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Serum metabolic profiling of human gastric cancer based on gas chromatography/mass spectrometry

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Abstract

Research on molecular mechanisms of carcinogenesis plays an important role in diagnosing and treating gastric cancer. Metabolic profiling may offer the opportunity to understand the molecular mechanism of carcinogenesis and help to non-invasively identify the potential biomarkers for the early diagnosis of human gastric cancer. The aims of this study were to explore the underlying metabolic mechanisms of gastric cancer and to identify biomarkers associated with morbidity. Gas chromatography/mass spectrometry (GC/MS) was used to analyze the serum metabolites of 30 Chinese gastric cancer patients and 30 healthy controls. Diagnostic models for gastric cancer were constructed using orthogonal partial least squares discriminant analysis (OPLS-DA). Acquired metabolomic data were analyzed by the nonparametric Wilcoxon test to find serum metabolic biomarkers for gastric cancer. The OPLS-DA model showed adequate discrimination between cancer and non-cancer cohorts while the model failed to discriminate different pathological stages (I-IV) of gastric cancer patients. A total of 44 endogenous metabolites such as amino acids, organic acids, carbohydrates, fatty acids, and steroids were detected, of which 18 differential metabolites were identified with significant differences. A total of 13 variables were obtained for their greatest contribution in the discriminating OPLS-DA model [variable importance in the projection (VIP) value > 1.0], among which 11 metabolites were identified using both VIP values (VIP > 1) and the Wilcoxon test. These metabolites potentially revealed perturbations of glycolysis and of amino acid, fatty acid, cholesterol, and nucleotide metabolism of gastric cancer patients. These results suggest that gastric cancer serum metabolic profiling has great potential in detecting this disease and helping to understand its metabolic mechanisms.

Key words: Metabolomics; Gastric cancer; Gas chromatography/mass spectrometry

Introduction

Gastric cancer is the fourth most common type of cancer and the second most frequent cause of cancer mortality in the world (1,2). It is one of the most prevalent and deadly forms of cancers with nearly a million new cases diagnosed each year worldwide. A majority of the gastric cancer patients diagnosed at an already advanced stage, is the result of the relatively asymptomatic nature in the early stages of the disease and the lack of adequate screening methods. The prognosis of advanced cancer still remains poor because of its high recurrence rate and metastatic features (3). Gastroscopy is widely used for early screening, but this methodology

is invasive, uncomfortable and costly. An effective way to improve the prognosis is to predict the carcinogenesis and metastasis at an early stage, to understand the molecular mechanism of carcinogenesis, and to identify the biomarkers at the early diagnosis of human gastric cancer. Although some serum biomarkers have been studied as noninvasive tools for screening gastric cancer (4,5), these serum tests are not available as screening or surveillance tests because of their low specificity and sensitivity (5). However, with the use of highly sensitive metabolomic techniques, serum metabolomic profiles may offer the opportunity to

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understand the molecular mechanism of carcinogenesis and help to noninvasively identify the potential biomarkers for an early diagnosis of human gastric cancer.

Metabolomics is a recently developed technology for the detection, identification and quantification of low molecular weight metabolites that are involved in the metabolism of an organism at a specified time under specific environmental conditions (6). This method has already been used as an effective tool for disease diagnosis (7), biomarker screening (8,9), and characterization of biological pathways (6). Recent technological advances in NMR spectroscopy and mass spectrometry (MS) have also further improved the sensitivity and spectral resolution for cancer metabolomic study (10). Among various techniques conventionally used for metabolic profiling, gas chromatography/MS (GC/MS) has proven to be a robust metabolomic tool widely applied to the identification and quantitation of cancer metabolites based on its high sensitivity, peak resolution, and reproducibility (11). Since cancers are known to possess highly unique metabolic profiles, identification of specific biomarkers in serum using a metabolomic approach could be noninvasive and useful for early cancer detection and prognosis.

In the present study, we hypothesized that the global analysis of metabolites in serum would define a metabolic signature that discriminates gastric cancer patients from healthy controls. To test the hypothesis, we conducted a comprehensive analysis of the serum metabolites using GC/MS. We aimed to determine whether variations in gastric cancer metabolites are reflected in serum, thereby possibly creating an alternative, noninvasive means for cancer detection. The study also intended to gain knowledge of potential metabolic biomarkers associated with gastric cancer morbidity, which can be used for early diagnosis, staging, and therapeutic strategies.

Material and Methods

Sample collection and reagents

The study protocol was approved by the Review Board of the Sixth Affiliated Hospital, Sun Yat-sen University and written informed consent was obtained from all participants before the study. Thirty patients aged from 39 to 88 years were diagnosed with gastric cancer. The pathological diagnosis was confirmed in routine histopathological H&E-stained specimens and categorized according to postoperative classification of malignant tumors (TNM) (12): stage I, 4 patients; stage II, 5 patients; stage III, 15 patients; stage IV, 6 patients. None of the patients were diagnosed with metabolic diseases such as diabetes mellitus or hyperthyroidism and none receive any medication before sample collection. The normal control group consisted of 30 healthy individuals aged 42 to 82 years, confirmed by gastroscopy. Each gastric cancer patient was matched

with a healthy control based on gender and age. Clinical information about the participants is provided in Table 1. Venous blood (4 mL) was collected in the morning after an 8-h fasting from a total of 30 gastric cancer patients and 30 healthy controls at the Sixth Affiliated Hospital, Sun Yat-sen University.

Pyridine and N, O-Bis (trimethylsilyl)trifluoro-acetamide (BSTFA) were purchased from Sigma-Aldrich (USA). Chromatography grade methanol, ethyl acetate (EA), chloroform, and reference standards such as amino acids (methionine, phenylalanine, proline, lysine, tyrosine, and glutamate), fatty acids (lauric acid, palmitic acid, stearic acid, oleic acid, arachidonic acid), glucose, and cholesterol were purchased from Sijia Chemicals Group Corporation (China).

Specimen preparation for GC/MS analysis

Individual 100- μ L serum samples were transferred to 2.0-mL Eppendorf tubes, lyophilized and extracted with 300 μ L of a mixture of methanol/chloroform (3:1, v/v) and vortexed for 30 s (13). After storing for 10 min at -20°C, the samples were centrifuged at 12,000 g for 10 min at 4°C. A 200- μ L aliquot of the supernatant was collected and transferred to 1.5-mL Eppendorf tubes. All samples were dried using liquid nitrogen and 50 μ L EA was added to each of the dried serum extracts, and vortex-mixed for 1 min. A widely used derivatization reagent, a mixture of BSTFA, pyridine and EA (3:1:1, v/v/v), was then added to the extract for derivatization for 16 h at room temperature (14). The solution thus obtained was then vortexed for 1 min and transferred to an amber glass vial for GC/MS analysis. In order to prevent a batch effect, all assays were conducted in a random- and double-blind manner.

One milligram of reference standards including amino acids (methionine, phenylalanine, proline, lysine, tyrosine, and glutamate), fatty acids (lauric acid, palmitic acid, stearic acid, oleic acid, arachidonic acid), glucose, and cholesterol

Table 1. Summary of the anatomical and clinicopathological characteristics of the gastric cancer patients and healthy controls studied.

	Gastric cancer patients	Healthy controls
Number	30	30
Male/female	15/15	15/15
Age (median, range)	63 (39-88)	62 (42-82)
TNM stage I	4	-
TNM stage II	5	-
TNM stage III	15	-
TNM stage IV	6	-
Poorly differentiated adenocarcinoma	23	-
Moderately differentiated adenocarcinoma	5	-
Well-differentiated adenocarcinoma	2	-

TNM = tumor node metastasis.

was dissolved in 5 mL pyridine to give a final concentration of 200 µg/mL. Each 200 µL reference standard was transferred to 1.5-mL Eppendorf tubes and derivatized as described above and prepared for GC/MS analysis according to the methodology described below.

GC/MS analysis

Analysis was performed on an Agilent 7890A gas chromatography system equipped with an Agilent 5975C Series autosampler (Agilent Technologies, USA). Separation was achieved on an Agilent DB-5MS capillary column (30 m x 0.25 mm ID x 0.25 µm film thickness). Each 2-µL aliquot of the derivatized solution or derivatized reference standard was injected in the splitless mode and helium was used as the carrier gas at a constant flow rate of 1.0 mL/min. The temperatures of inlet, transfer line, ion source, and quadrupole were maintained at 270°, 260°, 200°, and 150°C, respectively. The GC temperature programming was set to 2 min isothermal heating at 80°C, followed by 10°C/min oven temperature ramps to 180°C, 5° to 240°C/min, 20° to 290°C/min, and a final 10-min maintenance at 290°C. Data acquisition was achieved using MS in the electron impact mode at 70 eV and in the full-scan monitoring mode from m/z 30 to 600 with an acquisition rate of 20 spectra/s. Solvent delay time was set at 5 min.

Data processing and statistical analysis

GC/MS was employed to profile serum samples of gastric cancer patients and normal controls. Each sample or reference standard was represented by a GC/MS total ion current (TIC) chromatogram. Among the detected peaks, a multidimensional vector was constructed manually to characterize the biochemical profile (14). Peaks due to column bleeding and derivatization reagent were removed. Peaks were taken into account only when they were consistently detected in at least 80% of the samples. All the detected peaks were identified by comparing the MS spectra (15) with those available in the NIST mass spectral library (Wiley registry, 2008 edition) and customized reference mass spectral libraries (16). The mass spectra obtained were investigated manually and only those compounds with a matching probability of more than 80% were considered. The retention time and m/z data pairs were used as the identifier for each peak within each sample and the ion intensities for each peak were normalized to the sum of the peak intensities in that sample. To account for any difference in concentration between samples, all data were normalized to a total value of 100.

Statistical analysis was performed using multivariate statistics combined with univariate statistics. Normalized data were exported to SIMCA-P + (Version 11.5, Umetrics, Sweden) to perform orthogonal partial least squares discriminant analysis (OPLS-DA) and a model was built to identify variables that accounted for the differentiation of gastric cancer and non-gastric cancer cohorts, and group-

ing trends were observed in the data (17). Indication of significance was based on non-parametric two-tailed paired Wilcoxon analysis performed with SPSS 13.0 for Windows (SPSS, USA). P values of <0.05 were considered to be statistically significant. The fold change (FC) was based on the mean value of 30 gastric cancer patients relative to the mean of normal controls.

Results

GC/MS metabolic profiling and OPLS-DA modeling

Typical GC/MS TIC chromatograms of serum samples from a gastric cancer patient and the matched normal control are shown in Figure 1. Within one TIC chromatogram, over 60 signals were usually detected in a single specimen and some of these peaks were not investigated further as they were not consistently found in other sets of samples, too low in concentration, or of poor spectral quality to be confirmed as metabolites. As shown in Table 2, a total of 44 endogenous metabolites such as amino acids, organic acids, carbohydrates, fatty acids, and steroids were detected sequentially and these compounds are involved in many biochemical processes in biosystems, such as energy metabolism, lipid metabolism and amino acid metabolism. The first three fragment-ion m/z values with the highest abundance within each fragmentation pattern and the matching percentage to the NIST library are also listed in Table 2.

For the metabolic profile, the OPLS-DA model demonstrated satisfactory modeling and predictive abilities using one predictive component and three orthogonal components ($R^2Y_{cum} = 0.745$, $Q^2_{cum} = 0.671$). Distinct separation trends were achieved between the metabolite profiles of the two groups, as shown in Figure 2A. However, a separate OPLS-DA model using one predictive component and three orthogonal components ($R^2Y_{cum} = 0.575$, $Q^2_{cum} = 0.0379$) failed to discriminate different pathological stages (I-IV) of gastric cancer sera, although separation trends could be observed between the gastric cancer group and non-gastric cancer group as displayed in Figure 2B.

Analysis of highlighted metabolites

To identify which variables were accountable for such significant separation, variable importance in the projection (VIP) statistics from OPLS-DA modeling was first used to pre-select variables. As shown in Table 2, according to the criterion for VIP statistics ($VIP > 1$), a total of 13 variables were obtained, which mostly contributed to discriminating metabolic profiles between the two groups. The Wilcoxon test ($P < 0.05$) was used to investigate the differences of biomolecules between gastric cancer patients and normal controls. A total of 18 metabolites including carbohydrates, amino acids, fatty acids, and steroids were detected with significant differences, as displayed in Table 2. Only 3 of the metabolites detected were found to be higher in cancer

tissues, while 15 were lower. Valine, showing the greatest fold change (FC = 2.63), was the serum metabolite found to be most increased in gastric cancer patients compared to controls and hexanedioic acid was the metabolite most depleted (FC = -4.69) in gastric cancer patients. Eleven metabolites were identified using both VIP values (VIP >1) and the Wilcoxon test ($P < 0.05$).

Discussion

Metabolic profiling of digestive cancer using GC/MS has been reported (8,9,13,14,18), while few studies have focused on the investigation of the metabolic profile of the serum of gastric cancer patients. The present study explored the serum metabolic fingerprints of gastric cancer patients compared to healthy controls. Since the metabolomic data typically contain a large number of variables that are inter-related, a multivariate statistical method such as OPLS-DA coupled with a univariate statistical method such as the Wilcoxon test was used and selection of differential metabolites

was performed with a threshold of 1.0 by VIP and a P value set at 0.05, to identify variables with biological significance as endpoints of altered biochemical pathways.

Previous studies have indicated that a supervised model could be a robust model for discrimination in multivariate analysis and the OPLS-DA model has been adopted to separate cancer and non-cancer cohorts in human colorectal cancer tissue and serum specimen (13,14,18). The OPLS-DA model derived from our current metabolomic data showed satisfactory and adequate separation trends between the cancer and non-cancer groups, indicating the diagnostic potential of serum metabolic profile. It has been reported that the unsupervised principal component analysis model also could separate gastric cancer tissues from normal mucosa (19). Our attempt to stratify gastric cancer patients according to pathological stages (TNM I-IV) was not successful. A similar result has been reported for a serum study of colorectal cancer (13). We infer that metabolic perturbations may not be directly associated with the different pathological stages of gastric cancer, or that

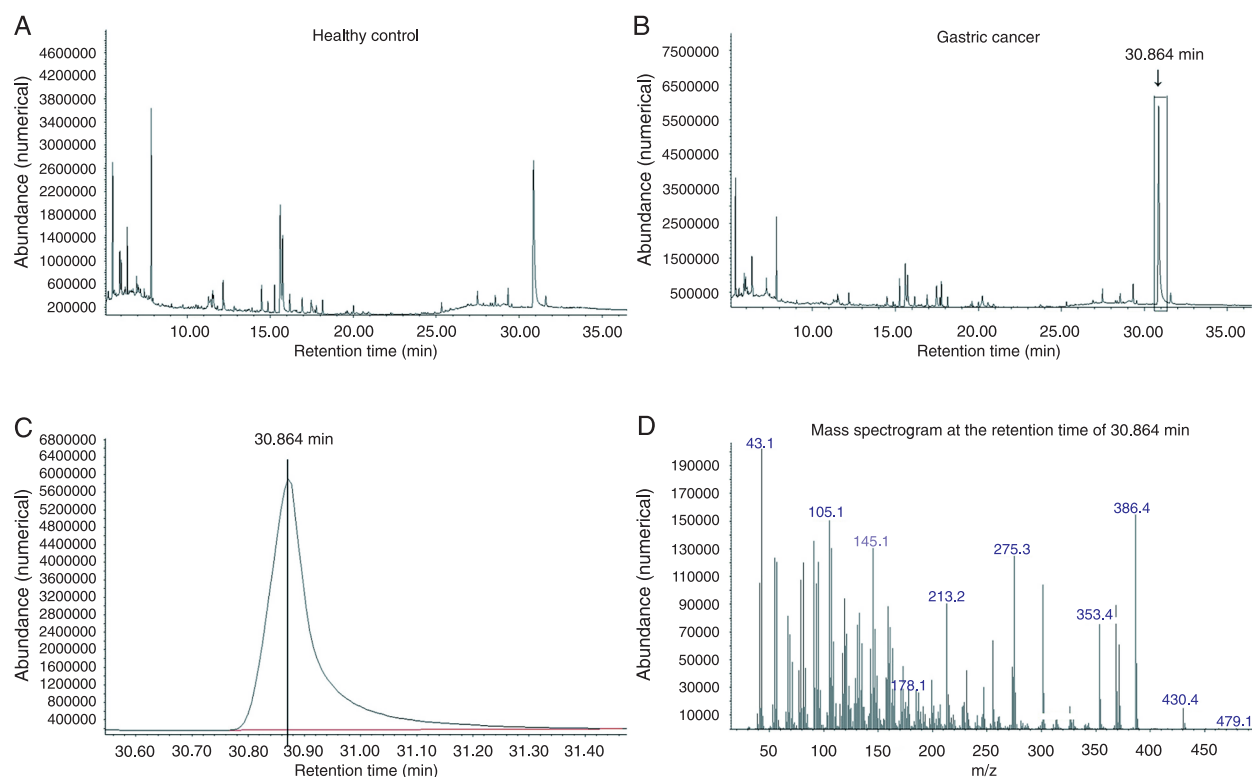


Figure 1. Typical gas chromatography/mass spectrometry (GC/MS) total ion current (TIC) chromatograms. *A* and *B* show that, within one TIC chromatogram, over 60 signals were usually detected in a single gastric cancer serum or normal control serum specimen. *C* shows a base peak at the retention time of 30.864 min that was extracted and magnified. *D* shows that the mass spectrogram at the peak summit was selected and compounds were identified via mass spectral match to the National Institute of Standards and Technology library. The m/z values of the first three highest abundance of fragmentation patterns were 43.1, 105.1, and 386.4.

Table 2. Serum differential metabolites derived from gas chromatography/mass spectrometry analysis.

Functional class	Retention time	m/z (No. 1) ^a	m/z (No. 2) ^a	m/z (No. 3) ^a	Match percent ^b	Compound ^c	VIP value ^d	Fold change ^e	P value ^e	Trend of gastric cancer
Amino acid metabolism										
1	10.695	69	41.1	97.1	83	dl-Ornithine	0.7922	1.93		
2	11.368	67	128	206	84	D-Alanine	0.032	1.03		
3	11.439	191.2	57.1	229.1	85	L-Proline	0.1954	1.17		
4	12.151	156.1	51	102.1	97	L-Glutamine	0.9197	-1.77	0.032	↓
5	12.222	187.1	156.1	69	80	L-Valine	1.0919	2.63	0.031	↑
6	12.817	188.1	60.1	40	80	L-Isoleucine	0.5739	-2.18		
7	12.888	188.1	69	110.1	82	Glycine	0.5828	-2.01		
8	12.92	69	41.1	188.1	86	L-Leucine	0.1553	1.14		
9	13.897	71.1	40	113.1	85	Valeric acid	0.1255	1.05		
10	22.261	73.1	32	225.1	83	Sarcosine	0.9688	1.93	0.049	↑
11	24.913	91.1	44	207.1	8	Glycyl-dl-alanine	0.5387	1.67		
12	24.234	57.1	98.1	207	80	Hexanedioic acid	1.3538	-4.69	0.022	↓
Fatty acid metabolism										
13	16.924	74.1	43.1	143.1	98	Hexadecanoic acid	0.0215	1.00		
14	17.688	67.1	95.1	40	82	9,12-Octadecadienoic acid	1.0384	-1.68	0.043	↓
15	17.778	69.1	111.1	32.1	87	cis-9-Hexadecenoic acid	0.8822	-1.47		
16	18.141	43.1	110.1	222.3	84	cis-13-Octadecenoic acid	1.1333	1.27		
17	19.518	67.1	96.1	40	96	10,13-Octadecadienoic acid	0.9204	-1.71		
18	19.622	55.1	97.1	264.3	97	9-Octadecenoic acid	1.2315	-1.69	0.017	↓
19	20.016	74.1	43.1	143.1	97	Heptadecanoic acid, 15-methyl ester	0.113	1.02		
20	20.256	55.1	83.1	111.1	99	trans-13-Octadecenoic acid	0.9642	-1.82	0.011	↓
21	20.605	73.1	43.1	129.1	93	Octadecanoic acid	0.333	1.20		
22	20.954	59.1	32	207	86	Octadecanamide	0.7332	-1.33		
23	23.775	59.1	32	95.1	85	9-Octadecenamide	0.5218	-1.46		
24	24.118	59	32	207.1	83	Tetradecanamide	0.2383	1.45		
25	25.541	55.1	97	276.3	80	Nonahexacontanoic acid	1.155	-2.35	0.021	↓
26	25.631	136.1	97.1	247	89	Docosanoic acid	0.8334	-2.04		
Cholesterol metabolism										
27	28.555	207	43.1	368.4	97	Cholesta-3,5-diene	2.0449	-2.14	0	↓
28	29.331	368.4	43.1	207	89	Cholesterol, pentafluoropropionate	2.092	-2.12	0	↓
29	30.864	43.1	105.1	386.4	99	Cholesterol	0.9811	-1.87	0.046	↓
30	31.595	368.4	207	81.1	81	Cholest-5-en-3-ol	1.5286	-2.68	0.001	↓
Nucleotide synthesis										
31	10.52	69	40	136.1	85	Adenine	0.615	1.93		
Glycometabolism										
32	9.046	73.1	43.1	267	85	Fumaric acid	0.7632	-1.87	0.03	↓
33	14.195	69	32	184.1	80	2-O-Mesyl arabinose	0.7436	-1.65	0.049	↓
34	15.255	43.1	110	194.2	85	d-Glucopyranoside	0.7927	1.16		
35	17.235	69.1	41.1	98.1	83	d-Fructopyranose	0.6557	-1.27		
36	17.468	73.1	43.1	129.1	91	L-(+)-Ascorbic acid	0.6204	1.27		
Others										
37	15.592	80.1	129.1	39.1	88	Barbituric acid	0.5453	1.25		
38	16.556	108	69	32.1	91	Hexadecanenitrile	0.7939	1.45	0.041	↑
39	19.227	69	40	113.1	81	Adipic dihydroxamic acid monohydrate	0.0687	-1.04		
40	19.706	73.1	40	281.1	84	Heptadecanenitrile	1.0089	1.91		
41	25.321	55.1	97.1	136.1	83	Oleanitrile	0.5013	1.17		
42	27.488	207.1	43.1	145.1	80	Benzeneacetonitrile,	2.0218	-2.03	0	↓
43	28.264	207	43.1	281	83	2-Amino-4-hydroxy-pteridinone	1.7826	-2.31	0.001	↓
44	29.545	207.1	43.1	281.1	80	1,2,4-Benzenetricarboxylic acid	1.9467	-2.72	0	↓

^aThe first three fragment-ion m/z values with the highest abundance within each fragmentation pattern are listed. ^bThe matching percentage to the NIST library is listed. ^cMetabolites are identified using available standard reference or NIST library databases. ^dVariable importance in the projection (VIP) was obtained by orthogonal partial least squares discriminant analysis (OPLS-DA) with a threshold of 1.0. ^eP value and fold change were calculated using the nonparametric Wilcoxon test (significance at P < 0.05). Fold change with a positive value indicates a relatively higher concentration present in gastric cancer serum, while a negative value means a relatively lower concentration as compared to the normal control.

a limited sample number may not be effective enough to discriminate different TNM stages in the model.

The metabolic profile also revealed several marker metabolites related to the metabolic disturbance in gastric cancer serum. First of all, perturbations of amino acid metabolism were noted in the present study. Valine was observed to be significantly increased in gastric cancer serum compared with healthy controls while glutamine was found to be significantly decreased. Other amino acids such as proline, isoleucine and leucine also varied, although no statistically significance could be demonstrated. Most cancer cells produce energy by glycolysis rather than oxidative phosphorylation via the tricarboxylic acid (TCA) cycle (20), termed as Warburg effect (21). As reported, an increase in valine has been detected in cancer tissue due to increased glycolysis (19). Increased levels of serum valine may be related to valine catabolism in cancer cells. In addition, branched chain amino acids including valine, leucine and isoleucine share the same enzyme systems and are considered as a group to maintain amino acid homeostasis (22). This explains the opposite altered trends of different branch chain amino acids. Previous studies have shown that tumors consume large amounts of glutamine as an energy source. Malignant cells transport glutamine across the cytoplasmic membrane to form glutamate at a faster rate than their non-malignant counterparts (23). Glutaminase, which catalyzes glutamine to glutamate, has been found to be overexpressed in tumor cells (24). Thus, our finding of decreased glutamine levels in serum is consistent with the hypothesis that rapidly growing tumor cells use glutamine from serum as an energy source.

Several essential and non-essential amino acids have been found to be related to abnormal protein synthesis in

the process of carcinogenesis in colorectal cancer (14). The present results suggest that abnormal protein synthesis also occurs in gastric cancer cells and the altered amino acid levels could be the phenotypic markers in serum. In the present study in gastric cancer, sarcosine, but not glycine, was found to be significantly elevated in gastric cancer serum. It has been recently reported that the prevalence of sarcosine increases with escalating severity of prostate cancer as it progresses towards metastatic disease and amino acid metabolism increases along nitrogen breakdown pathways (25). Sarcosine was further validated as a potential biomarker for early disease detection and as a predictor of aggressiveness because it was undetectable in any benign tissue samples. We infer that sarcosine may be a potential biomarker for the progression of gastric cancer metastasis.

Another notable feature of the analysis was alteration of fatty acid metabolism in serum from gastric cancer patients. The levels of a wide spectrum of fatty acids were found to be significantly decreased in cancer sera compared to normal controls. Fatty acid metabolic perturbation has been observed in several kinds of cancers. Saturated and monounsaturated fatty acids were found to be significantly elevated in serum of non-Hodgkin lymphoma patients, while linoleic acid and the levels of polyunsaturated fatty acids were significantly reduced (26). The levels of a wide spectrum of fatty acids, from C-12 to C-22, together with various glycerols were found to be significantly elevated in colorectal polyps and cancerous tissues compared to the adjacent normal mucosa (14). Decreased levels of palmitic acid, myristic acid and carnitine, the carrier of fatty acids, were observed in colorectal cancer sera compared to healthy controls (13). Fatty acids are used for both cell

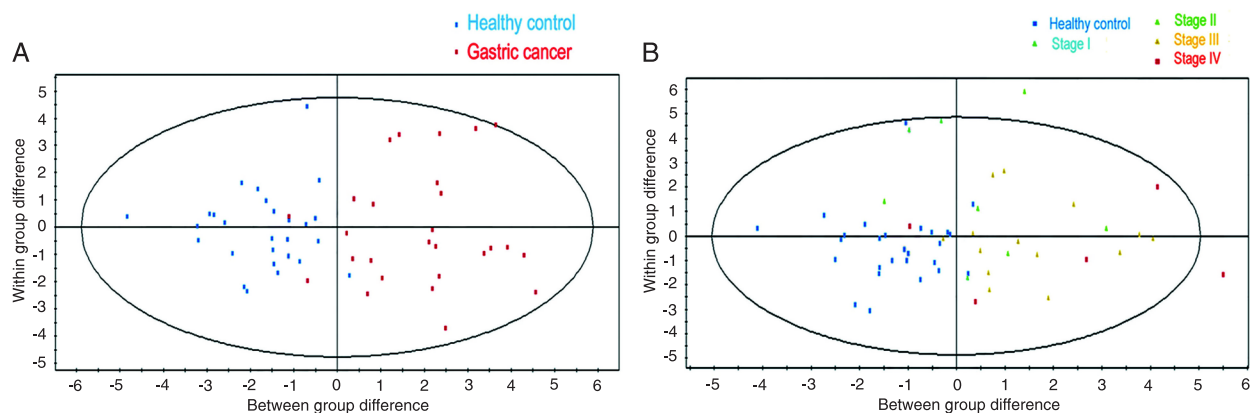


Figure 2. Establishment of orthogonal partial least squares discriminant analysis (OPLS-DA) models. *A* shows the score plot of OPLS-DA modeling for serum metabolomic data. It can be seen that healthy controls tended to cluster to the left, while the gastric cancer patients generally clustered to the right. The OPLS-DA model demonstrated satisfactory modeling and achieved a fairly distinct separation between the two groups. *B* illustrates the score plot of normal control and different TNM stages and the model could not separate gastric cancer patients in different TNM stages although separation trends could be observed between the gastric cancer group and non-gastric cancer group.

membrane structural purposes and energy production, such as β -oxidation. Fatty acid synthase is highly expressed in many human cancers (27), leading to transcriptionally up-regulated fatty acid synthesis. The production of polyunsaturated fatty acids has also been associated with tumor cell proliferation, apoptosis, and tumor angiogenesis (28,29). The present findings indicate that gastric cancer cells consume a lot of fatty acids to meet the demand of cell membrane synthesis and energy production, resulting in the depletion of serum fatty acids.

The levels of cholesterol and intermediate products of cholesterol synthesis such as cholesta-3,5-diene and cholest-5-en-3-ol were significantly decreased. Previous studies have confirmed increased levels of cholesterol in colorectal cancer tissue (18) and serum cholesterol levels were significantly lower in colon cancer, stomach cancer, and oral cancer patients (30). Many experimental studies have observed a positive association of tumor cell proliferation with cholesterol synthesis, which is needed for new membrane biogenesis (31). The present result suggests that a large amount of cholesterol is utilized for biomembrane synthesis and serum cholesterol may be ingested by gastric cancer cells. Promotion of glycolysis and disruption of the TCA cycle occur in gastric cancer cells and the intermediate products such as fumaric acid and α -ketoglutaric acid prominently increase in the metabolic profile of gastric cancer tissue (19). One crucial observation of the present study is that fumaric acid levels significantly decreased in the gastric cancer serum, suggesting that marker metabolites in serum may not change in parallel with metabolic perturbations of tumor tissue. This observation may indicate an overutilization of the organic acid and that tumor cells adopt a complementary pathway to scramble for energy-rich metabolites to 'fuel' tumor growth. Increased levels of adenosine, monophosphates, xanthine, and hy-

poanthine accompanied by a decrease of uric acid were found in colorectal cancer (14) while adenine was found to be about 1.93 times higher in concentration in gastric cancer patients compared to healthy controls in the present study. This suggests that the highly proliferative nature of cancer cells requires large quantities of these molecules for transmission of ATP and synthesis of nucleic acids. Our current study indicates that adenine, a compound for nucleotide synthesis, may be an indicator of abnormal cell proliferation.

With the use of highly sensitive metabolomic techniques, we were able to identify a metabolic signature in gastric cancer serum with multivariate and univariate statistical significance. The unique metabolic profiles generated by GC/MS revealed metabolic perturbations of amino acids, carbohydrate, fatty acids, cholesterol, and nucleotide. The results of this study also indicate the potential of this sufficiently robust profiling approach for the detection of gastric cancer. Metabolites such as valine, sarcosine, hexanedioic acid, and cholesterol could be possibly potential biomarkers and need to be validated. A limitation of the current study was that many differential endogenous biomarkers as well as macromolecular metabolites are worth exploring further and yet to be identified. Serum metabolic profile has great potential in detecting gastric cancer and helping to understand its underlying mechanisms.

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