Received: 24 December 2019 | Revised: 17 March 2020 | Accepted: 18 March 2020

DOI: 10.1002/jcla.23333

RESEARCH ARTICLE

WILEY

Identification of potential metabolite markers for colon cancer and rectal cancer using serum metabolomics

Jianping Wu | **Minyi Wu** | **Qianxia Wu**

Department of Clinical Laboratory, The First Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou, China

Correspondence
Qianxia Wu, Department of Clinical Laboratory, The First Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou 310003, Zhejiang, China.
Email: syjinfei100@sina.com; 1715318@zju.edu.cn

Funding information
Natural Science Foundation of China of Zhejiang Province, Grant/Award Number: LGC19H200004

Abstract

Background: To determine the metabolic characteristics of patients with colon cancer (CC) and rectal cancer (RC) using gas chromatography-mass spectrometry (GC-MS)-based metabolomics.

Methods: In this study, serum samples were collected from 22 CC patients and 23 RC patients preoperatively and postoperatively and 45 healthy volunteers (HVs), and subjected to metabolomics analysis by GC-MS. Differential metabolites in the preoperative RC and CC samples and HVs were identified as potential biomarkers and evaluated for their utilities by receiver operating characteristic analyses.

Results: The different metabolic markers between CC and RC patients were identified, which may assist in distinguishing the two types of cancers. The area under the curve (AUC) was 0.805 for combination of d-glucose and d-mannose for CC diagnosis, and 0.889 for combination of 2-aminobutanoic acid, 3-hydroxypyridine, d-glucose, d-mannose, isoleucine, l-tryptophan, urea, and uric acid for RC diagnosis. The combinations of metabolite markers showed a better predictability than CEA and CA199 two commonly used protein markers for CRC diagnosis in clinical practice. Combining the metabolite markers with these two protein markers effectively improved the diagnostic accuracy with the AUC reaching 0.936 and 0.937 for CC and RC diagnosis, respectively.

Conclusions: Metabolic profiles are different in the blood samples between CC and RC patients. The study has established a panel of metabolic markers as a predictive and multiplexing signature for CC and RC diagnosis.

KEYWORDS
biomarker, colon cancer, diagnosis, metabolomics, rectal cancer

Abbreviations: AUC, area under the curve; CC, colon cancer; CRC, colorectal cancer; GC-MS, gas chromatography-mass spectrometry; HVs, healthy volunteers; QC, quality control; RC, rectal cancer; ROC, receiver operating characteristic.

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.
© 2020 The Authors. *Journal of Clinical Laboratory Analysis* published by Wiley Periodicals LLC.

1 | INTRODUCTION

Colorectal cancer (CRC) is currently the third most common cancer worldwide. Occurrence of CRC is related to lifestyle, health management, and other genetic factors.[1,2] Its incidence and mortality have continued to increase in recent years.[3] The 5-year survival rate of CRC is still dismal (only 8%-9%) when cancer had metastasized.[4]

In the Unites States, it was estimated that the colon cancer (CC) ratio was almost 1:1 in men and women, while the rectal cancer (RC) ratio was 1.5:1 in 2015.[5] Because 40% of CC cases tend to be mistakenly classified as RC, it is difficult to obtain definitive numbers of deaths from CC and RC.[6] The overall 5-year survival rate of CRC patients in China was also only 66.3%.[7]

With effective diagnosis methods detecting the CRC at its early stage, the incidence and mortality of CRC could be significantly reduced and a better prognosis could also be obtained because it is still limited to the mucosa and submucosa.[8] The 5-year survival rate could be significantly increased to 90% when the CRC is diagnosed at its early stage and its treatment is performed at the in situ stage of cancer development.[9] In addition, the patterns of spread of lesions are different between RC and CC. When the CRC tumor identity is confirmed by both endoscopic and histopathologic examinations, different strategies shall be employed for RC and CC treatments. Thus, it is important to distinguish between these two types of tumors.

According to the National Comprehensive Cancer Network (NCCN) guideline of colorectal cancer screening, fecal occult blood tests and colonoscopy are the main screening options for CRC.[10] However, these two methods contain the limitation in weak compliance, missing diagnosis, and generating false positives in large-scale CRC screening.[11] At present, non-invasive screening methods involving tumor markers carcinoembryonic antigen (CEA) and sugar chain antigen (CA199) are widely used. However, these two serum biomarkers have relatively poor sensitivity and specificity, thereby producing high false-negative rates. They are inferior for diagnosing asymptomatic patients. In benign diseases such as pancreatitis, cholangitis, and other gallbladder diseases, CA199 level shows different degrees of elevation.[12] Moreover, these two markers cannot be used to differentiate CC and RC. Thus, it is necessary to identify efficient, non-invasive, and specific markers for CC and RC screening.

Metabolomics is a technique used to investigate life phenomena by exploring the changes in endogenous metabolites after stimulation or disturbance by external factors. Because the metabolome is downstream of the genome, transcriptome, and proteome, changes in the metabolome can reflect the real physical status in the functional level. Since the development of metabolomics, it has become increasingly widely used in research on diseases, including studies on disease subtypes, prognostic evaluation, and discovery of diagnostic markers.[13-16]

In this study, metabolomics was used to determine the metabolic differences among RC patients, CC patients, and healthy volunteers (HVs). The differentially expressed metabolites were screened to establish a diagnostic model for RC and CC.

2 | MATERIALS AND METHODS

2.1 | Participants

The trial was approved by the Ethics Committee of the First Affiliated Hospital of Zhejiang University. The informed consents were obtained when the patients were selected to enroll into the study. The study subjects were admitted to the hospital between December 2016 and May 2017 and comprised 22 CC patients and 23 RC patients in the middle-differentiation period. Samples were obtained from all patients preoperatively and postoperatively (3 days after surgery). In addition, 45 age- and sex-matched HVs were also enrolled as a control group and showed no significant changes in routine biochemical and tumor index test results. Fasting blood samples were collected in the morning into coagulation-promoting tubes, and serum was isolated after centrifugation. All samples were stored at −80°C for further analysis. The background characteristics of the study subjects are shown in Table 1.

2.2 | Reagents

Methoxyamine, pyridine, methyl-trimethyl-silyl-trifluoroacetamide (MSTFA), and deionized water were purchased from ANPEL Laboratory Technologies Inc. HPLC-grade methanol and acetonitrile were purchased from Merck. Other reagents employed were analytically pure grade and purchased from Sinopharm Chemical Reagent Co. Ltd.

2.3 | Sample preparation

After thawing in an ice-water bath, the serum samples were vortexed. A 50-μL aliquot of each sample was placed in a 1.5-mL

TABLE 1 Background characteristics of the subjects included in the study

		Healthy volunteers	Colon cancer	Rectal cancer
Number of subjects	Total	45	22	23
	Male	31	15	16
	Female	14	7	7
Age	Range	49-84	49-84	54-80
	mean	66.49	64.41	68.48

Eppendorf tube, and 200 μL of cold methanol containing 20 μg/mL tridecanoic acid was added. After vortexing for 30 seconds and centrifugation at 21 130 *g* for 15 minutes, 200 μL of supernatant was lyophilized in a centrifugal concentrator.

Before analysis, the sample was dissolved in 50 μL of methoxyamine-pyridine solution (20 mg/mL) and oximated for 90 minutes in a 37°C water bath. Silylation was then performed by addition of 40 μL of MSTFA and incubation in a 37°C water bath for 60 minutes. After centrifugation at 21 130 *g* for 15 minutes, 70 μL of supernatant was removed for GC-MS analysis. Quality control (QC) samples containing the same amount of each serum sample were processed in the same way as the actual samples and used to monitor the stability during the analysis.

2.4 | GC-MS analysis

The chromatographic separation was performed on an Agilent 7890A GC coupled to an Agilent 5975B MSD with electron ionization (EI) source. A DB-5 MS column (30 m × 250 μm × 0.25 μm; J&W Scientific) was used for separation with high-purity helium as the carrier gas at a consistent line flow rate of 40.0 cm/s. The initial oven temperature was 70°C for 3 minutes, followed by a temperature increase to 300°C in steps of 5°C/min, and maintenance at 300°C for 10 minutes. A 1-μL aliquot of sample was injected with a split ratio of 10:1 and an inlet temperature of 300°C. The transfer line and ion source temperatures were 280°C and 230°C, respectively. Electron ionization was used for the metabolic profiling analysis with an ionization energy of 70 eV. Mass spectra were acquired in the full scan mode using a mass scan range of 33-600 m/z. The solvent delay time was adjusted to 5.5 minutes, and the scan cycle was set at 0.2 seconds.

The QC samples were used to balance the GC-MS system before analysis of the actual samples. During the batch analysis, a QC sample was inserted every 10 samples to monitor the repeatability and stability of the pretreatment and instrument.

2.5 | Statistical analysis

The peak detection was performed with R version 3.3.2 software. The original data were exported into an online website (http://www.metaboanalyst.ca/) for peak matching by using XCMS for metaboanalyst. The algorithm was used for peak picking with a resolution of 25 ppm and a signal-to-noise threshold (snthr) set to 5. Features that appeared in less than 75% of the samples which underwent the same extraction method were removed (minfrac = 0.75). The peak width range was set to c (5,15), and bw was set to 4 seconds. The generated peak table was retained for multivariate statistical analysis. A principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA) were conducted with Simca-P 11.0 software (Umetrics AB). The differential metabolites were identified by using NIST 17 Mass Spectral Library. Further, the retention time and elution order of compounds were also used to assist in characterization of the compounds by using a database from Prof. Guowang Xu (Institute of Chemical and Physics, Chinese Academy of Sciences). R software was used to perform the Kruskal-Wallis test and receiver operating characteristic (ROC) curve analyses. SPSS 22.0 software (SPSS Inc) was used for binary logistic regression analyses.

3 | RESULTS

3.1 | Analytical characteristics

As a GC-MS instrument can show fluctuation during a runtime, tridecanoic acid was used as an internal standard to examine the stability of the analytical conditions. The retention time and peak area of tridecanoic acid in each QC sample were recorded. No drift of retention time was observed. The QC chart revealed that the internal standard in all QC samples showed small fluctuations (within 2 SD), indicating that the sample pretreatment, derivatization, and instrument analysis protocols adopted in the experiment were stable (Figure S1A).

In the study, a total of 638 peaks were detected, and the peak area was normalized by the internal standard. QC samples were employed to measure the variability of each peak during the analytical sequence. The relative standard deviation (RSD) of each peak was calculated based on the normalized peak area. In the 18 QC samples, 521 peaks had RSD < 30%, and only 38 peaks had RSD > 60%, indicating that most of the detected metabolites were relatively stable during the process (Figure S1B). Finally, 521 peaks were used for data processing.

3.2 | Metabolic characteristics revealed by statistical analysis

After peak extraction and normalization, the PLS-DA, with UV scaling, was performed to discriminate the potential marker candidates among the RC, CC, and HV groups. Preoperative and postoperative samples were investigated separately. The preoperative CC, RC, and HV samples were well discriminated in the PLS-DA score plot (Figure 1A). The cumulative R2Y and Q2 were 0.515 and 0.382, respectively, when the first three components were calculated. It indicated that the two types of patients had different metabolic characteristics. Cross-validation results (the intercepts of R2Y and Q2 are 0.204 and −0.217, respectively) indicated the model were not over-fitted (Figure 1C).

However, the postoperative samples from the three groups appeared to partially overlap in the model (Figure 1B), with R2Y = 0.409, Q2 = 0.16. Cross-validation results (the intercepts of R2Y and Q2 are 0.2 and −0.283, respectively) were showed in Figure 1D. The metabolic profiles of the patients tended to be closer to those of the HVs, suggesting that the levels of some metabolites recovered to the levels similar to HVs after surgery. Peaks with variance importance

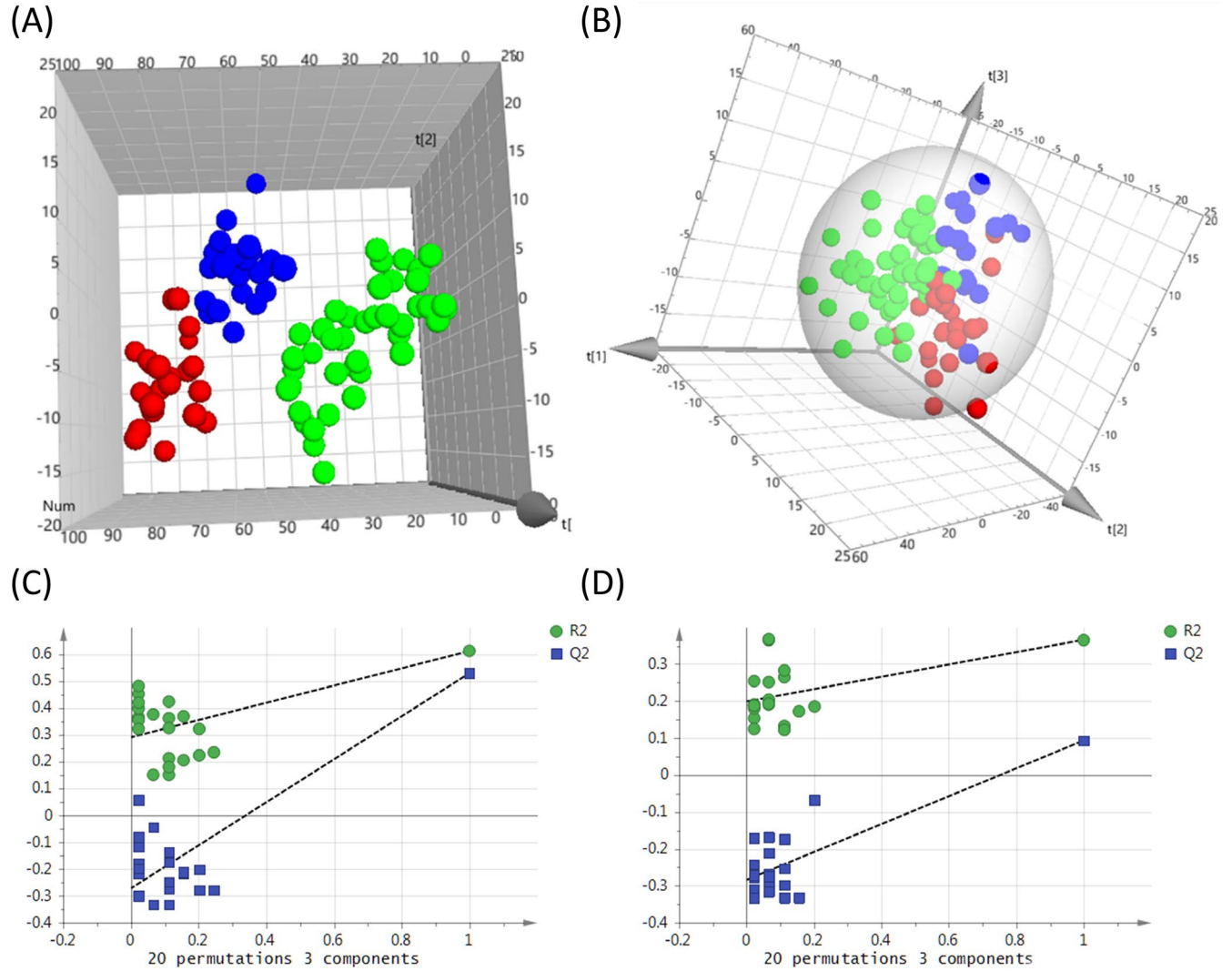

FIGURE 1 PLS-DA score plots for the CC, RC, and HV groups based on the metabolic data. A, Plot before surgery. B, Plot after surgery. C, Permutation plot of model A (before surgery). D, Permutation plot of model B (after surgery). Blue dots represent CC patients, red dots represent RC patients, and green dots represent HVs. The spherical area represents a 95% confidence interval based on Hotelling's T2 statistic

in projection (VIP) >1.0 in the PLS-DA model were selected, and the Kruskal-Wallis test was performed to identify the metabolites with significant differences between the groups.

After comparing the differences in metabolites among the CC, RC, and HV groups by the Kruskal-Wallis test, there were 10 metabolites with significant differences preoperatively and 13 metabolites with significant differences postoperatively ($P < .05$; Table 2). The differential metabolites, such as 2, 3-butanediol, 2-hydroxybutyric acid, isoleucine, L-alanine, and L-proline, were not identical before and after surgery, indicating that changes in the metabolic profiles were associated with the outcome of surgical treatment.

3.3 | Pathway analysis of differential metabolites

The related pathways for the differential metabolites were analyzed to reveal the potential contributors of the metabolic changes before and after surgery (Figure 2). According to the results of the pathway impact analyses, seven metabolic pathways were screened out. These pathways were related to biosynthesis, energy supply, and oxidative stress response, such as arginine and proline metabolism pathway, and starch and sucrose metabolism pathway. It is known that the pattern of energy metabolism changes markedly when cancer occurs, resulting in tremendous metabolic alterations to related small molecules, such as carbohydrates and amino acids. As proline acts as an important provider of ATP and is related to the TCA cycle and urea cycle, the arginine and proline metabolism pathway was highlighted in this study.

3.4 | Potential assessment of metabolites for diagnosis of CC and RC

The differential metabolites in the preoperative RC, CC, and HV groups were regarded as potential biomarkers and subjected to

TABLE 2 The differential metabolites between CC, RC, and HV groups before/after surgery

Metabolite	Retention time (min)	*P* value	
		preop	postop
2,3-Butanediol	7.51[a]		.00613
2-Aminobutanoic acid	11.27[a]	.01693	1.54E-08
2-Hydroxybutyric acid	9.96[a]		.00218
3-Hydroxybutyric acid	13.13[a]	.000123	.007597
3-Hydroxypyridine	10.67[a]		
Butanoic acid	7.26[b]		3.09E-09
D-Fructose	28.46[a]		5.44E-12
D-Glucose	28.97[a]	.0006573	1.33E-06
D-Mannose	28.75[a]	.0004288	5.59E-06
Glycine	9.86[a]	.06038	9.23E-08
Isoleucine	11.41[a]	.001992	
Lactic acid	8.07[a]	.081	.01688
L-Alanine	9.27[a]		5.33E-09
L-Proline	14.75[a]		6.98E-07
L-Tryptophan	34.59[a]	.0007671	
Urea	13.25[a]	.001023	
Uric acid	32.87[a]	.001717	5.49E-11

[a]Based on both of NIST database and elution time of DICP GC/MS database.

[b]Only based on NIST database.

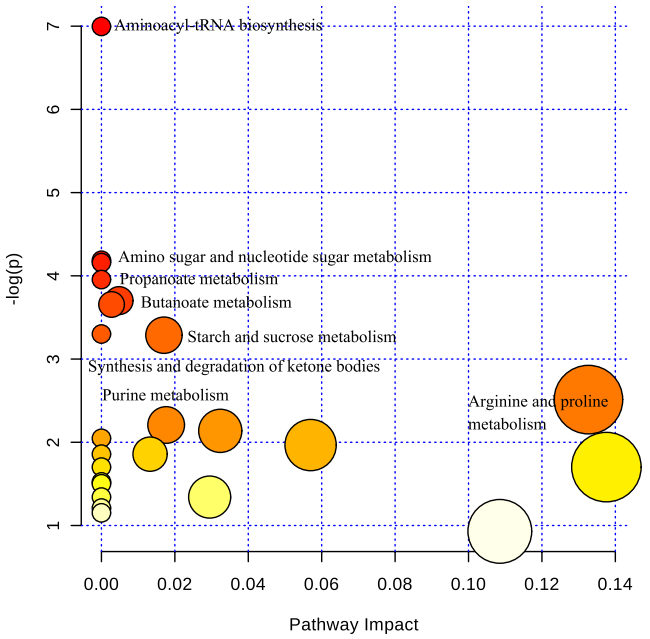

FIGURE 2 Metabolomics pathway analysis of differential metabolites among the CC, RC, and HV groups, *P*-value is encoded in intensity of red color of circles (representing pathways) and size of the circles represent the diversity of the pathway, based on the number of metabolites contained in the pathway

ROC analyses to identify their potential roles in early diagnosis. Paired comparisons were performed to eliminate the metabolites with no significant differences between the HV group and the patient groups. The area under the curve (AUC) was 0.805 for combination of d-glucose and d-mannose for CC diagnosis, and 0.889 for combination of 2-aminobutanoic acid, 3-hydroxypyridine, d-glucose, d-mannose, isoleucine, l-tryptophan, urea, and uric acid for RC diagnosis. In clinical practice, CEA and CA-199 are commonly used as tumor markers. In this study, the AUC for CEA and CA-199 was only 0.800 and 0.722 for CC diagnosis, and 0.744 and 0.765 for RC diagnosis, respectively. Combining the metabolite markers with protein markers effectively improved the diagnostic accuracy, and the AUC reached 0.936 and 0.937 for CC and RC diagnosis, respectively (Figure 3).

4 | DISCUSSION

In recent years, increasing numbers of studies have employed metabolomics to identify CRC markers.[17-20] However, to the best of our knowledge, this is the first study to investigate the differences of metabolite markers between CC and RC patients. In the present metabolomics study on preoperative samples, RC patients exhibited more changes in metabolites than CC patients (Table 3). Furthermore, nine metabolites differed significantly between CC and RC patients including 2-aminobutanoic acid, 3-hydroxypyridine, d-mannose, glycine, isoleucine, lactic acid, l-tryptophan, urea, and uric acid.

For example, isoleucine differed significantly between the HV and RC groups, and between the RC and CC groups ($P < .05$), but showed no significant difference between the HV and CC groups. Isoleucine is a branched-chain amino acid that can provide additional energy when the body consumes large amounts of energy. It may play an important role in the balance of body energy. These findings suggest that there may be differences in metabolic activity between the two cancers during the mid-differentiation stage, which may partly explain there are different metastatic patterns between CC and RC in different stage.

Diagnostic models for CC and RC were established based on the differential metabolites identified in the metabolomics analysis, and ROC analyses showed that the resultant prediction utilities were better than those of CEA and CA199, which are commonly used in clinical practice. The specificity of CEA and CA199 is poor, because they are not only altered in patients with gastrointestinal cancer but also in patients with other cancers such as breast cancer and lung cancer. Metabolomics is a technique that allows comprehensive exploration of altered metabolites in a particular disease, thus identifying metabolite markers with high specificity for the disease under investigation. Not a single marker might be sufficient to create a test with appropriate sensitivity and specificity for CRC as it is easily affected by a variety of factors, suggesting that a combination of multiple metabolite markers can better reflect the real state of patients.

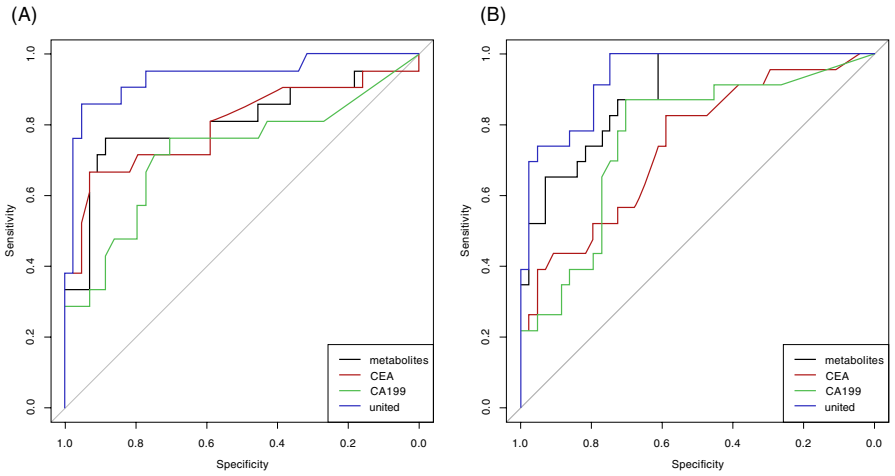

FIGURE 3 ROC curves. A, CC and HV groups. B, RC and HV groups. The blue lines show the ROC using the combinations of metabolites (d-glucose and d-mannose for CC; 2-aminobutanoic acid, 3-hydroxypyridine, d-glucose, d-mannose, isoleucine, l-tryptophan, urea, and uric acid for RC), the red lines show CEA, the green lines show CA199, and the violet lines show the combinations of metabolite markers and protein markers

TABLE 3 Paired comparisons of differential metabolites between HVs and preoperative RC or CC patients

	P value		
Metabolite	**HV VS RC**	**HV VS CC**	**RC VS CC**
2-Aminobutanoic acid	.006114↑[a]	.548	.03671↓[b]
3-Hydroxypyridine	.0008844↑	.1493	6.44E-05↓
D-Glucose	.0237↓	.0001418↓	.6015
D-Mannose	.4253↓	3.29E-05↓	.03281↑
Glycine	.05756	.35	.03472↓
Isoleucine	.008342↓	.08502	.001599↑
Lactic acid	.1245	.2244	.03881↓
L-Tryptophan	.002472↑	.9362	8.56E-05↓
Urea	7.91E-05↑	.5659	.05086
Uric acid	.002692↑	.165	.002529↓

[a]↑ means ConcA > ConcB(A VS B).

[b]↓ means ConcA < ConcB(A VS B).

In previous metabolomics studies of CRC, some metabolites were reported to be discrepantly altered in patients. For example, Mirnezami et al and Chan et al found that glycine was elevated in patients with CRC compared with healthy controls,[21,22] while Ma et al found that glycine was reduced.[23] In the present study, glycine was increased in serum from CC patients, but decreased in serum from RC patients.

Furthermore, our results showed that the tryptophan alteration in CC patients was opposite to that in RC patients, which is inconsistent with the previous studies.[24] The previous CRC studies did not distinguish among patients with CC and RC, and thus, differences in the proportions of these two types of patients may have led to the conflicting results. Thus, it is necessary to separate the two types of cancer patients and perform large-scale sample validation in future studies.

As can be seen from the present results, the differential metabolites of CRC are related to the progress of glycolysis (eg, glucose and lactate), gluconeogenesis (eg, 2-hydroxybutyric acid), and purine metabolism (eg, uric acid). According to the study of K. Satoh et.al, MYC gene regulates the cell metabolism of CRC and upregulates the proliferation of cancer cell. MYC regulates several metabolic pathways related to mitochondrial biogenesis,[25] which are also reflected from our results. The metabolic changes of glucose and metabolites in the TCA cycles have also been reported in other studies.[26,27] We also found that some metabolites from gut flora are included in the differential metabolites of CRC, such as Trp and butanoic acid. The deregulation of these metabolites indicated that the gut microbes may take part in the carcinogenesis of CRC. And the study of Hale VL et al provided some evidence from 16S rRNA gene sequencing and found some unique microbes for the occurrence of CRC.[27]

There are several limitations to the present study. First, two carbohydrates were combined to establish a diagnostic model for CC, which may be affected by high-sugar diets or other in vitro intakes. Second, the examined samples were all from cancer patients and were not compared with inflammatory samples and benign tumor samples. Third, the small sample size may have introduced potential interference. Fourth, a previous study found that early-stage CRC patients were easier to distinguish than mid-stage or late-stage patients, while mid-stage patients were the most difficult.[28] As the patients in the present study were all in the middle-differentiation period, we were unable to assess whether the candidate metabolite markers are suitable for early screening. In future studies, it will be necessary to include more samples to verify our findings.

In conclusion, a GC-MS-based metabolomics study was performed to identify the different metabolic characteristics of patients with CC and RC. Significantly changed metabolites were selected as potential markers for disease diagnosis. Combinations of metabolite markers, comprising d-glucose and d-mannose for CC diagnosis, and 2-aminobutanoic acid, 3-hydroxypyridine, d-glucose, d-mannose, isoleucine, l-tryptophan, urea, and uric acid for RC diagnosis, showed a better predictability than CEA and CA199. Furthermore, metabolic differences between CC and RC patients were identified, which may assist in distinguishing the two types of cancers.

ACKNOWLEDGMENTS

This work was supported by grant from the Natural Science Foundation of China of Zhejiang Province (LGC19H200004).

CONFLICT OF INTEREST

None of the authors have any commercial or other association that might pose a conflict of interest.

ORCID

Qianxia Wu https://orcid.org/0000-0002-8442-6476

REFERENCES

1. Aleksandrova K, Pischon T, Jenab M, et al. Combined impact of healthy lifestyle factors on colorectal cancer: a large European cohort study. *BMC Med*. 2014;12:168.
2. Desantis CE, Siegel RL, Sauer AG, et al. Cancer statistics for African Americans, 2016: progress and opportunities in reducing racial disparities. *CA Cancer J Clin*. 2016;66:290-308.
3. Ferlay J, Soerjomataram I, Dikshit R, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer*. 2015;136:E359-E386.
4. Meyerhardt JA, Mayer RJ. Systemic therapy for colorectal cancer. *N Engl J Med*. 2005;352:476-487.
5. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2015. *CA Cancer J Clin*. 2015;65:5-29.
6. Yin D, Morris CR, Bates JH, German RR. Effect of misclassified underlying cause of death on survival estimates of colon and rectal cancer. *J Natl Cancer Inst*. 2011;103:1130-1133.
7. Cai SR, Zheng S, Zhang SZ. Multivariate analysis of prognostic factors in colorectal cancer patients with different ages. *Zhonghua Zhong Liu Za Zhi*. 2005;27:483-485.
8. Lapointe LC, Pedersen SK, Dunne R, et al. Discovery and validation of molecular biomarkers for colorectal adenomas and cancer with application to blood testing. *PLoS ONE*. 2012;7:e29059.
9. Moghimi-Dehkordi B, Safaee A. An overview of colorectal cancer survival rates and prognosis in Asia. *World J Gastrointest Oncol*. 2012;4:71-75.
10. Provenzale D, Gupta S, Ahnen DJ, et al. Guidelines insights: colorectal cancer screening, version 1.2018. *J Natl Compr Canc Netw*. 2018;16:939-949.
11. Levin B, Lieberman DA, McFarland B, et al. Screening and surveillance for the early detection of colorectal cancer and adenomatous polyps, 2008: a joint guideline from the American Cancer Society, the US Multi-Society Task Force on Colorectal Cancer, and the American College of Radiology. *CA Cancer J Clin*. 2008;58:130-160.
12. Ballehaninna UK, Chamberlain RS. The clinical utility of serum CA 19–9 in the diagnosis, prognosis and management of pancreatic adenocarcinoma: an evidence based appraisal. *J Gastrointest Oncol*. 2012;3:105-119.
13. Chen Y, Richardson AD, Smith JW, Osterman A. Comparative metabolomics of breast cancer. *Pac Symp Biocomp*. 2007;181-192.
14. Claudino WM, Quattrone A, Biganzoli L, Pestrin M, Bertini I, Di LA. Metabolomics: available results, current research projects in breast cancer, and future applications. *J Clin Oncol*. 2007;25:2840-2846.
15. Kim K, Aronov P, Zakharkin SO, et al. Urine metabolomics analysis for kidney cancer detection and biomarker discovery. *Mol Cell Proteomics*. 2009;8:558-570.
16. Kobayashi T, Nishiumi S, Ikeda A, et al. A novel serum metabolomics-based diagnostic approach to pancreatic cancer. *Cancer Epidemiol Biomarkers Prev*. 2013;22:571-579.
17. Farshidfar F, Weljie AM, Kopciuk KA, et al. A validated metabolomic signature for colorectal cancer: exploration of the clinical value of metabolomics. *Br J Cancer*. 2016;115:848-857.
18. Ludwig C, Ward DG, Martin A, et al. Fast targeted multidimensional NMR metabolomics of colorectal cancer. *Magn Reson Chem*. 2010;47:S68-S73.
19. Nishiumi S, Kobayashi T, Ikeda A, et al. A novel serum metabolomics-based diagnostic approach for colorectal cancer. *PLoS ONE*. 2012;7:e40459.
20. Yan L, Ma C, Liu C, et al. NMR-based fecal metabolomics fingerprinting as predictors of earlier diagnosis in patients with colorectal cancer. *Oncotarget*. 2016;7:29454-29464.
21. Chan ECY, Koh PK, Mal M, et al. Metabolic profiling of human colorectal cancer using high-resolution magic angle spinning nuclear magnetic resonance (HR-MAS NMR) spectroscopy and gas chromatography mass spectrometry (GC/MS). *J Proteome Res*. 2009;8:352-361.
22. Mirnezami R, Jiménez B, Li JV, et al. Rapid diagnosis and staging of colorectal cancer via high-resolution magic angle spinning nuclear magnetic resonance (HR-MAS NMR) spectroscopy of intact tissue biopsies. *Ann Surg*. 2014;259:1138-1149.
23. Ma Yanlei, Zhang Peng, Wang Feng, Liu Weijie, Yang Jianjun, Qin Huanlong. An integrated proteomics and metabolomics approach for defining oncofetal biomarkers in the colorectal cancer. *Ann Surg*. 2012;255:720-730.
24. Huang A, Fuchs D, Widner B, Glover C, Henderson D, Allen-Mersh T. Serum tryptophan decrease correlates with immune activation and impaired quality of life in colorectal cancer. *Br J Cancer*. 2002;86:1691-1696.
25. Satoh K, Yachida S, Sugimoto M, et al. Global metabolic reprogramming of colorectal cancer occurs at adenoma stage and is induced by MYC. *Proc Natl Acad Sci USA*. 2017;114:E7697-E7706.
26. Jiménez B, Mirnezami R, Kinross J, et al. 1H HR-MAS NMR spectroscopy of tumor-induced local metabolic "field-effects" enables colorectal cancer staging and prognostication. *J Proteome Res*. 2013;12:959-968.
27. Qiu Y, Cai G, Zhou B, et al. A distinct metabolic signature of human colorectal cancer with prognostic potential. *Clin Cancer Res*. 2014;20:2136-2146.
28. Liesenfeld DB, Habermann N, Toth R, et al. Changes in urinary metabolic profiles of colorectal cancer patients enrolled in a prospective cohort study (ColoCare). *Metabolomics*. 2015;11:998-1012.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Wu J, Wu M, Wu Q. Identification of potential metabolite markers for colon cancer and rectal cancer using serum metabolomics. *J Clin Lab Anal*. 2020;34:e23333. https://doi.org/10.1002/jcla.23333