Correlation and clinical significance of serum amino acid level changes with TNM stage in patients with colorectal cancer

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Abstract

Purpose: To explore the correlation between the changes of serum amino acid levels in colorectal cancer patients and TNM staging from the perspective of amino acid levels in human peripheral blood.

Methods: The data of 723 colorectal cancer patients were collected and evaluated, and they were divided into T1, T2, T3, and T4 groups. The changes of amino acid levels in different stages and the correlation between serum amino acid levels and TNM stages were compared.

Results: There were significant differences in the levels of Ala, Pro, and Val in different TNM stages ($P<0.05$). Serum Ala, Pro, and Val were negatively correlated with TNM stages ($P<0.05$).

Conclusions: Serum Ala Pro Val level is correlated with TNM stage in patients with colorectal cancer. The higher TNM stage is, the lower serum Ala, Pro and Val level is. The identification of specific targeting factors required for tumor metabolism may lead to more effective therapies that limit the nutritional pathways of fatal cancers.

1 Introduction

Colorectal cancer (CRC) is the fourth leading cause of cancer death worldwide, accounting for approximately 10% of annual cancer-related deaths worldwide[1–3]. Studies have shown that identifying the specific stage of colorectal cancer patients and providing corresponding treatment measures to patients according to the stage is conducive to improving the treatment effect and improving the prognosis. Therefore, identifying the factors that change in the occurrence and development of colorectal cancer is of great significance for intervening in tumor growth. Metabonomics has been applied to the research of various tumors (such as oral cancer, thyroid cancer, gastric cancer, prostate cancer, and gynecological cancer) in recent years to study its role in cancer diagnosis, treatment, and prevention [4–7]. Metabolomics is a method for comprehensive analysis of metabolites in biological samples, which may provide an idea and method for inhibiting the occurrence and development of CRC tumors.

Amino acid is an important metabolite in metabolomics research. Amino acid is one of the most basic components of cell structure and an important part of protein synthesis in the process of cell proliferation. Amino acids are used in the synthesis of proteins and the formation of other low molecular weight compounds, and also provide energy for other biosynthetic pathways [3, 8, 9].

This study aims to explore the changes of serum amino acid metabolism in colorectal cancer patients with different TNM stages from the perspective of amino acid levels in human peripheral blood, combined with multivariate statistical analysis, and to analyze the amino acid metabolites and TNM with significant differences. relationship between periods.

2 Materials And Methods

2.1 Sample Collection

The study was approved by the Ethics Committee of the First Affiliated Hospital of Jinzhou Medical University (Liaoning, China) and conducted following the Declaration of Helsinki. During the period from 2018 to 2020, samples from 723 patients with CRC in the Second Colorectal Department of our hospital were collected, and fasting venous blood was taken in the morning and stored in a lithium vacuum blood vessel containing heparin. Shake well, temporarily stored in a 4°C refrigerator, and send it to the laboratory within 30 minutes.

2.2 Inclusion and exclusion criteria

The inclusion criteria of colorectal cancer cases were as follows: □ pathologically diagnosed with colorectal cancer; □ primary tumor; □ no neoadjuvant therapy.

Exclusion criteria: □Primary tumors in other parts; □Recurrent colorectal cancer; □Patients who have undergone preoperative radiotherapy and chemotherapy or targeted therapy; □Patients with serious diseases of other systems (blood system, metabolic system, etc.).

2.3 Grouping

□T1 group: patients with colorectal cancer with the postoperative pathological return of T1 stage (21 cases);□T2 group: patients with colorectal cancer whose postoperative pathological return was T2 stage (51 cases);□T3 group: patients with colorectal cancer whose postoperative pathological return was T3 (278 cases);□T4 group: patients with colorectal cancer whose postoperative pathological return was T4 stage (85 cases).

2.4 Statistical analysis

All data were entered into the MS EXCEL database, and SPSS26.0 software was used for data standardization. At the same time, metabolites with $P < 0.05$ were screened out by one-way analysis of variance. The normalized data were imported into SIMCA-P 14.1 (Umetrics, Umea, Sweden) software for principal component analysis (PCA) and orthogonal partial least squares discriminant analysis (OPLS-DA). Define the 95% confidence intervals for the model variables. The quality of the model is described by R2X and R2Y and Q2. Q2 represents the predictability of the model, and R2X or R2Y represents the fit of the model. Metabolites with VIP > 1 were screened by SIMCA-P, and metabolites satisfying VIP > 1 and U test $p < 0.05$ were regarded as distinguishable amino acid metabolites. Correlation analysis was performed using Spearman correlation analysis, and $P < 0.05$ was considered statistically significant.

3 Results

3.1 One-way ANOVA of serum amino acid levels in patients with different TNM stages

A total of 23 amino acids were included in the study analysis (Table 1), of which the levels of 6 amino acid metabolites were significantly different in different TNM stages, including Ala (F=3.229, P=0.0224), His (F=5.186, P =0.0016), Pro (F=3.897, P=0.0091), Ser (F=2.640, P=0.0490), Tyr (F=3.246, P=0.0219), Val (F=5.17, P=0.0020) (Fig 1).

Table 1

Anova of amino acid metabolites

Amino Acid	F value	P value	Amino Acid	F value	P value
Alanine (Ala)	3.229	0.022	Lysine (Lys)	0.667	0.573
Arginine (Arg)	0.641	0.589	Methionine (Met)	1.283	0.280
Asparagine (Asn)	0.847	0.469	Ornithine (Orn	0.195	0.900
Aspartic Acid (Asp)	0.692	0.557	Phenylalanine (Phe)	0.411	0.745
Citrulline (Cit)	1.709	0.164	Piperine (Pip)	1.246	0.293
Cysteine (Cys)	0.191	0.902	Proline (Pro)	3.897	0.009
Glutamine (Gln)	0.037	0.990	Serine (Ser)	2.640	0.049
Glutamic acid (Glu)	1.429	0.234	Threonine (Thr)	0.222	0.881
Glycine (Gly)	0.668	0.572	Tryptophan (Trp)	1.407	0.240
Homocysteine (Hcy)	0.526	0.665	Tyrosine (Tyr)	3.246	0.022
Histidine (His)	5.318	0.001	Valine (Val)	5.017	0.002
Leucine (Leu)	1.448	0.228			

3.2 Multivariate analysis of serum amino acid levels in patients with different TNM stages

Peripheral blood metabolites from CRC patients were analyzed using an LC-MS/MS-based metabolomics approach. First, unsupervised principal component analysis was performed on the metabolomic data of the normalized T1, T2, T3, and T4 groups. Fig 2a (PCA-X: R2X=0.759, Q2=0.748) shows that most of the four sets of data are scattered in different regions, and most of the samples are within the 95% confidence interval. Therefore, all samples can be included in the following analysis to ensure the maximum amount of information. In addition, we also performed OPLA-DA to analyze the data, as shown in Fig. 2b (OPLS-DA: R2X=0.846, R2Y=0.994, Q2=0.842), and found that the RCC group was significantly separated from the LCC group, indicating that The model is reliable for explaining the difference between the two. And the metabolites with VIP value>1 were obtained: Orn(VIP=3.5927), Pro(VIP=2.4046),

Ala(VIP=2.0572), Val(VIP=1.5241). Under the conditions of P<0.05 and VIP>1, three metabolites of Ala, Pro, and Val can be obtained (Table 2).

Table 2

VIP value and P value of differential metabolites

	VIP value	P value
Ala	2.0572	0.0224
Pro	2.4046	0.0091
Val	1.5241	0.0020

3.3 Correlation between differential metabolites and TNM staging

According to Spearman correlation analysis, Ala, Pro and Val were negatively correlated with TNM staging, among which Ala (r=-0.141, P=0.003), Pro (r=-0.119, P=0.013), Val (r=-0.177, P <0.01) (Table 3). All three amino acid metabolite levels showed a downward trend with increasing tumor stage (Fig 3).

Table 3

Correlation analysis of Ala, Pro, Val levels and TNM staging

	Ala	Pro	Val
r value	-0.141	-0.119	-1.77
P value	0.003	0.013	0.01

4 Discussion

CRC is one of the most common malignant tumors of the digestive system. The occurrence and development of CRC is a multi-factor and multi-gene cooperative process [10]. Therefore, it is of great significance to identify the factors that change in the occurrence and development of colorectal cancer for inhibiting tumor growth. Through analysis, we found that the levels of Ala, Pro, and Val were significantly different in different TNM stages, and serum Ala, Pro, Val were negatively correlated with TNM stages. The higher the TNM stage, the lower the serum Ala, Pro, and Val levels.

During periods of cellular stress, cancer cells alter their metabolism to increase survival, undergo uncontrolled proliferation, and progress toward metastasis formation [11]. Amino acids serve as substrates for protein synthesis and energy sources for protein production and metabolism, regulating

many cellular functions. Studies have shown that alanine supply supports metabolism, growth, and treatment resistance in pancreatic ductal adenocarcinoma (PDAC) [12]. Furthermore, PDAC cells need to meet their increased alanine requirements by upregulating SLC38A2. Alanine-deficient cancer cells experience a severe metabolic crisis that results in significantly impaired tumor growth[13].

The proliferation of cancer cells is dependent on biomass production, the biosynthesis of macromolecules such as DNA and proteins from amino acids and other metabolites, proline, which has been shown to promote the production of proteins, which are responsible for cell proliferation. Required [14, 15]. Proline is synthesized from ornithine or glutamate, and the specific role of proline in metabolic regulation is now accepted [16–18], and recent work not only suggests that proline metabolism is critical in cancer reprogramming [15, 19, 20], and its clinical significance has also been established [21]. In human gastrointestinal and kidney tumors, immunohistochemistry showed that proline oxidase levels were significantly reduced in nearly 80% of subjects compared to corresponding normal tissues [22]. Furthermore, proline extracted from collagen has been found to support the proliferation of pancreatic cancer cells [23]. The importance of proline for the proliferation of pancreatic cancer cells is manifested not only in the direct binding of proline to proteins but also in the catabolism of proline leading to the production of glutamine, glutamate, and aspartate [24]. Furthermore, it was found that down-regulation of proline biosynthesis in cancer cells expressing c-MYC resulted in a decrease in glycolysis and ATP production [15, 20]. Interestingly, recent studies have shown increased proline biosynthesis in isocitrate dehydrogenase 1 (IDH1)-mutated cancer cells compared to wild-type IDH cancer cells, which leads to electron transport in the TCA cycle Partial unhooking of the chain [25]. Thus, ATP-coupled oxygen consumption is increased in IDH1-mutated cancer cells under the effect of proline biosynthesis inhibition. Therefore, it is easy to speculate that both of these observations could affect cancer cell proliferation by disturbing cellular redox balance. Taken together, proline metabolism can support cancer proliferation and is, therefore, an interesting point of intervention to inhibit the growth of certain types of tumors.

Studies have shown that the biosynthesis of proline helps maintain intracellular nucleotide levels [15]. In some cancer cells, inhibition of proline biosynthesis was found to impair tumorigenic potential and reduce the growth of attached monolayers [19, 20]. Muscle atrophy and amino acid mobilization in the muscles of cancer patients may be driven by the secretion of different tumor-derived mediators. Therefore, a gradual decrease in muscle mass may be observed in some cancer patients. It has also been reported that the oxidation rate of branched-chain amino acids (BCAAs) in the muscle of cancer patients is higher [26]. There is increasing evidence that BCAAs are essential nutrients for cancer growth and are used by tumors as an energy source [27].

Molecularly targeted therapy refers to the use of drugs or other substances to target specific molecules (molecular targets) to stop the growth and spread of cancer cells. Identifying ideal targets is the key to the successful development of molecularly targeted therapies for cancer [28]. In this study, 11 amino acids and carnitine metabolites involved in the occurrence and development of CRC were analyzed. Whether they can be the target of targeted therapy? Targeted therapy of CRC with different metabolic pathways still needs more research to verify.

To the best of our knowledge, this study is the first to analyze the differences in the occurrence and development of CRC from the perspective of amino acids and obtained three metabolites with significant differences. More data analysis is needed to explore how the above-mentioned differential metabolites affect the occurrence and development of CRC. Of course, this study also has certain limitations. Since our data are data in the past 3 years, we cannot discuss the survival time and survival rate of patients, so it is difficult for us to understand the survival curve and prognosis of patients. More research is needed in the future to further understand the occurrence and development of CRC.

5 Conclusion

Through relevant studies and analysis, it was concluded that serum levels of Ala, Pro and Val were correlated with TNM stage in colorectal cancer patients. The higher TNM stage was, the lower serum levels of Ala, Pro and Val were, indicating that tumor growth requires consumption of Ala, Pro and Val amino acid metabolites. Identification of metabolic pathways and targeting factors of three amino acids can effectively prevent tumor growth and limit the development process of cancer.

Declarations

Author contribution

Study concept and design: Yang Feng, Shifeng Qiao. Acquisition of data: Chuanchao Yue, Xueqian Ma, Xiaoyu Song, Jinhao Liu, Yanlei Chen, Yanping Wang. Analysis and interpretation of data: Yang Feng, Yu Gao. Drafting of the manuscript: Yang Feng. Critical revision of manuscript: Shifeng Qiao. All authors read and approved the final manuscript.

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Declarations

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Footnotes

None.

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Figures

Figure 1

One-way ANOVA of amino acid metabolites.

a. Alanine(Ala); b. Histidine(His); c. Proline(Pro); d. Serine(Ser); e. Tyrosine(Tyr); f. Valine(Val)

Figure 2

Metabolic comparison between T1, T2, T3 and T4 groups

a. 3D Principal component analysis(PCA) scores of stage T1, T2, T3 and T4 colorectal cancer. b. Orthogonal partial least squares discriminant analysis(OPLS-DA) scores of stage T1, T2, T3, and T4 colorectal cancer

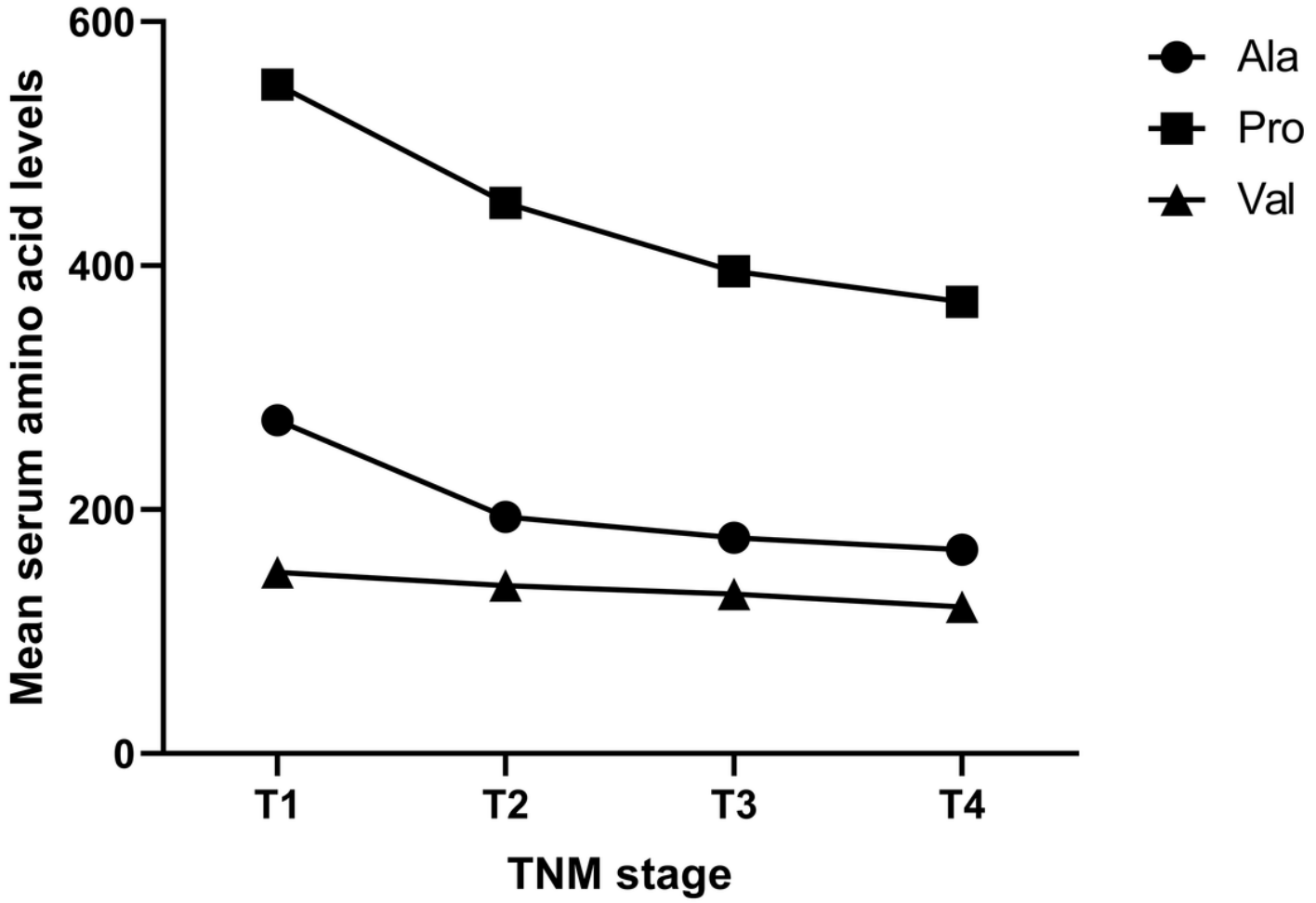

Figure 3

The metabolite levels of Ala, Pro and Val varied with TNM stage