Direct infusion mass spectrometry or liquid chromatography mass spectrometry for human metabonomics? A serum metabonomic study of kidney cancer

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Serum samples from kidney cancer patients and healthy controls were analyzed by both direct infusion mass spectrometry (DIMS) and liquid chromatography-mass spectrometry (LC-MS) with a high resolution ESI-Q-TOFMS. The classification and biomarker discovery capacities of the two methods were compared, and MS/MS experiments were carried out to identify potential biomarkers. DIMS had comparable classification and prediction capabilities to LC-MS but consumed only ~5% of the analysis time. With regard to biomarker discovery, twenty-three variables were found as potential biomarkers by DIMS, and 48 variables were obtained by LC-MS. DIMS is recommended to be a fast diagnostic method for kidney cancer, while LC-MS is necessary when comprehensive screening of biomarkers is required.

Introduction

Metabonomics has been defined by Nicholson as the quantitative measurement of the dynamic multiparametric response of a living system to pathophysiological stimuli or genetic modification.[1] It concerns low molecular weight compounds in biofluids and other complex matrixes, which are known as metabolites.[2,3] Many analytical techniques have been developed for metabonomic studies, including nuclear magnetic resonance spectroscopy (NMR), mass spectrometry (MS), fourier transform infrared spectroscopy (FT-IR), and Raman spectroscopy.[4–7] With the advantages of high sensitivity, high accuracy, wide dynamic range, robustness to molecular weight (MW) determination, and the ability to identify metabolites, mass spectrometry has become the workhorse of metabonomics research in recent years.[8–11]

There is a trade-off between comprehensive sample analysis and high sample throughput in MS-based metabonomics. Currently, liquid chromatography coupled to MS (LC-MS) is a reference tool for metabonomics analysis.[12,13] Although being beneficial to comprehensive analysis, the chromatographic step limits the throughput, especially when the number of sample sets is large.[14] Furthermore, the pretreatment of LC-MS data (peak alignment and retention time correction) before chemometrics analysis is problematic because of the possible loss of some relevant analytical signals or the generation of artifacts by erratic retention time shift correction or background subtraction.[15] On the contrary, direct infusion mass spectrometry (DIMS), by avoiding any prior chromatographic steps, has the greatest potential for high throughput and provides more concise raw data than LC-MS. DIMS has been used in targeted metabolite analysis and global metabolite profiling in the last decade.[8,16] A method using DIMS for the analysis of 23 amino acids in dry blood spots without chemical derivatization has been developed.[17] The quantitative analyses of metabolites by DIMS have been validated and were used in large newborn-screening programs to detect and diagnose inherited metabolic disorders in the neonatal period.[18,19] In general, DIMS applications for metabolite profiling are mainly concentrated in the microbial and plant arenas.[16,20] DIMS has been used for the global analysis of intracellular metabolites in different strains of the yeast S. cerevisiae.[21] A nontargeted metabolic analysis has been performed on strawberry fruit and tobacco flower extracts with direct infusion Fourier transform ion cyclotron resonance mass spectrometry (FTICR-MS).[22] Viant *et al.*[23] reported an optimized strategy for wide-scan direct infusion nanoelectrospray FTICR-MS. This method collected multiple adjacent selected ion monitoring (SIM) windows that were stitched together using novel algorithms. Using the SIM-stitching approach, an increase in the dynamic range and mass accuracy was achieved for metabonomic studies. Although DIMS has been explored in metabonomic analysis with low mass resolution analyzers, including quadrupole and ion-trap,[21,24,25] high mass resolution devices such as time of flight (TOF) mass spectrometers and FTICR-MS are more suited for such an approach, especially for global metabonomics analysis.[23,26–28] Mass spectrometry with high resolving power unambiguously discriminates isobaric ions; elemental compositions of low molecular mass analytes can be obtained by accurate mass measurements with ppm errors in mass spectra. Regardless of what devices used in DIMS, matrix effects are inevitable because the samples are infused together without separation, which may result in reduced sensitivity and deteriorated capability for metabolite identification.[29,30] Another limitation of DIMS is its inability to discriminate between isomeric compounds based solely on accurate mass.[31] Thus, a practical comparison of DIMS and LC-MS would be both interesting and useful. However, few studies have compared the two methods in real metabonomic analysis, especially using the same mass spectrometer.

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In this study, serum samples from kidney cancer patients and healthy controls were used as models to check the performance of LC-MS and DIMS with a high resolution ESI-Q-TOFMS. The resulting data were analyzed by multivariate data analysis. MS/MS experiments were carried out to identify potential biomarkers. The classification and biomarker discovery capacities of the two methods were compared.

Experimental

Reagents and materials

HPLC-grade methanol and acetonitrile were purchased from Merck (Darmstadt, Germany). Formic acid was obtained from Fluka (Switzerland). Distilled water (18.2 MΩ) was prepared using a Milli-Q water purification system (Millipore, MA, USA). All standards were purchased from Sigma-Aldrich (MO, USA).

Sample collection and storage

Thirty-one kidney cancer patients and 20 healthy volunteers from First Hospital of Xiamen were enrolled in this study. All of the patients were diagnosed using a histopathology examination and none had received chemotherapy, radiation or undergone nephrectomy before sample collection. The detailed demographic profiles of the participants are provided in Table 1. The *T* test was carried out to check whether the age and body mass index (BMI) distributions differed significantly between the two classes. The statistical test showed that the two classes were well matched. All of the blood samples were collected before breakfast with consent and then centrifuged at 3,000 × *g* for 10 min at 4 °C. The sera were stored at −80 °C prior to any further sample preparation or analysis. An in-house quality control (QC) sample was prepared by pooling and mixing the same volume of each sample.[32]

Sample preparation

The sera were thawed at room temperature before analysis. A volume of 600 μL of methanol was added to 200 μL of sera. After vortexing, the mixture was set aside at room temperature for 10 min and then centrifuged at 12,000 × *g* for 10 min at 4 °C. The supernatant was filtered through a 0.2-μm regenerated cellulose filter (Agilent Technologies, Inc., Boeblingen, Germany) before LC-MS analysis. For DIMS analysis, the filtered supernatant was further diluted 5-fold with 0.1% formic acid. To choose the most appropriate dilution, the same samples with different dilutions (2-fold, 5-fold, 10-fold, and 20-fold) were infused into the mass spectrometer for a pilot study. Five-fold dilution was ultimately selected because the maximum number of peaks (S/N > 5) were detected with this dilution, indicating the best overall response (low ion suppression and high sensitivity).

Table 1 Demographic and clinical data

	Kidney cancer patients	Healthy controls	*p* valve[a]
Number	31	20	—
Age (median, range)	56, 40–72	52, 43–71	0.15
Male/female ratio	19/12	12/8	—
BMI (median, range)	22.1, 16.4–27.6	23.4, 18.1–27.4	0.19
Race	Chinese	Chinese	—

[a] *p* values were calculated from student *T*-test.

Mass spectrometer

A high resolution electrospray mass spectrometer (MicrOTOF-Q II, Bruker Daltonics Corporation, USA) was operated in positive ion mode for both LC-MS and DIMS analysis. This mass spectrometer can provide accurate mass measurements with errors of less than 5 ppm using external calibration and mass resolving power of 20,000. The positive ion mode was used because more compounds can be ionized in this mode and because it is more widely used in serum metabolite profiling.[33,34] The capillary voltage was set at −4500 V with an end plate offset potential of −500 V. Data were collected from 50 to 1000 *m*/*z* with an acquisition rate of 1 spectrum per second. Because the sample flow rate differed between the two methods, the nebulizer gas and dry gas parameters were individually optimized. For LC-MS analysis, the dry gas was set to 6 L min^{-1} at 220 °C with a nebulization gas pressure of 0.7 bar, while for DIMS, the dry gas was set to 1.2 L min^{-1} at 120 °C with a nebulization gas pressure of 0.4 bar. In the MS/MS experiments, argon was used as the collision gas and the collision energy was adjustable from 10 eV to 30 eV.

LC-MS analysis

LC separation was performed on a 2.1 × 150-mm Acclaim C18 3-μm column (Dionex, USA) using a high performance liquid chromatography system (Ultimate-3000, Dionex, USA). The column was maintained at 30 °C and the mobile phase was 0.1% formic acid (A) and acetonitrile with 0.1% formic acid (B). The gradient started with 15% (B) for 2 min, and increased to 40% (B) over 7 min, and then 98% (B) over 7–30 min. After holding at 98% (B) for 5 min, the composition was returned to its initial conditions and maintained for three different column volumes for equilibration. The sample injection volume was 15 μL. The chromatograph was coupled directly to the mass spectrometer at a flow rate of 200 μL min^{-1} without splitting. A QC sample and a blank were analyzed after every 5 samples to identify the sample carryover and check for stability.

DIMS analysis

A syringe pump (Razel, Connecticut, USA) was used at a flow rate of 3 μL min^{-1} to infuse samples directly into the mass spectrometer. The infusion time was 30 s for each sample. Including the manual operation time, the whole analysis time was about 2 min for each sample. A longer infusion time was not used because the signal was sufficiently stable within 30 s. Longer infusion time does not significantly improve the S/N or the number of detected features. To avoid the cross-contamination, a blank run was inserted between sample runs. A QC sample was also used after every 5 samples to check for reproducibility.

Data pretreatment

The raw data acquired from LC-MS were pretreated by the DataAnalysis 4.0 software (Bruker Daltonics Corporation) to find compounds with molecular features. Next, the data were exported into the ProfileAnalysis 1.1 software (Bruker), which allowed peak alignment, background noise subtraction and data reduction, yielding a table of mass and retention time pairs with associated intensities for all detected peaks. The main parameters were set as follows: retention time range 1–40 min, mass range 50–1000, mass window 0.5, retention time window 1 min, and noise elimination level 5. Variables that did not exist in 80% of participants in one group were filtered out. For DIMS, the data pretreatment is much easier because there is no need to take account of retention time. ProfileAnalysis 1.1 software was also used to convert the mass spectra into a variable table that displayed mass and associated intensities as columns for all samples. The DIMS main parameters were set as follows: mass range 50–1000, mass window 0.5, and noise elimination level 5. Variables were also filtered as mentioned above.

Chemometrics analysis

The data were exported to the SIMCA-P 11.5 demo version (Umetrics AB, Umeå, Sweden) for multivariate data analysis. Both principle component analysis (PCA) and partial least squares-discriminant analysis (PLS-DA) were used for modeling the differences between the kidney cancer patients and the healthy controls. PCA is an unsupervised data analysis technique that reduces original data to a few principal components while retaining the features that contribute most to the variance. PLS-DA, in contrast, is a supervised extension of PCA that uses class information to maximize the separation among classes of observations. Pareto (Par) scaling was used in all models to avoid chemical noise.

Results and discussion

Metabonomic profile of LC-MS and DIMS

Typical LC-MS base peak chromatograms (BPC) of serum samples from a cancer patient and a healthy control are shown in Fig. 1. Several base peaks are marked on the chromatogram to provide an intuitive display. Fig. 2 presents the typical metabolic fingerprints of the same samples by DIMS analysis. About 2000 peaks were detected in the mass spectrum, with intensities ranging from 2×10^2 to 1×10^6 (arbitrary units) and S/N larger than 5. Obvious differences can be observed between the fingerprints of a cancer patient and a healthy control using DIMS analysis.

To give an overview of the differences between LC-MS and DIMS in serum metabonomic profiles, a summed mass spectrum from the LC-MS analysis is presented in Fig. 3(a). The ion of *m*/*z* 149.01 was a major solvent peak in LC-MS analysis. Comparing the main peaks in the mass spectra, most of the ions in the DIMS mass spectrum have a *m*/*z* difference of 21.98 compared to those of LC-MS, corresponding to the mass of [Na–H]. Thus, the compounds were apt to form sodium ion adducts in the DIMS analysis. It is worth mentioning that because the summed mass spectrum of the LC-MS is the summation of all the ions during the entire analysis time, the signal of low abundance ions can be overlaid by the summed background. Therefore, direct comparison of the number of ions between the spectra is not meaningful.

The stability of the analytical system is one of the most important factors in obtaining valid data for metabonomic

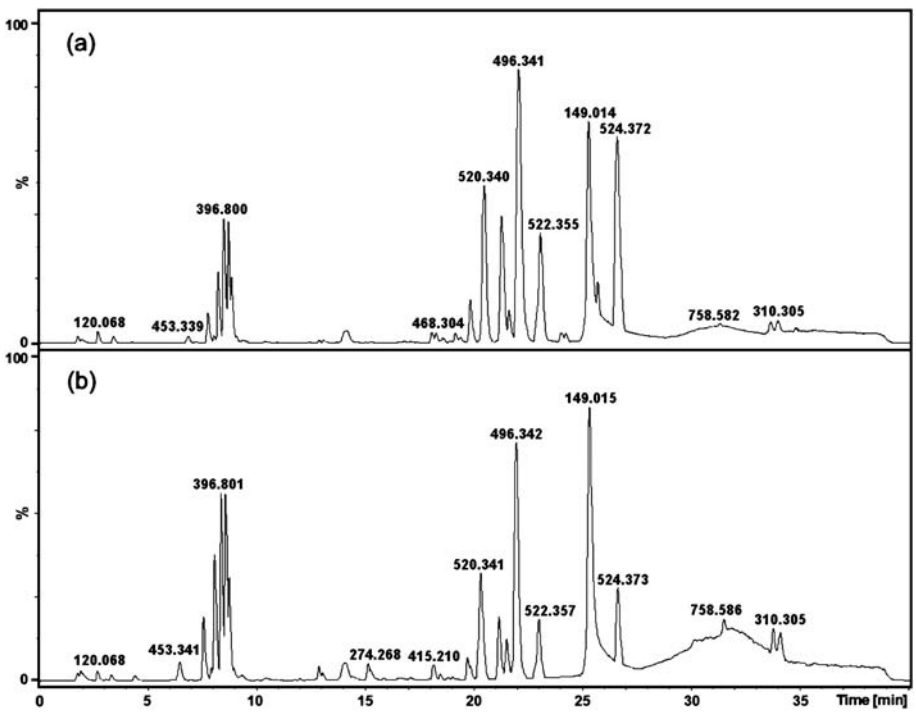

Fig. 1 Typical LC-MS base peak chromatograms (BPC) obtained from serum samples of (a) a healthy control and (b) a kidney cancer patient.

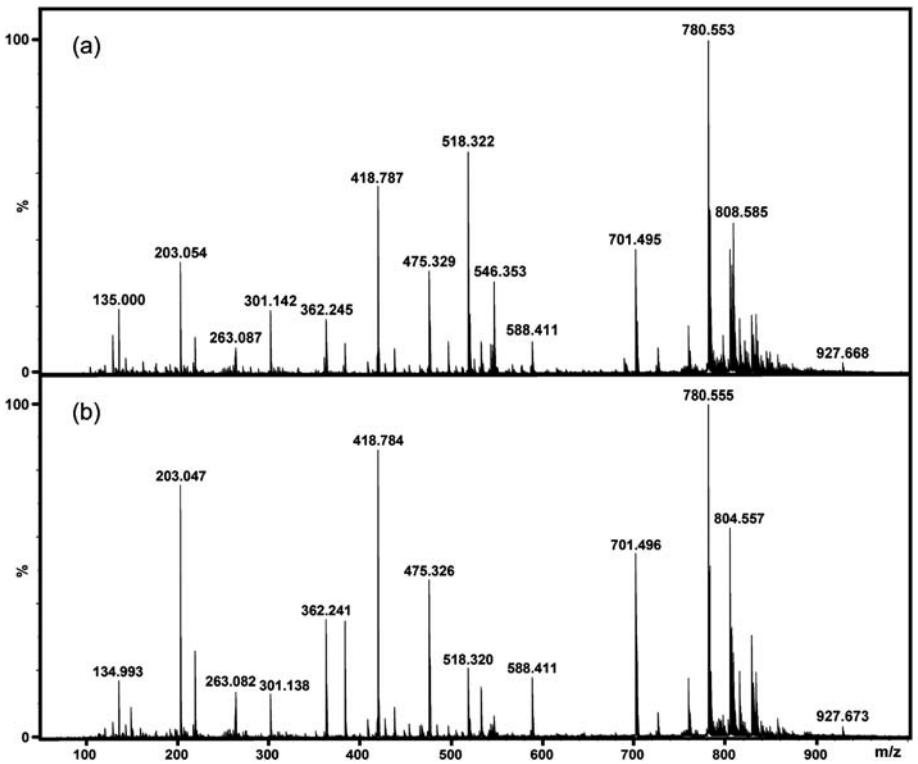

Fig. 2 Typical metabolic fingerprints obtained from serum samples of (a) a healthy control and (b) a kidney cancer patient.

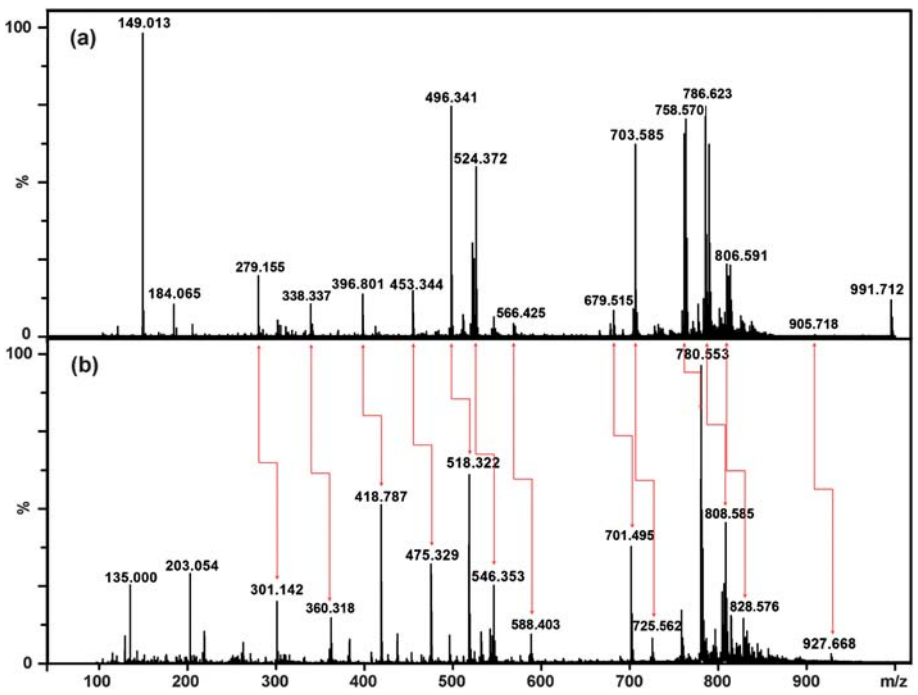

Fig. 3 Comparison of a summed mass spectrum from (a) LC-MS analysis with (b) a mass spectrum from DIMS analysis.

analysis. A QC sample was injected every 5 samples to monitor and evaluate the stability of the two methods. In order to evaluate the method stability and repeatability roundly, quality assurance of all detected peaks across the QC samples was performed.[35] The variations in retention times of all the peaks for LC-MS were less than 0.2 min, and the variations of *m*/*z* values for both methods were less than 10 mDa. The relative standard deviations (RSDs) of the peak areas (for LC-MS) and peak intensities (for DIMS) were calculated across the QC samples. In LC-MS analysis, 68% (using peak area) of all the peaks have RSDs less than 15%; and 82% and 90% of the peaks have RSDs less than 20 and 30%, respectively. For DIMS, the number of

peaks showing repeatability at RSDs of 15, 20 and 30% was 79%, 87% and 93%, respectively. These results demonstrate the excellent stability and reproducibility of both methods.

Comparison of classification and prediction abilities

The datasets from LC-MS and DIMS, containing 8360 and 1801 variables respectively, were imported to SIMCA-P for multivariate statistical analysis. PCA was used as an unbiased statistical method to detect any inherent trends within the data and to identify any potential outliers that could affect subsequent discriminant analysis. As shown in Fig. 4, obvious separation trends can be observed between the two groups by both methods, indicating inherent metabolic changes of the kidney cancer patients compared to the controls. According to the R^2X (cum) parameter in PCA, 40.1% and 55.3% of the variables can be explained by the first two components of the models built on LC-MS and DIMS datasets, respectively.

To further study the differences between the kidney cancer patients and the healthy controls, and to find out potential biomarkers, supervised PLS-DA was subsequently used. 80% of the data were randomly extracted from each group to create a training set for building the PLS model. The remaining data formed the independent prediction set and were used to evaluate the developed model. The classification and prediction results are shown in Fig. 5. Distinct clustering between the patients and controls was achieved with both methods. All of the samples in the prediction set were classified to the area in which they were supposed to be. In PLS-DA, R^2Y (cum) and Q^2 (cum) parameters were used for the evaluation of the models, indicating fitness and prediction ability, respectively.[36,37] The R^2Y (cum) and Q^2 (cum) values obtained by DIMS are 0.969 and 0.931, respectively, which are comparable to the values of 0.986 and 0.928 achieved by LC-MS (summarized in Table 2). It should be noticed that although fewer variables were obtained, DIMS analysis generated as robust a model as LC-MS. Considering that the analysis time for each sample in DIMS is only 2 min, compared with several tens of minutes for LC-MS, DIMS undoubtedly provides a much higher sample throughput. DIMS could be developed as a fast prognostic or diagnostic method for kidney cancer.

Table 2 Comparison of the PLS-DA models built on data from LC-MS and DIMS analysis

	R^2Y(cum)	Q^2(cum)	Imported Var No.[a]	Discovered Marker No.[b]
LC-MS	0.986	0.928	8360	48
DIMS	0.969	0.931	1801	23

[a] Imported Var No.: number of the variables imported to build the model. [b] Discovered Marker No.: number of variables discovered as potential markers.

Comparison of biomarker discovery abilities

To discover the potential biomarkers among thousands of variables, parameter VIP (Variable Importance in the Projection) was employed to reflect variable importance. A variable is considered important for the model when its VIP is above 1.0.[38,39] To focus on highly significant variables, only those with VIP above 2.0 were considered here. The *T*-test was performed in succession, and the variables without significant differences between the patients and the controls ($p > 0.01$) were eliminated.

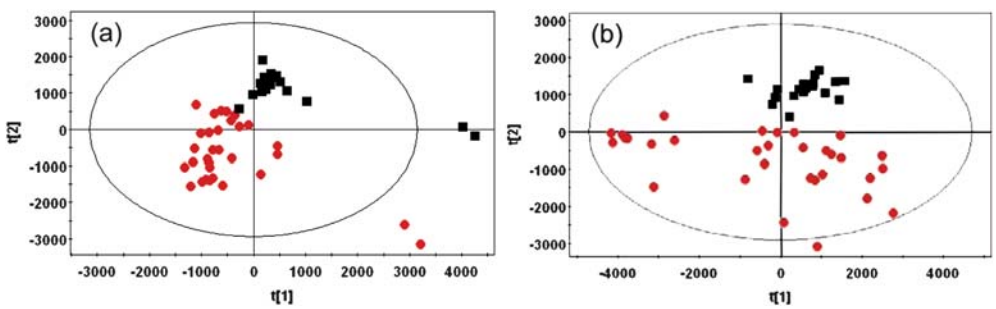

Fig. 4 PCA scores plots based on the (a) LC-MS dataset and (b) DIMS dataset (● kidney cancer patients, ■ controls).

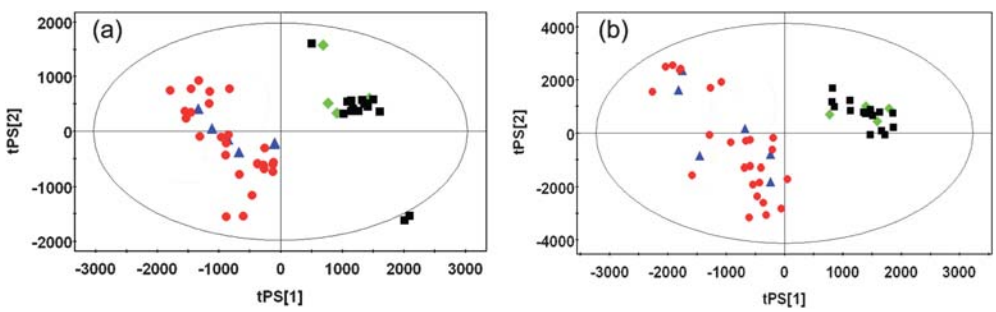

Fig. 5 PLS-DA scores plots for response variables (● patient training set, ■ control training set, ▲ patient prediction set, and ◆ control prediction set): (a) LC-MS training set with prediction set overlaid and (b) DIMS training set with prediction set overlaid.

Table 3 Potential biomarkers identified from LC-MS and DIMS analysis

LC-MS					DIMS					
No.	*m/z*	Adduct	Fold change[a]	*p* value[b]	No.	*m/z*	Adduct	Fold change[a]	*p* value[b]	Identified results
1	524.38	M + H [1+]	−2.18	7.12E-11	1	546.35	M + Na [1+]	−4.09	2.21E-21	LPC[c] (18 : 0)[e]
2	991.71	2M-H [1+]	−2.70	2.68E-17						LPC (16 : 0)[e]
3	496.35	M + H [1+]	−1.65	2.47E-14	2	518.32	M + Na [1+]	−3.36	3.09E-21	LPC (16 : 0)[e]
4	522.36	M + H [1+]	−1.47	3.02E-08	3	544.34	M + Na [1+]	−2.19	1.17E-12	LPC (18 : 1)[e]
5	520.34	M + H [1+]	−1.42	1.01E-03	4	542.32	M + Na [1+]	−2.10	1.92E-10	LPC (18 : 2(9*Z*,12*Z*))[f]
6	310.31	M + H [1+]	−8.61	9.56E-16	5	332.29	M + Na [1+]	−3.21	1.12E-20	UN[g]
7	274.27	M + H [1+]	+6.34	5.80E-12	6	274.27	M + H [1+]	+3.08	8.73E-07	C16 sphinganine[f]
8	482.36	M + H [1+]	−3.53	1.81E-17						LPC (15 : 0)[f]
9	510.36	M + H [1+]	−3.14	2.27E-17						LPC (17 : 0)[e]
10	508.38	M + H [1+]	−3.62	7.56E-17						LPC (*P*-18 : 0))[f]
11	480.35	M + H [1+]	−2.83	5.25E-13						LPC (*P*-16 : 0)[f]
12	466.33	[1+]	−6.05	7.94E-18						UN[g]
13	184.07	[1+]	−1.85	2.31E-09	7	184.07	[1+]	−1.25	1.21E-05	Fragment of LPC[e]
14	338.34	M+ ACN +H[1+]	−3.52	1.94E-07	8	360.32	M+ ACN +Na[1+]	−6.23	5.70E-21	Thromboxane[f]
15	774.59	[1+]	−8.35	3.30E-14	9	796.55	M + Na [1+]	−2.04	1.49E-07	UN[h]
16	387.17	2M + H[1+]	+11.38	7.40E-09	10	409.16	2M + Na [1+]	+4.85	7.83E-06	Phenylacetylglycine [e]
17	468.31	M + H [1+]	−2.21	1.09E-07						LPC (14 : 0)[e]
18	548.37	M + H [1+]	−2.24	2.31E-12	11	548.37	M + H [1+]	−2.22	8.05E-13	LPC (20 : 2(11*Z*,14*Z*))[f]
19	293.27	[1+]	−8.68	1.04E-21						UN[h]
20	510.39	M + H [1+]	−3.47	2.06E-15						LPC (*O*-18 : 0)[f]
21	464.31	[1+]	−5.04	3.04E-15						UN[g]
22	550.39	M + H [1+]	−2.23	1.77E-08	12	550.39	M + H [1+]	−2.12	9.08E-09	LPC (20 : 1(11*Z*))[f]
23	438.30	[1+]	−3.54	2.97E-18						UN[g]
24	376.32	[1+]	−6.65	8.25E-19						UN[g]
25	105.06	[1+]	+8.85	1.45E-09						UN[h]
26	506.36	[1+]	−3.38	4.83E-13						UN[g]
27	317.25	[1+]	−2.13	4.74E-18						UN[h]
28	404.21	[1+]	+9.88	1.75E-04						UN[h]
29	280.09	[1+]	−3.36	2.91E-23	13	280.09	[1+]	−3.06	6.73E-25	UN[h]
30	838.58	[1+]	−7.64	1.82E-21	14	860.58	M + Na[1+]	−1.06	9.00E-17	UN[g]
31	822.58	[1+]	−4.53	9.13E-16	15	844.55	M + Na[1+]	−3.20	8.21E-18	UN[g]
32	538.39	M + H [1+]	−2.98	2.11E-15						LPE[d] (22 : 0/0 : 0)[f]
33	293.24	[1+]	−5.72	1.11E-26						UN[h]
34	319.29	M + Na[1+]	−3.20	1.03E-16	16	319.29	M + Na[1+]	−2.73	7.73E-25	Thromboxane[f]
35	219.02	[1+]	−1.36	9.65E-07	17	219.02	[1+]	−1.36	9.80E-03	UN[h]
36	558.32	M + H [1+]	−3.41	4.30E-15						LPE (22 : 1/0 : 0)[f]
37	205.09	M + H [1+]	−1.58	2.34E-06						L-Tryptophan[e]
38	357.24	M + Na[1+]	−3.21	1.44E-20						Tetrahydrodeoxy-corticosterone[e]
39	373.23	[1+]	−3.58	3.01E-22						UN[h]
40	640.44	M + 2H[2+]	+13.88	4.33E-04						Ganglioside GM3 (d18:1/25 : 0)[f]
41	291.26	[1+]	−3.16	4.68E-17						UN[h]
42	397.23	[1+]	−3.56	3.07E-19						UN[g]
43	618.42	M + 2H[2+]	+9.24	5.05E-04	18	640.41	M + H + Na[2+]	+3.03	1.39E-05	Ganglioside GM3 (d18:1/22 : 1)[f]
44	327.23	[1+]	−3.53	1.10E-19						UN[g]
45	182.07	M + H [1+]	−1.69	4.53E-15						L-Tyrosine[e]
46	333.24	[1+]	−2.19	5.73E-25						UN[h]
47	104.09	[1+]	−1.54	7.46E-14	19	104.09	[1+]	−2.27	5.31E-23	Fragment of LPC[e]
48	488.32	[1+]	−2.94	5.74E-19						
					20	381.30	[1+]	−4.02	1.30E-14	UN[h]
					21	576.28	[1+]	−2.58	2.53E-20	UN[h]
					22	361.33	[1+]	−2.34	1.08E-19	UN[h]
					23	754.55	[1+]	+1.53	8.20E-03	UN[h]

[a] Fold change was calculated from the arithmetic mean values of each group. Fold change with a positive value indicates a relatively higher concentration present in kidney cancer patients while a negative value means a relatively lower concentration compared to the healthy controls. [b] *p* values were calculated from student *T*-test. [c] LPC: Lysophosphatidylcholine. [d] LPE: Lysophosphatidylethanolamine. [e] Metabolites formally identified by standard samples. [f] Metabolites putatively annotated. [g] Unknown: Compounds which MS/MS spectra were not interpretable or not informative. [h] Unknown: Compounds lacked of enough intensity for MS/MS experiments.

As a result, 23 variables were found as potential markers by DIMS, while 48 variables were obtained by LC-MS (Table 3).

The method of identifying compounds is as follows. First, the quasi-molecular ion of the corresponding variable was found in the mass spectrum. Second, the exact mass of the quasi-molecular ion was searched on the HMDB or METLIN website to find possible compounds within a certain mass range. Because the variation of the measured *m/z* value in our experiment was below 10 mDa, the accurate mass cutoff was set as 10 mDa. Third, the scope was further narrowed with some obvious

structure information obtained from the MS/MS data, and the standard MS/MS spectra of possible compounds were searched on the website. Finally, the result was confirmed with commercial standard compounds if available. Taking the variable of *m*/*z* 524 as an example, the MS/MS results from LC-MS and DIMS were shown in Fig. 6. For LC-MS, the corresponding quasi-molecular ion peak was found according to the retention time in the extracted ion chromatogram of *m*/*z* 524. For DIMS, the sum intensity of the variable *m*/*z* 524 and its sodium adduct *m*/*z* 546 was approximately one order of magnitude lower than that of LC-MS, and the intensity of *m*/*z* 524 was only 20% of the sum intensity. Nevertheless, the *m*/*z* 524 ion was still chosen for the MS/MS experiment in DIMS analysis because it can be conveniently compared with the MS/MS spectrum of the commercial standard or the spectra from the literature. All of the identification results are shown in Table 3. Unfortunately, about 40% of the potential biomarkers were unidentifiable due to insufficient intensity for MS/MS experiments or the restrictions of metabolite databases.

As shown in Table 3, most biomarkers found by DIMS were also obtained by LC-MS. Instead of forming $[M + H]^+$ ions in LC-MS, the compounds prefer to form $[M + Na]^+$ adducts in DIMS. The complicated serum matrix not only affects the ion adduct forms, but also reduces the biomarker detection ability. When thousands of metabolites are infused together into the mass spectrometer, ion suppression effects cause some biomarkers of low abundance, low volatility, or low ionization efficiencies to be undetectable in the DIMS analysis. Thus, the LC-MS method is a better choice for comprehensive screening of potential biomarkers. On the other hand, although the DIMS method is less information-rich, it requires only ~5% of the analysis time of the LC-MS approach and still provides roughly half as much biomarker information, allowing quality statistical data generation.

It is worthy to mention that more compounds can be detected and less analysis time is needed if ultra performance liquid chromatography (UPLC) is used instead of conventional LC.[40] However, the general throughput of UPLC is not comparable to that of DIMS. Very fast gradient elution may be used in UPLC to obtain a throughput closer to that of DIMS, but it would sacrifice both separation performance and detection capability.

The biological changes of the potential biomarkers

The alteration of potential biomarkers in kidney cancer patients compared to healthy controls is also presented in Table 3. A fold change with a positive value indicates a relatively higher concentration in kidney cancer patients, while a negative value indicates a relatively lower concentration compared to the healthy controls. The alteration trends (up- or down-regulation) of corresponding biomarkers obtained by the two methods were consistent with one another although the fold values were not equal. The differences in the concentration ratios between the two methods may have been due to the different data

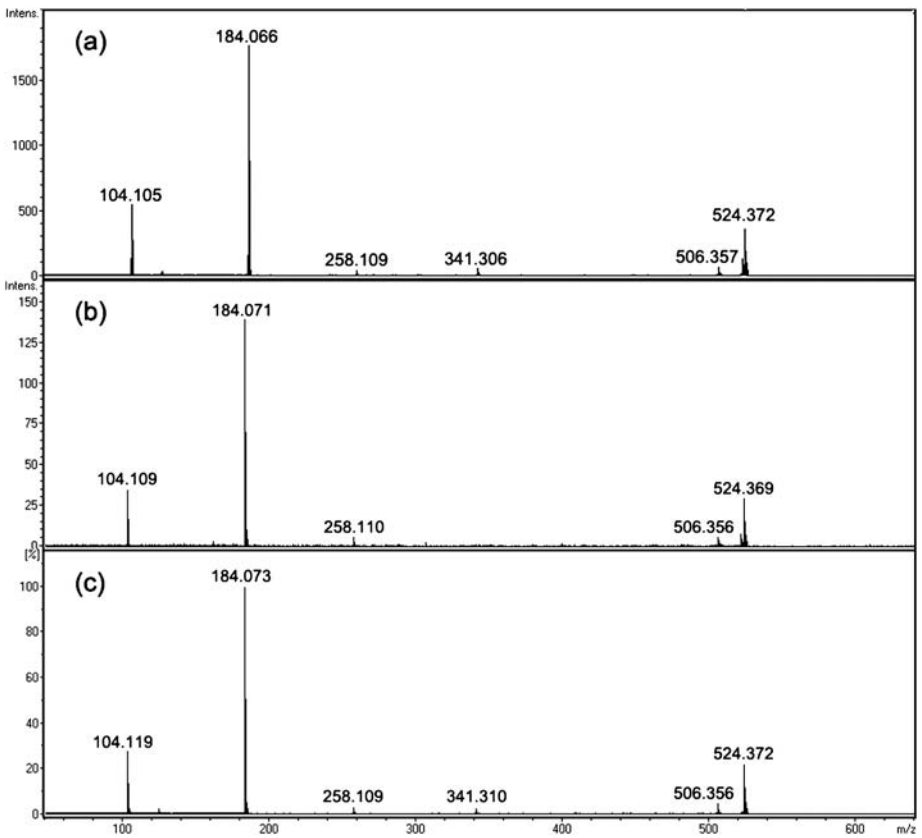

Fig. 6 Identification of a selected marker (*m*/*z* 524): (a) MS/MS spectrum in LC-MS analysis; (b) MS/MS spectrum in DIMS analysis; (c) MS/MS spectrum of the commercial standard Lysophosphatidylcholine C18:0. The collision energy was 20 eV.

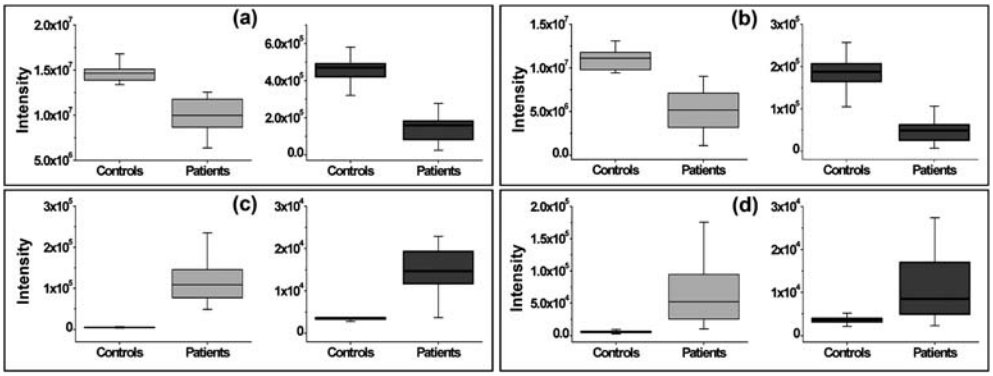

Fig. 7 Variation of serum (a) LPC (16 : 0), (b) LPC (18 : 0), (c) phenylacetylglycine and (d) Ganglioside GM3 levels in kidney cancer patients compared to controls. Light gray and dark gray bars correspond to LC-MS and DIMS acquisitions, respectively. The boxes are drawn from the 25th to 75th percentiles in the intensity distribution. The median, or 50th percentile, is drawn as a black horizontal line inside the box. The whiskers extend from the upper inner to lower inner fence values.

pretreatment and different matrix complexities. The pretreatment of LC-MS data, including peak alignment and data reduction, is still problematic and increases the uncertainty of the quantitative result. In DIMS, ion suppression may hinder its ability to reflect true concentration differences.

As shown in Fig. 7, an obvious decline of LPC (16 : 0) and LPC (18 : 0) and an increase of phenylacetylglycine and Ganglioside GM3 were observed by both methods in kidney cancer patients compared to controls. LPC is formed by the hydrolysis of phosphatidylcholine by the enzyme phospholipase A2 or lecithin-cholesterol acyltransferase (LCAT). It regulates many biological process, including cell proliferation, tumor cell invasiveness, and inflammation.[33] A distinct decline of LPC was observed in our study, which is in agreement with the earlier study on kidney cancer by ^{31}P-NMR spectroscopy.[41] Similar trends were also found in other malignant diseases, such as leukemia, malignant lymphomas, gastrointestinal and liver cancer.[38,42] Serum LPC concentrations might be developed as a general indicator for malignant disease.

The obvious increase of phenylacetylglycine revealed a disordered fatty acid metabolism in kidney cancer patients. Phenylacetylglycine is an acyl glycine, which is normally a minor metabolite of fatty acids. In certain cases, the measurement of acyl glycines in body fluids can be used to diagnose disorders associated with mitochondrial fatty acid beta-oxidation.[43,44] Similar changes were reported in a recent study on chemically-induced precancerous colorectal lesions.[45] Phenylacetylglycine is also a putative biomarker of phospholipidosis.[46] Urinary phenylacetylglycine concentration was reported to increase in animals exhibiting abnormal phospholipid accumulation in many tissues.[47]

Gangliosides (sialic acid-containing glycosphingolipids) are synthesized from ceramide and have attracted considerable interest for more than 20 years as potential targets for cancer diagnosis because of their relevance in tumor growth and metastasis.[48,49] The ganglioside GM3 is one of the main components of the total gangliosides in many cell types.[50] It inhibits proliferation of epidermoid carcinoma cells and neuroblastoma cells by suppressing activity of the relevant cell growth factor receptor.[51,52] Over-expression of ganglioside GM3 has been found in several kinds of tumors,[49,53] which is in agreement with our kidney cancer study.

The discovery of these biomarkers not only gives a better understanding of the pathophysiological changes of kidney cancer but also indicates possible chemotherapy for patients. The quantification of these metabolites could be significant in further studies as an aid in the prognostication of kidney cancer.

Conclusion

The performance of LC-MS and DIMS on serum metabonomic study was compared using the same mass spectrometer. Patients with kidney cancer and healthy volunteers were used as real models. DIMS had comparable classification and prediction capability to LC-MS. With regard to biomarker discovery, 23 variables were found as potential markers by DIMS, while 48 variables were obtained by LC-MS. Most biomarkers obtained by DIMS were also observed by LC-MS, and the trends of alteration of the corresponding biomarkers were similar. Considering that a much higher throughput can be obtained without a chromatographic step, DIMS could be developed as a fast prognostic or diagnostic method for kidney cancer. The LC-MS method is necessary when comprehensive screening of biomarkers is required.

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