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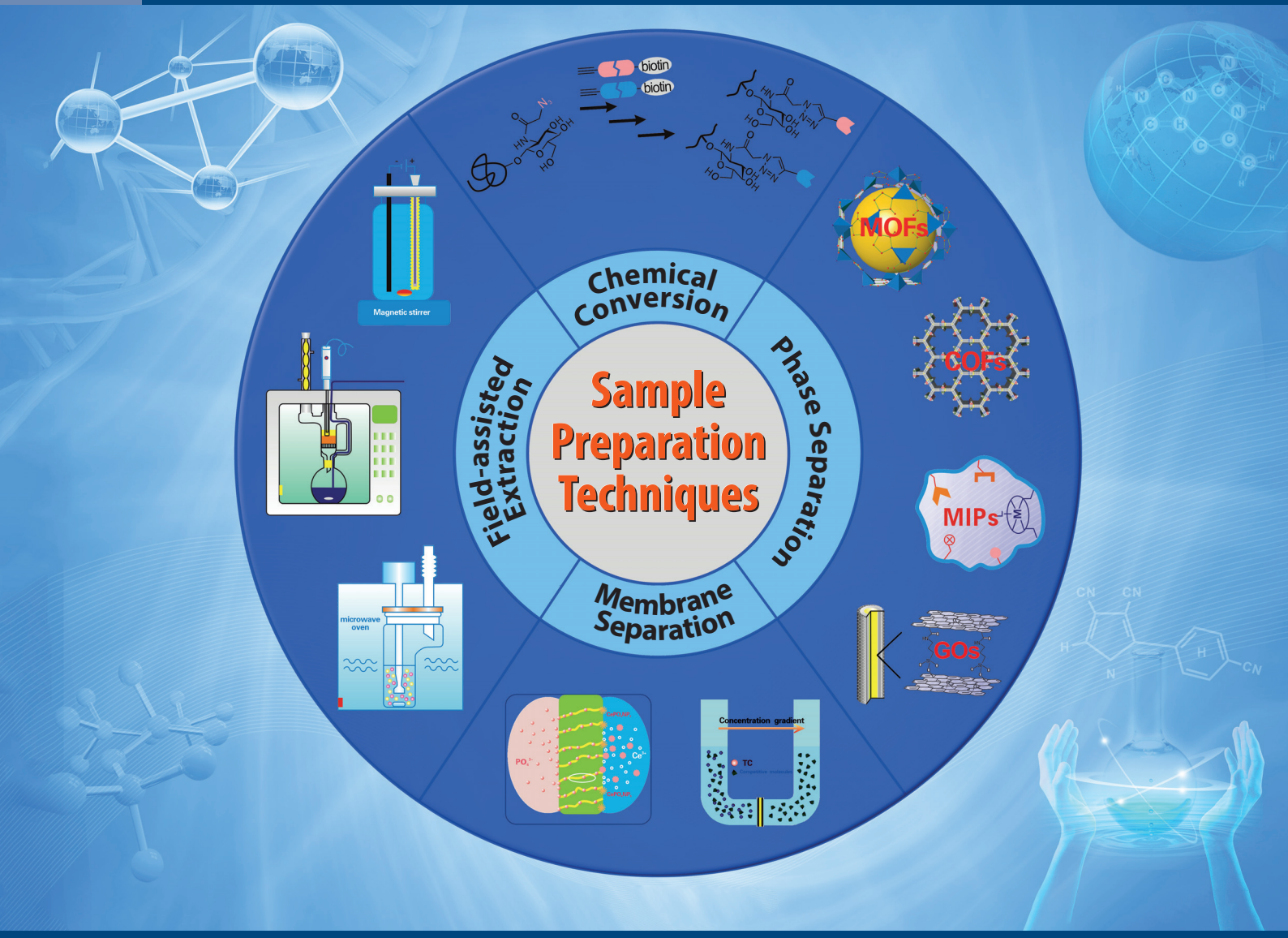

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REVIEW ARTICLE

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Metabolomics of lung cancer: Analytical platforms and their applications

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Lung cancer is the leading type of cancer worldwide in terms of the number of new cases and is responsible for the largest number of deaths due to poor prognosis and difficult early detection. Due to its ability to detect numerous small molecular metabolites simultaneously, metabolomics has been widely used for the assessment of global metabolic changes in a living organism to discover candidate biomarkers for cancer diagnosis, investigate the development of cancer, and provide insights into the underlying pathophysiology. This review will mainly describe recent developments in lung cancer metabolomics in terms of early-stage detection, biomarker discovery and mechanism exploration by using nuclear magnetic resonance, liquid chromatography–mass spectrometry, gas chromatography–mass spectrometry, and capillary electrophoresis–mass spectrometry in the last 10 years. The sample collection and metabolite extraction methods are also summarized.

KEYWORDS

capillary electrophoresis–mass spectrometry, gas chromatography–mass spectrometry, liquid chromatography–mass spectrometry, lung cancer, metabolomics

1 | INTRODUCTION

Lung cancer is the most commonly diagnosed cancer and the leading cause of cancer deaths worldwide [1]. By sex, it is the most commonly diagnosed cancer and the leading cause of cancer death in males; among females, lung cancer ranks third for incidence and mortality [1]. Lung cancer can be classified into four main subtypes: small cell lung cancer, accounting for approximately 15% and non-small cell lung cancer (NSCLC), including squamous cell carcinoma, adenocarcinoma (AC), and large cell carcinoma, accounting for approximately 85% of all lung cancers [2–4]. Currently, there are several types of diagnostic methods for lung cancer, including the imaging examination (X-ray, CT scan, magnetic resonance imaging], histopathological examination, cytological examination, and biochemical examination of tumor markers [5–7]. Medical developments have been helpful in reducing deaths; however, deaths caused by lung cancer are still increasing due to some reasons, such as (1) the early detection of lung cancer is not easy because the symptoms in the early-stage are not entirely clear; (2) the complexity of lung cancer pathogenicity and molecular subtyping; (3) the absent targeted therapy for a specific subtype and individual patient; and (4) the unclear pathological mechanism of this disease [8]. Thus, there is an urgent need to reveal the metabolic changes and pathogenesis of lung cancer and to screen for useful diagnostic biomarkers, especially for early-stage detection.

Metabolomics aims to perform a comprehensive analysis of all metabolites in a biological system. Thus, this analysis reveals what exactly takes place inside organisms due to pathophysiologic stimuli, environmental stress, and genetic modification [9,10]. Endogenous metabolites have a good ability to provide information about physiological functions and pathological status in detail [11]. Therefore, metabolomics has become a promising tool in disease

Article Related Abbreviations: AC, adenocarcinoma; LPC, lysophosphatidylcholine; NSCLC, non-small cell lung cancer; PC, phosphatidylcholine; PE, phosphatidylethanolamine; SCC, squamous cell carcinoma; VOC, volatile organic compound.

biomarker screening, pathological mechanism interpretation, and drug efficacy evaluation. NMR spectroscopy and MS are the two main tools for metabolic profiling. The improved sensitivity and resolution of NMR spectroscopy and MS, as well as the construction of efficient and large-scale metabolite annotation databases, have promoted the progress of metabolomics [8].

Current metabolomics studies of lung cancer mainly focus on early-stage detection, the differentiation between subtypes and biomarker discovery, metabolite alterations in different lung cancer stages, mechanism exploration, and the effective therapy evaluation of lung cancer [4,8]. This review aims to summarize the analytical platforms and their applications in metabolomics studies of lung cancer, including sample pretreatment methods and analytical tools based on NMR spectroscopy and MS, especially in the last 10 years. Additionally, the advantages and disadvantages of different analytical platforms are also given.

2 | SAMPLE COLLECTION AND METABOLITE EXTRACTION METHODS FOR LUNG CANCER METABOLOMICS STUDY

Biofluids (such as blood and urine), tissues, and cells were mostly used as samples in metabolomics studies. Since the lungs are related to the respiratory tract, samples of sputum, expiration concentrate, and bronchoalveolar lavage fluid have also been included [8].

Sample collection is a very important step, and the quality of the collected samples significantly affects the experimental results; thus, paying more attention to the preanalytical factors is very necessary [12–14]. Our previous study reported that (4E,14Z)-sphingadienine-C18-1-phosphate can be used for determining the quality changes in serum and plasma due to long-term exposure at room temperature, it is also suitable for determining whether the blood samples in biobanks should be excluded from metabolomics investigations [12]. Besides exposure to room temperature, blood collection tubes, and batch effects should also be carefully checked to avoid the chemical noise in MS [13–15].

On the other hand, hemolysis should be avoided when collecting blood sample. Hemolysis means when blood cells are broken and their contents are released into the surrounding fluid (serum or plasma), following cell membrane injury. It happens in vivo and more often in vitro because of a collection of high hematocrit [16] and improper operation. There are many examples of improper operation such as incorrect needle size, unneeded mixing, excessive force during sample filling [17]. Hemolysis can also be caused by strong shaking of the tube, transportation by pneumatic post within the clinic, vigorous high-speed centrifugation [14]. Yin et al. reported that hemolysis caused numerous changes in the metabolic profile, including 69 ion features and some clinical parameters, such as lysophosphatidylcholines C16:0, C18:0, hemoglobin, AST, and potassium, which are significantly increased in moderate and strong hemolytic plasma samples [13]. Hemolysis can be avoided by drawing and handling carefully for the whole blood samples. The most reliable method of detecting slight hemolysis is the measurement of free hemoglobin in the samples [18].

To obtain high-quality data, researchers have suggested paying more attention to sample collection procedures and adopting standard operation procedures [13,19,20] and defined validation procedures from surgical section to sample analysis [21]. Table 1 shows preanalytical recommendations for tissue, plasma/serum, and urine collection.

Several studies were carried out to characterize the differences between plasma and serum. For overall coverage, the numbers of serum features in LC–MS positive and negative modes and GC–MS were 2877, 2469, and 170, respectively, and the numbers in plasma were 3080, 2258, and 178, respectively [22] (Supporting Information Table S1). These results indicated plasma and serum could offer similar analytical opportunities. However, some specific metabolites, such as erythritol, creatinine, glycerophosphocholines, glutamine, and/ hexadecanoic acid, were unique to one biofluid instead of another in the case of plasma, but for serum, only thromboxane B2 was unique. Moreover, the levels of leucine, tryptophan, malonic acid, galactose, and serine, exhibited more differences among serum than among plasma samples. Tyrosine and *R*-hydroxybutyric acid exhibited more variation among plasma than among serum samples. This finding suggested that one of these biofluids is more suitable for one specific mission than for another. Thus, in the case of plasma, the choice of anticoagulant agent should be carefully taken into account. Additionally, in the proteomic analysis, plasma is more preferable [22,23]. The anticoagulant choice is another important issue to be considered and until now its application in metabolomics is still a subject of discussion [18]. EDTA, besides its ability as a coagulation inhibitor, is also an inhibitor for enzymes (e.g. glycolytic enzyme hexokinase), which depend on Mg^{2+}. This makes EDTA plasma suitable for metabolomics [19]. Another study debated that citrate or EDTA can cause ion suppression or enhancement of metabolites coeluting with anticoagulant peaks due to cluster formation of sodium and potassium [24]. For sodium citrate, its ionic strength and pH are not preferred for lipid extraction or LC–MS analysis [25]. However, little differences were found between the effects of different anticoagulants (Li-heparin, sodium citrate, K2-EDTA) in metabolite coverage by LC–MS [26], also, the same in case of EDTA and heparin [27].

An appropriate sample preparation procedure should be selected for different sample types to ensure metabolite coverage and metabolic profile quality [4]. The general

TABLE 1 Pre-analytical recommendation for tissue, plasma/serum and urine collection

Sample type	Recommendation	Ref.
General recommendation	1. Study design should be defined carefully in advance. 2. Matching well for sex, age, and body mass index. 3. Keep normality for circadian rhythm (if metabolite level will be affected). 4. Fasting (in some case). 5. Avoid exercise, stress, drugs, and nutritional supplements (before the clinical test by a specific time). 6. Using the same and good quality collection tubes. 7. Avoid repeating of freeze-thaw cycles and stepwise of thawing. 8. Freezing prefers to be at −80°C freezers. 9. Choosing the suitable extraction method according to the purpose of the study.	[13,14,106]
Special for tissue	1. A tissue sample is harvested essentially through biopsies or surgery. 2. Tissue sampling should be performed as soon as possible, then immediately frozen (for enzymatical reactions avoiding which lead to metabolite changes). 3. Blood removing before storage (rising the tissue with a buffer) to avoid the contamination by blood metabolites. 4. Quenching of the metabolism for stopping all enzymatic activities ASAP by using liquid nitrogen or denaturing the enzymes with acid or cold solvent addition.	[107]
Special for plasma/serum	1. Avoiding chemical noise coming from plastic collection tubes and plastic pipette tips. 2. Collection samples on iced water during drawing in case of plasma and on iced water after clotting formation (>30 min) for serum. 3. The clotting time should be rigorously controlled (serum). 4. Choosing the suitable anticoagulants agent (plasma) 5. For plasma is preferred to immediately separation of blood cells by centrifugation at 4°C. 6. Separate cells ASAP (within 0.5 h). 7. Avoid exposing at room temperature. 8. Biofluids should be split into small aliquots 9. Storing at −80°C or until analysis, and the stepwise freezing are recommended. 10. Consistent standard operation procedures through one sample set.	[13,14,17]
Special for urine	1. A combined use of a mild pre-centrifugation (RCF between 1000 and 3000 at 4°C) and filtration. 2. Storage in liquid nitrogen; usually, without additives. 3. Quick processing. 4. Storage at 4°C between collection and processing.	[108]

protocol for metabolomics studies has been discussed in detail by Yin et al. [28]. For the pretreatment of biofluids, the major steps are protein precipitation and concentration by freeze drying [4,8,20,29]. For cell line pretreatment in metabolomics studies, quenching and harvesting are the two critical steps for ending intracellular enzymatic reactions to avoid metabolite degradation. For a tissue sample that is also rich in enzymes, quenching and homogenization are important procedures [30–35]. Supporting Information Table S1 shows some metabolite extraction techniques that have been used for lung cancer metabolomics studies.

Ten milligrams of tissue sample and 25–50 μL of serum/plasma are usually required for metabolomics study [36–39]. Methanol, acetonitrile, isopropanol, water, and their mixture have been commonly used as extraction solvents in organic solvent-based protein precipitation methods. Ammonium acetate, formic acid, ammonium carbonate, and ammonium bicarbonate have been used as volatile modifiers [8]. LLE can separate polar metabolites and nonpolar metabolites into aqueous and organic phases, respectively. In the case of CE–MS analysis, a 5 kDa cutoff filter was used to remove residual proteins (9100 g, 3 h at 4°C)

after LLE [40]. In NMR spectroscopy analysis, deuterated solvents and internal standards of trimethylsilyl-propanoic acid or 4,4-dimethyl-4-silapentane-1-sulfonic acid were used (Supporting Information Table S1).

It is worth mentioning that the metabolome was affected by different sample preparation procedures. A study was carried out by using LC–MS for the quantification of 27 metabolites, including glycolysis and tricarboxylic acid (TCA), in addition to the quantification of approximately 700 unknown features. A new quenching approach was employed in this study, liquid nitrogen was added to cell culture to halt metabolism, and the sample was stored at −80°C for at least 7 days prior to extraction. Additionally, using methanol:chloroform (9:1) as a solvent extraction, better recovery and stability of the extract were achieved than that obtained using acetonitrile, ethanol, and methanol [41].

The effect of freezing delay time on the metabolome of cancer tissue was also examined by using high-resolution magic angle spinning NMR spectroscopy. The study revealed that the samples were stable within the first 30 min. However, over time, an increase in the level of choline was observed, while levels of the ascorbate, glutathione, and creatine were reduced. Moreover, the levels of glycine, glucose, and choline were affected by increasing the experiment time [21].

3 | ANALYTICAL PLATFORMS OF METABOLOMICS

Different kinds of endogenous metabolites have different polarities and physicochemical properties. A single analytical platform cannot detect the alterations in these metabolites at the same time [42]. Hence, the combination of different analytical platforms is a necessity to achieve meaningful results.

3.1 | NMR-based metabolomics

NMR spectroscopy is one of the earliest platforms applied in metabolomics studies and has the advantages of nondestructive, highly reproducible (>98%), simple sample pretreatment (high-resolution magic-angle spinning NMR spectroscopy does not need any pretreatment), and very few matrix effects. [43–45]. ^{1}H NMR spectroscopy has long been the most common tool used in NMR-based metabolomics, but ^{13}C NMR spectroscopy has been increasingly applied in metabolomics studies and is considered a powerful tool for the structural elucidation of metabolites because it can provide backbone structure information for metabolites [46]. Moreover, NMR spectroscopy could provide a good quantification property for compounds existing in complex mixtures due to the direct relationship between the peak area and the concentration of specific nuclei [47]. However, the major drawback of NMR spectroscopy is its relatively low sensitivity compared with MS, although the increase in magnetic field strengths and low-volume microprobes can be used to enhance the sensitivity of NMR spectroscopy [48–50].

One-dimensional NMR spectroscopy has been frequently used for studying the difference between lung cancer patients and healthy controls, as well as the difference in different lung cancer subtypes. Carrola et al. reported that the levels of trigonelline and hippurate decreased, while the levels of *N*-acetylglutamine, *R*-hydroxyisobutyrate, and creatinine increased in urine samples [51]. Plasma analysis demonstrated that pyruvate, lactate, higher VLDL + LDL, and lower HDL were elevated, whereas the levels of glucose, citrate, acetate, formate, methanol, histidine, glutamine, tyrosine, and alanine were reduced [52]. Rocha et al. reported the metabolic difference between tumor and matched control tissues as well as the difference between squamous cell carcinomas (SCC) and AC by using high-resolution magic angle spinning (HRMAS) NMR spectroscopy. The results revealed that 13 metabolites (amino acids, organic acids, glucose, and peptide) had significant differences between tumor tissues and matched controls, and a multivariate model based on these 13 metabolites allowed for good discrimination between tumors from control tissue with an accuracy rate of approximately 97%. Moreover, this study also demonstrated that major metabolic disturbances in AdC were related to phospholipid metabolism and protein catabolism, while in SqCC, the disturbances were in glycolysis and the metabolism of some amino acids [53]. Moreover, Deja et al. reported that isoleucine, acetoacetate, creatine, *N*-acetylated glycoproteins, and glycerol had distinct differences between advanced stage (III–IV) and early-stage (I–II) of NSCLC, and can be used as biomarkers for early diagnosis [35]. Stable isotope-resolved metabolomic analysis indicated that activation of pyruvate carboxylation may be critical for refilling intermediates in the TCA cycle, which can be used for lipid, nucleic acid, and protein biosynthesis to adapt the high demands for lung tumor growth [54].

Although 1-D ^{1}H-NMR spectroscopy is frequently used in lung cancer metabolomics, there are many overlappings in this spectrum due to a small spectral width and a narrow chemical shift range. Thus, this causes many difficulties in the metabolite identification. While 1-D carbon NMR spectroscopy can overcome the problem of small spectral width because it has a wide spectral dispersion, unfortunately, it is less sensitive and the natural abundance of ^{13}C nuclei is very rare [55]. The problem of overlapping in spectrum can be solved by using 2D-NMR spectroscopy, which improves signal dispersion and elucidates the signal connectivity; it can offer higher resolution detection of metabolites than 1D-NMR spectroscopy [56,57]. The latter has been applied to investigate the difference between lung cancer tissue and its surrounding noncancerous tissue. The results showed the upregulation of some primary metabolites related to glycolysis and

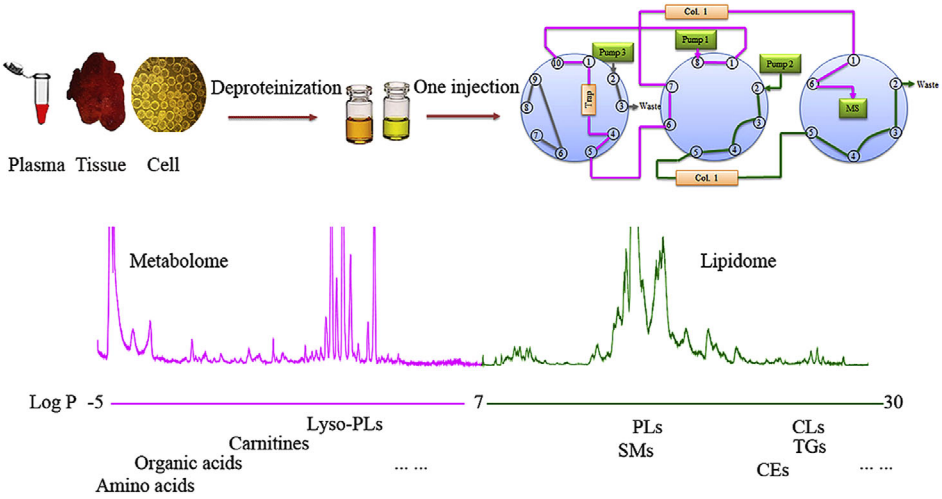

FIGURE 1 Analysis of metabolomics and lipidomics by using 2-D LC–MS. Reproduced from Ref. 58 with permission

the TCA cycle, such as lactate, alanine, succinate, glutamate, aspartate, and citrate [54]. 2D-NMR spectroscopy can offer higher resolution than 1D-NMR spectroscopy, as shown in Supporting Information Figure S1 [54], and provide useful information on the chemical structure of compounds that can be used for metabolite identification [56]. The longer time consumed for both experimental and data acquisition is the common challenge in 2D-NMR spectroscopy compared to 1D-NMR spectroscopy [4].

3.2 | LC–MS

With the development of MS techniques, LC combined with MS has become a pillar for metabolomics studies due to its high resolution and high sensitivity. The combination of LC and MS markedly reduced ion suppression and provided good chromatographic separation. Currently, the use of UHPLC–MS improves column efficiency, peak resolution, and sensitivity compared to LC–MS [39]. Compared with NMR spectroscopy, LC–MS has larger metabolite coverage due to its diverse types of chromatographic columns, ionization modes, MS detectors, etc. To measure metabolites with weak polarity or/and non-polarity, RP columns, such as C_8 and C_{18}, are commonly used. For polar metabolites, a HILIC column will be a better choice. Currently, RPLC–MS is more widely used in metabolomics studies due to its good reproducibility and stability. However, to analyze complex samples, 2-D LC, which consists of HILIC and RPLC columns, has been applied to obtain higher peak capacity and better resolution (Figure 1) [58].

The ionization method should be properly selected according to the characteristics of specific metabolites to be detected. For instance, atmospheric pressure chemical ionization is much more suitable for cholesterol when compared with ESI mode [4]. However, ion suppression from matrix is another disadvantage of LC–MS ionization, which can be relieved by nanospray ionization.

At present, LC–MS-based metabolomic studies of lung cancer have mainly focused on metabolic changes in lung cancer patients, biomarker discovery, and mechanism exploration. A previous study reported that free amino acids (serine, proline, isoleucine, ornithine, asparagine, glutamine, citrulline, methionine, leucine, histidine, and tryptophan), free fatty acids, fatty acid amide, acyl-carnitines, lysophosphatidylcholines (LPCs), and phospholipids exhibited notable differences in lung cancer patients [33,59,60]. Calderon-Santiago et al. reported significant differences in trisaccharide phosphate, suberic acid, tetrahexose, trihexose, nonanedioic acid, and the monoglyceride (22:2) between lung cancer patients and smokers/high-risk factor individuals [61].

Metabolite biomarkers for distinguishing benign from malignant patients, as well as for lung cancer early diagnosis and early warning were performed by LC–MS. Gao et al. reported that *N*-succinyl-2,6-diaminopimelate, deoxycholic acid glycine conjugate, octanoylcarnitine, LPCs, lysophosphatidylethanolamine (20:2), phosphatidylcholine (PC) (34:1), phosphatidylserines, and cholesteryl acetate can be used as a combinational candidate biomarker to distinguish benign from malignant patients. These metabolites also indicated disturbances in amino acid metabolism, lipid metabolism, and cholesterol metabolism in plasma from malignant patients [62]. Mathé et al. reported a biomarker for lung cancer early detection. In this study, the urinary metabolite profiling of 1000 cases of healthy control and lung cancer was performed by a nontargeted metabolomics method. The results demonstrated that creatine riboside and *N*-acetylneuraminic acid were significantly increased in NSCLC, and these molecules were found to be associated with poor prognosis. Furthermore, creatine riboside could also serve as a classifier biomarker for early lung cancer detection and showed a close relationship with poor

prognosis in stage I–II lung cancer [63]. Xiang et al. analyzed metabolic changes in the plasma of lung cancer patients by using UHPLC–MS technology. The results showed that 28 metabolites had significant differences when NSCLC patients were compared with healthy controls. Cortisol, cortisone, and 4-methoxyphenylacetic acid can be used as potential plasma biomarkers for the early detection of non-small cell lung cancer with good specificity and sensitivity [64].

Furthermore, the hydrophilic interaction method coupled with MS was used to determine the prediagnostic biomarker for NSCLC. Serum samples were collected from a prospective cohort of high-risk patients from the beta-carotene and retinol efficacy trial. This study demonstrated that diacetylspermine can be used as a novel prediagnostic biomarker for NSCLC and has additive performance when combined with prosurfactant protein B, a marker identified from a proteomics study [65].

The LC–MS-based metabolomics method combined with biological approaches could be a powerful tool for mechanism exploration. A study from Zhang et al., which integrated approaches of metabolomics, biochemistry and genetics, demonstrated that phosphoglycerate dehydrogenase defines a metabolic subtype in lung adenocarcinomas with poor prognosis. In particular, serine is utilized differentially in lung adenocarcinomas, and serine metabolism is linked to DNA stability in the phosphoglycerate dehydrogenase-high subtype [66]. Faubert et al. found that metabolic enzyme glycine decarboxylase could induce impressive changes in glycine/serine metabolism, glycolysis, and pyrimidine metabolism and then regulate the proliferation of cancer cells and drive NSCLC tumorigenesis [67].

Alterations in lipid and fatty acid metabolism have also been observed in cancer and lung cancer. Fatty acids and lipids are required for membrane biosynthesis and act as signaling molecules, which may explain the shifting of these metabolites toward de novo fatty acid synthesis [4]. Therefore, the limitation in fatty acid bioavailability can suppress cancer cell proliferation [68]. Supporting Information Table S2 shows several examples of fatty acid and lipid metabolism alterations in lung tumor tissue compared with nontumor tissue. Many lipid derivatives increase in lung cancer. A study carried out to understand the role of LPCs in lung cancer revealed alterations in five LPC metabolites with different fatty acyl positions, including sn-1 lyso16:0, sn-2 LPC 16:0, sn-1 LPC 18:0, sn-1 LPC 18:1, and sn-1 LPC 18:2 [69]. However, sphingosine shows a decrease, and this metabolite is important for proliferation and apoptosis through pathways involving ceramidase and sphingosine kinase [33].

3.3 | GC–MS

Gas chromatography is mainly used for the separation and analysis of volatile analytes [70]. The advantage of this method is that the column can be easily designed to separate specific analytes of interest. But the major disadvantage is this technique is that it is limited on the volatile and thermally stable compounds. Thermolabile metabolites, such as ATP, NADPH, and arginine, are not suitably separated by this technique due to possible decomposition by heating after injection [56,71]. Chemical derivatization can be introduced to help in the analysis of nonvolatile and thermally unstable compounds [72], especially for those with one or several polar functional groups (hydroxyl, carboxyl or amino functional groups, etc.) [73,74]. However, for some metabolites such as amino acids, their derivatized products are not very stable and easy to degrade during injection and separation, in this case the internal quality markers can be added for monitoring the performance [75]. *N*,*O*-Bis(trimethylsilyl)trifluoroacetamide and *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide have been extensively used in metabolomics for silylation reactions [76]. *N*-methyl-*N*-(tert-butyldimethylsilyl)trifluoroacetamide with a tert-butyldimethylsilyl group exhibits the unique fragmentation of [M-57] due to the loss of a tert-butyl group, which is useful for the identification of unknown metabolites [77,78]. Figure 2 shows the general protocol for using GC–MS in metabolomics.

GC–MS has been used for the analysis of volatile organic compounds (VOCs) in lung cancer patients. Poli et al. quantified 13 VOCs (seven aliphatic and six aromatic compounds) in the exhaled air of NSCLC patients, healthy controls, and chronic obstructive pulmonary disease patients, and the combination of 13 VOCs allowed the classification of different groups [79]. Caprolactam, propanoic acid, hexanal, and heptanal were regarded as biomarkers of lung cancer. Additionally, VOCs in breath originating from blood could be a feasible tool for screening noninvasive biomarkers of lung cancer [80,81].

GC–MS analysis has advantages in revealing metabolic disturbances in the TCA cycle, glycolysis, organic acids, amino acids, and sugars. The results demonstrated that the levels of ethanolamine, glutamic acid, maltose, glycerol, and lactic acid were increased in lung cancer, while some amino acids, such as tryptophan, lysine, and histidine, were decreased. These studies confirmed that alterations in endogenous metabolites were useful for declaring the characteristics of lung cancer at the metabolic level and establishing novel diagnostic tools based on GC–MS [11,82,83]. Wikoff et al. studied the variations between the early-stage of adenocarcinoma and normal lung tissues and found fluctuations in some metabolites involved in nucleotide biosynthesis, such as 5,6-dihydrouracil 5′-deoxy-5′-methylthioadenosine, methylthioadenosine, and xanthine. This study also showed different metabolic disorders associated with early-stage lung adenocarcinoma, which may contribute to molecular targets for personalizing therapeutic and treatment efficacy monitoring [84].

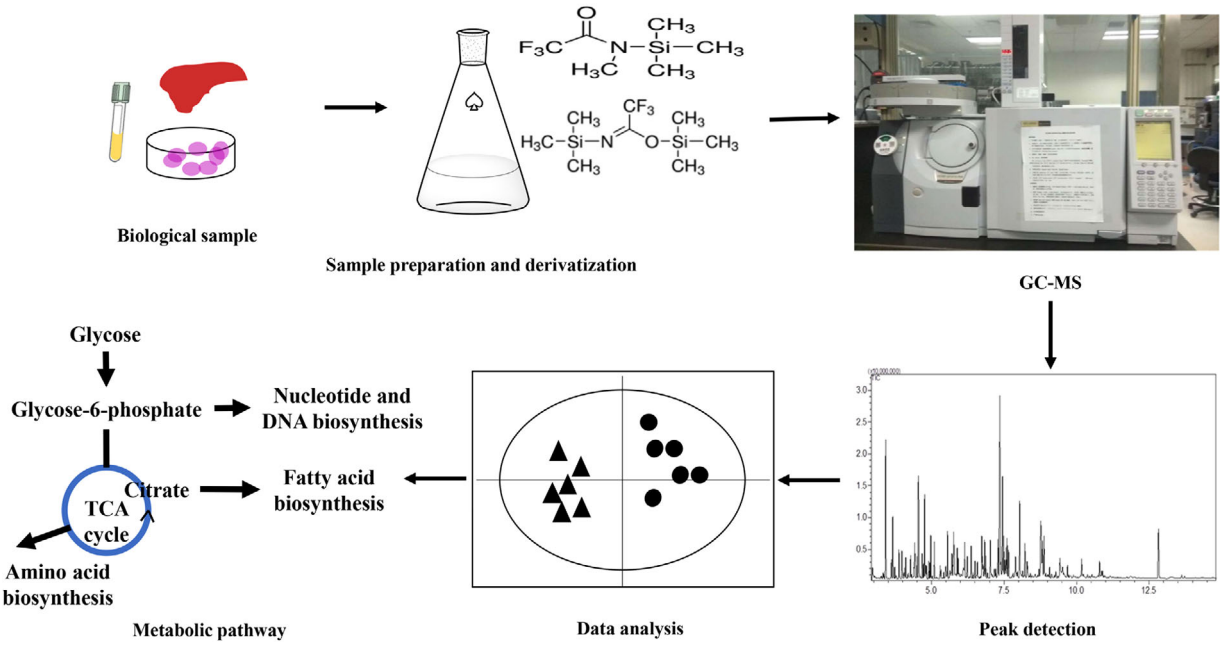

FIGURE 2 General protocol for GC–MS-based metabolomics, including the different derivatization and injection methods

3.4 | CE–MS

CE–MS is not a pillar technique in metabolomics. However, this technique has been widely used for the analysis of charged (ionic) and highly polar compounds, such as nucleic acids, amino acids, carboxylic acids, and sugar phosphates. The separation in CE depends on the migration of charged compounds forced by an electric field between the two electrodes [9,85]. Although it has high separation efficiency, the main drawbacks are the less sensitivity and higher variability in the migration time of CE–MS than those of GC–MS and LC–MS [75,86].

Kami et al. revealed that the significant difference between lung cancer tissue and normal tissue from the same patient was related to the amino acid pool, glycolysis, the TCA cycle, and energy production. The levels of related enzymes, such as phosphofructokinase and pyruvate kinase, were also found to be upregulated. This study provided an understanding of tissue-specific tumor microenvironments, which may be used to guide the improvement of targeted cancer therapy [40].

3.5 | MALDI-MS

MALDI-MS enables the rapid profiling of different biomolecular species from biofluids and tissues. The major advantage of MALDI over other types of ionization (e.g. ESI) is that MALDI can detect the analytes as singly charged ion at increased mass/charge ratios, which make the result easy to interpret, especially for higher molecular weight protonated molecules. While in metabolomics, the result will be complicated due to the presence of matrix ions in the low mass range (<500 Da). Nowadays there are still many studies performed by using MALDI, especially for lipidomics analysis [87,88].

MALDI-MS has also been used for lung cancer metabolomics and proteomics studies [88–90]. Lee at al. investigated whether direct tissue MALDI-MS analysis on lipid may assist with the histopathologic diagnosis of NSCLCs. The results demonstrated that MALDI-MS based lipid profile was able to distinguish lung cancer tissue from adjacent normal tissue with excellent accuracy of 92.9%, and several phospholipids including PC (34:1) presented increase in lung cancer. Additionally, SCC and AC were found to have different lipid profiles, and PC (32:0) $[M+Na]^+$ (m/z 756.68) showed increase in adenocarcinomas [88].

4 | DISTURBED METABOLISM IN LUNG CANCER

Previous studies have shown that many metabolic disturbances occur in lung cancer patients. This information is helpful for defining potential biomarkers. Supporting Information Table S2 lists the many potential biomarkers discovered for lung cancer. Figure 3 shows the main metabolic pathways in lung cancer, including the tricarboxylic acid cycle, glycolysis, fatty acid metabolism, and amino acid metabolism [36]. The following paragraphs summarize the findings in the tricarboxylic acid cycle and glycolysis, lipid metabolism, and amino acid metabolism.

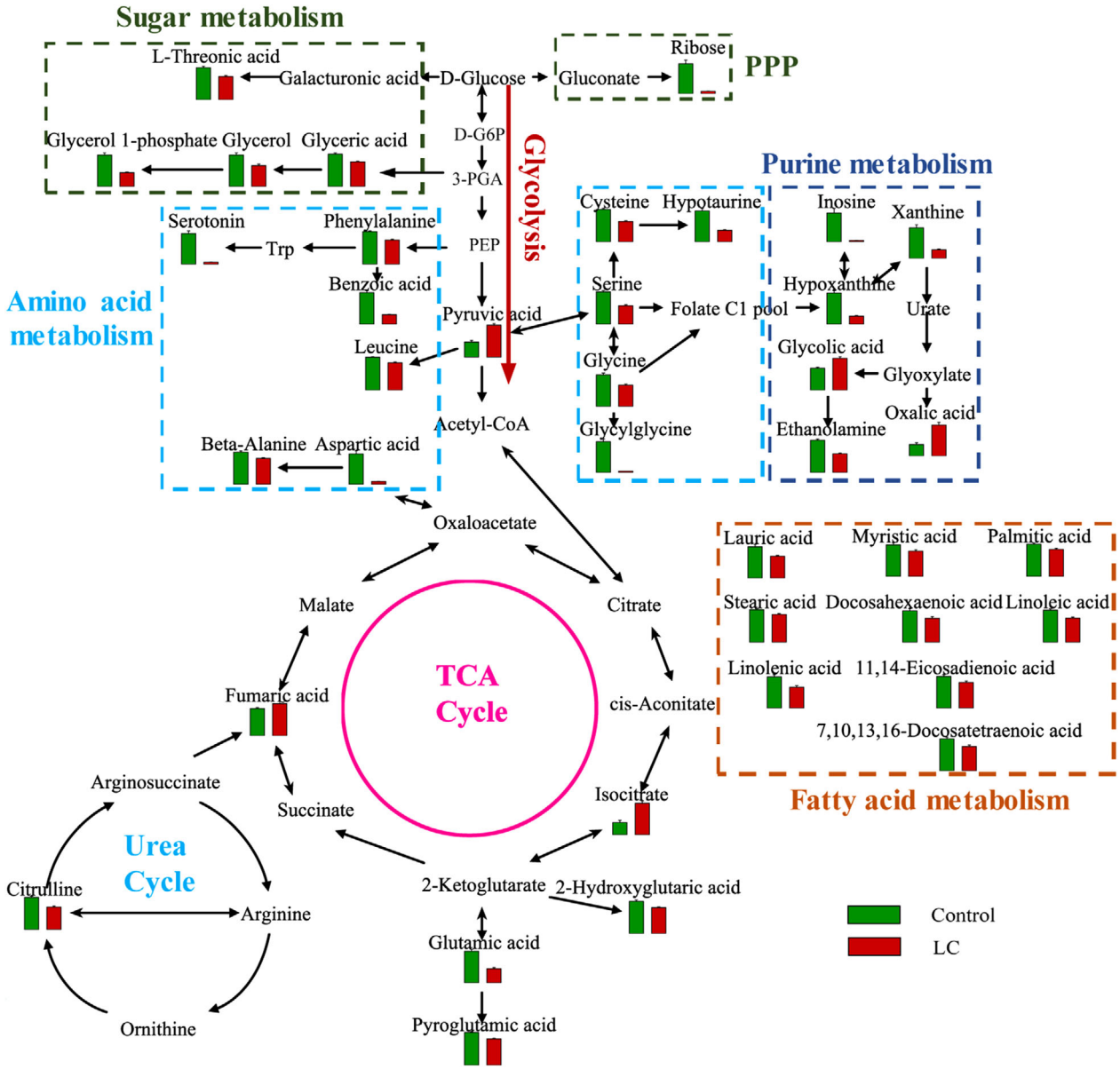

FIGURE 3 Map of the pathways of significantly different metabolites. Reproduced from Ref. 36 with permission

4.1 | TCA cycle and glycolysis

The TCA cycle and glycolysis are central pathways that provide energy and essential metabolic substrates for cell biosynthesis and maintain redox balance in cells. Dysfunction of the TCA cycle is involved in a wide variety of cancer diseases. Fan et al. found that ^{13}C enrichment in lactate, succinate, and citrate was higher in lung tumors, indicating that glycolysis and the TCA cycle were significantly changed in lung tumor tissues [54]. Davidson et al. performed metabolic flux analysis by using stable isotope tracing of [1,2-^{13}C] glucose and [U-^{13}C] glutamine to investigate whether glucose converts to lactate, and glutamine is the major source of the TCA cycle carbon in cancer tissue as found in cultured cell. The result revealed that glucose converts to lactate and its contribution to the TCA cycle increased in lung cancer. Thus, glucose is required for cancer formation. However, in the case of glutamine, its utilization was insignificant in both sides. This study declares that nutrient and environment are very important for the determination of the metabolic phenotype of cancer cells [91]. Furthermore, Yang et al. reported that lung cancer cells have higher glycolysis; in particular, the activity of pyruvate carboxylase was upregulated in lung cancer [92]. The quantification of glycolytic metabolites revealed that the overexpression of pyruvate kinase isoform (M2) leads to an accumulation of glycolytic intermediates that are subsequently incorporated into the serine metabolism pathway. Thus, cancer cells offer higher concentrations of phosphoenolpyruvate and 3-phosphoglycerate [93,94]. Furthermore,

new evidence on human non-small cell lung cancers (NSCLC) suggested that lactate can be converted to pyruvate then to the TCA cycle; thus, lactate can be used as a fuel and carbon source in vivo [95].

4.2 | Amino acid metabolism

Fluctuations in amino acid concentrations and their biosynthetic pathways are needed for tumor growth and proliferation [4]. An analysis of the metabolome and proteome revealed that glycosylation, glutaminolysis, and polyamine biosynthesis were increased in lung adenocarcinoma [96]. According to the existing literature, amino acids and their derivatives could serve as candidate biomarkers and these molecules are closely related to disease occurrence. Moreover, cysteine and glutamic acid are important components of glutathione and are significantly increased in lung adenocarcinoma compared to control tissue. Many enzymes related to glutathione biosynthesis, glutathione recycling, heterologous biological metabolism, and redox balance have increased significantly [96]. Furthermore, the serum levels of histidine and threonine were decreased in lung cancer patients due to increased utilization in the glycine/serine/threonine and pyrimidine pathways in lung tumors [67,97].

4.3 | Lipid metabolism

Lipids include many different types of molecule classes and have important biological functions in energy production and cell membrane composition and act as signaling molecules [4]. Disrupted lipid metabolism contributed to the occurrence and development of many diseases [88,98]. Abnormal lipid metabolism has been demonstrated in lung cancer. A global lipidomics study was performed on early NSCLC, adenocarcinoma, and SCC patients and healthy controls. The results suggested that some lipid species, including sphingomyelin, lysophosphatidylcholine (LPC), and fatty acid derivatives, were prominently changed and were associated with lung cancer progression [99,100]. Moreover, decreased choline levels in serum were observed in lung cancer and were speculated to be associated with the increased requirements of cancer cells for proliferation due to its role as a building block for membrane phospholipids [97]. Additionally, the lung cancer pathway analysis showed that the major alteration was in sphingolipid metabolism, and the ROC curve analysis showed that glycerophospho-narachidonoyl ethanolamine and sphingosine could be regarded as potential biomarkers for diagnosis and prognosis in lung cancer. Additionally, glycerophospho-narachidonoyl ethanolamine and sphingosine displayed more convenience for the detection of lung cancer [34].

Alterations in the distribution of phospholipids have been shown to decline with cancer progression [101], meaning that early alterations in lipid metabolism accompany carcinogenesis and may lead to a change in post transformation [102]. NSCLC tumors showed dramatic alterations in lipid profiles, including increases in phosphatidylethanolamines (PEs) compared to healthy control tissue [103,104]. Additionally, the elevation of PEs was shown in plasma of malignant nodules [62]. Moreover, the secretion of phosphatidylethanolamine-binding protein increased in lung adenocarcinoma [105]. It was interesting to note that phosphatidylethanolamine-binding protein was an important factor in the development and metastasis of cancer [106,107]. PEs were found to be promising biomarkers for differentiation between benign and lung cancer samples [102].

Phospholipase A2 catalyzes oxidized phospholipids to form lysophospholipid platelet-activating factor (e.g. lysophosphatidylcholines) and free fatty acids [108–110], which play a central role in cancer development, proliferation, angiogenesis, and metastasis [111–113]. Notably, the levels of phospholipase A2 and PAF were increased in lung cancer patients [114]. A previous study revealed that the levels of arachidonic acid, linoleic acid, and their eicosanoid derivatives in NSCLC adenocarcinoma were greater in lung cancer patients than in healthy controls [110]. Additionally, other studies have shown that free fatty acids and the oxidized derivative lead to lung cancer development, progression, and metastases in cell culture [115], tissues [116], and animal models [115]. For instance, 5-HETE and 12-HETE increased survival and improved adhesion lung cancer, respectively. Furthermore, 15-HETE induced lung cancer metastasis and angiogenesis. Moreover, 9-HODE and 13-HODE played an important role in receptor activation, which promotes lung cancer development and metastasis [110]. Furthermore, the fatty acids coming from phospholipid species of erythrocytes can be used as biomarkers in diagnosis and management of lung cancer [117]. Polyunsaturated fatty acids are an important target associated with alterations in blood cells in malignant diseases, including lung cancer [118–121].

5 | CONCLUDING REMARKS

Metabolomics plays an increasingly important role in biomarker discovery and mechanism interpretation of lung cancer. Disrupted metabolism in lung cancer will be helpful for mechanism exploration at the metabolic level and provide a rich basis for biomarker screening, especially for early diagnosis and classifying different stages of lung cancer. Improvement should be made mainly in analytical methods and platforms, as well as the statistical analysis of metabolomics data for efficiently screening biomarkers with high sensitivity and specificity. Furthermore, a sufficient sample size in the discovery phase and a large-scale sample set in the validation phase are needed. The discovery and validation phases should include lung cancer subtypes and other related

diseases. Although the application of metabolic biomarkers in clinical hospitals is still far away, metabolomics is a promising tool for disease biomarker discovery and mechanism exploration at the metabolic level.

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CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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