**ARTICLE**

Alteration of Ornithine Metabolic Pathway in Colon Cancer and Multivariate Data Modelling for Cancer Diagnosis

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ABSTRACT

It is noteworthy that colon cancer is the fourth place in new cases and the fifth in mortalities according to global cancer statistics 2018. Tumorigenesis displays specific correlation with metabolic alterations. A variety of metabolites, including ornithine (Orn), are related to colon cancer according to sources of disease metabolic information retrieval in human metabolome database. The metabolic regulation of Orn pathway is a key link in the survival of cancer cells. In this study, the plasma Orn levels in colon cancer patients and healthy participants were measured by liquid chromatography tandem mass spectrometry, and the metabolic disturbances of Orn in colon cancer were identified. Based on exploring the pathway structure of Orn metabolism via MetaboAnalyst and Kyoto Encyclopedia of Genes and Genomes database, we found that the upstream and downstream metabolites included arginine (Arg) and N1, N12-diacetylspermine (DiAcSpm). We observed that the Arg-Orn-DiAcSpm metabolic pathway was up-regulated in colon cancer through pathway analysis. We used multivariate data modelling to build a regression diagnosis model based on the three metabolites for colon cancer, and the diagnosis capability of this model was analyzed via receiver operating characteristic curve. This study provides a theoretical basis for further feature description of tumor metabolic pathway, which may lead to discover new therapeutic targets and drugs against colon cancer. Multivariate data modelling is expected to be a novel technology for developing noninvasive screening tool of cancer.

KEYWORDS

Metabolic pathway; colon cancer; ornithine; multivariate data modelling

1 Introduction

According to the statistics, more than 1.8 million new colorectal cancer cases were diagnosed worldwide in 2018, and colorectal cancer is the third leading cause of cancer deaths in females and the fourth in males. Furthermore, the colon cancer is the fourth most common cancer in new cases [1].



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Metabolic alterations are the key links in occurrence and development of tumors, which are driven by the genetically relevant mutations that promote oncogenesis [2]. Cell metabolic change is the idiosyncrasy of carcinoma, which is helpful for malignant transformation and tumor maintenance [3]. Metabonomics technique can be used for global profiling of metabolites in human body, and provides specific metabolic profiles to guide in-depth understanding of metabolic reaction endpoint information of diseases. Metabolite quantitative analysis technology has great potential in drug development and disease diagnosis [4]. Plasma is a collection area of metabolites in the human body. The changes of plasma metabolites in cancer patients may reflect the metabolic regulations in the process of cell carcinogenesis [5]. Recent studies show the significant differences in serum glycolysis, protein, and lipid metabolism between colorectal cancer patients and healthy participants [6]. By identifying the key metabolic pathways, the related mechanisms of tumorigenic process could be further studied.

Human Metabolome Database (HMDB) is a Web-based metabolomic database. It is the most complete and comprehensive database to collect human metabolites and metabolism data in the world. Through data retrieval of the biomedical information describing metabolites and diseases of human body in this database, we observed that the levels of various metabolites including ornithine (Orn) changed significantly in colorectal cancer.

Orn that is a non-proteinogenic amino acid is involved in many biosynthesis pathways in the human body. Some researches have shown that Orn metabolism is remarkably altered in many cancers. Orn plays a special role in tumor growth, invasion and metastasis [7,8]. Tan et al. [9] has found that Orn is down-regulated in colorectal cancer via gas chromatography time-of-flight mass spectrometry. Uchiyama et al. [10] has shown that Orn concentration is lower in serum of patients with colorectal cancer through capillary electrophoresis-time-of-flight mass spectrometry. However, the interesting question of why the metabolic perturbation of Orn appears in colorectal cancer has not yet been answered. In addition, the metabolic alteration of Orn pathway plays specific roles in survival of human esophageal chemotherapy-resistant cancer stem cells [11]. But there are limited data on the regulations of Orn metabolic pathway in colon cancer.

Liquid chromatography tandem mass spectrometry (LC-MS/MS) is a superior high-resolution biomolecular analysis technology, which has the advantages of fast scanning speed, strong selectivity and high sensitivity. It can realize rapid, micro and high-throughput analysis of metabolites. This technology has been widely applied to analyze the levels of metabolites in blood and urine samples of cancer patients and in tumor tissues [12–14].

Recent studies have demonstrated that many low-molecular metabolites may be markers for displaying biological mechanisms during the tumorigenic process and for diagnosing cancer diseases [15–17]. However, there is a lack of appropriate analytical methods to further evaluate the application potential of these molecular markers. Multivariate correlation analysis of metabolites would be helpful for clarifying their values in the pathogenesis, diagnosis and treatment of cancer. Accordingly, it is very desirable to develop an efficient mathematical model of multivariate correlation analysis for cancer diagnosis using low-molecular metabolites.

In this study, we used LC-MS/MS technology to verify the plasma Orn metabolic perturbation in patients with colon cancer. The metabolic pathway of Orn in human body was identified by MetaboAnalyst and Kyoto Encyclopedia of Genes and Genomes (KEGG) database, and the upstream and downstream relevant metabolites of Orn metabolism were analyzed to reveal the mechanisms of the alterations in Orn level of colon cancer. We developed a mathematical model based on the biomolecules in Orn metabolic pathway for diagnosing colon cancer through multivariate data modelling. Our study showed that the metabolic pathway of Orn participated in human colonic carcinogenesis and the combined metabolic profile of biomolecules in this pathway might be a novel diagnosis tool for early screening colon cancer.

2 Methods and Materials

2.1 Clinical Sample Collection

This study was approved by the Ethics Committee of the First Affiliated Hospital of Jinzhou Medical University and conducted in accordance with the principles of Helsinki Declaration. Each patient or healthy person involved in this study signed an informed consent. We collected blood and urine samples of 63 patients with colon cancer and 63 healthy participants from the First Affiliated Hospital of Jinzhou Medical University. There were no age, gender and body mass index (BMI) differences between the two groups. The inclusion criteria of all the subjects were as follows: blood samples and urine samples provided by all subjects were taken before surgery, chemotherapy, radiotherapy or other relevant treatments; the diagnosis of cancer patients was confirmed by histopathology, and cancer stage was classified according to the Tumor-Node-Metastasis (TNM) classification of American Joint Committee on Cancer (AJCC); tests of the liver and kidney functions of subjects showed normal findings; weight gain or loss of all subjects with normal BMI was less than 10%. All subjects had no hyperthyroidism, diabetes, hyperlipidemia and tuberculosis. The detailed baseline characteristics of subjects were shown in [Tab. 1](#).

Table 1: Clinical characteristics of the subjects included in the study

	Colon cancer	Healthy control	<i>P</i> -value
Number of samples	63	63	
Sex			0.476
Male	33	29	
Female	30	34	
Age (years)			0.623
Median (range)	56.5 (27–78)	52.0 (25–71)	
BMI (kg/m²)			0.781
Median (range)	20.6 (18.3–22.8)	22.7 (19.2–23.8)	
TNM stage			
I	26		
II	18	\	
III	11	\	
IV	8		

Specimens from fasting blood samples of cancer patients and healthy participants were collected in vacuum blood collection tubes and immediately stored at 4°C. Subsequently, the specimens were produced into dried blood spot (DBS) samples on filter papers and kept at –80°C until measurement. The urine samples of all the subjects were stored at –20°C until the sample preparation.

2.2 Blood Sample Preparation

A disc with a diameter of 3 mm was punched out from each DBS paper at room temperature. Adding 100 µL working solution into each well of 96-well plates containing DBS discs, shaking for 20 min and centrifuging at 1500 × *g* for 2 min were used to remove interferences from samples. After dried in nitrogen airflow, the filtrate was collected using new 96-well plates containing 60 µL 1-butanol/acyetyl chloride mixture and 100 µL mobile phase (80% acetonitrile aqueous solution) for LC-MS/MS detection.

2.3 LC-MS/MS Detection

LC-MS/MS analysis was used to detect the concentrations of Orn and arginine (Arg) in plasma, which was conducted with AB Sciex 4000 QTrap (AB Sciex, Framingham, MA, USA) and LC-20A (Shimadzu, Kyoto, Japan) as detectors.

2.4 Urine Sample Preparation

Urine samples adjusted to room temperature were centrifuged for 5 min at 3000 rpm, and the supernatant was diluted with distilled deionized water in the ratio of 1:4. The collected samples were incubated with anti-diacetylspermine antibody in 96-well plates at room temperature for 1 h. After incubation, the wells were washed with wash solution. The incubation process was repeated with horseradish peroxidase (HRP)-conjugated anti-rabbit antibody, then wells were washed again. Subsequently, incubation with ortho-phenylenediamine was 10 min. The 1 mol/l sulfuric acid was added to stop the reaction, and the absorbance was measured at a wavelength of 490 nm. Correction of N1, N12-diacetylspermine (DiAcSpm) concentration with urine creatinine concentration (mg/dl) was used to be the actual measurement.

2.5 DiAcSpm Measurement

Concentration of urinary DiAcSpm was measured by the enzyme-linked immunosorbent assay (ELISA) in 7600-020 automated biochemical analyser (HITACHI, Tokyo, Japan).

2.6 Statistical Analysis

Student's *t*-test was performed to analyze the metabolic differences of Orn, Arg and DiAcSpm between colon cancer and healthy control groups. Metabolic profile discrimination of the three metabolites between the two groups was presented via partial least squared discriminant analysis (PLS-DA) of multivariate correlation analysis (SIMCA-P software v13.0, Umetrics, Umea, Sweden). The diagnosis model of colon cancer was established by logistic regression analysis, and its diagnostic ability was confirmed via the receiver operating characteristic (ROC) curve.

2.7 Metabolism Data Retrieval and Pathway Analysis

HMDB (<http://hmdb.ca/>) was performed for online retrieval of metabolic information related to colorectal cancer. The metabolic pathway of Orn was identified by database sources of MetaboAnalyst (<http://www.metaboanalyst.ca/>) and KEGG (<http://www.genome.jp/kegg/>).

3 Results

3.1 Metabolic Information Related to Colon Cancer in HMDB

Data sets of human colorectal cancer metabolism in HMDB were downloaded and analyzed. All the differential expression metabolites in blood samples from adult patients with colorectal cancer were listed in [Fig. 1](#), one of which was Orn.

3.2 Orn Metabolic Alterations in Colon Cancer

LC-MS/MS analysis of blood samples showed that the metabolic level of Orn significantly decreased in colon cancer patients as compared to the healthy controls ([Fig. 2A](#)). The result of this study was similar to the previous reports.

We further analyzed the correlation between Orn and clinicopathological factors in colon cancer group. [Tab. 2](#) presented that Orn was remarkably associated with tumor invasion depth and its level decreased in T3-4 stage cases compared with the T1-2 stage (29.46 ± 16.07 vs. 36.12 ± 20.09 , $P = 0.025$). There was a negative correlation between Orn and lymphatic invasion status. Lymphatic invasion-positive group showed significantly lower level of Orn than the negative group (28.49 ± 12.63 vs. 35.54 ± 17.85 , $P = 0.018$). Analysis of the association between Orn and TNM stage demonstrated that its level in Stage IV cases was lower than that in Stage I (27.36 ± 14.27 vs. 37.24 ± 20.61 , $P < 0.001$).

Colorectal cancer				
Metabolite	Biospecimen	Patient Status	Age	Sex
1-Methyladenosine (HMDB0003331)	Blood	Abnormal	Adult (>18 years old)	Both
L-Lysine (HMDB0000182)	Blood	Abnormal	Adult (>18 years old)	Both
L-Alanine (HMDB0000161)	Blood	Abnormal	Adult (>18 years old)	Both
L-Aspartic acid (HMDB0000191)	Blood	Abnormal	Adult (>18 years old)	Both
Glycine (HMDB0000123)	Blood	Abnormal	Adult (>18 years old)	Both
L-Histidine (HMDB0000177)	Blood	Abnormal	Adult (>18 years old)	Both
L-Isoleucine (HMDB0000172)	Blood	Abnormal	Adult (>18 years old)	Both
L-Methionine (HMDB0000696)	Blood	Abnormal	Adult (>18 years old)	Both
Sarcosine (HMDB0000271)	Blood	Abnormal	Adult (>18 years old)	Both
L-Threonine (HMDB0000167)	Blood	Abnormal	Adult (>18 years old)	Both
L-Valine (HMDB0000883)	Blood	Abnormal	Adult (>18 years old)	Both
Pyruvic acid (HMDB0000243)	Blood	Abnormal	Adult (>18 years old)	Both
L-Lactic acid (HMDB0000190)	Blood	Abnormal	Adult (>18 years old)	Both
L-Tryptophan (HMDB0000929)	Blood	Abnormal	Adult (>18 years old)	Both
L-Tyrosine (HMDB0000158)	Blood	Abnormal	Adult (>18 years old)	Both
Uridine (HMDB0000296)	Blood	Abnormal	Adult (>18 years old)	Both
L-alpha-Aminobutyric acid (HMDB0000452)	Blood	Abnormal	Adult (>18 years old)	Both
2-Hydroxybutyric acid (HMDB0000008)	Blood	Abnormal	Adult (>18 years old)	Both
3-Hydroxybutyric acid (HMDB0000357)	Blood	Abnormal	Adult (>18 years old)	Both
4-Hydroxyproline (HMDB0000725)	Blood	Abnormal	Adult (>18 years old)	Both
6-Phosphogluconic acid (HMDB0001316)	Blood	Abnormal	Adult (>18 years old)	Both
Acetic acid (HMDB0000042)	Blood	Abnormal	Adult (>18 years old)	Both

Figure 1: (continued)

Colorectal cancer					
Metabolite	Biospecimen	Patient Status	Age	Sex	
L-Acetylcarnitine (HMDB0000201)	Blood	Abnormal	Adult (>18 years old)	Both	
L-Alanine (HMDB0000161)	Blood	Abnormal	Adult (>18 years old)	Both	
L-Arginine (HMDB0000517)	Blood	Abnormal	Adult (>18 years old)	Both	
L-Asparagine (HMDB0000168)	Blood	Abnormal	Adult (>18 years old)	Both	
L-Aspartic acid (HMDB0000191)	Blood	Abnormal	Adult (>18 years old)	Both	
Betaine (HMDB0000043)	Blood	Abnormal	Adult (>18 years old)	Both	
L-Carnitine (HMDB0000062)	Blood	Abnormal	Adult (>18 years old)	Both	
Citric acid (HMDB0000094)	Blood	Abnormal	Adult (>18 years old)	Both	
Creatine (HMDB0000064)	Blood	Abnormal	Adult (>18 years old)	Both	
L-Cystine (HMDB0000192)	Blood	Abnormal	Adult (>18 years old)	Both	
Elaidic acid (HMDB0000573)	Blood	Abnormal	Adult (>18 years old)	Both	
Fumaric acid (HMDB0000134)	Blood	Abnormal	Adult (>18 years old)	Both	
L-Glutamic acid (HMDB0000148)	Blood	Abnormal	Adult (>18 years old)	Both	
L-Glutamine (HMDB0000641)	Blood	Abnormal	Adult (>18 years old)	Both	
Glycerol (HMDB0000131)	Blood	Abnormal	Adult (>18 years old)	Both	
Glycine (HMDB0000123)	Blood	Abnormal	Adult (>18 years old)	Both	
Hippuric acid (HMDB0000714)	Blood	Abnormal	Adult (>18 years old)	Both	
L-Histidine (HMDB0000177)	Blood	Abnormal	Adult (>18 years old)	Both	
Kynurenic acid (HMDB0000715)	Blood	Abnormal	Adult (>18 years old)	Both	
L-Lactic acid (HMDB0000190)	Blood	Abnormal	Adult (>18 years old)	Both	
L-Leucine (HMDB0000687)	Blood	Abnormal	Adult (>18 years old)	Both	
Tetracosanoic acid (HMDB0002003)	Blood	Abnormal	Adult (>18 years old)	Both	
L-Lysine (HMDB0000182)	Blood	Abnormal	Adult (>18 years old)	Both	
Malic acid (HMDB0000744)	Blood	Abnormal	Adult (>18 years old)	Both	
L-Methionine (HMDB0000696)	Blood	Abnormal	Adult (>18 years old)	Both	
Oleamide (HMDB0002117)	Blood	Abnormal	Adult (>18 years old)	Both	
Oleic acid (HMDB0000207)	Blood	Abnormal	Adult (>18 years old)	Both	
Ornithine (HMDB0000214)	Blood	Abnormal	Adult (>18 years old)	Both	
Palmitic acid (HMDB0000220)	Blood	Abnormal	Adult (>18 years old)	Both	
L-Phenylalanine (HMDB0000159)	Blood	Abnormal	Adult (>18 years old)	Both	

Figure 1: (continued)

Colorectal cancer				
Metabolite	Biospecimen	Patient Status	Age	Sex
L-Proline (HMDB0000162)	Blood	Abnormal	Adult (>18 years old)	Both
Pyruvic acid (HMDB0000243)	Blood	Abnormal	Adult (>18 years old)	Both
Ribitol (HMDB0000508)	Blood	Abnormal	Adult (>18 years old)	Both
L-Serine (HMDB0000187)	Blood	Abnormal	Adult (>18 years old)	Both
Sphinganine (HMDB0000269)	Blood	Abnormal	Adult (>18 years old)	Both
L-Threonine (HMDB0000167)	Blood	Abnormal	Adult (>18 years old)	Both
Threonic acid (HMDB0000943)	Blood	Abnormal	Adult (>18 years old)	Both
Trimethylamine N-oxide (HMDB0000925)	Blood	Abnormal	Adult (>18 years old)	Both
L-Tryptophan (HMDB0000929)	Blood	Abnormal	Adult (>18 years old)	Both
L-Tyrosine (HMDB0000158)	Blood	Abnormal	Adult (>18 years old)	Both
Urea (HMDB0000294)	Blood	Abnormal	Adult (>18 years old)	Both
Uridine (HMDB0000296)	Blood	Abnormal	Adult (>18 years old)	Both
L-Valine (HMDB0000883)	Blood	Abnormal	Adult (>18 years old)	Both
L-Alanine (HMDB0000161)	Blood	Abnormal	Adult (>18 years old)	Both
L-Glutamine (HMDB0000641)	Blood	Abnormal	Adult (>18 years old)	Both
Phenol (HMDB0000228)	Blood	Abnormal	Adult (>18 years old)	Both
Linoleic acid (HMDB0000673)	Blood	Abnormal	Adult (>18 years old)	Both

Figure 1: Expression of metabolites related to colon cancer in HMDB (version 4.0; hmdb.ca). Data sets showed all the differential expression metabolites in blood samples from adult patients with colorectal cancer. Orn was labeled in red

There have been evidences that left-sided colon cancer (LCC) represents differences in biology, pathology and epidemiology as compared to right-sided colon cancer (RCC) [18]. On the basis of this finding, our study also examined the metabolic levels of Orn in the tumor location of colon cancer, but no significant difference between RCC and LCC groups was evident (28.92 ± 15.38 vs. 34.05 ± 14.77 , $P = 0.174$). Moreover, none of the other clinicopathological factors including age ($P = 0.438$), sex ($P = 0.701$), and histological type ($P = 0.603$) were significantly correlated to Orn.

3.3 Determination of Orn Metabolic Pathway

The functional enrichment analysis of Orn was performed by MetaboAnalyst. Fig. 3A presented the affected pathways of Orn based on all the previous studies data. Two of the most important Orn metabolic pathways were urea cycle and polyamine metabolism. A network of Orn metabolism was drawn according to the metabolic pathway information provided by KEGG database (Fig. 3B). The pathways inside the network, which involved urea cycle and polyamine metabolism, joined the

metabolites. We observed that the upstream metabolites of Orn metabolic pathway contained Arg and the downstream metabolites included DiAcSpm.

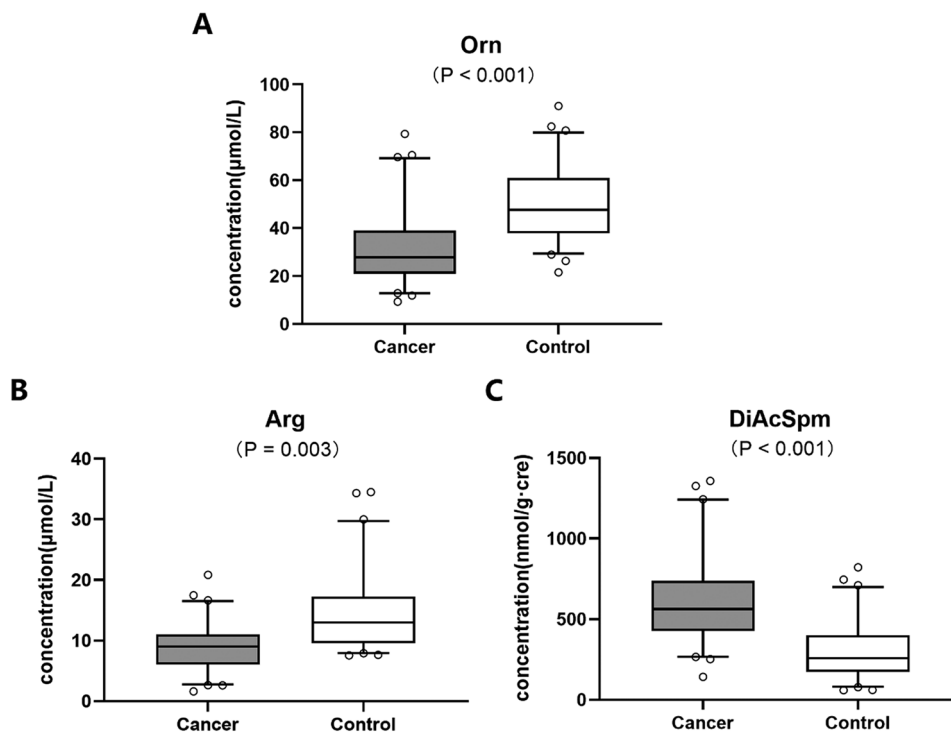


Figure 2: Metabolic levels of biomolecules including (A) Orn, (B) Arg and (C) DiAcSpm in Orn pathway between colon cancer and healthy control groups

3.4 Analysis of Metabolites in Orn Metabolic Pathway

The results based on the analysis of Arg and DiAcSpm metabolisms exhibited that the concentration of plasma Arg decreased (Fig. 2B) while urine DiAcSpm increased (Fig. 2C) in colon cancer group compared with the controls.

We examined the correlation between the two metabolites and clinicopathological factors of the patients with colon cancer (Tab. 3). Lymphatic invasion-positive group showed significantly lower level of Arg than the negative group (7.71 ± 1.95 vs. 9.18 ± 2.97 , $P = 0.020$). This study also proved that Arg level was lower in Stage IV than in Stage I cases (7.11 ± 1.78 vs. 9.32 ± 3.13 , $P = 0.006$).

As shown in Tab. 3, DiAcSpm was positively associated with the lymphatic invasion status. DiAcSpm level in lymphatic invasion-positive group was significantly higher than that in the negative group (702.38 ± 269.47 vs. 557.00 ± 258.55 , $P = 0.007$). Analysis of the association between DiAcSpm and TNM stage indicated that its level increased with the elevation of TNM stage giving values of 528.89 ± 256.43 (Stage I), 612.52 ± 270.38 (Stage II), 666.37 ± 303.57 (Stage III), and 730.61 ± 212.68 (Stage IV), respectively. There was a significant difference in the level of DiAcSpm between Stages I and IV cases ($P < 0.001$).

The other clinicopathological factors including age, sex, histological type, depth of invasion, and side of tumor did not significantly correlate with Arg and DiAcSpm.

Table 2: Correlation between Orn and clinicopathological factors

Factors	N	Orn ($\mu\text{mol/L}$)	<i>P</i> -value
Age (years)			0.438
≤56.5	22	34.53 ± 19.96	
>56.5	41	30.60 ± 14.16	
Sex			0.701
Male	33	30.20 ± 16.54	
Female	30	33.85 ± 18.66	
Histological type			0.603
Adenocarcinoma	51	33.37 ± 12.32	
Non-adenocarcinoma	12	29.01 ± 15.50	
Depth of invasion			0.025
T1-2	37	36.12 ± 20.09	
T3-4	26	29.46 ± 16.07	
Lymphatic invasion			0.018
Negative	44	35.54 ± 17.85	
Positive	19	28.49 ± 12.63	
TNM stage			<0.001 (I vs. IV)
I	26	37.24 ± 20.61	
II	18	30.85 ± 18.33	
III	11	31.07 ± 17.20	
IV	8	27.36 ± 14.27	
Side of tumor			0.174
LCC	35	34.05 ± 14.77	
RCC	28	28.92 ± 15.38	

Note: LCC: left-sided colon cancer; RCC: right-sided colon cancer; Data were presented as mean ± SD.

3.5 Multivariate Analysis of Metabolites

The PLS-DA model was constructed based on the metabolic data of the three metabolites. Score scatter 3D plot presented a discrimination of the combined metabolic profile of Arg, Orn and DiAcSpm between colon cancer and control groups (Fig. 4A). The model displayed satisfactory modeling ($R^2_{Ycum} = 0.837$) and predictive ($Q^2_{cum} = 0.751$) abilities. Chance permutation test at 200 times (Fig. 4B) indicated that no over-fitting appeared in this model with y-axis intercept values of -0.0214 (R2) and -0.135 (Q2), respectively.

We used Arg, Orn and DiAcSpm to construct a regression diagnosis model for diagnosing colon cancer. The levels of these three metabolites showed significant differences between the two groups in logistic regression analysis giving values of $P < 0.001$ (Arg), $P = 0.023$ (Orn) and $P < 0.001$ (DiAcSpm), respectively. The regression equation was $y = 0.044 \times \text{Orn} + 0.408 \times \text{Arg} - 0.009 \times \text{DiAcSpm} - 2.013$.

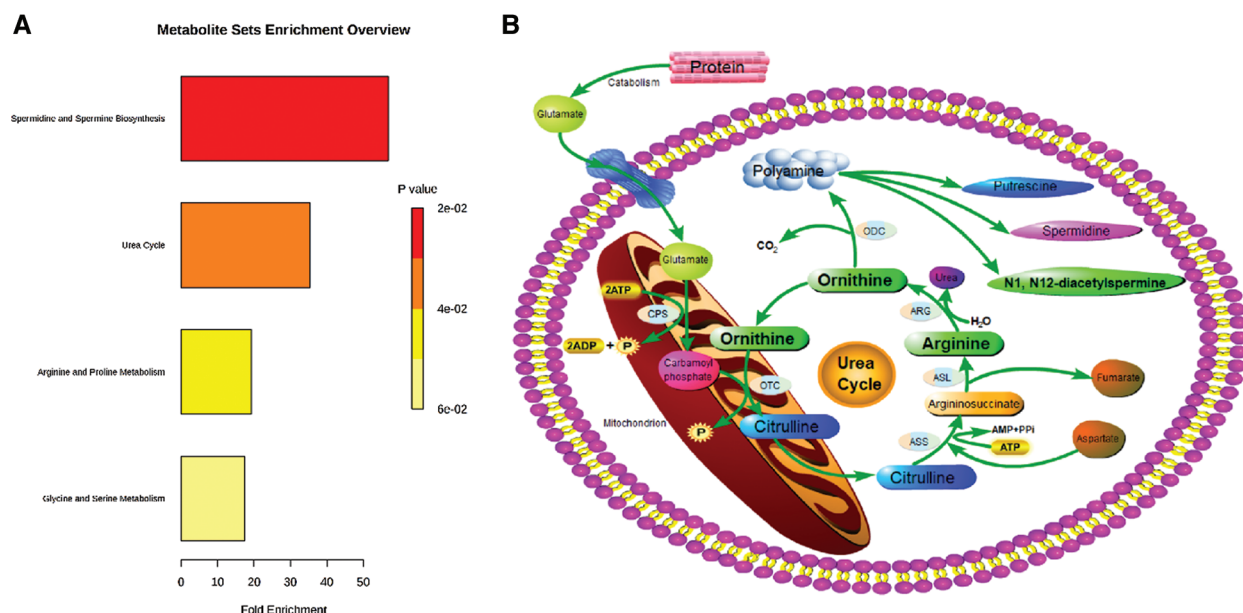


Figure 3: Affected metabolic pathways of Orn in human body. (A) Functional enrichment analysis of Orn in MetaboAnalyst (version 4.0; www.metaboanalyst.ca). (B) The network of Orn metabolic pathways. CPS: carbamoyl phosphate synthetase; OTC: ornithine transcarboxylase; ASS: arginine succinate synthetase; ASL: argininosuccinate lyase; ARG: arginase; ODC: ornithine decarboxylase

Table 3: Arg and DiAcSpm metabolic levels in clinicopathological factors

Factors	N	Arg ($\mu\text{mol/L}$)		DiAcSpm (nmol/g-cre)	
		mean \pm SD	<i>P</i> -value	mean \pm SD	<i>P</i> -value
Age (years)			0.832		0.260
≤ 56.5	22	8.57 \pm 2.53		574.48 \pm 251.68	
> 56.5	41	8.89 \pm 2.11		668.27 \pm 263.50	
Sex			0.622		0.724
Male	33	9.20 \pm 3.09		641.03 \pm 267.45	
Female	30	8.65 \pm 2.34		610.67 \pm 282.36	
Histological type			0.183		0.519
Adenocarcinoma	51	8.99 \pm 3.10		597.39 \pm 246.78	
Non-adenocarcinoma	12	7.84 \pm 1.87		645.52 \pm 273.44	
Depth of invasion			0.365		0.388
T1-2	37	8.92 \pm 2.66		603.77 \pm 266.28	
T3-4	26	8.33 \pm 2.74		675.83 \pm 290.14	
Lymphatic invasion			0.020		0.007
Negative	44	9.18 \pm 2.97		557.00 \pm 258.55	
Positive	19	7.71 \pm 1.95		702.38 \pm 269.47	

(Continued)

Table 3 (continued).

Factors	N	Arg ($\mu\text{mol/L}$)		DiAcSpm (nmol/g:cre)	
		mean \pm SD	<i>P</i> -value	mean \pm SD	<i>P</i> -value
TNM stage			0.006 (I vs. IV)		<0.001 (I vs. IV)
I	26	9.32 \pm 3.13		528.89 \pm 256.43	
II	18	8.31 \pm 2.85		612.52 \pm 270.38	
III	11	8.50 \pm 2.04		666.37 \pm 303.57	
IV	8	7.11 \pm 1.78		730.61 \pm 212.68	
Side of tumor			0.256		0.433
LCC	35	8.17 \pm 1.95		582.57 \pm 234.30	
RCC	28	9.03 \pm 2.46		639.15 \pm 301.73	

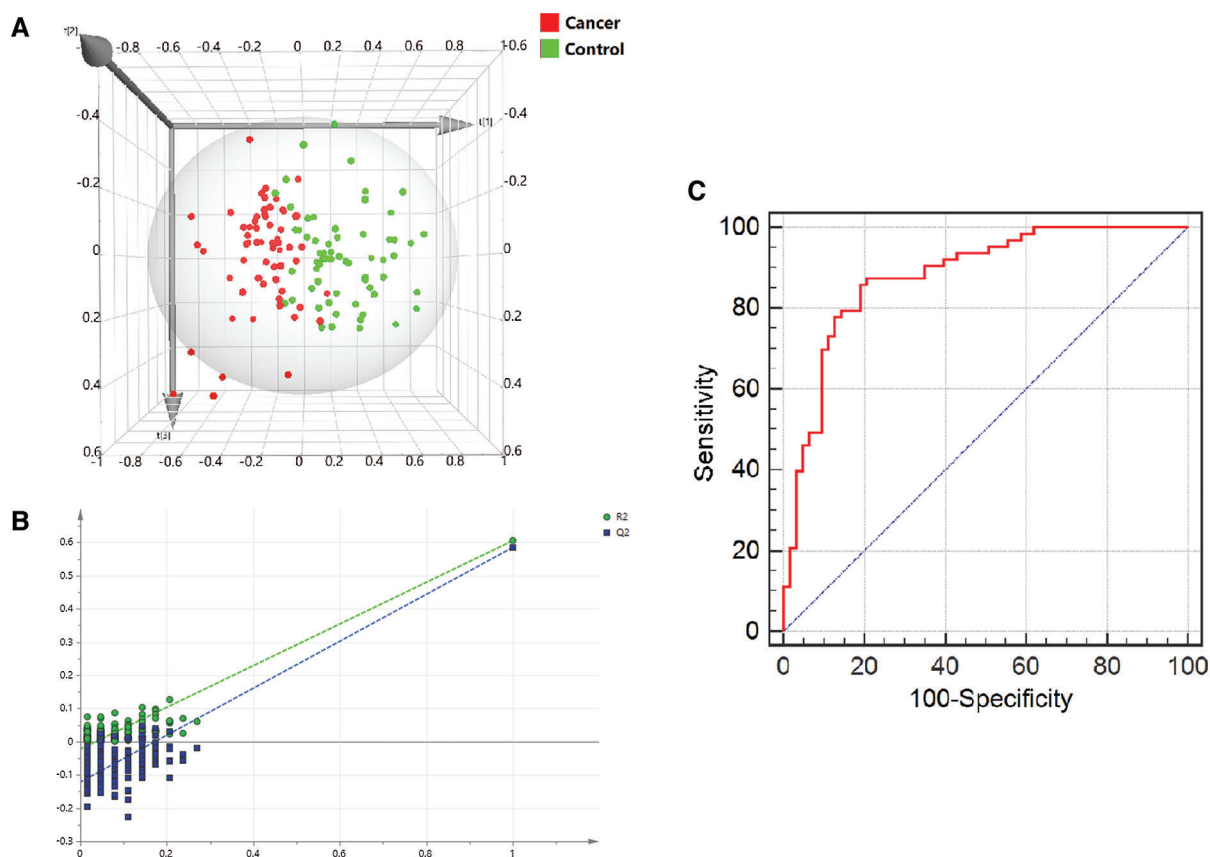


Figure 4: Multivariate correlation analysis of metabolites. (A) 3D score plot presented the discrepancy of a combined metabolic profile of Arg, Orn and DiAcSpm between the two groups. The x, y and z axes were the first 3 principal calculated components for the PLS-DA model. (B) A 200-time permutation test was used to validate the corresponding model. There was no over-fitting in the model. (C) ROC curve based on logistic regression analysis showed the efficiency of the diagnosis model

Efficiency of the diagnosis model based on logistic regression analysis was assessed via ROC curve. The area under the curve (AUC) was 0.880, and the model displayed sensitivity and specificity of 85.7% and 81.0%, respectively. The cut-off value was 0.667 (Fig. 4C).

4 Discussion

This study further verified that the concentration of Orn significantly decreased in plasma of patients with colon cancer compared with the healthy participants. Through the identification of correlation between Orn and clinicopathological factors in colon cancer group, we demonstrated that Orn was closely associated with the depth of tumor invasion, lymphatic invasion status and TNM stage. The results showed that the level of Orn in advanced colon cancer tended to be lower than that in early stage carcinoma, suggesting that Orn was negatively correlated with the tumor burden in colon cancer.

In order to further reveal the possible mechanisms of Orn metabolism changes in colon cancer, we used MetaboAnalyst and KEGG database to find the Orn metabolic pathway in human body, and observed the upstream and downstream metabolites including Arg and DiAcSpm. We analyzed the metabolic alterations of Arg and DiAcSpm in colon cancer. The results proved that Arg in plasma was down-regulated and DiAcSpm in urine was up-regulated in patients with colon cancer.

As the precursor of Orn synthesis, Arg is converted into Orn and urea through urea cycle. Arg can inhibit the growth of tumor cells by downregulating the expression of anti-apoptotic genes Survivin and Bcl-2 [19]. Arg depletion may selectively inhibit the motility and invasion of colon cancer cells [20]. In addition, Arg intake can improve the prognosis of colorectal cancer patients [21]. Arginine succinate synthetase 1 (ASS1) is the key enzyme in the progress of Arg biosynthesis. Recent studies have shown that the ability of tumor cells synthesizing Arg is reduced because of the ASS1 deficiency. The low concentration of Arg in cancer patients may contribute to the growth of tumors [22,23]. Therefore, these metabolic changes could lead to a decrease in the source of Orn synthesis in colon cancer patients.

Orn is converted into polyamines by ornithine decarboxylase (ODC), and polyamines mainly include putrescine, spermine and spermidine in human body. DiAcSpm with little individual variation is the derivative of spermine. DiAcSpm has strong correlation with the tumorigenesis, and its concentration is significantly higher in various tumors. DiAcSpm appears to accumulate in tissues at a particular stage during the development of cell carcinogenesis [24–26]. Moreover, activity of ODC which is a key enzyme in the biosynthesis of polyamines is elevated in colorectal cancer. Overexpression of ODC could cause enhanced proliferation to promote tumor formation [27]. Thus, it suggests that the ODC-DiAcSpm pathway is overexpressed in colon cancer patients and this change may enhance the Orn catabolism.

In this study, we further proved that the source of Orn synthesis was reduced and the catabolism was enhanced in patients with colon cancer. These metabolic regulations might explain the reason of a decrease in the level of Orn metabolism during the colonic carcinogenesis.

In the urea cycle, arginase catalyzes the synthesis of Orn from Arg. Ma et al observed that arginase was overexpressed in colon cancer, and this change was remarkably associated with Stages III–IV tumors [28]. In the polyamine metabolism, it has been demonstrated that ODC activity is elevated obviously in tumor tissue and the alteration is accompanied with elevation of TNM stage in colorectal cancer patients [29]. We examined the metabolic levels of Arg and DiAcSpm in different pathological stages of colon cancer patients. The results showed that compared with those in Stage I group, the Arg level decreased whereas DiAcSpm increased in Stage IV colon cancer. Thus, we could conclude that Arg-Orn-DiAcSpm pathway which might play important roles in the development and progression of carcinoma is up-regulated in colon cancer and this up-regulation is greater in advanced stage tumor.

The derived polyamine metabolites in Orn pathway play a certain role in the biosynthesis of cancer stem cells, and polyamines can promote the growth of cancer cells. The Orn metabolic pathway of cancer stem

cells exposed to chemotherapy drugs changes significantly, which may produce a marked effect by inhibiting the activity of histone demethylase. This inhibition can regulate gene expression level and then affects cell survival to resist drug therapy [11]. Hence, the change of Orn metabolic pathway is a key link in the process of cancer cell proliferation.

Through multivariate data modelling, we investigated the characteristic of a combined metabolic profile of biomolecules in Orn pathway. The results of PLS-DA in multivariate statistical analysis presented significant differences in the metabolic profile of a combination of Arg, Orn and DiAcSpm between colon cancer and healthy control groups. We further used these three metabolites to construct a regression analysis model for diagnosing colon cancer. ROC curve analysis of the regression model showed its good diagnostic ability. As compared to a combination of carcinoembryonic antigen (CEA), carbohydrate antigen 19-9 (CA19-9) and alpha fetoprotein (AFP) for diagnosing colorectal cancer with sensitivity of 80.43%, 69.57% and 73.91%, respectively, our regression diagnostic model had preferable predictive capacity [30]. It suggests that the three metabolites' quantitative analysis can successfully discriminate colon cancer patients and healthy participants. Arg, Orn and DiAcSpm may form an efficient diagnosis model of colon cancer.

5 Conclusion

In short, utilizing the metabolome database combined with clinical sample test, we verified the significant alterations in the level of Orn metabolism in patients with colon cancer. Through analysis of metabolic pathway, we observed the metabolic perturbations of Arg-Orn-DiAcSpm pathway and revealed the reason of the decrease in Orn level of colon cancer. The up-regulation of Orn metabolic pathway might be the key event in pathogenesis of colon cancer, which could provide theoretical support to discover new therapeutic targets and drugs against cancer. Based on the results from multivariate analysis, we found that the combined metabolic profile of Arg, Orn and DiAcSpm might be an excellent noninvasive screening tool of colon cancer. Multivariate data modelling is expected to offer new technical support for cancer diagnosis. The high standard deviation of the measurements in this study may be related to the limited sample size and individual differences. We will conduct prospective studies involving a large cohort of clinical samples from colon cancer patients and healthy controls to determine the validity of this diagnosis model. Further investigations should be completed to elucidate the oncological and biological significances of Orn metabolic pathway in cancer. It is necessary to research the effect of down-regulation of Orn metabolic pathway on cancer cells.

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