease in the amount of that involving the tricarboxylic acid cycle (TCA cycle).

The second pathway is alanine, asparate, and glutamate metabolism as shown in Fig. 5. Zhang et al. reported that L-glutamine, L-glutamic, and aspartic acid were higher in the circulation of lung cancer patients than in healthy controls [22]. L-glutamine is essential for cancer cell proliferation and survival and is involved in the synthesis of metabolism, proteins, lipids, and nucleotides. The demand for glutamine depends on the energy requirements of cancer cells. Aspartic acid is one of the most important intermediates in the oxidation of L-glutamine and is an important metabolite of tumor cell energy metabolism. Glutamine can be converted to aspartic acid, which forms oxaloacetate, malate, and pyruvate via the TCA cycle [70,71]. First, the activity of malate-aspartate shuttle cells depends on the concentration of aspartate in certain tumor cell lines. Second, the purine nucleotide cycle is another important energy metabolism pathway that may require a large amount of aspartate in tumors. Aspartic acid continues to metabolize from L-alanine and pyruvic acid, resulting in higher concentrations in lung cancer patients than in healthy controls. Yong et al. also reported a

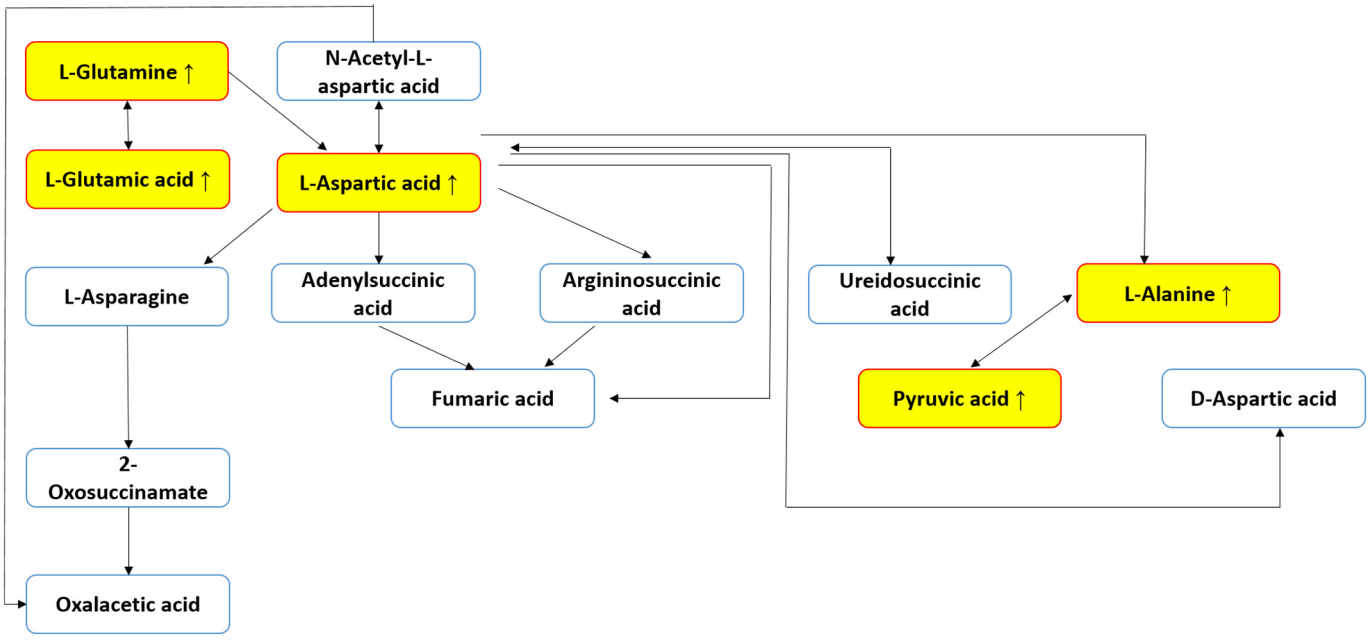

Fig. 5. Alanine, asparate, and glutamate metabolism.

higher level of alanine in the lung cancer group than in normal controls [66]. Another possible reason for the increase in L-alanine and pyruvic acid might be related to the Warburg effect. In the Warburg effect, a large amount of pyruvate is produced by aerobic glycolysis, resulting in an increase in pyruvate. Furthermore, a large amount of pyruvate is further converted to alanine, causing an increase in alanine [72]. Therefore, the significant increase in L-glutamine, L-glutamic acid, and aspartate observed in lung cancer tissues indicates that tumor cell energy metabolism may be significantly increased during malignant tumor progression.

The last pathway is glycine, serine, and threonine metabolism as shown in Fig. 6. This pathway focuses on the upregulation of choline. Compounds containing choline are the major components of the cell membrane, and the increase in these metabolites reflects cell death (apoptosis or necrosis). Yokota et al. [73] reported that choline changes can be an important parameter for the prognosis of small cell carcinoma by in vitro ^{1}H-NMR studies. Abnormal choline phospholipid metabolism is associated with carcinogenesis and tumor progression. Changes in choline phospholipid metabolism have been observed in many types of cancer [74]. Choline is involved in the methylation reaction after oxidation to betaine, which is not only required for the methionine/homocysteine cycle, but also plays an important role in choline-

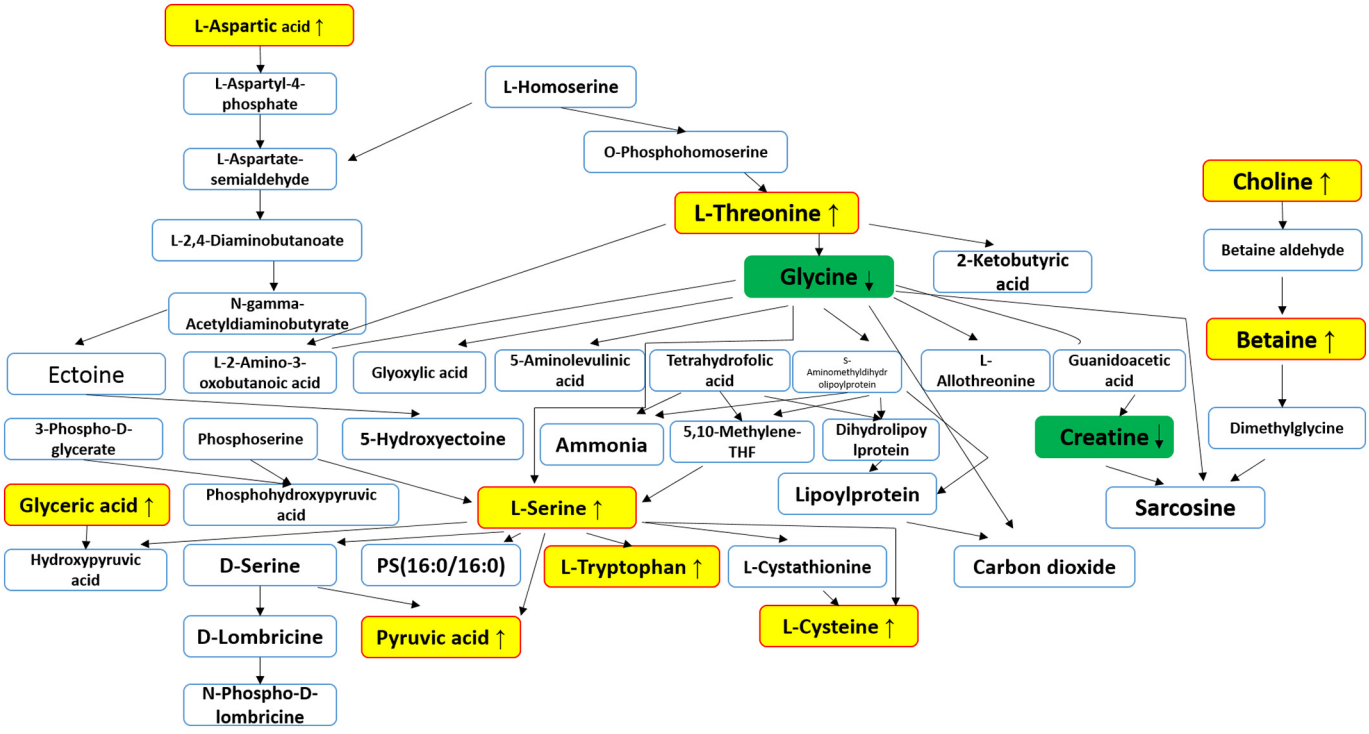

Fig. 6. Glycine, serine, and threonine metabolism.

mediated single carbon metabolism. Tumors typically exhibit altered choline phospholipid metabolism characteristics compared to normal tissues and are characterized by abnormally high levels of choline-containing compounds. This suggests that choline might also be an important biomarker for lung cancer diagnosis in the future. An et al. reported tyrosine and tryptophan had a higher level in a lung cancer group than in controls. The unusual increase in two essential aromatic amino acids of tyrosine and tryptophan in urinary excretion might be caused by the derangement of protein metabolism in cancer patients. Hu et al. reported the level of glycine was lower in a lung cancer group [29]. Glycine, a simple and non-essential amino acid, was involved in the production of DNA, phospholipids, and collagen as well as the release of energy. Given that cancer cells reprogram their metabolisms comprehensively, the decreased level of glycine in lung cancer patients may be related to accelerated DNA synthesis. A decrease in creatine may be caused by glycine downregulation.

8. Conclusion and perspective

Lung cancer is one of the most common cancers in the world. However, the diagnostic techniques and methods still have significant shortcomings. One of the major limitations in diagnosis is differentiating the stage of lung cancer, as it is difficult to determine the stage using current approaches. Therefore, the metabolomics method can be used to study the stage of lung cancer by determining the changes in the biomarkers in pneumonia, lung cancer, and other different degrees of lung disease. Metabolomics is considered a powerful tool to identify potential candidate biomarkers for early diagnosis, prognosis, and treatment of lung cancer. According to multi-faceted studies of lung cancer, the patient's survival rate and quality of life might be significantly improved.

This paper reviewed the metabolomics workflow for lung cancer biomarker discovery, which is summarized in Fig. 7. According to previous reports, the metabolomics workflow for the study of lung cancer can be summarized in six steps. Three highly credible metabolic pathway diagrams were selected for the analysis, and upregulated substances in these three pathways were closely related to the energy metabolism and proliferation of lung cancer cells. The results suggest that these potential biomarkers might be useful for the early diagnosis of lung cancer. However, for clinical application, these results are sufficient. Many proof tests are necessary to verify these results.

The goal of metabolomics research is to identify markers that can help distinguish between lung cancer and healthy patients, various lung cancer types and stages, and also aid in tumor detection. This review focused on potential metabolism-associated biomarkers that have been identified as either upregulated or downregulated in lung cancer patients compared to healthy individuals. All of these potential biomarkers were further analyzed by MetaboAnalyst for pathway interpretation. Three significant pathways were noted: valine, leucine, and isoleucine biosynthesis; alanine, asparate, and glutamate metabolism; and glycine, serine, and threonine metabolism. Metabolites belonging to these pathways, including valine, leucine, isoleucine, glutamine, aspartic acid, alanine, pyruvic acid, choline, glycine, tyrosine, and tryptophan, may help distinguish lung tumors from benign or chronic lung diseases. These biomarkers also are indicative of subsequent progression toward cancer in patients with lung diseases.

To search biomarkers for lung cancer, many metabolomics studies focus on determining the changes in small molecule metabolites, but these changes will be influenced by many factors, including individual differences, interaction of small molecules in the body or the environment, methods, sample collection, preparation, storage, experimental process, data analysis, data description, and database standardization [75,76]. Under the influence of these factors, the experimental data will show significant differences, which is definitely not conducive to the creation of comparable data in metabolomics research. Therefore, the standardization of the whole process especially lung cancer metabolomics experiments, data analyses, and reports are desperately needed to make data from different metabolomics laboratories comparable, which helps to ascertain more accurate biomarkers in a standard condition. Limitations and challenges remain in many metabolomics studies of lung cancer, although metabolomics were widely used in lung cancer research over the past decade. Different analytical techniques have their own weaknesses and limitations in sensitivity, reproducibility, or equipment costs. No matter which analytical method is used, metabolomics research involves the following common problems [77–80]: (1) Metabolomics is very sensitive to many internal and

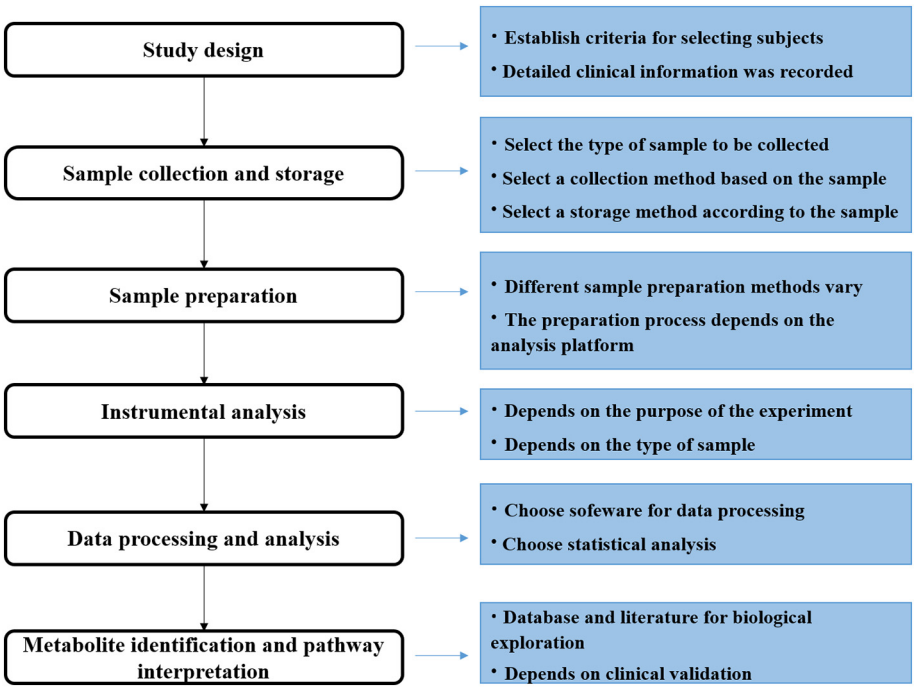

Fig. 7. The workflow of metabolomics for lung cancer.

external factors, and small differences may affect the metabolic profile. Therefore, it is urgent to standardize experimental methods and data analysis techniques. (2) The composition of metabolites in samples is very complex, and it is currently impossible to accurately measure the entire metabolome group with a single analysis. (3) Metabolomics analysis produces a large amount of data, and it is difficult to use the existing metabolite database to comprehensively identify all of the changed metabolites. In addition, the mechanism of identified metabolite changes is difficult to explain. (4) Although metabolomics is now widely used to identify biomarkers in lung cancer, the number of patient samples is not enough to for the translation of these laboratory achievements into clinical applications. (5) Moreover, the results focused mostly on the qualitative but not quantitative approach, which also limits the clinical application of these markers.

Therefore, it is necessary to overcome the aforementioned limitations and realize the standardization of metabolomics through the continuous development of metabolomics research technology, which will make the results of different studies more comparable and help identify biomarkers. If metabolic biomarkers can successfully overcome research and clinical barriers, they will help improve the diagnosis, staging, prognosis, and treatment of many diseases. Metabolites are important as potential biomarkers for several reasons. Metabolites undergo earlier and more significant changes than genes or proteins, and these changes can be measured in absolute terms, while changes in the activity of genes and proteins differ from changes in concentration. Metabolites can be distributed through biochemical pathways, which can be explained biologically to enhance their significance. This approach has emerging potential either on its own or in combination with other more mature “omics” technologies. Ideally, the combination of genomic, proteomic, metabolomics, and microbiome markers could provide an optimal panel of information to effectively diagnose and successfully treat lung cancer patients.

Therefore, further studies of targeted metabolomics based on our review should be conducted to assess the differences between the cases and controls and to compare the metabolomics variations between patients with different lung cancer grades.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cca.2019.05.012.

Acknowledgments

This work was financially supported by the Ningbo Natural Science Foundation, China (No. 2017A610258), the National Natural Science Foundation of China, China (No. 81603266), and the Zhejiang Key Laboratory of Pathophysiology, China (No. 201810). This study was also sponsored by a K.C. Wong Magna Fund from Ningbo University, China.

References

[1] P. Anand, A.B. Kunnumakara, C. Sundaram, K.B. Harikumar, S.T. Tharakan, O.S. Lai, B. Sung, B.B. Aggarwal, Cancer is a preventable disease that requires major lifestyle changes, Pharm. Res. 25 (9) (2008) 2097–2116, https://doi.org/10.1007/s11095-008-9661-9.

[2] J. Spratlin, N.J. Serkova, S.G. Eckhardt, Clinical applications of metabolomics in oncology: a review, Clin. Cancer Res. 15 (2) (2009) 431–440, https://doi.org/10.1158/1078-0432.CCR-08-1059.

[3] C.C. Tseng, C.C. Wang, C.C. Hsiao, H. Lu, S. Leu, H.C. Chang, K.T. Huang, W.F. Fang, Y.M. Chen, S.F. Liu, Time courses and value of circulating microparticles in patients with operable stage non-small cell lung cancer undergoing surgical intervention, Tumor Biol. 11 (1) (2016) 1–10, https://doi.org/10.1007/s13277-016-5047-5.

[4] M. Pirozynski, 100 years of lung cancer, Respir. Med. 100 (12) (2006) 2073–2084, https://doi.org/10.1016/j.rmed.2006.09.002.

[5] M.A. Rasmussen, R.M. Kannangara, B.M. Jespersen, L. Munck, S.B. Engelsen, From metabolome to phenotype: GC-MS metabolomics of developing mutant barley seeds reveals effects of growth, temperature and genotype, Sci. Rep. 7 (1) (2017) 8195, https://doi.org/10.1038/s41598-017-08129-0.

[6] P.Y. Yin, G.W. Xu, Metabolomics for tumor marker discovery and identification based on chromatography–mass spectrometry, Expert. Rev. Mol. Diagn. 13 (4) (2013) 339–348, https://doi.org/10.1586/erm.13.23.

[7] A.I. Robles, C.C. Harris, Integration of multiple "OMIC" biomarkers: a precision medicine strategy for lung cancer, Lung Cancer 107 (2017) 50–58, https://doi.org/10.1016/j.lungcan.2016.06.003.

[8] J. Tumas, K. Kvederaviciute, M. Petrulionis, B. Kurlinkus, A. Rimkus, G. Sakalauskaite, J. Cicenas, A. Sileikis, Metabolomics in pancreatic cancer biomarkers research, Med. Oncol. 33 (12) (2016) 133, https://doi.org/10.1007/s12032-016-0853-6.

[9] G.A. Nagana Gowda, N. Shanaiah, A. Cooper, M. Maluccio, D. Raftery, Visualization of bile homeostasis using (1)H-NMR spectroscopy as a route for assessing liver cancer, Lipids 44 (1) (2009) 27–35, https://doi.org/10.1007/s11745-008-3254-6.

[10] W.M. Claudino, A. Quattrone, L. Biganzoli, M. Pestrin, I. Bertini, L.A. Di, Metabolomics: available results, current research projects in breast cancer, and future applications, J. Clin. Oncol. 25 (19) (2007) 2840–2846, https://doi.org/10.1200/JCO.2006.09.7550.

[11] E.M. Defeo, C.L. Wu, W.S. Mcdougal, L.L. Cheng, A decade in prostate cancer: from NMR to metabolomics, Nat. Rev. Urol. 8 (6) (2011) 301–311, https://doi.org/10.1038/nrurol.2011.53.

[12] T.W. Fan, A.N. Lane, R.M. Higashi, M.A. Farag, H. Gao, M. Bousamra, D.M. Miller, Altered regulation of metabolic pathways in human lung cancer discerned by (13)C stable isotope-resolved metabolomics (SIRM), Mol. Cancer 8 (2009) 41, https://doi.org/10.1186/1476-4598-8-41.

[13] S. Deja, I. Porebska, A. Kowal, A. Zabek, W. Barg, K. Pawelczyk, I. Stanimirova, M. Daszykowski, A. Korzeniewska, R. Jankowska, P. Mlynarz, Metabolomics provide new insights on lung cancer staging and discrimination from chronic obstructive pulmonary disease, J. Pharm. Biomed. Anal. 100 (2014) 369–380, https://doi.org/10.1016/j.jpba.2014.08.020.

[14] M. Calderón-Santiago, F. Priego-Capote, N. Turck, X. Robin, B. Jurado-Gámez, J.C. Sanchez, M.D. Luque de Castro, Human sweat metabolomics for lung cancer screening, Anal. Bioanal. Chem. 407 (18) (2015) 5381–5392, https://doi.org/10.1007/s00216-015-8700-8.

[15] S.J. Cameron, K.E. Lewis, M. Beckmann, G.G. Allison, R. Ghosal, P.D. Lewis, L.A. Mur, The metabolomic detection of lung cancer biomarkers in sputum, Lung Cancer 94 (2016) 88–95, https://doi.org/10.1016/j.lungcan.2016.02.006.

[16] M. Gottschalk, G. Ivanova, D.M. Collins, A. Eustace, R. O'Connor, D.F. Brougham, Metabolomic studies of human lung carcinoma cell lines using in vitro 1H NMR of whole cells and cellular extracts, NMR Biomed. 21 (8) (2008) 809–819, https://doi.org/10.1002/nbm.1258.

[17] I. Horváth, Z. Lázár, N. Gyulai, M. Kollai, G. Losonczy, Exhaled biomarkers in lung cancer, Eur. Respir. J. 34 (1) (2009) 261–275, https://doi.org/10.1183/09031936.00142508.

[18] Y. Li, X. Song, X. Zhao, L. Zou, G. Xu, Serum metabolic profiling study of lung cancer using ultra high performance liquid chromatography/quadrupole time-of-flight mass spectrometry, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 966 (1) (2014) 147–153, https://doi.org/10.1016/j.jchromb.2014.04.047.

[19] Y.M. Guo, X.M. Wang, L. Qiu, X.Z. Qin, H. Liu, Y.Y. Wang, F. Li, X.D. Wang, G.Q. Chen, G.G. Song, Probing gender-specific lipid metabolites and diagnostic biomarkers for lung cancer using Fourier transform ion cyclotron resonance mass spectrometry, Clin. Chim. Acta 414 (1) (2012) 135–141, https://doi.org/10.1016/j.cca.2012.08.010.

[20] X.M. Zhou, C.C. He, Y.M. Liu, Y. Zhao, D. Zhao, Metabonomic classification and detection of small molecule biomarkers of malignant pleural effusions, Anal. Bioanal. Chem. 404 (10) (2012) 3123–3133, https://doi.org/10.1016/10.1007/s00216-012-6432-6.

[21] A.D. Pamungkas, C.Y. Park, S.Y. Lee, H.J. Sun, Y.H. Park, High resolution metabolomics to discriminate compounds in serum of male lung cancer patients in South Korea, Respir. Res. 17 (1) (2016) 100, https://doi.org/10.1186/s12931-016-0419-3.

[22] X. Zhang, X. Zhu, C. Wang, H. Zhang, Z. Cai, Non-targeted and targeted metabolomics approaches to diagnosing lung cancer and predicting patient prognosis, Oncotarget 7 (39) (2016) 63437–63448, https://doi.org/10.18632/oncotarget.11521.

[23] B. Álvarez-Sánchez, F. Priego-Capote, L.D. Castro, Metabolomics analysis I. Selection of biological samples and practical aspects preceding sample preparation, TrAc Trends Anal. Chem. 29 (2) (2010) 111–119, https://doi.org/10.1016/j.trac.2009.12.003.

[24] R. Dong, X. Wang, A. Lian, C. Chi, C. Ke, L. Guo, S. Liu, W. Zhao, G. Xu, E. Li, Exhaled volatile organic compounds as lung cancer biomarkers during one-lung ventilation, Sci. Rep. 4 (8) (2014) 7312, https://doi.org/10.1038/srep07312.

[25] A. Peralbo-Molina, M. Calderón-Santiago, F. Priego-Capote, B. Jurado-Gámez, M.D. Luque de Castro, Metabolomics analysis of exhaled breath condensate for discrimination between lung cancer patients and risk factor individuals, J. Breath Res. 10 (1) (2016) 016011, , https://doi.org/10.1088/1752-7155/10/1/016011.

[26] H.G. Gika, G.A. Theodoridis, J.E. Wingate, I.D. Wilson, Within-day reproducibility of an HPLC−MS-based method for metabonomic analysis: application to human urine, J. Proteome Res. 6 (8) (2007) 3291–3303, https://doi.org/10.1021/pr070183p.

[27] E.J. Want, A. Nordström, H. Morita, G. Siuzdak, From exogenous to endogenous: the inevitable imprint of mass spectrometry in metabolomics, J. Proteome Res. 6 (2) (2007) 459–468, https://doi.org/10.1021/pr060505+.

[28] C.P. Wen, F. Zhang, D. Liang, C. Wen, J. Gu, H. Skinner, W.H. Chow, Y. Ye, X. Pu, M.A. Hildebrandt, M. Huang, C.H. Chen, C.A. Hsiung, M.K. Tsai, C.K. Tsao, S.M. Lippman, X. Wu, The ability of bilirubin in identifying smokers with higher risk of lung cancer: a large cohort study in conjunction with global metabolomic profiling, Clin. Cancer Res. 21 (1) (2015) 193–200, https://doi.org/10.1158/1078-0432.CCR-14-0748.

[29] J.M. Hu, H.T. Sun, Serum proton NMR metabolomics analysis of human lung cancer following microwave ablation, Radiat. Oncol. 13 (1) (2018) 40, https://doi.org/10.1186/s13014-018-0982-5.
[30] S.M. Rocha, M. Caldeira, J. Carrola, M. Santos, N. Cruz, I.F. Duarte, Exploring the human urine metabolomic potentialities by comprehensive two-dimensional gas chromatography coupled to time of flight mass spectrometry, J. Chromatogr. A 1252 (24) (2012) 155–163, https://doi.org/10.1016/j.chroma.2012.06.067.
[31] M. Katajamaa, M. Orešič, Data processing for mass spectrometry-based metabolomics, J. Chromatogr. A 1158 (1) (2007) 318–328, https://doi.org/10.1016/j.chroma.2007.04.021.
[32] K.H. Liland, Multivariate methods in metabolomics – from pre-processing to dimension reduction and statistical analysis, TrAc Trends Anal. Chem. 30 (6) (2011) 827–841, https://doi.org/10.1016/j.trac.2011.02.007.
[33] J. Zhuang, X. Tang, Z. Du, M. Yang, Y. Zhou, Prediction of biomarkers of therapeutic effects of patients with lung adenocarcinoma treated with gefitinib based on progression-free-survival by metabolomic fingerprinting, Talanta 160 (1) (2016) 636–644, https://doi.org/10.1016/j.talanta.2016.08.007.
[34] Y.R. Chen, Z.H. Ma, L.S. Min, H.w. Li, B. Wang, J. Zhong, L.C. Dai, Biomarker identification and pathway analysis by serum metabolomics of lung cancer, Biomed. Res. Int. 2015 (7) (2015) 183624, https://doi.org/10.1155/2015/183624.
[35] Y.Q. Li, Y.F. Liu, D.D. Song, Y.P. Zhou, L. Wang, S. Xu, Y.F. Cui, Particle swarm optimization-based protocol for partial least-squares discriminant analysis: application to 1H nuclear magnetic resonance analysis of lung cancer metabonomics, Chemom. Intell. Lab. Syst. 135 (15) (2014) 192–200, https://doi.org/10.1016/j.chemolab.2014.04.014.
[36] Q. Yang, X. Shi, Y. Wang, W. Wang, H. He, X. Lu, G. Xu, Urinary metabonomic study of lung cancer by a fully automatic hyphenated hydrophilic interaction/RPLC-MS system, J. Sep. Sci. 33 (10) (2010) 1495–1503, https://doi.org/10.1002/jssc.200900798.
[37] H. Xu, R. Liu, B. He, C.W. Bi, K. Bi, Q. Li, Polyamine metabolites profiling for characterization of lung and liver cancer using an LC-tandem MS method with multiple statistical data mining strategies: discovering potential Cancer biomarkers in human plasma and urine, Molecules 21 (8) (2016) 1040, https://doi.org/10.3390/molecules21081040.
[38] Q. Wu, Y. Wang, X. Gu, J. Zhou, H. Zhang, W. Lv, Z. Chen, C. Yan, Urinary metabolomic study of non-small cell lung carcinoma based on ultra high performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry, J. Sep. Sci. 37 (14) (2014) 1728–1735, https://doi.org/10.1002/jssc.201400222.
[39] M. Wu, Y. Xu, W.L. Fitch, M. Zheng, R.E. Merritt, J.B. Shrager, W. Zhang, D.L. Dill, G. Peltz, C.D. Hoang, Liquid chromatography/mass spectrometry methods for measuring dipeptide abundance in non-small-cell lung cancer, Rapid Commun. Mass Spectrom. 27 (18) (2013) 2091–2098, https://doi.org/10.1002/rcm.6656.
[40] W.R. Wikoff, S. Hanash, B. DeFelice, S. Miyamoto, M. Barnett, Y. Zhao, G. Goodman, Z. Feng, D. Gandara, O. Fiehn, A. Taguchi, Diacetylspermine is a novel prediagnostic serum biomarker for non-small-cell lung Cancer and has additive performance with pro-surfactant protein B, J. Clin. Oncol. 33 (33) (2015) 3880–3886, https://doi.org/10.1200/jco.2015.61.7779.
[41] W.R. Wikoff, D. Grapov, J.F. Fahrmann, B. DeFelice, W. Rom, H. Pass, K. Kim, U. Nguyen, S. Taylor, D.R. Gandara, K. Kelly, O. Fiehn, S. Miyamoto, Metabolomic markers of altered nucleotide metabolism in early stage adenocarcinoma, Cancer Prev. Res. (Phila.) 8 (5) (2015) 410–418, https://doi.org/10.1158/1940-6207.CAPR-14-0329.
[42] W. Chen, S. Lu, J. Ou, G. Wang, Y. Zu, F. Chen, C. Bai, Metabonomic characteristics and biomarker research of human lung cancer tissues by HR1H NMR spectroscopy, Cancer Biomark. 16 (4) (2016) 653, https://doi.org/10.3233/CBM-160607.
[43] W.X. Chen, Y.k. Zu, Q. Huang, F.E. Chen, G.F. Wang, W.X. Lan, C.X. Bai, S.H. Lu, Y. Yue, F. Deng, Study on Metabonomic characteristics of human lung Cancer using high resolution magic-angle spinning 1H NMR spectroscopy and multivariate data analysis, Magn. Reson. Med. 66 (6) (2011) 1531–1540, https://doi.org/10.1002/mrm.22957.
[44] T. Wen, L. Gao, Z. Wen, C. Wu, C.S. Tan, W.Z. Toh, C.N. Ong, Exploratory investigation of plasma metabolomics in human lung adenocarcinoma, Mol. BioSyst. 9 (9) (2013) 2370–2378, https://doi.org/10.1039/c3mb70138g.
[45] Q. Wang, P. Gao, X.Y. Wang, Y.X. Duan, The early diagnosis and monitoring of squamous cell carcinoma via saliva metabolomics, Sci. Rep. 4 (4) (2014) 6804, https://doi.org/10.1038/srep06802.
[46] M. Suzanne, T. Sandra, B. Dinesh, A. Wohlgemuth, Systemic metabolomic changes in blood samples of lung cancer patients identified by gas chromatography time-of-flight mass spectrometry, Metabolites 5 (2) (2015) 192, https://doi.org/10.3390/metabo5020192.
[47] C.L. Silva, M. Passos, J.S. Câmara, Investigation of urinary volatile organic metabolites as potential cancer biomarkers by solid-phase microextraction in combination with gas chromatography-mass spectrometry, Br. J. Cancer 105 (12) (2011) 1894–1904, https://doi.org/10.1038/bjc.2011.437.
[48] M. Roå-Mazurczyk, A. Wojakowska, A. Marczak, K. PolaåSki, M. Pietrowska, J. Polanska, R. Dziadziuszko, J. Jassem, W. Rzyman, P. Widlak, Panel of serum metabolites discriminates cancer patients and healthy participants of lung cancer screening - a pilot study, Acta Biochim. Pol. 64 (3) (2017) 513–518, https://doi.org/10.18388/abp.2017_1517.
[49] M. Ros-Mazurczyk, K. Jelonek, A. Marczyk, F. Binczyk, M. Pietrowska, J. Polanska, R. Dziadziuszko, J. Jassem, W. Rzyman, P. Widlak, Serum lipid profile discriminates patients with early lung cancer from healthy controls, Lung Cancer 112 (2017) 69–74, https://doi.org/10.1016/j.lungcan.2017.07.036.
[50] C.M. Rocha, J. Carrola, A.S. Barros, A.M. Gil, B.J. Goodfellow, I.M. Carreira, J. Bernardo, A. Gomes, V. Sousa, L. Carvalho, I.F. Duarte, Metabolic signatures of lung cancer in biofluids: NMR-based metabonomics of blood plasma, J. Proteome Res. 10 (9) (2011) 4314–4324, https://doi.org/10.1021/pr200550p.
[51] Q.B. Song, W.G. Hu, P. Wang, Y. Yao, H.Z. Zeng, Identification of serum biomarkers for lung cancer using magnetic bead-based SELDI-TOF-MS, Acta Pharmacol. Sin. 32 (12) (2011) 1537–1542, https://doi.org/10.1038/aps.2011.137.
[52] K. O'Shea, S.J. Cameron, K.E. Lewis, C. Lu, L.A. Mur, Metabolomic-based biomarker discovery for non-invasive lung cancer screening: a case study, Biochim. Biophys. Acta 1860 (11) (2016) 2682–2687, https://doi.org/10.1016/j.bbagen.2016.07.007.
[53] Y. Niu, Y. Jiang, C. Xu, X. Wang, Y. Liu, H. Zhao, B. Han, L. Jiang, Preliminary results of metabolite in serum and urine of lung cancer patients detected by metabolomics, Chin. J. Lung Cancer 15 (4) (2012) 195–201, https://doi.org/10.3779/j.issn.1009-3419.2012.04.01.
[54] S.G. Musharraf, S. Mazhar, M.I. Choudhary, N. Rizi, Atta-ur-Rahman, Plasma metabolite profiling and chemometric analyses of lung cancer along with three controls through gas chromatography-mass spectrometry, Sci. Rep. 5 (2015) 8607, https://doi.org/10.1038/srep08607.
[55] E. Louis, F.X. Cantrelle, L. Mesotten, G. Reekmans, L. Bervoets, K. Vanhove, M. Thomeer, G. Lippens, P. Adriaensens, Metabolic phenotyping of human plasma by H-NMR at high and medium magnetic field strengths: a case study for lung cancer, Magn. Reson. Chem. 55 (8) (2017) 706–713, https://doi.org/10.1002/mrc.4577.
[56] P.G. Lokhov, O.P. Trifonova, D.L. Maslov, A.I. Archakov, Blood plasma metabolites and the risk of developing lung cancer in Russia, Eur. J. Cancer Prev. 22 (4) (2013) 335–341, https://doi.org/10.1097/CEJ.0b013e32835b3898.
[57] Y.F. Liu, S. Xu, H. Gong, Y.F. Cui, D.D. Song, Y.P. Zhou, G.F. Wang, Partial least-squares discriminant analysis optimized by particle swarm optimization: application to 1H nuclear magnetic resonance analysis of lung cancer metabonomics, Chemom. Intell. Lab. Syst. 29 (10) (2015) 537–546, https://doi.org/10.1002/cem.2737.
[58] A. Klupczynska, P. Dereziński, T.J. Garrett, V.Y. Rubio, W. Dyszkiewicz, M. Kasprzyk, Z.J. Kokot, Study of early stage non-small-cell lung cancer using Orbitrap-based global serum metabolomics, J. Cancer Res. Clin. Oncol. 143 (4) (2017) 649–659, https://doi.org/10.1007/s00432-017-2347-0.
[59] A. Klupczynska, P. Dereziński, W. Dyszkiewicz, K. Pawlak, M. Kasprzyk, Z.J. Kokot, Evaluation of serum amino acid profiles' utility in non-small cell lung cancer detection in polish population, Lung Cancer 100 (2016) 71–76, https://doi.org/10.18388/abp.2017_1517.
[60] J.F. Fahrmann, K. Kim, B.C. DeFelice, S.L. Taylor, D.R. Gandara, K.Y. Yoneda, D.T. Cooke, O. Fiehn, K. Kelly, S. Miyamoto, Investigation of metabolomic blood biomarkers for detection of adenocarcinoma lung cancer, Cancer Epidemiol. Biomark. Prev. 24 (11) (2015) 1716–1723, https://doi.org/10.1158/1055-9965.EPI-15-0427.
[61] D.C. Wedge, J.W. Allwood, W. Dunn, A.A. Vaughan, K. Simpson, M. Brown, L. Priest, F.H. Blackhall, A.D. Whetton, C. Dive, Is serum or plasma more appropriate for intersubject comparisons in metabolomic studies? An assessment in patients with small-cell lung cancer, Anal. Chem. 83 (17) (2011) 6689–6697, https://doi.org/10.1021/ac2012224.
[62] Y. Chen, Z. Ma, A. Li, H. Li, B. Wang, J. Zhong, L. Min, L. Dai, Metabolomic profiling of human serum in lung cancer patients using liquid chromatography/hybrid quadrupole time-of-flight mass spectrometry and gas chromatography/mass spectrometry, J. Cancer Res. Clin. Oncol. 141 (4) (2015) 705–718, https://doi.org/10.1007/s00432-014-1846-5.
[63] C. Joana, C.M. Rocha, A.S. Barros, A.M. Gil, B.J. Goodfellow, I.M. Carreira, B. Joao, G. Ana, S. Vitor, C. Lina, Metabolic signatures of lung cancer in biofluids: NMR-based metabonomics of urine, J. Proteome Res. 10 (1) (2011) 221, https://doi.org/10.1021/pr100899x.
[64] B. Callejón-Leblic, T. García-Barrera, J. Grávalos-Guzmán, A. Pereira-Vega, J.L. Gómez-Ariza, Metabolic profiling of potential lung cancer biomarkers using bronchoalveolar lavage fluid and the integrated direct infusion/ gas chromatography mass spectrometry platform, J. Proteome 145 (2016) 197–206, https://doi.org/10.1016/j.jprot.2016.05.030.
[65] Z. An, Y. Chen, R. Zhang, Y. Song, J. Sun, J. He, J. Bai, L. Dong, Q. Zhan, Z. Abliz, Integrated ionization approach for RRLC-MS/MS-based metabonomics: finding potential biomarkers for lung cancer, J. Proteome Res. 9 (8) (2010) 4071–4081, https://doi.org/10.1021/pr100265g.
[66] A.Y. Jin, C. Hye Rim, K. Tae Min, K. Bhumsuk, K.J. Wook, W. He, P. Chul-Kee, L. Se-Hoon, I. Seock-Ah, K. Jeong Eun, An NMR metabolomics approach for the diagnosis of leptomeningeal carcinomatosis in lung adenocarcinoma cancer patients, Int. J. Cancer 136 (1) (2015) 162–171, https://doi.org/10.1002/ijc.28949.
[67] H.L. Eley, S.T. Russell, M.J. Tisdale, Effect of branched-chain amino acids on muscle atrophy in cancer cachexia, Biochem. J. 407 (1) (2007) 113–120, https://doi.org/10.1042/BJ20070651.
[68] T.M. O'Connell, The complex role of branched chain amino acids in diabetes and cancer, Metabolites 3 (4) (2013) 931–945, https://doi.org/10.3390/metabo3040931.
[69] H. Nicastro, L.C. Da, D.F. Chaves, L.R. Bechara, V.A. Voltarelli, M.M. Rogero, Does branched-chain amino acids supplementation modulate skeletal muscle remodeling through inflammation modulation? Possible mechanisms of action, J. Nutr. Metab. 2012 (1) (2012) 136937, , https://doi.org/10.1155/2012/136937.
[70] M.H. Kim, H. Kim, Oncogenes and tumor suppressors regulate glutamine metabolism in cancer cells, J. Cancer Prev. 18 (3) (2013) 221–226, https://doi.org/10.15430/JCP.2013.18.3.221.
[71] D. Daye, K.E. Wellen, Metabolic reprogramming in cancer: unraveling the role of glutamine in tumorigenesis, Semin. Cell Dev. Biol. 23 (4) (2012) 362–369, https://doi.org/10.1016/j.semcdb.2012.02.002.
[72] O. Ben-Yoseph, R.S. Badar-Goffer, P.G. Morris, H.S. Bachelard, Glycerol 3-

phosphate and lactate as indicators of the cerebral cytoplasmic redox state in severe and mild hypoxia respectively: a 13C- and 31P-n.m.r. study, Biochem. J. 291 (3) (1993) 915–919, https://doi.org/10.1590/S0100-83582009000300014.
[73] Y. Hajime, G. Jianfei, M. Munetaka, H. Kotaro, T. Hisao, N. Yosinobu, Lactate, choline, and creatine levels measured by vitro 1H-MRS as prognostic parameters in patients with non-small-cell lung cancer, J. Magn. Reson. Imaging 25 (5) (2010) 992–999, https://doi.org/10.1002/jmri.20902.
[74] G. Kristine, Z.M. Bhujwalla, S.M. Ronen, Choline metabolism in malignant transformation, Nat. Rev. Cancer 11 (12) (2011) 835–848, https://doi.org/10.1038/nrc3162.
[75] M.S. Monteiro, M. Carvalho, M.L. Bastos, P. Pinho, G. De, Metabolomics analysis for biomarker discovery: advances and challenges, Curr. Med. Chem. 20 (2) (2013) 257–271, https://doi.org/10.2174/092986713804806621.
[76] A.H. Emwas, C. Luchinat, P. Turano, L. Tenori, R. Roy, R.M. Salek, D. Ryan, J.S. Merzaban, R. Kaddurah-Daouk, A.C. Zeri, Standardizing the experimental conditions for using urine in NMR-based metabolomic studies with a particular focus on diagnostic studies: a review, Metabolomics 11 (4) (2015) 872–894, https://doi.org/10.1007/s11306-014-0746-7.
[77] A.H.M. Emwas, The strengths and weaknesses of NMR spectroscopy and mass spectrometry with particular focus on metabolomics research, Methods Mol. Biol. 1277 (2015) 161, https://doi.org/10.1007/978-1-4939-2377-9_13.
[78] A. Scalbert, L. Brennan, O. Fiehn, T. Hankemeier, B.S. Kristal, B.V. Ommen, E. Pujos-Guillot, E. Verheij, D. Wishart, S. Wopereis, Mass-spectrometry-based metabolomics: limitations and recommendations for future progress with particular focus on nutrition research, Metabolomics 5 (4) (2009) 435–458, https://doi.org/10.1007/s11306-009-0168-0.
[79] M.E. Bollard, J.C. Stanley, E.G. Lindon, J.K. Nicholson, E. Holmes, NMR-based metabonomic approaches for evaluating physiological influences on biofluid composition, NMR Biomed. 18 (3) (2005) 143–162, https://doi.org/10.1002/nbm.935.
[80] R.A. Spicer, R. Salek, C. Steinbeck, A decade after the metabolomics standards initiative it's time for a revision, Sci. Data 4 (2017) 170138, , https://doi.org/10.1038/sdata.2017.138.