OncoTargets and Therapy

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ORIGINAL RESEARCH

Discovering Biomarkers in Peritoneal Metastasis of Gastric Cancer by Metabolomics

This article was published in the following Dove Press journal:
OncoTargets and Therapy

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Background and Objective: Metabolomics has recently been applied in the field of oncology. In this study, we aimed to use metabolomics to explore biomarkers in peritoneal metastasis of gastric cancer.

Methods: Peritoneal lavage fluid (PLF) of 65 gastric cancer patients and related clinical data were collected from the First Hospital of Jilin University. The metabolic components were identified by liquid chromatography-mass spectrometry (LC-MS). Total ion current (TIC) spectra, principal component analysis (PCA), and the Student's *t*-test were used to identify differential metabolites in PLF. A support vector machine (SVM) was used to screen the differential metabolites in PLF with a weight of 100%. Cluster analysis was used to evaluate the similarity between samples. Receiver operating characteristic (ROC) curve analysis was used to assess the diagnostic ability of the metabolites. Univariate and multivariate logistic regression analyses were used to identify potential risk factors for peritoneal metastasis of gastric cancer.

Results: We found the differential levels of PLF metabolites by LC-MS, TIC spectra, PCA and the *t*-test. Cluster analysis showed the co-occurrence of metabolites in the peritoneal metastasis group ($p<0.05$). ROC analysis showed the diagnostic ability of metabolites ($p<0.05$). Univariate and multivariate logistic regression analyses showed the potential independent risk factors for peritoneal metastasis in gastric cancer patients ($p<0.05$).

Conclusion: Through the statistical analysis of metabolomics, we found that TG (54:2), G3P, α-aminobutyric acid, α-CEHC, dodecanol, glutamyl alanine, 3-methylalanine, sulfite, CL (63:4), PE-NMe (40:5), TG (53:4), retinol, 3-hydroxysterol, tetradecanoic acid, MG (21:0/0:0/0:0), tridecanoic acid, myristate glycine and octacosanoic acid may be biomarkers for peritoneal metastasis of gastric cancer.

Keywords: gastric cancer, metabolomics, peritoneal metastasis, diagnosis

Introduction

Gastric cancer, one of the most common malignant tumours after lung cancer and liver cancer, occurs in the upper digestive tract.[1,2] Approximately 20% of gastric cancer patients are diagnosed with peritoneal metastasis before surgery.[3] More than half of advanced gastric cancer patients have peritoneal metastasis after surgery, which leads to poor prognosis.[3] The 5-year survival rate of patients with positive peritoneal lavage cytology is approximately 12%, and the median survival time of patients with peritoneal metastasis is approximately 6–7 months.[4,5] However, the sensitivity of gastric cancer peritoneal metastasis imaging and tumour marker detection is low. Therefore, the need to find sensitive diagnostic markers of gastric cancer with peritoneal metastasis is urgent.

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http://doi.org/10.2147/OTT.S245663

Metabolomics can accurately discover the basic characteristics and material basis of life activities.[6–10] It can enlarge small changes in the genome and proteome, reflecting the endpoint of gene functional activities and changes in the biochemical phenotype of organisms, and is also directly related to the final effect of these activities.[11] Therefore, metabolomics is considered the final direction of omics research.[12] Yue et al[13] found 43 arginine metabolites helpful for the accurate diagnosis of small cell lung cancer by LC-MS. Zhang et al[14] found that the levels of 9 metabolites, such as glutamic acid and glutamine, were significantly different in the cancerous tissues and normal tissues of 40 patients with oesophageal squamous cell carcinoma by LC-MS and that this difference was closely related to the pathological characteristics of lymph node metastasis and postoperative survival time. However, the application of metabolomics to peritoneal metastasis of gastric cancer is still unclear.

In this study, we collected the PLF of 65 gastric cancer patients and related clinical data from the First Hospital of Jilin University. The metabolic components of the PLF were determined by LC-MS. TIC spectra, PCA, and the *t*-test were used to identify differential metabolites in PLF samples. An SVM was used to screen the differential metabolites with a weight of 100%. Cluster analysis was used to evaluate the similarity between samples. ROC analysis was used to assess the diagnostic ability of the metabolites. Univariate and multivariate logistic regression analyses were used to identify potential risk factors for peritoneal metastasis in gastric cancer patients. We found the differential levels of PLF metabolites by LC-MS, TIC spectra, PCA and the *t*-test. Cluster analysis showed the co-occurrence of metabolites in the peritoneal metastasis group. ROC analysis showed the diagnostic ability of metabolites. Univariate and multivariate logistic regression analyses showed the potential independent risk factors for peritoneal metastasis in gastric cancer patients. In the end, we found that TG (54:2), G3P, α-aminobutyric acid, α-CEHC, dodecanol, glutamyl alanine, 3-methylalanine, sulfite, CL (63:4), PE-NMe (40:5), TG (53:4), retinol, 3-hydroxysterol, tetradecanoic acid, MG (21:0/0:0/0:0), tridecanoic acid, myristate glycine and octacosanoic acid may be biomarkers for peritoneal metastasis of gastric cancer.

Patients and Methods

Patient Source and Sample Collection

From August 2018 to December 2018, 62 patients with gastric cancer (45 males and 17 females) underwent laparoscopic exploration or laparoscopic radical gastrectomy. Informed consent was obtained from patients and their families before surgery, and the study was approved by the Ethics Committee of The First Hospital of Jilin University, together with confirmation of patient written informed consent, and compliance with the Declaration of Helsinki. The inclusion criteria for patients with gastric cancer were as follows: samples obtained from the First Hospital of Jilin University; a pathological diagnosis of gastric cancer; an age of no more than 75 years; the presence of primary tumours; good liver function, heart function, renal function and bone marrow function; and no other serious immunosuppressive diseases or simultaneous malignant tumours. The exclusion criteria for patients with gastric cancer were as follows: congenital diseases; poor general condition; severe organic diseases; previous radical or palliative surgery, radiotherapy, chemotherapy or biotherapy; complications of gastrointestinal haemorrhage; perforation; and serious infection.

Two hundred millilitres of lavage fluid were collected from the subphrenic space, subhepatic space and Douglas fossa of 62 patients with gastric cancer.

Exfoliative Cytology

After centrifugation, the supernatants of PLF samples from 62 gastric cancer patients were discarded, and the precipitates were retained. After smearing, the exfoliative cytology was detected by pasteurization.

qRT-PCR

The total RNA was extracted from the PLF samples by TRIzol™ reagent (Invitrogen Thermo Science) and then reverse transcribed to produce cDNA. Finally, the cDNA was amplified by PCR. Table 1 lists the sequences of the primers used. We used TransStart TIP Green qPCR SuperMix (cat. No. AQ131, TransGen Biotech Co., Ltd., Beijing, China) for RT qPCR analysis. The analysis mixture contained 0.2 g of DNA, 0.2 M forward primers, 0.2 M reverse primers, and 10 μL of qPCR SuperMix in a total volume of 20 μL. The conditions were as follows: 94.0°C for 30 seconds, followed by 40 cycles of 94.0°C for 5 seconds and 60.0°C for 30 seconds. Three replicates of each sample were analysed in a CFX 96 Touch Real-time Polymerase Chain Reaction Detection System (Bio-Rad Laboratory Ltd.). The relative expression of actin and CEA in the different experimental groups was calculated by the 2-ΔΔCq method.

Table 1 CEA mRNA Results of Peritoneal Lavage

	Invasion of Serosa	No Invasion of Serosa	Total
CEA Positive	16	9	25
CEA Negative	8	29	37
Total	24	38	62

LC-MS

Four microliters of each sample were chromatographed onto a C18 reverse-phase column (2.1 × 100 mm, 1.8 μm, Waters, Milford, MA) using an Agilent 1290 Infinity liquid chromatography system (Agilent, Santa Clara, CA). During chromatographic separation, the column was maintained at 40°C. Elution was performed at a flow rate of 400 μL/min, with 5% acetonitrile in water for the first 2 min, a linear gradient of 5% to 95% acetonitrile over the next 15 min, and 95% acetonitrile in water for the last 2 min. Both acetonitrile and water contained 0.1% formic acid.

Statistical Analysis

The *t*-test was carried out to analyse the positive ion mode and negative ion mode data, and differential metabolites were screened by $p<0.05$. An SVM was used to precisely identify different metabolites. The BRB array tool was used for cluster analysis to uncover the distributions of poorly metabolised foreign bodies in patients with gastric cancer.[15] ROC curves were drawn with SPSS based on a series of different binary classifications (demarcation value or determination threshold) as well as the true-positive rate (sensitivity), the ordinate and the false-positive rate (1-specificity) according to the abscissa. Univariate and multivariate logistic regression analyses were used in SPSS to identify risk factors for peritoneal metastasis of gastric cancer.

Results

Different Levels of Metabolites in the PLF of Gastric Cancer Patients

According to the pathological data, the patients were divided into two groups. Patients in group A had serous invasion, and those in group B did not have serous invasion. According to the results of exfoliative cytology of the PLF, findings during surgery and pathological data, a group positive for peritoneal metastasis (group C) and a group negative for peritoneal metastasis of gastric cancer (group D) were found. According to qRT-PCR analysis of CEA mRNA in PLF, patients were divided into a CEA-positive group (group E) and a CEA-negative group (group F; Table 1).

Mass spectral data of the metabolites were obtained by LC-MS. Moreover, we found a difference in the expression of metabolites between groups A and B, groups C and D, and groups E and F by the TIC spectra (Figure 1). Differences in the levels of metabolites between groups A and B, groups C and group D, and groups E and group F were further verified by PCA (Figure 2).

The results of a *t*-test to analyse data in positive and negative ion mode found 213 differential metabolites in positive ion mode (supplemental material Table 1) and 174 differential metabolites in negative ion mode between groups A and B (supplemental material Table 2). In addition, 190 differential metabolites (supplemental material Table 3) between groups C and D were screened under cation mode, and 115 differential metabolites (supplemental material Table 4) were screened in negative ion mode. Screening of groups E and F revealed 501 differential metabolites in positive ion mode (supplemental material Table 5) and 246 differential metabolites in negative ion mode (supplemental material Table 6).

Differential Metabolites in the PLF of Patients with Peritoneal Metastasis Screened with a Weight of 100%

We used an SVM to carry out discrimination analysis to distinguish the patients in each group and further screened differential metabolites with a weight of 100%. Four differential metabolites in positive ion mode and 4 differential metabolites in negative ion mode were identified between groups A and B (Table 2). Two differential metabolites in positive ion mode and 2 differential metabolites in negative ion mode were identified between groups C and D (Table 3). Ten differential metabolites in positive ion mode and 4 differential metabolites in negative ion mode were identified between groups E and F (Table 4). The mass to charge ratios (M/Z) were used to screen the human metabolome database (HMDB) to find the corresponding substances. The differential metabolites between groups A and B were sulfite, TG (54:2), G3P, α-aminobutyric acid, α-CEHC, dodecanol, alanine glutamyl, and 3-methylpropionic acid (Table 2). The differential metabolites between groups C and D were sulfite, G3P, Cl (63:4), and PE-NMe (40:5) (Table 3). The differential metabolites between groups E and F were sulfite, G3P, TG (54:2), α-aminobutyric acid, α-CEHC, glutamyl alanine,

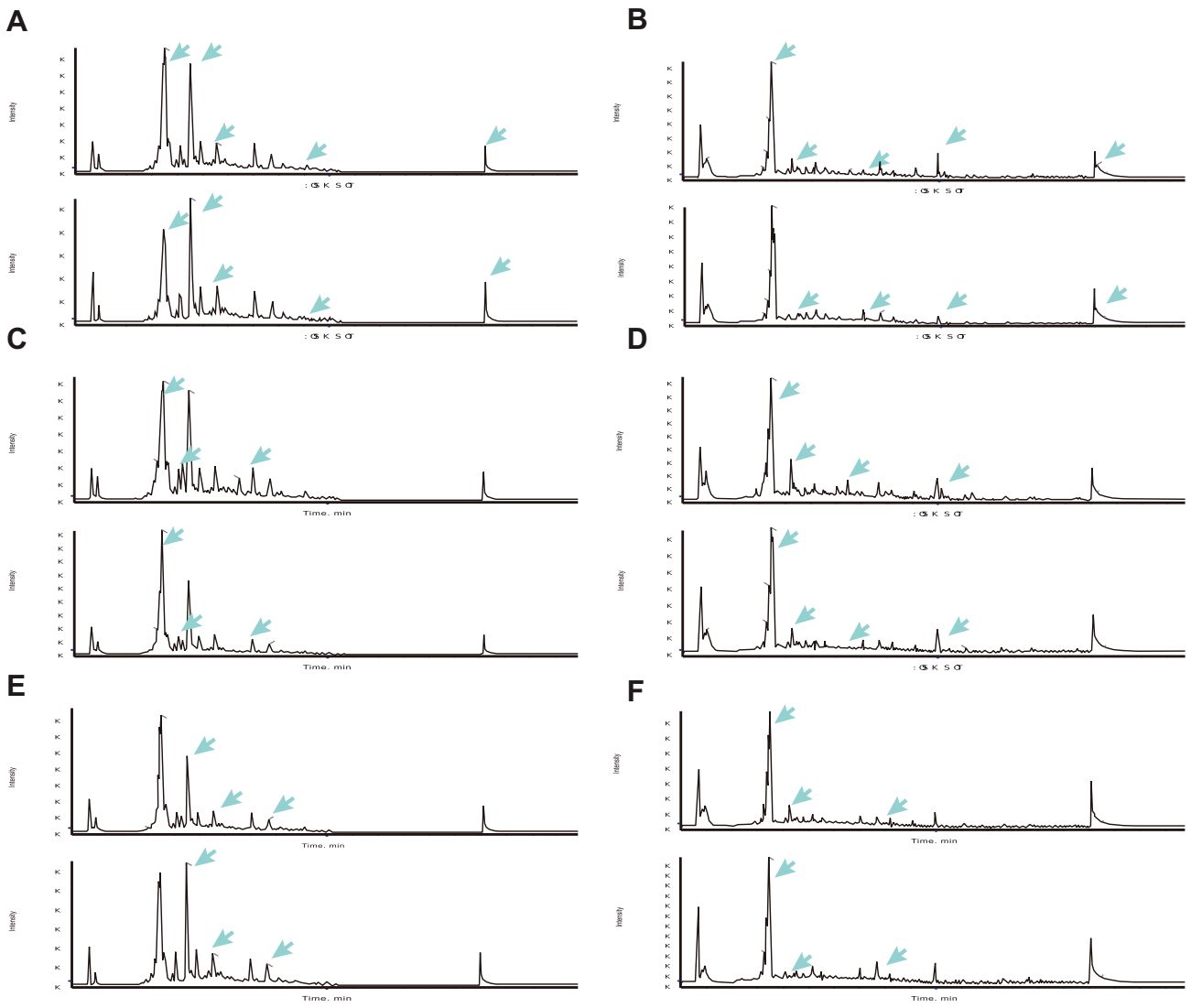

Figure 1 TIC spectra of the different groups. TIC analysis of groups A and B (**A** and **B**), groups C and D (**C** and **D**), and groups E and F (**E** and **F**).

retinol, 3-hydroxysterol, tetradecanoic acid, MG (21:0/0:0/0:0), tridecanoic acid, myristate glycine, octadecanoic acid, and TG (53:4) (Table 4).

Cluster analysis showed the co-occurrence of metabolites in groups A, C and E. As shown in Figure 3, the levels of TG (54:2), sulfite, G3P, α-aminobutyric acid, α-CEHC, glutamyl alanine and 3-methylpropionic acid in group A were similar. In addition, the levels of CL (63:1), PE-NMe (10:5), sulfite and G3P in group C were similar. The levels of sulfite, TG (54:2), G3P, α-aminobutyric acid, pyrite, TG (53:4), retinal, α-CEHC, 3-hydroxysterol, tetradecanoic acid, Mg (21:0/0:0/0:0), tridecanoic acid, and octadecanoic acid in group E were similar.

Differential Metabolites Have Good Diagnostic Ability for Peritoneal Metastasis in Gastric Cancer Patients

ROC analysis showed that sulfite, TG (54:2), G3P, α-aminobutyric acid, α-CEHC, dodecanol, glutamyl alanine and 3-methylalanine had good diagnostic ability in groups A and B (Table 5; Figure 4). In groups C and D, sulfite, G3P, Cl (63:4), and PE-NMe (40:5) had good diagnostic ability (Table 6; Figure 4). In groups E and F, sulfite, G3P, TG (54:2), α-aminobutyric acid, TG (53:4), α-CEHC, glutamyl alanine, retinol, 3-hydroxysterol, tetradecanoic acid, MG (21:0/0:0/0:0), tridecanoic acid, myristate glycine and octacosanoic acid had good diagnostic ability (Table 7; Figure 4).

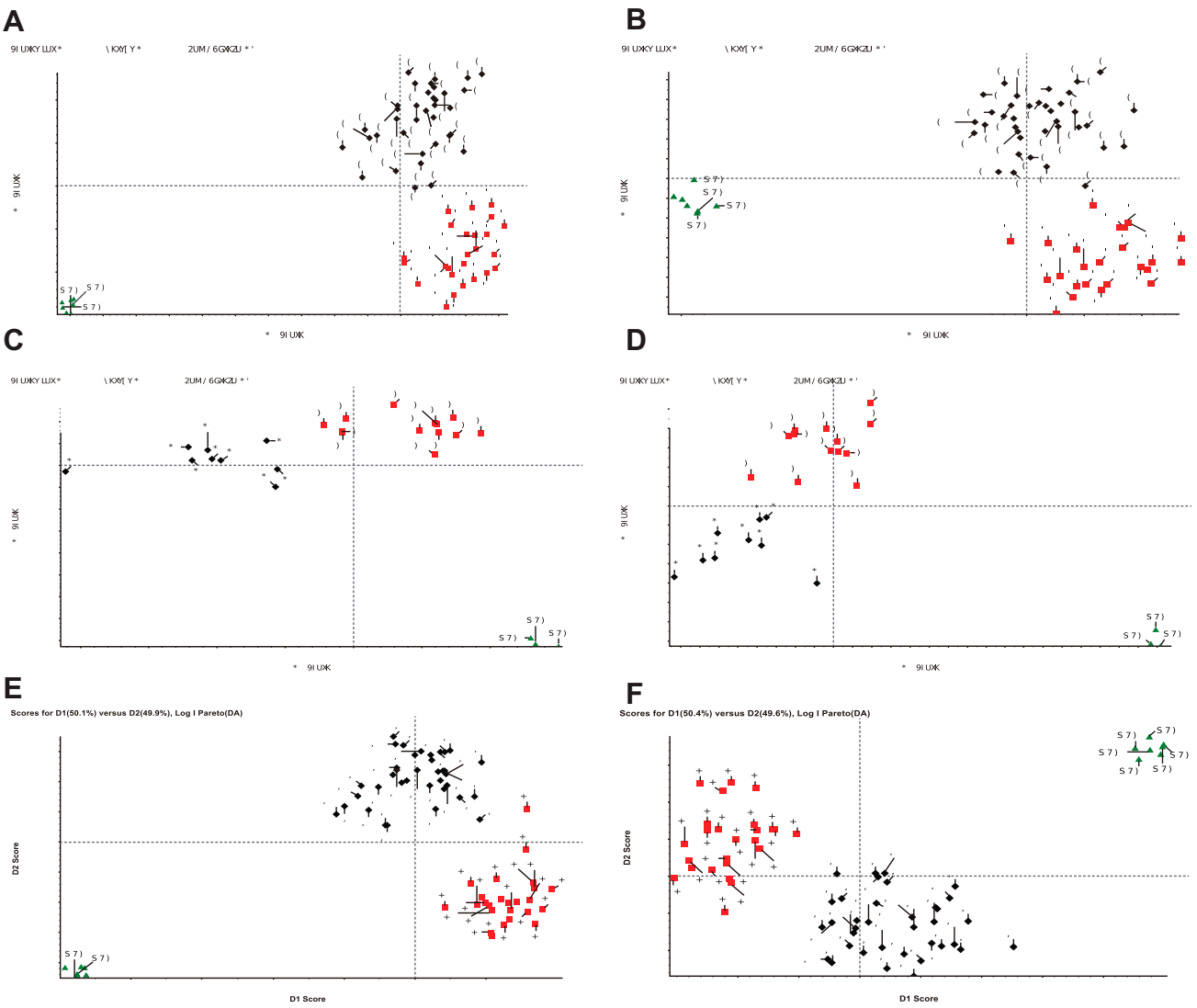

Figure 2 PCA of the different groups. PCA of groups A and B (**A** and **B**), groups C and D (**C** and **D**), and groups E and F (**E** and **F**).

Metabolites are Independent Risk Factors for Peritoneal Metastasis in Gastric Cancer Patients

Univariate regression analysis showed that sulfite, TG (54:2), G3P, α-aminobutyric acid, α-CEHC, dodecanol, glutamyl alanine and 3-methylpropionic acid were risk factors for peritoneal metastasis of gastric cancer in groups A and B (Table 8). Sulfite, G3P, Cl (63:4), and PE-NMe (40:5) were risk factors for peritoneal metastasis of gastric cancer in groups C and D (Table 9). Sulfite, glyceraldehyde 3-phosphate, TG (54:2), α-aminobutyric acid, TG (53:4), α-CEHC, glutamyl alanine, retinol, 3-hydroxysterol, tetradecanoic acid, Mg (21:0/0:0/0:0), tridecanoic acid, myristate glycine and octacosanoic acid were risk factors for peritoneal metastasis of gastric cancer in groups E and F (Table 10).

Multivariate regression analysis showed that sulfite, TG (54:2), G3P, α-aminobutyric acid, α-CEHC, dodecanol, glutamyl alanine and 3-methylalanine were independent risk factors for peritoneal metastasis of gastric cancer in groups A and B (Table 11). Sulfite, CL (63:4), and PE-NMe (40:5) were independent risk factors for peritoneal metastasis of gastric cancer in groups C and D (Table 12). Sulfite, G3P, TG (54:2), α-aminobutyric acid, TG (53:4), α-CEHC, glutamyl alanine, retinol, 3-hydroxysterol, tetradecanoic acid, MG (21:0/0:0/0:0), tridecanoic acid, myristate glycine and octacosanoic acid were independent risk factors for peritoneal metastasis of gastric cancer in groups E and F (Table 13).

Discussion

Gastric cancer, which has a high incidence and poor prognosis, seriously threatens human health.[2,16,17] Peritoneal

Table 2 Groups A and B Screened Out Different Metabolites

Differential Substance	Mass Charge Ratio (m/z)	Retention Time	*p*	Group A Responsiveness	Group B Responsiveness	Weight
Sulfite	116.9282	16.23	8.46E-05	151.6±20.7	120.6±32	100%
TG (54:2)	289.937	18.62	2.19E-11	397.6±94.1	238.8±58.6	100%
G3P	190.9289	19.98	1.32E-09	232.2±49	104.6±78	100%
α - aminobutyric acid	181.8979	16.27	5.76E-13	71.6±17.2	29.6±17.9	100%
α-CEHC	279.1593	4.65	3.75E-06	1324.9±630.9	725.7±287.6	100%
dodecanol	228.2326	8.03	3.42E-06	1411.6±354.9	1008.1±264.5	100%
Glutamyl alanine	302.1447	5.44	6.12E-13	1805.8±369.9	903±385.4	100%
3-methylpropionic acid	106.9899	16.15	9.49E-10	1220.3±471.9	437.6±374	100%

Table 3 Groups C and D Screened Out Different Metabolites

Differential Substance	Mass Charge Ratio (m/z)	Retention Time	*p*	Group C Responsiveness	Group D Responsiveness	Weight
CL(63:4)	685.4379	16.1	0.0005	845.2±339	219.1±361.1	100%
PE-NMe(40:5)	808.5853	9.75	0.00045	3907.2±2355.3	535±534.3	100%
Sulfite	116.9282	16.26	2.38E-06	683.7±70.3	365.2±156.1	100%
G3P	190.9289	19.98	1.72E-07	235.9±43.3	72.5±54.9	100%

Table 4 Groups E and F Screened Out Different Metabolites

Differential Substance	Mass Charge Ratio (m/z)	Retention Time	*p*	Group E Responsiveness	Group F Responsiveness	Weight
Sulfite	116.9282	16.26	3.05E-21	698.8±83.6	310±115.2	100%
G3P	190.9289	19.98	3.12E-22	197.2±36.6	59±34.2	100%
TG(54:2)	289.937	18.62	3.03E-13	316.2±73	161.1±58	100%
α - aminobutyric acid	181.8979	16.27	5.88E-14	72.9±16.7	28.5±18.2	100%
TG(53:4)	476.4127	6.13	3.61E-10	181.3±56.7	62.3±64.3	100%
Alpha-CEHC	279.1593	4.65	1.45E-15	1724.8±444.2	723.9±292.1	100%
Glutamyl alanine	302.1447	5.44	5.36E-12	1935.8±224.8	1064.5±472.9	100%
Retinal	596.4488	8.81	3.78E-11	105.4±47	26.1±30.5	100%
3-hydroxysterol	432.3875	6.07	1.21E-09	568.5±162.9	233.8±190.3	100%
Tetradecenoic acid	268.2266	7.09	1.42E-09	276.4±87	132.7±70.9	100%
MG(21:0/0:0/0:0)	242.2115	5.76	1.54E-09	875.1±212.3	501.6±195.7	100%
Tridecanoic acid	214.2174	7.54	1.16E-09	690.6±180.2	390.5±147	100%
Myristoyl glycine	308.2202	7.09	5.15E-10	372.8±112.3	179.1±92.9	100%
Octadecanoic acid	254.248	4.6	2.27E-10	510.1±93.2	337.2±83.9	100%

metastasis is an important factor in the death of gastric cancer patients. Most patients diagnosed with peritoneal metastasis of gastric cancer have already had cancerous ascites and metastasis and lost the opportunity for treatment.[18,19] However, there are no obvious symptoms or signs of the early stage of peritoneal metastasis of gastric cancer, and conventional ultrasound, CT and other detection methods cannot diagnose peritoneal metastasis precisely. Therefore, the need to find more sensitive diagnostic markers for peritoneal metastasis of gastric cancer is urgent. Compared with some single molecular markers, metabolic diagnostic markers are more comprehensive and accurate.[20–33] Metabolomics plays an important role in the screening of tumour biomarkers. A large number of studies have found potential biomarkers of gastric cancer, colorectal cancer, oesophageal cancer, liver cancer, ovarian

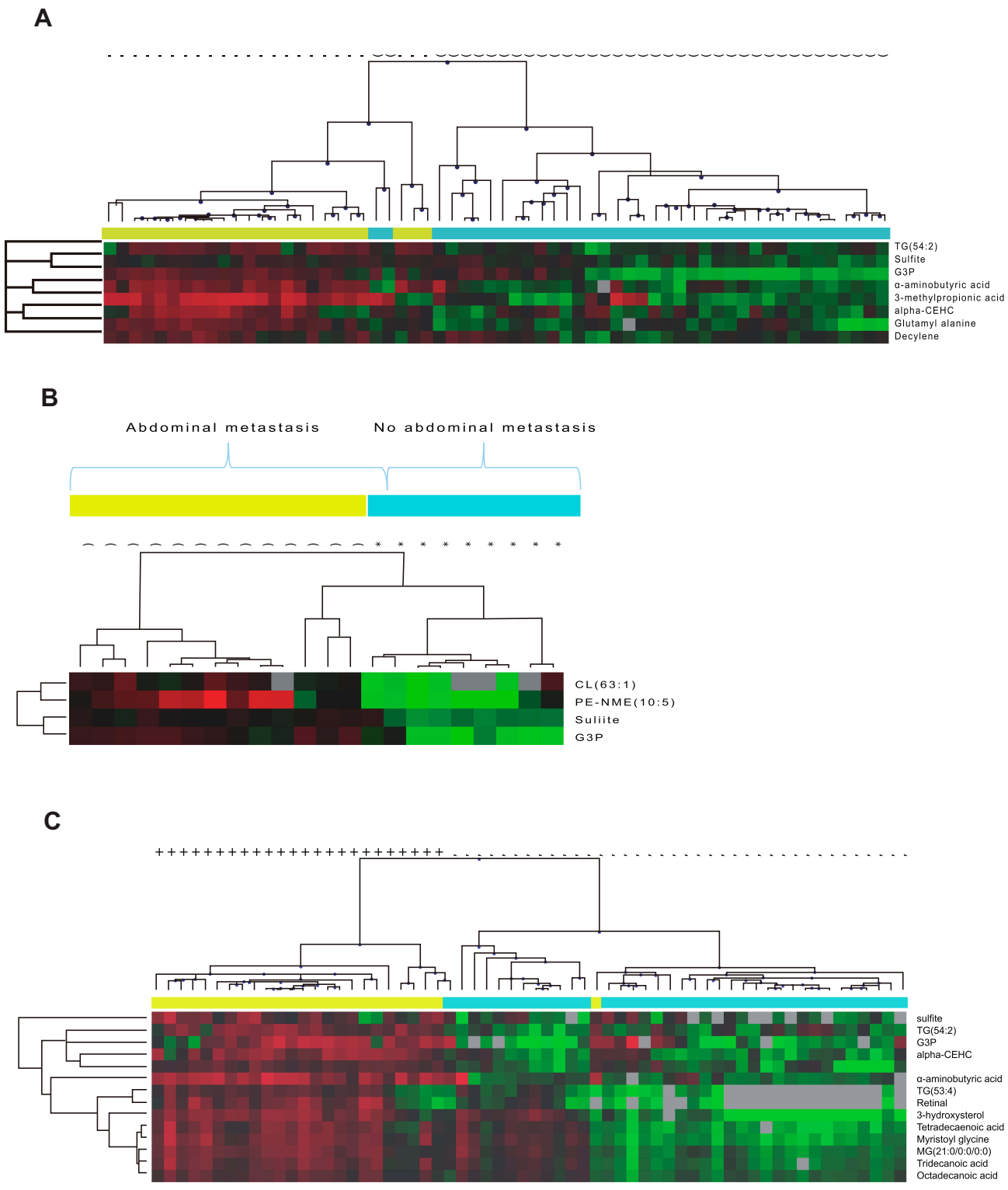

Figure 3 Cluster analysis of the different groups. Cluster analysis of groups A and B (**A**), groups C and D (**B**), and groups E and F (**C**).

cancer and other malignant tumours in blood, urine, tissue and other samples through metabolomics.[34–37] In this study, we found that TG (54:2), G3P, α-aminobutyric acid, α-CEHC, dodecanol, glutamyl alanine, 3-methylalanine, sulfite, CL (63:4), PE-NMe (40:5), TG (53:4), retinol, 3-hydroxysterol, tetradecanoic acid, MG (21:0/0:0/0:0), tridecanoic acid, myristate glycine and octacosanoic acid have good diagnostic ability for gastric cancer metastasis and are

Table 5 Area Under ROC Curve of Differential Metabolites in Groups A and B

Differential Metabolites	Area	Standard Error	Sig.	95% CI	
				Lower	Upper
Sulfite	0.782	0.06	0	0.664	0.899
TG(54:2)	0.742	0.072	0.001	0.601	0.884
G3P	0.879	0.044	0	0.794	0.965
α - aminobutyric acid	0.955	0.029	0	0.897	1
Alpha-CEHC	0.786	0.062	0	0.665	0.907
Decylene	0.806	0.059	0	0.69	0.922
Glutamyl alanine	0.956	0.023	0	0.911	1
3-methylpropionic acid	0.868	0.05	0	0.771	0.966

potential independent risk factors for gastric cancer patients with peritoneal metastasis.

Metabonomics is a hot topic in recent years. It has been reported that glucose metabolism plays a key role in the growth of gastric cancer.[38] However, we found that some lipid metabolites play a key role in peritoneal metastasis of gastric cancer, which may be caused by different pathological processes of gastric cancer. Sulfite is mainly produced from the metabolism of sulfur-containing amino acids (cysteine, methionine) in the human body.[39] Current research shows that the level of homocysteine in the sera of patients with oesophageal cancer, gastric cancer, colorectal cancer and other malignant tumours is significantly increased. Sulfite was found to have an antitumour effect by affecting cell cycle arrest, apoptosis, invasion and colony formation in SH-SY5Y tumour cells.[40] Xu et al[41] used Mendel randomization to analyse 27 case–control studies on the relationship between the level of blood homocysteine and the risk of gastric cancer and proved that the level of blood homocysteine had a significant impact on the risk of gastric cancer. An increase in sulfite content in peritoneal lavage fluid may indicate an increase in homocysteine levels, which is consistent with previous research results.

G3P is an important metabolite of glycolysis and the pentose phosphate pathway.[42] Glycolysis is the main energy source of tumour cells.[43,44] The pentose phosphate pathway not only provides 5-ribonucleic acid for the rapid proliferation of tumour cells; in addition, the p53 protein has been reported to inhibit the pentose phosphate pathway by binding glucose-6-phosphate dehydrogenase. In tumour cells, p53 is mutated, enhancing the pentose phosphate pathway.[45] Studies have shown that G3P plays an important role in tumour cell survival, tumour angiogenesis, tumour cell gene expression regulation and mRNA post-transcriptional regulation.[46]

Lipid metabolism plays an important role in cancer.[47] TG (54:2), PE-NMe, Cl (63:4), and TG (53:4) are triglycerides. MG (21:0/0:0/0:0:0:0) belongs to the glycerol monoester family. Myristate glycine, tridecanoic acid, octadecanoic acid, 3-methylpropionic acid and tetradecanoic acid are fatty acids. Dodecanol is a fatty alcohol in body fluids. It has been proven that the consumption of lipids and the levels of lipid metabolites are increased in gastric cancer, while the

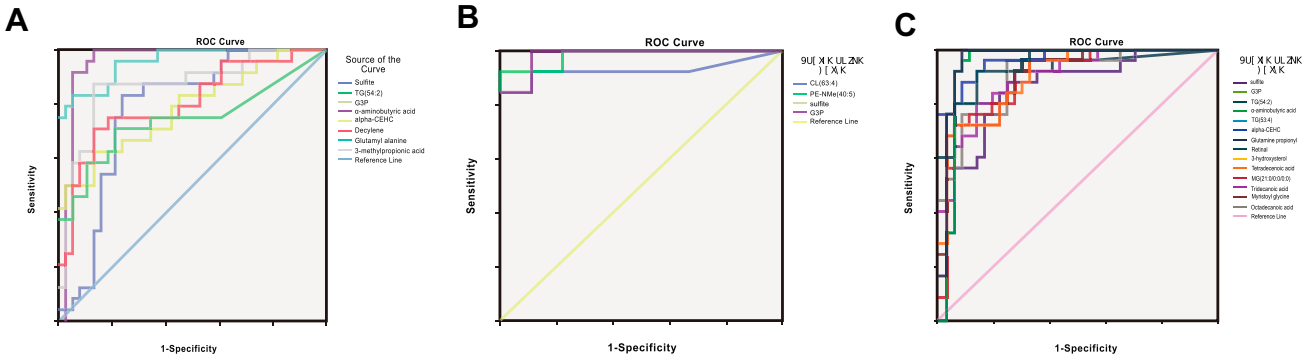

Figure 4 ROC curve analysis of metabolites in the different groups. ROC curve analysis of differential metabolites between groups A and B (**A**), groups C and D (**B**), and groups E and F (**C**).

Table 6 Area Under ROC Curve of Differential Metabolites in Groups C and D

Differential Metabolites	Area	Standard Error	Sig.	95% CI	
				Lower	Upper
Sulfite	0.906	0.09	0.002	0.73	1
G3P	0.983	0.022	0	0.939	1
CL (63:4)	0.85	0.101	0.006	0.653	1
PE-NMe(40:5)	0.983	0.022	0	0.94	1

Table 7 Area Under ROC Curve of Differential Metabolites in Groups E and F

Differential Metabolites	Area	Standard Error	Sig.	95% CI	
				Lower	Upper
Sulfite	0.859	0.048	0	0.765	0.954
G3P	0.874	0.046	0	0.784	0.963
TG(54:2)	0.949	0.025	0	0.901	0.997
α - aminobutyric acid	0.963	0.028	0	0.909	1
TG(53:4)	0.909	0.037	0	0.837	0.981
Alpha-CEHC	0.964	0.02	0	0.924	1
Glutamyl alanine	0.968	0.024	0	0.92	1
Retinal	0.913	0.04	0	0.835	0.99
3-hydroxysterol	0.902	0.038	0	0.827	0.976
Tetradecenoic acid	0.904	0.037	0	0.831	0.977
MG(21:0/0:0/0:0)	0.899	0.04	0	0.822	0.977
Tridecanoic acid	0.909	0.036	0	0.838	0.98
Myristoyl glycine	0.904	0.038	0	0.83	0.978
Octadecanoic acid	0.905	0.038	0	0.83	0.98

plasma levels of lipids are decreased in gastric cancer.[48,49] Fatty acids can be used as a diagnostic marker of gastric cancer.[50] In this study, we found that an increase in these lipid metabolites may indicate that lipid metabolism in the peritoneal environment in gastric cancer with peritoneal metastasis has changed significantly.

Table 9 Univariate Logistic Regression for Group C and Group D

Differential Metabolites	B	S.E.	Wals	*p*	95% CI	
					Lower	Upper
CL(63:4)	0.004	0.002	7.23	0.007	0.895	1.546
PE-NMe(40:5)	0.003	0.002	4.628	0.031	0.989	2.345
Sulfite	0.015	0.005	7.333	0.007	0.502	1.992
G3P	0.055	0.032	2.91	0.088	0.325	2.445

Amino acid metabolism and cholesterol metabolism play an important role in the occurrence and development of cancer. Glutamyl alanine is a naturally occurring dipeptide composed of glutamate and alanine. α-Aminobutyric acid is a nonessential amino acid mainly in the cytoplasm that is mainly produced from the catabolism of methionine, threonine and serine. 3-Hydroxysterol is the intermediate of cholesterol biosynthesis. Some studies showed that the levels of serum cholesterol and low-density lipoprotein were lower in patients with gastric cancer metastasis than in normal controls.[51] Retinal, also known as vitamin A aldehyde, is a derivative of retinol after its oxidation. Retinol can be irreversibly oxidized to retinoic acid, which is involved in the regulation of some cellular functions, such as cell growth, proliferation and differentiation. The relationship between retinol intake and blood retinol concentration and the risk of gastric cancer were shown to be controversial in past case-control and cohort studies. Some studies have shown that retinol can reduce the risk of gastric cancer, while others have not found this relationship. A meta-analysis[52] of 31 studies showed a slight negative correlation between retinol intake (with RR = 0.94, 95% CI: 0.87–1.03) or blood retinol level (with RR = 0.87, 95% CI: 0.73–1.05) and the risk of gastric cancer by comparing the highest and lowest intervals of the blood retinol level. Subgroup analysis showed a slight

Table 8 Univariate Logistic Regression for Group A and Group B

Differential Metabolites	B	S.E.	Wals	*p*	95% CI	
					Lower	Upper
Sulfite	0.043	0.013	10.845	0.001	0.003	1.762
TG (54:2)	0.009	0.003	10.491	0.001	0.458	1.078
G3P	0.023	0.006	16.788	0	0.121	2.233
α - aminobutyric acid	0.105	0.024	18.908	0	0.089	2.231
Alpha-CEHC	0.003	0.001	12.344	0	0.212	2.234
Dodecanol	0.004	0.001	13.709	0	1.002	1.007
Glutamyl alanine	0.007	0.002	12.934	0	0.989	3.345
3-hydroxysterol	0.003	0.001	19.937	0	0.502	2.992

Table 10 Univariate Logistic Regression for Group E and Group F

Differential Metabolites	B	S.E.	Wals	p	95% CI	
					Lower	Upper
Sulfite	0.009	0.002	14.183	0	0.325	2.445
G3P	0.056	0.014	16.812	0	0.623	2.762
TG (54:2)	0.032	0.008	16.288	0	0.895	1.546
α - aminobutyric acid	0.104	0.024	19.293	0	0.989	2.345
TG(53:4)	0.027	0.006	17.919	0	0.502	1.992
Alpha-CEHC	0.006	0.002	12.788	0	0.325	2.445
Glutamyl alanine	0.011	0.003	10.324	0.001	0.889	1.231
Retinal	0.047	0.011	17.718	0	1.001	2.233
3-hydroxysterol	0.009	0.002	17.279	0	0.983	1.078
Tetradecenoic acid	0.022	0.005	17.068	0	1.012	1.234
MG(21:0/0:0/0:0)	0.008	0.002	17.66	0	0.001	2.233
Tridecanoic acid	0.011	0.003	17.063	0	0.502	1.992
Myristoyl glycine	0.016	0.004	18.137	0	0.925	1.445
Octadecanoic acid	0.02	0.005	16.757	0	0.088	1.078

Table 11 Multivariate Logistic Regression of Group A and Group B

Differential Metabolites	B	S.E.	Wals	p	95% CI	
					Lower	Upper
Sulfite	0.287	1.639	2.227	0.008	0.523	3.762
TG (54:2)	−0.472	0.549	3.001	0	0.008	1.078
G3P	0.206	0.736	0.045	0	2.001	5.233
α - aminobutyric acid	0.979	0.447	0.102	0.005	1.089	4.231
Alpha-CEHC	0.319	0.155	1.112	0.009	2.344	4.234
Dodecanol	0.953	0.424	3.221	0	5.234	8.23
Glutamyl alanine	0.414	0.573	2.874	0	0.989	2.345
3-hydroxysterol	−0.497	0.581	0.022	0	0.502	1.992

Table 12 Multivariate Logistic Regression of Group C and Group D

Differential Metabolites	B	S.E.	Wals	p	95% CI	
					Lower	Upper
CL(63:4)	0.015	9.005	7.333	0.007	0.623	2.762
PE-NMe(40:5)	−7.596	3.167	5.752	0.016	0	0.078
Sulfite	0.156	4.198	8.342	0.007	1.001	2.233

negative correlation between serum retinol level and gastric cancer risk in only Western countries.

To the best of our knowledge, this is the first study to identify the diagnostic role of metabolites in gastric cancer metastasis. Through our work, we can better help in the search for new methods to detect gastric cancer metastasis. However, the in-depth molecular mechanism has not been fully explored. In the future, we will continue to explore the molecular mechanism of metabolites in vitro and in vivo.

Conclusion

In this study, we discovered the role of metabolites in peritoneal metastasis of gastric cancer. TG (54:2), G3P, α-aminobutyric acid, α-CEHC, dodecanol, glutamyl alanine, 3-methylalanine, sulfite, CL (63:4), PE-NMe (40:5), TG (54:2), TG (53:4), retinol, 3-hydroxysterol, tetradecanoic acid, MG (21:0/0:0/0:0), tridecanoic acid, myristate glycine and octacosanoic acid have good diagnostic ability and are potential markers of peritoneal metastasis in gastric cancer. In the future, we will continue to explore the

Table 13 Multivariate Logistic Regression of Group E and Group F

Differential Metabolites	B	S.E.	Wals	p	95% CI	
					Lower	Upper
Sulfite	0.01	1.259	30.518	0	0.502	1.992
G3P	0.042	7.651	21.012	0	0.925	1.445
TG (54:2)	0.064	2.859	32.242	0	0.088	1.078
α-aminobutyric acid	0.67	3.242	14.166	0.009	1.001	2.233
TG(53:4)	0.052	1.152	24.379	0	0.889	1.231
Alpha-CEHC	0.014	2.248	21.59	0	1.012	1.234
Glutamyl alanine	0.001	5.447	23.234	0	0.895	1.546
Retinal	0.152	6.394	22.084	0	0.989	2.345
3-hydroxysterol	−0.043	2.52	23.55	0	0.502	1.992
Tetradecenoic acid	−0.099	1.844	21.238	0	0.325	2.445
MG(21:0/0:0/0:0)	−0.072	3.518	13.682	0	0.623	1.762
Tridecanoic acid	0.018	1.012	30.986	0	0.983	2.078
Myristoyl glycine	0.117	0.243	22.123	0	1.001	2.233
Octadecanoic acid	0.051	4.979	26.147	0	0.802	1.992

specific molecular mechanism of these metabolites in peritoneal metastasis of gastric cancer.

Abbreviations

PLF, peritoneal lavage fluid; LC-MS, liquid chromatograph-mass spectrometry; TIC, total ion current; SVM, support vector machine; ROC, receiver operating characteristic; G3P, glyceraldehyde-3-phosphate; M/Z, mass to charge ratio; CI, confidence interval; PCA, principal component analysis; TG, triglyceride; MG, monoglyceride; α-EHEC, (S) −3,4-dihydro-6-hydroxy-2,5,7,8-tetramethyl-2H-1-benzopyran-2-propanoic acid.

Ethics Statement

The patient sample comes from the First Affiliated Hospital of Jilin University. All patients have the right of written informed consent, and compliance with the declaration of Helsinki.

Disclosure

The authors declare that they have no conflict of interest.

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