Original Article

Plasma and tissue free amino acid profiles and their concentration correlation in patients with lung cancer

Qihong Zhao PhD[1], Ye Cao MM[1], Ying Wang MM[1], Chuanlai Hu MD[1], Anla Hu MD[1], Liang Ruan MM[1], Qingli Bo MM[1], Qifei Liu MB[1], Wenjun Chen MM[1], Fangbiao Tao MD[1], Min Ren MD[2], Yongsheng Ge MD[3], Anguo Chen MD[2], Li Li MD[1]

[1]*Department of Food and Nutrition Hygiene, School of Public Health, Anhui Medical University, Anhui, China*
[2]*Department of General Surgery, the First Affiliated Hospital, Anhui Medical University, Anhui, China*
[3]*Department of General Surgery, Shengli Hospital, Anhui Medical University, Anhui, China*

Variation of plasma free amino acids (PFAAs) is an essential feature of protein metabolic abnormalities in cancer patients. But there still little data about the cancer tissue free amino acid (TFAAs) profiles, including their patterns and correlations with PFAAs. To evaluate the variation in PFAAs and cancer TFAAs in patients with lung cancer, including their patterns and correlations, we investigated the concentrations of free amino acids in lung cancer tissues (n=27), paired lung paracarcinomous tissues (n=27) and plasma (n=27) using an automatic amino acid analyzer after pre-treatment. Within the PFAAs, the concentrations of five amino acids (tryptophan, glycine, citrulline, ornithine and proline) were significantly decreased, while that of phenylalanine was markedly increased compared with control subjects. Within the TFAAs, the concentrations of three amino acids (taurine, glutamic acid and glycine) were increased, while the concentrations of two amino acids (lysine and ornithine) were decreased significantly in lung cancer tissues compared with the paracarcinomous tissues. The amino acid patterns in PFAAs and TFAAs had similar trends, but percentage variations were diverse. Additionally, the concentrations of five amino acids (lysine, phenylalanine, threonine, serine, and alanine) in PFAAs correlated with those in lung cancer TFAAs, but no amino acids in PFAAs were correlated with those in lung paracarcinomous TFAAs. Thus, PFAA profiles may reflect the status of cancer tissues, which may provide more information about the metabolic statuses and prognoses of patients with lung cancer.

Key Words: plasma free amino acids, tissue free amino acids, lung cancer, amino acid pattern, concentration correlations

INTRODUCTION

Lung cancer is one of the most aggressive malignant tumours worldwide, and most lung cancers are not easily detected until late stages. According to global cancer statistics,[1] 2.16 million of the total new cancer cases in males in 2008 were lung cancer. Furthermore, lung cancer is also becoming increasingly common among females. Although many treatment methods and anti-cancer drugs have been developed to improve its prognosis, the overall survival rate of patients with lung cancer is still very low. In 2008, approximately 1.75 million deaths occurred in patients with lung cancer.[1] Based on the Chinese cancer registry annual report, 3.12 million new cancer cases occurred in 2012 with a 180.54/100,000 cancer mortality ratio, and lung cancer ranked first in the list of cancer incidence and cancer mortality in China.[2]

Redistribution of plasma free amino acids (PFAAs) is an essential feature of protein metabolic abnormalities in cancer patients.[3,4] This redistribution is mediated by the tumour itself and is characterized by increased whole-body protein turnover, which can result from malnutrition or may be related to the stages or types of cancer. Body weight loss is likely to be the most obvious clinical sign long before cancer-related cachexia for anorexia or other nutritional changes.[5,6]

Meanwhile, levels of PFAAs represent the net effect of all factors that influence the total flux of amino acids in the body.[7,8] The alteration of plasma amino acid patterns depends on their metabolism during the development of cancer. Many previous studies have described changes in PFAA profiles in cancer patients; some of these focused on the differences in PFAAs between cancer patients and healthy individuals, as well as the application to early detection for cancer patients.[9-11] Other studies concentrated on the intravenous supplementation of amino acids

Corresponding Author: Dr Li Li, Department of Food and Nutrition Hygiene, School of Public Health, Anhui Medical University, Meishan Road, Hefei, Anhui 230601, China.
Tel: +86-551-63869176; Fax: +86-551-63869176
Email: li1964li@163.com
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to cancer patients based on the results of the differences of the PFAAs between cancer patients and healthy individuals.[12,13] However, there remains little data about the profiles of the free amino acids of human cancer tissues, and little was known about the patterns and correlations of tissue free amino acids (TFAAs) and PFAAs.

In the present study, we measured PFAAs and TFAAs in patients with lung cancer as well as PFAAs in healthy volunteers, focusing on analysis of the alterations of PFAA and TFAA profiles and the patterns and correlations between PFAAs and TFAAs.

METHODS

Study population

All patients (n=27) who underwent surgical resection of primary lung cancer at Anhui Medical University were recruited with written informed consent before they participated in the study. Plasma was taken before the surgery and fresh tumour specimens were obtained immediately after surgery from all patients according to protocols approved by the ethics committees of Anhui Medical University. The healthy control samples were also obtained from Anhui Medical University with written informed consent at the same period before plasma sampling. All data were analyzed anonymously throughout the study.

All patients recruited in this study were diagnosed with primary lung cancer according to the tumour site and tumour stages based on UICC classification. None of the study patients had received prior radiotherapy or antineoplastic treatment and they had no clinical or biochemical evidence of liver or renal failure. Pathological diagnoses prior to TFAA determinations of clinical lung cancer tissues after surgical resection were conducted if the dissected tumour block was not large enough for both. Exclusion criteria for healthy controls were: malignancy, recent surgery, infection, or severe diseases with alteration of PFAAs, such as endocrine, hepatic, gastrointestinal, and renal disorders. The patients' and healthy controls' characteristics, including age, BMI, stages of tumour, weight loss presence and other blood biochemical indexes in this study are listed in Table 1. Smoking status, as one of the important risk factors for lung cancer, was also recorded and analyzed in relation to the PFAAs and TFAAs (Supplementary tables S1 and S2). Finally, gender and UICC staging were recorded and analyzed (Supplementary tables S1 and S2).

Sampling

After an overnight fasting before surgery, 4 mL blood samples were obtained by venipuncture from the forearm, were kept in heparinised glass syringes, immediately put on ice, and processed using the following procedures within 2 h: centrifuging at 2,000 g for 15 mins at 4℃ to obtain plasma, followed by deproteinization with 10% sulfosalicylic acid for 1 h. Supernatants were frozen in liquid nitrogen and stored at -80℃ until analyzed.

Tissue specimens were sampled immediately after surgery and areas of necrosis or liquefied parts were excised. These specimens were also frozen in liquid nitrogen and stored at -80℃ until analyzed.

Amino acid analysis

The plasma supernatants were re-centrifuged at 2,000 g for 5 mins at 4℃ and diluted (1:4) by the sample diluents (membraPure, Germany), then subsequently filtered using a 0.22 μm membrane. The clear supernatants with sample diluents were used for individual amino acid analysis by an automatic amino acid analyzer (A300, membraPure, Germany).

The tissue specimens were weighed, cut into small pieces, levigated by mortar with liquid nitrogen, and deproteinized with 10% sulfosalicylic acid. The following procedures were similar with plasma samples in the present study.

Amino acid analysis was performed with a kit consisting of packaged and QC-tested columns and reagents. The amino acid levels were calculated by means of a modified version of software supplied by the manufacturer (A300, membraPure, Germany). Recoveries, detection limits and linearity of all amino acids were established by analyzing plasma samples before and after standard addition method with reference compounds to define these parameters. Exact retention times and response factors for each amino acid were determined at two wavelengths (λ=570 nm and λ=440 nm). The signal ratios at these wavelengths were determined for proper identification of the individual amino acids and detection of co-eluting interferences.

The free amino acids were composed of two groups: the essential amino acids (EAAs) and the non-essential amino acids (NEAAs). EAAs measured were lysine (Lys), methionine (Met), isoleucine (Ile), leucine (Leu), tryptophan (Trp), phenylalanine (Phe), histidine (His), valine (Val), and threonine (Thr); the non-essential amino acids (NEAAs) tested included taurine (Tau), aspartic acid (Asp), serine (Ser), glutamic acid (Glu), glycine (Gly), alanine (Ala), α-Aminobutyric acid, cystein (Cys), tyrosine (Tyr), hydroxylysine (Hylys), ornithine (Orn), arginine (Arg), and proline (Pro) for the PFAAs. For TFAA measurement, the hydroxylysine and arginine content was under the detection limits.

Aromatic amino acid (AAA) levels were calculated as the sum of phenylalanine (Phe), tryptophan (Trp), histidine (His) and tyrosine (Tyr), and the plasma branched chain amino acid (BCAA) levels were calculated as the sum of the values for Val, Leu, and Ile concentrations. The plasma total nonessential amino acid (NEAA) levels were the sum of the levels for all non-essential amino acids. A coefficient, which was automatically generated by the calculating system supplied with the automatic amino acid analyzer, was used to adjust these values.

Percentage variation is a ratio of the difference of baseline concentration and final/changed concentration after some stress or interference to the baseline concentration.[14] We calculated the percentage variation here using the mean PFAAs concentration of the healthy controls as the baseline concentration, and the PFAAs concentration in the patients with lung cancer as the changed concentration. As for TFAAs, the concentration of TFAAs in paracarcinomous tissues were considered as the baseline concentration, and the concentration of TFAAs in the cancer tissues were calculated as the final concentration.

Statistical analysis

PFAA and TFAA concentrations are given as the mean± SE. After Levene's test for equality of variances, independent t tests were performed to assess the differences in TFAAs and PFAAs between groups. Simple correlation analysis was conducted to investigate the relationships between PFAAs and TFAAs using Spearman's correlation coefficient (r-values). Statistical analysis was performed using SPSS 16.0. A p-value<0.05 was considered as statistically significant.

RESULTS

PFAAs in lung cancer patients and the healthy

The PFAA concentrations of lung cancer patients and healthy controls are shown in Table 2 as the mean±SE.

Overall, the sum of EAAs in PFAAs was decreased significantly (p<0.01) in lung cancer patients compared with the healthy controls. Among the EAAs, tryptophan was lower in the patients than in the controls (p<0.01), but phenylalanine was higher in the patients than in the controls (p<0.05).

The sum of NEAAs was also decreased markedly in the patients compared with the healthy controls. Among the NEAAs, notable decreases in glycine, citrulline and proline (p<0.001 for all), accompanied by a moderate decrease in ornithine (p<0.05), were observed in the patient plasma compared with the healthy controls. In contrast, aspartic acid and hydroxylysine were increased significantly in the patients compared with the controls (p<0.001 for both).

There was no difference in AAA and BCAA between these two groups, but the BCAA/AAA ratio was decreased in patient plasma compared with the healthy donors (p<0.001).

TFAAs in lung cancer patients

The TFAA concentrations of the lung cancer and paracarcinomous tissues are shown in Table 3 as the mean±SE. Overall, there was no difference in the sum of EAAs between these two groups (p=0.727), but among these EAAs within the TFAAs, one amino acid, namely lysine, was decreased significantly compared with that in the paracarcinomous tissues.

The sum of NEAAs in cancer tissues was increased significantly compared with the paracarcinomous tissues (p<0.05). Among these NEAAs, taurine, glutamic acid and glycine were increased markedly compared with the paracarcinomous tissues (p<0.001, p<0.001 and p<0.05, respectively). Only ornithine was decreased markedly compared to the paracarcinomous tissues (p<0.05).

Patterns of TFAAs and PFAAs in lung cancer patients

TFAA and PFAA patterns in lung cancer patients are shown in Figure 1 and 2. The TFAAs and PFAAs had similar trends, despite overall increases or decreases in the content of all amino acids.

We also observed a significant difference in the percentage variation of BCAA/AAA, EAA and NEAA within the TFAAs compared with the PFAAs, as shown in Figure 3 (p<0.001, p<0.001 and p<0.05, respectively). There were no significant differences in the percentage variation of BCAA and AAA between the two groups.

Table 1. Age, BMI, stages of tumour and weight loss presence of all participants included in this study†

Participants	Lung cancer patients for TFAA and PFAA assays	Healthy controls for PFAA assay
n	27	22
Male/Female	18/9	13/9
Age	60.5	61
(median, 2.5th–97.5th)	(50, 71)	(39, 79)
BMI	19.2	21.2
(median, 2.5th–97.5th)	(16.6, 25.9)	(17.7, 25.7)
Weight loss	None	None
UICC staging (1/2/3/4)	8/13/5/1	—
Hemoglobin‡	135±3	147±3
Creatinine‡	62±4	65±2
Alanineaminotransferase‡	17±4	24±2
Aspartateaminotransferase‡	28±2	31±1
Alkalinephosphatase‡	78±5	88±4

† $\bar{x}$ ±SE unless otherwise noted in the table. The units for the blood biochemical indexes were: g/L for hemoglobin, μ/L for alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase, and μmol/L for creatinine.
‡There was no statistically difference between groups by independent t test.

Table 2. Plasma free amino acid profile (μmol/L) in patients with lung cancer compared with controls†

PFAAs	Control (n=22)	Patients (n=27)	p value
Essential			
Lysine	279±23	325±32	0.258
Methionine	61±8	57±12	0.826
Isoleucine	190±34	158±25	0.454
Leucine	166±14	188±11	0.244
Tryptophan*	298±110	53±3	0.038
Phenylalanine**	109±4	139±8	0.003
Histidine	184±27	238±80	0.511
Valine	281±29	241±24	0.307
Threonine	214±35	270±50	0.362
Sum of EAA**	1422±40	965±132	0.003
Non-essential			
Taurine	313±138	104±21	0.143
Aspartic acid***	54±4	105±14	0.001
Serine	258±27	253±28	0.893
Glutamic acid	210±11	211±41	0.989
Glycine***	454±29	298±29	0.001
Alanine	611±32	643±88	0.730
Citrulline***	82±6	39±9	0.001
α-aminobutyric acid	62±3	55±7	0.478
Cystein	68±4	84±13	0.237
Tyrosine	71±5	74±4	0.639
Hydroxylysine**	11±4	33±3	0.002
Ornithine*	210±14	156±18	0.025
Arginine	96±12	101±11	0.782
Proline***	339±51	129±11	0.001
AAA	282±13	311±16	0.157
BCAA	575±37	582±29	0.890
BCAA/AAA***	2.21±0.87	1.65±0.90	0.000
NEAA**	2784±215	1860±173	0.002

† $\bar{x}$ ±SE. EAA, essential amino acids; AAA, aromatic amino acids; BCAA, branched-chain amino acids; NEAA, nonessential amino acids.
*p<0.05, **p<0.01, ***p<0.001，indicating a significant difference by independent t test after Levene's test for equality of variances between groups.

Table 3. Tissue free amino acid profile (μmol/kg wet wt) in lung cancer and paracarcinomous tissues from patients[†]

TFAAs	Paracarcinomous tissues (n=27)	Cancer tissues (n=27)	*p* value
Essential			
Lysine**	2023±192	1385±142	0.010
Methionine	258±35	267±28	0.276
Isoleucine	362±38	454±39	0.103
Leucine	869±85	1087±92	0.091
Tryptophan	135±25	100±17	0.240
Phenylalanine	455±47	462±38	0.905
Histidine	312±27	341±31	0.500
Valine	655±68	627±68	0.773
Threonine	666±65	764±66	0.295
EAA	5430±473	5216±385	0.727
Non-Essential			
Taurine***	1131±115	1804±156	0.001
Aspartic acid	913±86	1236±145	0.063
Serine	1224±112	1320±102	0.534
Glutamic acid***	2178±168	3147±197	0.000
Glycine*	2387±177	2891±166	0.043
Alanine	1887±157	2254±155	0.104
Citrulline	136±20	116±16	0.453
α-aminobutyric acid	239±24	184±28	0.148
Cystein	162±17	178±19	0.534
Tyrosine	425±48	426±39	0.992
Ornithine*	220±22	145±24	0.028
Proline	1363±160	1580±171	0.361
AAA	966±112	982±90	0.912
BCAA	1887±160	2168±133	0.184
BCAA/AAA	2.38±0.19	2.44±0.15	0.796
NEAA*	12236±978	15276±915	0.027

[†] $\bar{x}$ ±SE. EAA: essential amino acids; AAA: aromatic amino acids; BCAA: branched-chain amino acids; NEAA: nonessential amino acids.
*p<0.05, **p<0.01, ***p<0.001 indicating a significant difference by independent t test after Levene's test for equality of variances between groups.

Correlations of TFAAs and PFAAs in lung cancer patients

We also analyzed the correlation of free amino acids between lung cancer tissues and lung paracarcinomous tissues with plasma from lung cancer patients. The results, shown in Table 4, indicated statistically significant positive correlations of lysine, phenylalanine, threonine, serine, alanine and BCAA/AAA between TFAAs from the lung cancer tissue group and the PFAAs from the same subjects. Only NEAAs had a significant inverse correlation between TFAAs from the lung cancer tissue group and the PFAAs from the same subjects. Only one statistically significant positive correlation of BCAA/AAA was observed between TFAAs from the lung paracarcinomous tissue group and the PFAAs from the same subjects.

DISCUSSION

Cancer metabolism has been an area of increasing concern because understanding the differences between healthy and cancer cell metabolism could help to develop therapy to bring patients to metabolic homeostasis and could also identify new therapeutic targets for clinical intervention. In the present study, we focused on the evaluation of the PFAA and TFAA profiles in patients with lung cancer, which probably reflected protein metabolism disturbances. We also analyzed the patterns and correlations of PFAAs and TFAAs in lung cancer patients compared with corresponding control patients with similar blood biochemical indexes for metabolism.

Many previous studies have observed a number of significant variations in PFAA profiles in patients with different types of cancer[5,15-17] and in laboratory animals with different stages of cancer.[18] Recent studies have suggested that PFAA patterns could be used for early detection or screening for cancers.[9,10] To confirm this hypothesis and detect the free amino acid levels inside cancer tissues, we conducted this study with lung cancer patients just after their surgical resection.

In the evaluation of BCAA in plasma, we found no significant difference in valine, leucine and isoleucine between patient and control samples, which does not agree with previous studies.[10,19] This is likely because leucine and isoleucine are required for muscle protein synthesis, in particular during the late-stage of cancer with cachexia occurring.[12] In TFAAs, we also did not observe intrinsic differences in BCAA between lung cancer tissues and lung paracarcinomous tissues because the lung was not the main metabolic site.[6,20] Additionally, the sampling time and the physical status of the patients may also contribute to the lack of differences in BCAA between groups. In the present study, we sampled the blood and the tissues before the surgery or just after the surgical resection when the BMI and body weight of patients were still at normal levels, which could also explain why gender, smoking status and UICC staging had no effect on the differences in PFAA and TFAA levels between the patients with lung cancer.

Although AAA and BCAA were not statistically different in PFAAs and TFAAs, we found that the BCAA/AAA ratio was statistically significantly decreased in PFAAs in patients, but not in TFAAs, which means that the utilization of BCAA in lung cancer patients was strengthened because the BCAA/AAA ratio showed a significant positive correlation in the TFAAs compared with the PFAAs. These variations might be related to the nutrition status because there was no weight loss or anorexia in these patients.[21-24]

Many previous studies have suggested that the amino acid metabolism in cancer cells varied notably, and a similar variation could be observed in PFAA patterns.[9,25,26] In PFAAs, Nainiet al observed that the content of arginine was decreased markedly in lung cancer patients.[16] Other studies found tryptophan, glutamic acid and ornithine increased significantly in PFAAs from lung cancer patients.[3,22] The TFAAs profiles in lung cancer patients, however, were unknown. In the present study, we detected a significant increase in the glutamic acid content in lung cancer tissues relative to the lung paracarcinomous tissues. However, in PFAAs, glutamic acid was not statistically different between the lung cancer patients and the healthy donors. This phenomenon may be explained by that fact that glutamic acid may improve the growth of cancer in lung tissues, but in PFAAs, glutamic acid could be inhibited by being absorbed for the extensive oxidization performed by mammalian intestine epithelium cells.[27,28]

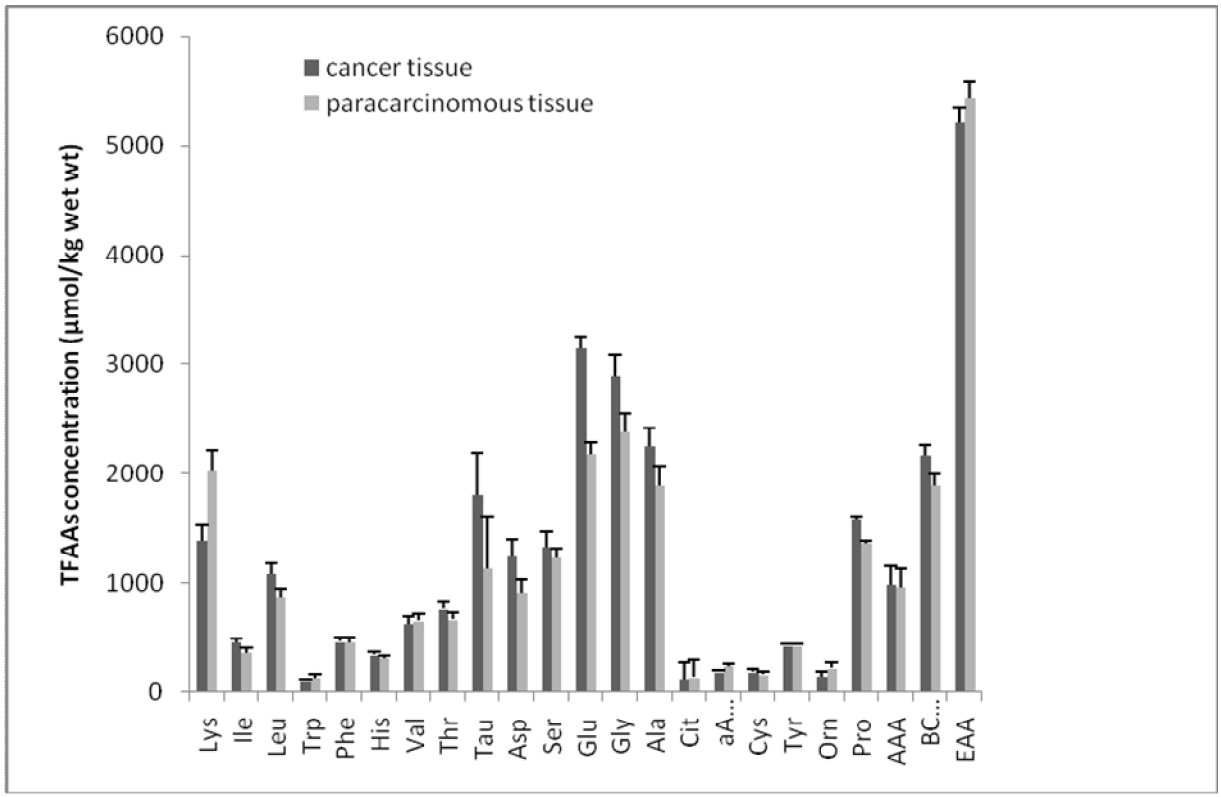

Figure 1. Patterns of TFAAs in patients with lung cancer. The abbreviations in this figure are: lysine (Lys), isoleucine (Ile), leucine (Leu), tryptophan (Trp), phenylalanine (Phe), histidine (His), valine (Val),and threonine (Thr) and the essential amino acids (EAAs) in the EAA section; and taurine (Tau), aspartic acid (Asp), serine (Ser), glutamic acid (Glu), glycine (Gly), alanine (Ala), citrulline (Cit), α-Aminobutyric acid (αAAA), cystein (Cys), tyrosine (Tyr), ornithine (Orn), proline (Pro), aromatic amino acids (AAA) and branched chain amino acid (BCAA) in the nonessential amino acids (NEAAs) section.

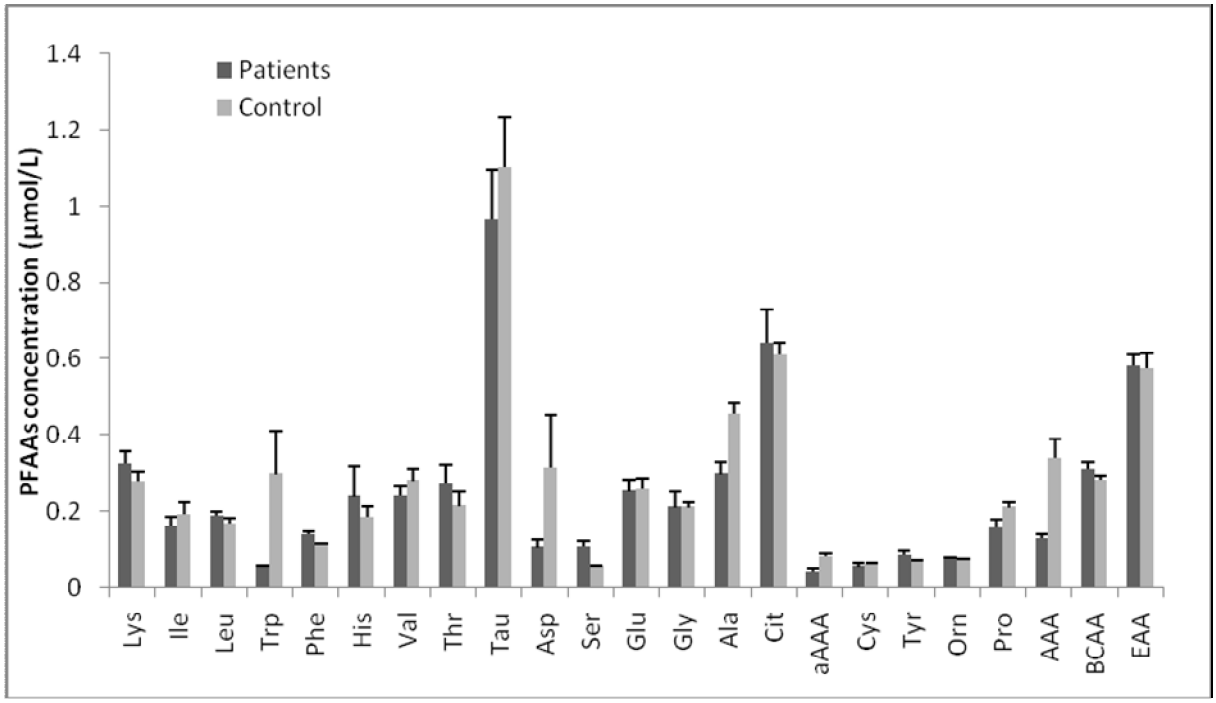

Figure 2. Patterns of PFAAs in patients with lung cancer and the healthy control. The abbreviations in this figure are: lysine (Lys), isoleucine (Ile), leucine (Leu), tryptophan (Trp), phenylalanine (Phe), histidine (His), valine (Val), and threonine (Thr) and the essential amino acids (EAAs) in the EAA section; and taurine (Tau), aspartic acid (Asp), serine (Ser), glutamic acid (Glu), glycine (Gly), alanine (Ala), citrulline (Cit), α-Aminobutyric acid (αAAA), cystein (Cys), tyrosine (Tyr), ornithine (Orn), proline (Pro), aromatic amino acids (AAA) and branched chain amino acid (BCAA) in the nonessential amino acids (NEAAs) section.

Table 4. Correlation of TFAAs in lung cancer tissues and lung paracarcinomous tissues with the PFAAs from the same batch of subjects

	TFAAs in lung cancer tissues		TFAAs in lung paracarcinomous tissues	
	Correlation coefficients (r)	*p* value	Correlation coefficients (r)	*p* value
Lysine	0.547	0.019*	0.229	0.318
Isoleucine	-0.350	0.120	0.210	0.361
Leucine	-0.314	0.166	-0.375	0.094
Tryptophan	-0.007	0.985	0.190	0.759
Phenylalanine	0.878	0.000***	-0.377	0.166
Histidine	0.339	0.217	0.389	0.111
Valine	-0.374	0.095	0.293	0.197
Threonine	0.622	0.003**	0.167	0.469
Sum of EAA	-0.404	0.107	-0.311	0.170
Taurine	-0.121	0.623	-0.323	0.177
Aspartic acid	-0.023	0.922	0.129	0.577
Serine	0.450	0.041*	-0.047	0.840
Glutamic acid	-0.077	0.784	-0.187	0.504
Glycine	-0.166	0.554	-0.231	0.407
Alanine	0.450	0.041*	0.014	0.951
Citrulline	0.306	0.267	-0.081	0.836
α-Aminobutyric acid	0.161	0.485	0.319	0.229
Cystein	0.265	0.288	0.070	0.764
Tyrosine	-0.373	0.095	-0.512	0.018
Ornithine	0.330	0.144	-0.093	0.733
Proline	0.186	0.508	-0.234	0.308
AAA	-0.253	0.268	-0.441	0.099
BCAA	-0.264	0.248	-0.144	0.534
BCAA/AAA	0.457	0.037*	0.439	0.046*
NEAA	-0.442	0.045*	-0.300	0.241

The values shown are correlation coefficients (r). *p<0.05; **p<0.01; ***p<0.001 *vs*. the PFAAs in patients with lung cancer.

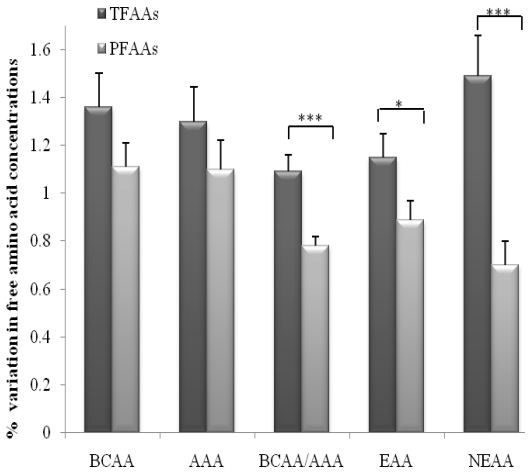

Figure 3. Percent variation in TFAAs and PFAAs. The abbreviations in this figure are: branched chain amino acids (BCAA), aromatic amino acids (AAA), essential amino acids (EAA) and nonessential amino acids (NEAA). *p< 0.05, ***p<0.001.

Most of the gluconeogenic amino acids in PFAAs, such as glycine, were significantly decreased compared with the healthy controls due to the Warburg effect in cancer patients.[29] Other gluconeogenic amino acids, such as serine, were also decreased, but there was no statistical significance between groups. In contrast, within the lung cancer tissues, glutamic acid, serine and valine were increased notably compared with the lung paracarcinomous tissues, suggesting an increase in glucogenes is in the cancer tissues, not only in liver or kidney.[30,31]

In the present study, we measured PFAAs and TFAAs in patients with lung cancer as well as PFAAs in healthy volunteers in order to analyze the alterations in the PFAA and TFAA profiles between the cancer patients and the corresponding controls, as well as the patterns and the correlations between the PFAAs and TFAAs. We observed marked variation in the concentration of EAAs in PFAAs, but little in TFAAs. Among the EAAs, lysine, phenylalanine and threonine showed significant concentration correlation between lung cancer tissues and the plasma from patients with lung cancer, but no significant concentration correlation was found between lung paracarcinomous tissues and the plasma from the same batch of subjects. This concentration correlation may provide us some information of the metabolic status inside the tumour tissues, but this information still needs more validation in the future clinical researches. We also noted that gender, smoking status and UICC staging had no effect on the differences in PFAAs and TFAAs in the patients with lung cancer. However, these patients were still nutritionally healthy at the time of sample collection. Because the patients enrolled in this study were all studied over only 2 to 3 years, further investigation will be focused on the relationship of the PFAAs and TFAAs to the prognosis and will also include the evaluation of more cases.

Conclusions

This study provides evidence for a difference in PFAAs between patients with lung cancer and healthy individuals. We observed a difference in TFAAs between the cancer tissues and the paracarcinomous tissues of patients with lung cancer. Furthermore, we found that the amino acid patterns in PFAAs and TFAAs had similar trends, but the percentage variations of BCAA/AAA, EAA and NEAA were notably different. The concentration correlations of

five amino acids were shown to be statistically significant between PFAAs and lung cancer TFAAs, but no concentration correlations of amino acids were observed between PFAAs with lung paracarcinomous TFAAs. Thus, we conclude that PFAAs are likely to be more informative for the early detection of patients with lung cancer due to their great percentage variation and stronger correlation with cancer TFAAs than paracarcinomous TFAAs.

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AUTHOR DISCLOSURES

The authors declare that they have no competing interests.

REFERENCES

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin. 2011;61:69-90. doi: 10.3322/caac.20107.
2. CCDC. Chinese cancer registry annual report 2012, The center of Chinese cancer registry, Chinese Center For Disease Control and Prevention (CCDC). 2012. (In Chinese)
3. Heber D, Byerley LO, Tchekmedyian NS. Hormonal and metabolic abnormalities in the malnourished cancer patient: effects on host-tumor interaction. JPEN J Parenter Enteral Nutr. 1992;16:60S-64S. doi: 10.1177/014860719201600605.
4. Heber D, Byerly LO, Chlebowski RT. Metabolic abnormalities in the cancer patient. Cancer. 1985;55:S225-9. doi: 10.1002/1097-0142(19850101)55:1+<225::AID-CNCR2820551304>3.0.CO;2-7.
5. Lee JC, Chen MJ, Chang CH, Tiai YF, Lin PW, Lai HS et al. Plasma amino acid levels in patients with colorectal cancers and liver cirrhosis with hepatocellular carcinoma. Hepatogastroenterology. 2003;53:1269-73.
6. Tsoli M, Robertson G. Cancer cachexia: malignant inflammation, tumorkines, and metabolic mayhem. Trends Endocrinol Metab. 2013;24:174-83. doi: 10.1016/j.tem.2012.10.006.
7. Moyano D, Vilaseca MA, Artuch R, Lambruschini N. Plasma amino acids in anorexia nervosa. Eur J Clin Nutr. 1998;52:684-9. doi: 10.1038/sj.ejcn.1600625.
8. Abumrad NN, Miller B. The physiologic and nutritional significance of plasma-free amino acid levels. J Parenter Enteral Nutr. 1983;7:163-70. doi: 10.1177/014860718300702163.
9. Maeda J, Higashiyama M, Imaizumi A, Nakayama T, Yamamoto H, Daimon T et al. Possibility of multivariate function composed of plasma amino acid profiles as a novel screening index for non-small cell lung cancer: a case control study. BMC Cancer. 2010;22:690. doi: 10.1186/1471-2407-10-690.
10. Miyagi Y, Higashiyama M, Gochi A, Akaike M, Ishikawa T, Miura T et al. Plasma free amino acid profiling of five types of cancer patients and its application for early detection. PLoS One. 2011;6:e24143. doi: 10.1371/journal.pone.0024143.
11. Leichtle AB, Nuoffer JM, Ceglarek U, Kase J, Conrad T, Witzigmann H, Thiery J, Fiedler GM. Serum amino acid profiles and their alterations in colorectal cancer. Metabolomics. 2012;8:643-53. doi: 10.1007/s11306-011-0357-5.
12. Durham WJ, Dillon EL, Sheffield-Moore M. Inflammatory burden and amino acid metabolism in cancer cachexia. Curr Opin Clin Nutr Metab Care. 2009;12:72-7. doi: 10.1097/MCO.0b013e32831cef61.
13. Dillon EL, Volpi E, Wolfe RR, Sinha S, Sanford AP, Arrastia CD et al. Amino acid metabolism and inflammatory burden in ovarian cancer patients undergoing intense oncological therapy. Clin Nutr. 2007;26:736-43. doi: 10.1016/j.clnu.2007.07.004.
14. Cenarro A, Puzo J, Ferrando J, Mateo-Gallego R, Bea AM, Calmarza P et al. Effect of Nicotinic acid/Laropiprant in the lipoprotein(a) concentration with regard to baseline lipoprotein(a) concentration and LPA genotype. Metabolism. 2014; 63:365-71. doi: 10.1016/j.metabol.2013. 10.014.
15. Clarke EF, Lewis AM, Waterhouse C. Peripheral amino acid levels in patients with cancer. Cancer. 1978;42:2909-13. doi: 10.1002/1097-0142(197812)42:6<2909::AID-CNCR2820420654>3.0.CO;2-#.
16. Naini AB, Dickerson JW, Brown MM. Preoperative and postoperative levels of plasma protein and amino acid in esophageal and lung cancer patients. Cancer. 1988;62:355-60. doi: 10.1002/1097-0142(19880715)62:2<355::AID-CNCR2820620221>3.0.CO;2-E.
17. Cascino A, Muscaritoli M, Cangiano C, Conversano L, Laviano A, Ariemma S et al. Plasma amino acid imbalance in patients with lung and breast cancer. Anticancer Res. 1995;15:507-10.
18. Kawamura I, Moldawer LL, Keenan RA, Batist G, Bothe A Jr, Bistrian BR et al. Altered amino acid kinetics in rats with progressive tumor growth. Cancer Res. 1982;42:824-9.
19. Proenza AM, Crespi C, Roca P, Palou A. Gender related differences in the effect of aging on blood amino acid compartmentation. J Nutr Biochem. 2001;12:431-40. doi: 10.1016/S0955-2863(01)00157-7.
20. Eley HL, Russell ST, Tisdale MJ. Effect of branched-chain amino acids on muscle atrophy in cancer cachexia. Biochem J. 2007;407:113-20. doi: 10.1042/BJ20070651.
21. Zhou X, Wang JL, Lu J, Song Y, Kwak KS, Jiao Q et al. Reversal of cancer cachexia and muscle wasting by ActRIIB antagonism leads to prolonged survival. Cell. 2010;142:531-43. doi: 10.1016/j.cell.2010.07.011.
22. Giordano KF, Jatoi A. The cancer anorexia/weight loss syndrome: therapeutic challenges. Curr Oncol Rep. 2005;7: 271-6. doi: 10.1007/s11912-005-0050-9.
23. Jatoi A Jr, Loprinzi CL. Current management of cancer-associated anorexia and weight loss. Oncology (Williston Park). 2001;15:497-502.
24. Lindsey AM, Piper BF. Anorexia and weight loss: indicators of cachexia in small cell lung cancer. Nutr Cancer. 1985;7: 65-76. doi: 10.1080/01635588509513841.
25. Proenza AM, Oliver J, Palou A, Roca P. Breast and lung cancer are associated with a decrease in blood cell amino acid content. J Nutr Biochem. 2003;14:133-8. doi: 10.1016/S0955-2863(02)00225-5.
26. Wang F, Permert J. Specific amino acid profile in culture media conditioned by human pancreatic cancer cell lines. Pancreatology. 2002;2:402-6. doi: 10.1159/000065088.
27. Wu G. Synthesis of citrulline and arginine from proline in enterocytes of postnatal pigs. Am J Physiol. 1997;272: G1382-90.
28. Stoll B, Henry J, Reeds PJ, Yu H, Jahoor F, Burrin DG. Catabolism dominates the first-pass intestinal metabolism of dietary essential amino acids in milk protein-fed piglets. J Nutr. 1998;128:606-14.
29. Chen Z, Lu W, Garcia-Prieto C, Huang P. The Warburg effect and its cancer therapeutic implications. J Bioenerg Biomembr. 2007;39:267-74. doi: 10.1007/s10863-007-9086-x.

30. Leij-Halfwerk S, Dagneli PC, Kappert P, Oudkerk M, Sijens PE. Decreased energy and phosphorylation status in the liver of lung cancer patients with weight loss. J Hepatol. 2000;32: 887-92. doi: 10.1016/S0168-8278(00)80092-6.

31. Stumvoll M, Meyer C, Perriello G, Kreider M, Welle S, Gerich J. Human kidney and liver gluconeogenesis: evidence for organ substrate selectivity. Am J Physiol. 1998; 274:E817-26.

Original Article

Plasma and tissue free amino acid profiles and their concentration correlation in patients with lung cancer

Qihong Zhao PhD[1], Ye Cao MM[1], Ying Wang MM[1], Chuanlai Hu MD[1], Anla Hu MD[1], Liang Ruan MM[1], Qingli Bo MM[1], Qifei Liu MB[1], Wenjun Chen MM[1], Fangbiao Tao MD[1], Min Ren MD[2], Yongsheng Ge MD[3], Anguo Chen MD[2], Li Li MD[1]

[1]*Department of Food and Nutrition Hygiene, School of Public Health, Anhui Medical University, Anhui, China*
[2]*Department of General Surgery, the First Affiliated Hospital, Anhui Medical University, Anhui, China*
[3]*Department of General Surgery, Shengli Hospital, Anhui Medical University, Anhui, China*

肺癌病人血浆和组织中游离氨基酸分析以及它们的浓度相关性

肿瘤病人体内血浆游离氨基酸变化是其蛋白质代谢异常的一个基本特征。但是目前很少有研究关注肿瘤组织内部的氨基酸情况，包括它们的模式以及与血浆氨基酸的浓度相关性。为了评价肺癌病人血浆和肿瘤组织中游离氨基酸的变化情况，我们使用氨基酸分析仪测定了 27 位肺癌肿瘤病人治疗前的血浆、肿瘤组织以及癌旁组织中的游离氨基酸浓度。跟正常人血浆游离氨基酸浓度相比较，我们发现肿瘤病人血浆游离氨基酸浓度中色氨酸、甘氨酸、瓜氨酸、鸟氨酸和脯氨酸显著下降，而蛋氨酸显著上升；与癌旁组织相比，牛磺酸、谷氨酸和甘氨酸浓度显著增加，而赖氨酸和鸟氨酸浓度显著下降。在血浆和组织中游离氨基酸有相似的浓度趋势，但是百分比变化是各异的。另外，我们发现血浆和肺癌组织中的赖氨酸、蛋氨酸、苏氨酸、丝氨酸和丙氨酸有浓度相关性，但是在血浆中和癌旁组织中没有发现一种氨基酸具有浓度相关性。因此，血浆游离氨基酸变化可能反应了肿瘤组织中氨基酸水平，这或许会对肺癌病人未来的代谢状态和预后提供更多信息。

关键词：血浆游离氨基酸、组织游离氨基酸、肺癌、氨基酸模式、浓度相关性