International Scholars Journals

African Journal of Internal Medicine ISSN: 2326-7283 Vol. 9 (8), pp. 001-008, August, 2021. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Diagnostic modeling with differences in plasma amino acid profiles between non-cachectic colorectal/breast cancer patients and healthy individuals

Naoyuki Okamoto[1]*, Yohei Miyagi[2], Akihiko Chiba[3], Makoto Akaike[4], Manabu Shiozawa[4], Akira Imaizumi[5], Hiroshi Yamamoto[6], Toshihiko Ando[5], Minoru Yamakado[7] and Osamu Tochikubo[8]

[1]Cancer Prevention and Cancer Control Division, Kanagawa Cancer Center, 1-1-2, Nakao, Asahi-ku, Yokohama-city, 241-0815 Japan.
[2]Moleculer Pathology and Genetics Division, Kanagawa Cancer Center, 1-1-2, Nakao, Asahi-ku, Yokohama-city, 241-0815 Japan.
[3]Department of Breast Surgery, Kanagawa Cancer Center, 1-1-2, Nakao, Asahi-ku, Yokohama-city, 241-0815 Japan.
[4]Department of Gastrointestinal Surgery, Kanagawa Cancer Center, 1-1-2, Nakao, Asahi-ku, Yokohama-city, 241-0815 Japan.
[5]Institute of Life Sciences, Ajinomoto CO., Inc., Japan.
[6]HI Department, Ajinomoto CO., Inc. , Japan.
[7]Center for Multiphasic Health Testing and Services, Mitsui Memorial Hospital, Japan.
[8]Department of Public Health, Yokohama City University, Graduate School of Medicine, Japan.

Accepted 22 December, 2020

Metabolic changes in patients with cancer lead to alterations in their amino-acid balances. Thus, amino-acid profiles may be useful as biomarkers of cancers. This study was conducted to analyze amino-acid profiles in plasma by multivariate analysis, in order to elucidate differences between cancer patients and controls. Venous blood samples were taken from colorectal and breast cancer patients, and healthy controls. Plasma free amino acids were measured by liquid chromatography/mass spectrometry. No weight loss was observed in any of the cancer patients. Multiple logistic regression models were used to discriminate between cancer patients and controls. The area under the curve (AUC) of the receiver operating characteristic (ROC) curve for each discriminant score was calculated as 0.860 (95% confidence interval: 0.784 to 0.937) for colorectal cancer and 0.906 (95% CI: 0.845 to 0.967) for breast cancer. The performance of these discriminants was independent of the cancer stage. This study revealed significant differences in plasma amino acid profiles between cancer patients and controls. The development of a cancer alters plasma amino-acid profiles without cachexia or weight loss, and the pattern of change differs between two cancers. Plasma amino-acid profiling might therefore be useful for the early detection of cancer.

Key words: amino acid profiles, plasma, screening, cancer, multivariate analysis

INTRODUCTION

Recent developments in metabolomic approaches enable investigators to measure amino acids and various other metabolites in humans by inexpensive methods with high throughput (Yoshida et al., 2007). Lee et al. (2004) have described how metabolic profiling data can be used to define biological status for diagnostic purposes through multivariate analysis. A great deal of knowledge on human amino-acid metabolism has also been collected over the last three decades through the monitoring of plasma amino-acid levels. Metabolic changes alter the amino-acid balance in patients with various diseases. Because of this, physicians can use indexes such as

*Corresponding author. E-mail: okamoto@gancen.asahi.yokohama.jp. Tel: +81 45 391 5761 ext.4030. Fax: +81 45 366 3157.

Fischer's ratio, that is, the ratio between branched-chain amino acids (BCAAs) and aromatic amino acids (Fisher, 1975, 1976; Rosen, 1977), to track the progression of liver fibrosis and the effectiveness of drug treatment.

The changes in the balance of plasma free amino acids (PFAA) may only be slight during the early stage, whereas later-stage patients may become cachectic due to malnourishment, depending on the deficiency of PFAA (Heber et al., 1985; Mackenzie and Baracos, 2004; Lai et al., 2005).

Investigators can also use post-genomic technologies to derive multivariate functions easily with the aid of a computer. Noguchi et al. (2006) used multivariate functions made up of plasma amino-acid profiles as indexes of diabetes, while Zhang et al. (2006) used them to diagnose liver fibrosis in patients infected with chronic heaptitis C. Shikata et al. (2007), meanwhile, described the structure of the amino-acid metabolism network. The reports by these authors demonstrate that multivariate analysis of plasma free amino-acid profiles is a promising and versatile method for diagnosing various diseases.

Investigators have often found noticeable alterations in the metabolism of cancer cells (Heber et al., 1985; Mackezie and Baracos, 2004; Sido et al., 1998; Rodriguez et al., 2004; Yamaguchi et al., 2005) and changes in the plasma amino-acid profiles of cancer patients (Norton et al., 1985; Naini et al., 1988; Cascino et al., 1991, 1995; Kubota et al., 1991; Lviano et al., 2003; Proenza et al., 2003; Vissers et al., 2005). Cascino et al. (1991, 1995) for example, described significant increases in tryptophan (Trp), glutamic acid (Glu), and ornithine (Orn) in lung cancer patients. Proenza et al. (2003) reported an increased level of Orn in lung cancer patients. Kubota et al. (1991) first used plasma amino-acid profiles to discriminate between breast cancers, gastrointestinal tract cancers, head and neck cancers, and healthy individuals within small sample populations partly made up of malnourished patients. Their results indicated that the amino acid profiles may be useful for cancer diagnosis by site.

The detection of metabolic changes using amino-acid profiles is a promising approach for detecting the presence of various cancers.

Colorectal cancer is a major cause of morbidity and mortality worldwide, and one of the most common causes of cancer deaths in Japan (Saito, 1996). Breast cancer, meanwhile, is the most common cancer among Japanese women (Ohnuki, 2006). Both types of cancer can be eliminated by surgical or endoscopic excision if diagnosed at an early stage, without recurrence or metastases in most patients. The development of technologies for early detection is thus a crucial strategy for decreasing cancer deaths. In this study we investigated differences in the plasma amino-acid profiles between non-cachectic colorectal cancer patients, non-cachectic breast cancer patients, and controls, then examined whether these cancers could be detected by plasma free amino-acid profiling using multivariate analysis.

MATERIALS AND METHODS

Subjects

Sixty-three patients with colorectal cancer and 61 patients with breast cancer diagnosed histologically and hospitalized at the Kanagawa Cancer Center, Yokohama, Japan, between February 2006 and December 2007 were recruited as cases.

The recruitment criteria for the selection of cases were as follows: clinical stage be 0, I, II, or III, no metastasis, tumor marker (CEA, CA19-9, P53) concentrations in serum be under the cut-off levels, and no weight-loss before hospitalization.

All of the patients were fully informed by the chief physician at admission and agreed to participate in this study. One-hundred and fifty-four healthy individuals who had undergone medical examinations at the Center for Multiphasic Health Testing and Services, Mitsui Memorial Hospital, Tokyo, between May and June 2006 were recruited as controls. None of the controls had abnormal tumor markers (CEA, CA19-9).

Of these cases, 49 patients with colorectal cancer and 45 with breast cancer were chosen at random to form the training data set. Age (+/- 5 years)-gender matched controls were chosen for each cancer and the remaining controls were used for a test data set to evaluate the performance of the estimated discriminants. Blood samples were collected from all of the colorectal cancer patients, breast cancer patients, and healthy controls. None of the patients underwent medical interventions such as surgery, chemotherapy, or radiotherapy before their blood was sampled. The baseline characteristics of the patients and controls are summarized in Table 1-A, B.

Analytical methods

Blood samples (5 ml) from all cases and controls were taken from forearm veins after an overnight fast, placed in tubes containing ethylenediaminetetraacetic acid disodium salt (EDTA-2Na; Termo, Japan), and immediately cooled with ice. Blood was drawn from the cancer patients before any operation or treatment. Plasma samples were separated by centrifugation at 3,000 rpm and 4°C for 15 min, and then stored at –80°C. The plasma samples were deproteinized in a final concentration of 80% acetonitrile before sample preparation. The amino-acid concentrations in the plasma samples were measured by high-performance liquid chromatography (HPLC)- electrospray ionization (ESI)-mass spectrometry (MS), followed by derivatization. An MSQ Plus LC/MS system (Thermo Fischer Scientific, Waltham, MA, USA) equipped with an ESI source was used in positive-ionization mode for selected ion monitoring (SIM). Xcalibur™ version 1.4 SR1 software (Thermo Fisher Scientific, Yokohama, Japan) was used for data collection and processing. The HPLC separation system consisted of an L-2100 pump, L-2200 autosampler, and L-2300 column oven (Hitachi High-Technologies Corporation, Tokyo, Japan). A Wakosil-II 3C8-100HG column (100, 2.1, 3 mm; Wako Pure Chemical Industries, Osaka, Japan) was used for the separation, and the mobile phase consisted of eluent A (25 mM ammonium formate in water) and eluent B (water:acetonitrile = 40:60). The following amino acids and related molecules (24 compounds) were measured and used in the analysis: alanine (Ala), alpha-aminobutyric acid (a-ABA), arginine (Arg), asparagine (Asn), citruline (Cit), glutamic acid (Glu), glutamine (Gln), glycine (Gly), histidine (His), isoleucine (Ile), leucine (Leu), lysine (Lys), methionine (Met), ornithine (Orn), phenylalanine (Phe), proline (Pro), serine (Ser), threonine (Thr), tryptophan (Trp), tyrosine (Tyr), and valine (Val). The plasma levels of amino acids were expressed in M.

Statistical analysis

The amino-acid concentrations are given as means ± standard

Table 1. The characteristics of the cancer patients and the controls by training/test data.

A) Colorectal cancer patients and controls						
	Training data		**Test data**		**Total**	
	Case	**Control**	**Case**	**Control**	**Case**	**Control**
Num of data	49	49	13	54	62	103
(Male, Female)	(38,11)	(38,11)	(4,9)	(14,40)	(42,20)	(52,51)
Age (Mean±SD)	64.1±8.2 *)	59.6±5.9	57.5±12.4	55.8±5.7	62.7±9.5 *)	57.6±6.1
(min~max)	(40~78)	(40~69)	(33~75)	(40~69)	(40~78)	(40~69)
BMI (Mean±SD)	23.1±4.3	23.8±4.1	22.2±3.6	22.8±3.0	22.9±3.5	23.3±3.6
(min~max)	(13.8~34.9)	(17.8~42.2)	(13.1~26.9)	(17.4~31.6)	(13.1~34.9)	(17.4~42.2)
Stage0	2	-	0	-	2	-
I	7	-	2	-	9	-
II	19	-	3	-	22	-
III	14	-	8	-	22	-
IV	6	-	0	-	6	-
Uncharacterized	1	-	0	-	1	-
B) Breast cancer patients and controls						
	Training data		**Test data**		**Total**	
	Case	**Control**	**Case**	**Control**	**Case**	**Control**
Num of data	45	45	16	6	61	51
Age (Mean±SD)	56.4±12.6	57.5±6.0	62.6±9.8	58.3±4.2	58.0±12.1	57.6±5.8
(min~max)	(28~81)	(40~69)	(41~77)	(54~65)	(28~81)	(40~69)
BMI (Mean±SD)	22.0±4.2	22.9±4.2	22.3±3.1	21.8±2.4	22.0±2.9	22.8±4.0
(min~max)	(16.4~30.2)	(17.4~42.2)	(17.0~27.6)	(18.5~25.2)	(16.4~30.2)	(17.4~42.2)
Stage 0	5	-	3	-	8	-
I	22	-	8	-	30	-
II	14	-	4	-	18	-
III	4	-	1	-	5	-
Uncharacterized	0	-	0	-	0	-

*):Significant (p<0.05) at t-test .

deviation (SD). Student's t -tests and Mann-Whitney U-tests were used to assess differences between patients and controls for each cancer. A probability of 5% or less was considered significant. Principal component analysis (PCA) was performed using the standardized z-score for all the training data. Discriminant functions for each cancer were predicted by multiple logistic-regression analysis. The multiple logistic- regression model was applied to all combinations of the 21 amino acids, and the maximum number of variables was restricted to below seven. To avoid multicolinearity, combinations of variables with a variance inflation factor (VIF) exceeding 10 were omitted from the analysis. The best model was defined as the candidate formula with the minimum Akaike's Information Criterion (AIC; Steyerberg et al., 2000). The efficiency of discrimination of the discriminants was estimated from the receiver-operator characteristic (ROC) curve of the estimated probability score of each cancer. The area under the curve (AUC) was calculated for the ROC curve (ROC_AUC; Baker, 2003). To confirm the performance of the estimated discriminants, ROC_AUC values were also calculated using the probability scores obtained from the test data. The Kruskal-Wallis test was used to estimate the effect of the cancer stage on the probability score. All of the statistical and multivariate analyses were performed with MATLAB (The Mathworks, MA, USA) and GraphPad Prism (GraphPad Software, CA, USA).

This study and study protocol were reviewed and approved in advance by the institutional review board of the Kanagawa Cancer Center.

RESULTS

Characteristics of patients and control subjects

The training group included 49 colorectal cancer patients (two at pathological stage 0, seven at stage I, nineteen at stage II, fourteen at stage III, six at stage IV, and one uncharacterized; Table 1A) who had been diagnosed by low- dose helical computed tomography (CT) and biopsy, and 45 breast cancer patients (five at pathological stage 0, twenty-two at stage I, fourteen at stage II, and four at stage III; Table 1B) who had been diagnosed by mammography and biopsy before any symptoms were noticed. There was no significant difference in body-mass index (BMI) between the patients and control subjects for either type of cancer (Table 1A, B).

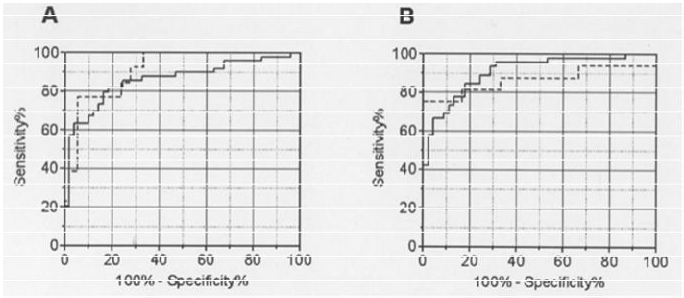

Figure 1. ROC curves of discriminant-1 for colorectal cancer (A) and discriminant-2 for breast cancer (B) of training data (solid line) and test data (dotted line).

Altered amino-acid profiles in colorectal and breast cancers

The plasma concentrations of several amino acids in the colorectal cancer patients were significantly different from those observed in the controls. The levels of Thr, Cit, Val, Met, Ile, Leu, Tyr, and Phe were reduced in the colorectal patients, while that of Glu was increased (Table 2A). The total essential amino acids (EAAs) therefore declined (especially the BCAAs, Val, Leu, and Ile), whereas the total plasma amino acids (SUM AA), including the non-essential amino acids, were unchanged (Table 2A). In contrast, the concentrations of the BCAAs, Tyr, and Phe decreased, whereas the Fischer's ratio (BCAA/(Tyr+Phe); (Rosen et al., 1977) remained unchanged (Table 2A).

The alteration of the plasma amino-acid profile in breast cancer differed from that in colorectal cancer, with fewer changes observed. The levels of Met, Ile, Phe, and Arg decreased in the breast cancer patients, while those of Thr, Ser, Glu, a-ABA and Orn increased (Table 2B). The breast cancer patients were similar to the colorectal cancers in exhibiting no significant change in EAA and BCAA, but they did exhibit an increase in the Fischer's ratio, a change not observed in colorectal cancer (Table 2B).

To summarize the changes of the plasma amino-acid profiles, we performed a principal component analysis (PCA) using the standardized z-score for each amino acid and five principal components (PCs) with eigenvalues of more than 1 (Table 3). To analyze the contributions of three background factors, that is, age, gender, and the presence (=1) or absence (=0) of cancer, to each PC, we used the Spearman's correlation coefficients between these factors and the five PC scores. Our analysis revealed that PC3 was mainly correlated with the presence or absence of colorectal cancer, that PC4 was mainly correlated with the presence or absence of breast cancer, and that gender and age contributed principally to PC1 and PC2 (Table 3). These findings suggest that the altered amino-acid profiles would be useful for detecting the onset of these cancers. They also imply that the two kinds of cancer have different effects on the plasma amino-acid profile.

Multivariate functions for discriminating colorectal and breast cancers

The above- mentioned results suggest that it should be possible to improve discrimination by deriving multivariate functions with the amino- acid profiles as explanatory variables. Noting this, we performed multiple logistic-regression analyses with selected variables using each training datum (as described in the Subjects and Methods section). For colorectal cancer, discriminant-1 consisted of the six amino acids Val, Glu, Thr, a-ABA, Gln, and Pro. For breast cancer, discriminant- 2 consisted of the six amino acids Thr, Ala, a-ABA, Ile, Orn, and Arg. The estimated coefficients, standard errors, and p-values for each model are summarized in Table 4.

To evaluate the performance of the discriminants, we calculated ROC curves for each of the discriminant scores. When using training data for this calculation, we obtained ROC _AUC values of 0.860 (95% confidence interval: 0.784 to 0.937) for colorectal cancer and 0.906 (95% confidence interval: 0.845 to 0.967) for breast cancer (Figure 1A and B, solid lines). To confirm the performance of the discriminants, we derived ROC curves from the split test data. These reproduced the good diagnostic performance, with values of 0.910 (95% confidence interval: 0.834 to 0.986) for colorectal cancer, and 0.865 (95% confidence interval: 0.765 to 1.000) for breast cancer (Figure 1A and B, broken lines).

Discriminant scores are independent of cancer stage

If the above-mentioned discriminants are to be used in diagnosis for early cancers, they must have adequate predictive power. To determine whether they met this condition, we compared all of the predicted values (both the training data and test data) of each discriminant with the pathological stage (Figure 2A and B) . In the case of colorectal cancer, about 60% of the patients were categorized as early stage (stages 0, I, and II, Table 1A). The Kruskal-Wallis test revealed no significant correlation between the probability of colorectal cancer and the pathological stage (Figure 2A). In the case of breast cancer included training and test data, most of the patients were categorized as early stage (stages 0, I, and II, Table 1B) and only five of the patients were defined as advanced stage (stage III, Table 1B). The discriminant score in the stage 0 or I group was not significantly different from those in the stage II or III groups (Figure 2B). These results suggest that the discriminants obtained are equally predictive for early and later stage.

DISCUSSION

Cancer-associated cachexia is a well known cause of alterations in the plasma amino-acid profiles of cancer

Table 2. The differences of plasma amino-acid profiles between colorectal cancer patients (A), breast cancer patients (B), and controls using the Mann-Whitney U-test.

A) Colorectal cancer			
Amino acids	**Case (mean±SD)**	**Control (Mean ± SD)**	**Significance**
Thr	102.1 ± 23.8	116 ± 23.8	*p*<0.01
Ser	115.8 ± 16.8	121.6 ± 23.4	
Asn	42.1 ± 7.3	45.3 ± 9.5	
Glu	45 ± 18.6	36.9 ± 13.2	*p*<0.05
Gln	596.7 ± 83.9	590.1 ± 94.4	
Pro	163.3 ± 41.7	164.2 ± 39	
Gly	243.2 ± 49.2	251.3 ± 57.6	
Ala	391.8 ± 111	378.4 ± 86.5	
Cit	21.5 ± 4.8	24.4 ± 4.9	*p*<0.01
a-ABA	20.5 ± 7.3	19.1 ± 6	
Val	243.5 ± 50.6	276.8 ±46.1	*p*<0.01
Met	28.1± 5.3	31.4 ± 5.7	*p*<0.01
Ile	68.9 ± 13.3	80.9 ± 16.2	*p*<0.001
Leu	119.8 ± 23.3	136.1 ± 24	*p*<0.001
Tyr	77.5 ± 20.4	84.2 ± 13.6	*p*<0.01
Phe	63.1 ± 11.7	72 ± 10.8	*p*<0.001
His	77.2 ± 17.7	80.3 ± 14	
Trp	59.4 ± 9.8	62.2 ± 12.1	
Orn	56.9 ± 18	59.5 ± 15.6	
Lys	214.3 ± 34.5	217.4 ± 30.6	
Arg	101.2 ± 19	107.6 ± 25	
SUM AA	2851.8 ± 357	2956 ± 255.7	
EAA	976.4 ± 141.5	1073 ± 134.7	*p*<0.01
BCAA	432.2 ± 79.3	493.8 ± 81.5	*p*<0.01
Fisher ratio	3.1 ± 0.5	3.2 ± 0.4	
B) Breast cancer			
Amino acids	**Case (Mean ± SD)**	**Control (Mean ± SD)**	**Significance**
Thr	119.7 ± 22.8	111.5± 25.2	*p*<0.05
Ser	126.4 ± 26.9	114.5 ± 21.4	*p*<0.05
Asn	42.4 ± 7.1	43.6 ± 8.6	
Glu	34.1 ± 14.3	24.3 ± 10.9	*p*<0.001
Gln	594.5 ± 97.1	591.9 ± 75.3	
Pro	146.3 ± 32.9	150.6 ± 38.2	
Gly	291.6 ± 80.6	270.8 ± 75.1	
Ala	395.6 ± 81.4	373.3 ± 93.1	
Cit	19.6 ± 4.7	21.8 ± 5.1	
a-ABA	20 ± 5	17.2 ± 4.4	*p*<0.05
Val	237.2 ± 39	235.5 ± 45.5	
Met	24.9 ± 5.3	26.7 ± 4.3	*p*<0.05
Ile	55.2 ± 12.5	64.8 ± 15.9	*p*<0.01
Leu	108.5 ± 17	115.6 ± 25	
Tyr	69.6 ± 12.4	75.8 ± 15	
Phe	60.6 ± 12.1	68 ± 12.1	*p*<0.01
His	78.3 ± 15.1	82 ± 11.8	
Trp	57.8 ± 9.9	62.2 ± 12.2	
Orn	61.7 ± 17.1	49.4 ± 13.4	*p*<0.001
Lys	205.1 ± 34.8	205.5 ± 29.7	
Arg	90 ± 23.8	107.3 ± 19.4	*p*<0.001

Table 2B Contd**.**

SUM AA	2839.2 ± 285.9	2812.1 ± 264.7	
EAA	947.4± 104.4	971.7± 126.5	
BCAA	400.9 ± 61.3	415.8 ± 82.6	
Fisher ratio	3.1± 0.4	2.9± 0.3	p<0.05

Table 3. Correlation between subjects and PCA of the amino-acid profiles in the training data.

Principal components			**PCA1**	**PCA2**	**PCA3**	**PCA4**	**PCA5**
Eigenvalue (% of total variance)			6.2 29.50%	2.9 13.80%	1.6 7.60%	1.5 7.00%	1.1 5.20%
Spearman's correlation coefficient	Gender		-0.3293	0.3515	0.0775	-0.1224	-0.0485
	Age		0.0084	-0.1116	0.0486	0.1039	0.2349
	Presence (=1) or absence (=0) of;						
		Colorectal cancer	-0.0635	-0.1368	0.229	-0.0501	-0.0563
		Breast cancer	-0.1748	0.2117	0.1402	-0.2416	0.0109

Table 4. The estimated coefficients, standard errors, and p-values for each model.

	Coefficient	**SE**	***p*_value**
A) Colorectal cancer			
Constant	-0.898	3.034	0.767
Val	-0.042	0.011	0.000
Glu	0.096	0.025	0.000
Thr	-0.039	0.017	0.023
a-ABA	0.177	0.056	0.002
Gln	0.009	0.004	0.027
Pro	0.018	0.009	0.041
B) Breast cancer			
Constant	3.714	3.021	0.219
Thr	0.045	0.021	0.031
Ala	0.010	0.005	0.048
a-ABA	0.210	0.090	0.020
Ile	-0.120	0.039	0.002
Orn	0.061	0.025	0.016
Arg	-0.133	0.037	0.000

patients (Heber et al., 1985; Mackenzie and Baracos patients., 2004; Lai et al., 2005). Cachectic characteris-tics are thought to result from semi-starvation, decreased muscle protein synthesis, increased muscle protein degradation, increased protein synthesis and turnover, increased gluconeogenesis, and other processes (Heber et al., 1985; Mackenzie and Baracos, 2004). Given the possible effects of colorectal cancer on the absorption of amino acids and other nutrients, some suspect that the plasma amino-acid concentrations, particularly those of EAAs, may decrease even when no weight loss is detected (Table 1A, B). It thus appears that the change of plasma amino- acid profiles observed in colorectal cancer may result in part from malnutrition. Indeed, plasma amino-acid profiles are also altered in patients with inflammatory bowel disease (IBD; Papadia et al., 2007). Several studies, however, have demonstrated significant changes in the plasma amino-acid profiles of cancer patients without cachexia (Cascino, 1995; Proenza, 2003; Vissers, 2005). This suggests that multivariate analysis of amino-acid profiles may be useful for the early detection of cancer.

This work therefore recruited the earlier-stage cancer cases before clinical stage IV, although we also included 6 patients with colorectal cancer of pathological stage IV (Table 1).

Our group found no apparent signs of cachexia in the patients of the current study, as none of them had lost significant amounts of body weight (Table 1A, B). We thus speculate that the changes in the amino-acid profiles of breast cancer patients cannot be accounted for by malnutrition or cachexia. Vissers et al. (2005) observed decreases in the plasma levels of several amino acids in breast cancer patients and colon cancer patients with little or no weight loss. These findings suggest that the differences in amino-acid profiles observed are due not to malnutrition, but to cancer- specific alterations of amino-acid metabolism, especially in cases of breast cancer.

There has recently been accumulating evidence of important effects on Arg levels in association with activities of the immune system in cancer patients. Several reports on cancer have mentioned increased production of arginase I, which catalyzes the conversion of Arg into Orn and urea, and increased production of NO synthase, which catalyzes the conversion of Arg into Cit and nitric oxide (NO) (Sido et al., 1998; Rodriguez et al., 2004; Yamaguchi et al., 2005; Vissers et al., 2005). Cascino et al. (1995) reported a decrease in the plasma Arg level and an increase in the Orn level in breast and lung cancers, albeit not to statistically significant degrees. Our group also found a slight, less than significant, decrease of plasma Arg levels in both cancer types examined (Table 2A, B). Based on this finding, we incorporated Arg

into our logistic-regression model for breast cancer (Table 3).

In addition to the possible immunological effects, there is evidence that cancers originating from different organs might lead to different alterations of the amino-acid profile (Norton et al., 1985; Naini et al., 1988; Cascino et al., 1991, 1995; Kubota et al., 1991; Lviano et al., 2003; Proenza et al., 2003; Vissers et al., 2005). The altered plasma amino-acid profiles identified in our study differed between colorectal and breast cancers. As amino acids are produced and assimilated in an organ- specific manner, we were encouraged to find that the indices screened for the two cancers differed in the compositions of the plasma amino acids.

In general, histological diagnostic methods such as colonoscopy and biopsy are established means for obtaining definite diagnoses. Yet the invasiveness and considerable expense render them unfeasible for many individuals who undergo medical examinations. Instead, faecal occult blood testing is used to screen for colorectal cancer (Saito, 1996) and mammography is used to screen for breast cancer (Ohnuki et al., 2006; Moss et al., 2006). Both of these methods have disadvantages. In faecal occult blood testing, for example, the appearance of haemoglobin in stool is not specific for neoplasms (Ito et al., 2002). Mammography, meanwhile, exposes individuals to radiation and would not be cost-effective for annual examination (Ohnuki et al., 2006). Even if no one test is invasive or expensive, a battery of various tests performed in an annual medical examination can be burdensome and expensive. The discovery of new tumor markers using genomic and proteomic technologies is being pursued vigorously (Cho, 2007). Yet few, if any, of the markers identified so far are sensitive or specific enough to be clinically useful for early detection. To name just two examples, the major tumor marker for colorectal cancer, carcinoembryonic antigen (CEA), and the major marker for breast cancer, cancer antigen 15-3 (CA15-3), are both thought to lack adequate sensitivity for early detection (Ito et al., 2002; Cho, 2007).

Nevertheless, the results presented here demonstrate that plasma free amino-acid profiling is useful for detecting both colorectal cancer and breast cancer. Unlike conventional tumor markers, the discriminants developed in this study identify patients equally well at any stage of cancer. Thus, they may used for screening.

This study had important limitations. First, the samples were limited in number, particularly those for testing data, and they were taken from only two cancer sites. Second, the number of cases was too low to permit analyses of confounding factors such as sex, age, liver disease, and other chronic diseases. Third, the study was not a randomized controlled case-control study and a cohort study, but a non randomized controlled study. Hereafter, we will be planning a randomized controlled cohort study to establish a new cancer screening method with plasma free amino acid profiles.

If it ever becomes possible to evaluate the incidence of every type of cancer using only one plasma sample, the reduction in cancer mortality will be tremendous.

ACKNOWLEDGEMENTS

This work has been supported by a Grant-in-Aid for Scientific Research on Basic Research B (No. 17390195) from the Ministry of Education, Culture, Sports, Science and Technology of Japan. We thank Dr. H Miyano, Mr. K. Shimbo, Mr. H. Yoshida, Ms. M. Amao, and Ms. M. Nakamura for amino- acid analyses. We also thank Ms. T. Kasakura and Ms. Y. Osawa for data acquisition.

REFERENCES

Baker SG (2003). The central role of receiver operating characteristic (ROC) curves in evaluating tests for the early detection of cancer. J. Nat. Cancer Inst. 95: 511-515.

Cascino A, Cangiano C, Ceci F (1991). Increased plasma free tryptophan levels in human cancer; a tumor related effect? Anticancer Res. 11: 1313-1316.

Cascino A, Muscaritoli M, Cangiano C (1995). Plasma amino acid imbalances in patients with lung and breast cancer. Anticancer Res. 15: 507-510.

Cho WCS (2007). Contribution of oncoproteomics to cancer biomarker discovery. Mol. Cancer. 6: 25-37.

Fischer JE, Funovics JM, Aguirre A (1975). The role of plasma amino acids in hepatic encephalcopathy. Surgery. 78: 276-290.

Fischer JE, Rosen HM, Ebeid AM, James JH, Keane JM, Soeters PB (1976). The effect of normalization of plasma amino acids on hepatic encephalcopathy in man. Surgery 80: 77-91.

Heber D, Byerly LO, Chlebowski RT (1985). Metabolic abnormalities in cancer patient. Cancer 55: 225-229.

Ito S, Hibi K, Nakayama H (2002). Detection tumor DNA in serum of colorectal cancer patients. Jpn. J. Cancer Res. 93:1266-1269.

Kubota A, Meguid MM, Hitch DC (1991). Amino acid profiles correlate diagnostically with organ site in three kinds of malignant tumors. Cancer 69: 2343-2348.

Lai HS, Lee JC, Lee PH (2005). Plasma free amino acid profile in cancer patients. Seminars in Cancer Biol. 15: 267-276.

Lee K, Hwang D, Yokoyama T (2004). Identification of optimal classification functions for biological sample and state discrimination from metabolic profiling data. Bioinformatics 20: 959-969.

Lviano A, Cascino A, Muscaritoli M, Fanfarillo F, Fanelli R (2003). Tumor induced changes in host metabolism: a possible role for free tryptophan as a marker of neoplastic disease. Adv. Exp. Med. Biol. 527: 363-366.

Mackenzie M, Baracos VE (2004). Cancer associated cachexia: altered metabolism of protein and amino acids. In: Cynober LA editor. Metabolic and therapeutic aspects of amino acids in clinical nutrition. FL: CRC Press. pp. 339-354.

Moss SM, Cuckle H, Evans A, Johns L, Waller M, Bobrow L (2006). Effect of mammographic screening from age 40 years on breast cancer mortality at 10 year's follow-up: a randomised controlled trial. Lancet 368: 2053-2060.

Naini AB, Dickerson JWT, Brown MM (1988). Preoperative and postoperative levels of plasma protein and amino acid in esophageal and lung cancer patients. Cancer 62: 355-360.

Noguchi Y, Zhang QW, Sugimoto T (2006). Network analysis of plasma and tissue amino acid and the generation of an amino index for potential diagnostic use. Am. J. Clin. Nutr. 83: 513S-519S.

Norton JA, Gorchboth CM, Wesley RA, Burt ME, Brennan MF (1985). Fasting plasma amino acid levels in cancer patients. Cancer 56: 1181-1186.

Ohnuki K, Kuriyama S, Shoji N, Nishino Y, Tsuji I, Ohuchi N (2006). Cost-effectiveness analysis of screening modalities for breast cancer in Japan with special reference to women aged 40-49 years. Cancer Sci. 97: 1242-1247.

Papadia C, Shereood RA, Kalantzis C (2007). Plasma citrulline con-

centration: a reliable marker of small bowel absorptive capacity independent of intestinal inflammation. Am. J. Gastroenterol. 102: 1474-1482.
Proenza AM, Olivier J, Palou A, Roca P (2003). Breast and lung cancer are associated with a decrease in blood cell amino acid content. J. Nutr. Biochem. 14: 133-138.
Rodriguez PC, Quiceno DG, Zabaleta J (2004). Arginase I production in the tumor microenvironment by mature myeloid cells inhibits T-cell receptor expression and antigen-specific T-cell responses. Cancer Res 64: 5839-5849.
Rosen HM, Yoshimura N, Hodgman JM, Fischer JE (1977). Plasma amino acid patterns in hepatic encephalcopathy of differing etiology. Gastroenterol. 72: 483-487.
Saito H (1996). Screening for colorectal cancer by immunochemical focal occult blood testing. Jpn. J. Cancer Res. 87: 1011-1024.
Shikata N, Maki Y, Noguchi Y (2007). Multi-layered network structure of amino acid (AA) metabolism characterized by each essential AA-deficient condition. Amino acids. 33: 113-1121.
Sido B, Hack V, Hochlehnert A, Lipps H, Herfarth C, Drage W (1998). Impairment of intestinal glutathione synthesis in patients with inflammatory bowel disease. Gut. 42: 485-482.
Steyerberg EW, Eijkemans MJC, Harrell Jr FE, Habbema JDF (2000). Prognostic modelling with logistic regression analysis: a comparison of selection and estimation method in small data sets. Stat. Med. 19: 1059-1079.
Vissers YLJ, Dejong CHC, Luiking YC, Fearon LCH, von Meyenfeldt MF, Deutz NEP (2005). Plasma arginine concentration are reduced in cancer patients: evidence for arginine deficiency? Am. J. Clin. Nutr. 81: 1142-1146.
Yamaguchi K, Saito H, Oro S, Tatebe S, Ikeguchi M, Tsujitani S (2005). Expression of inducible nitric oxide synthase is significantly correlated with expression of vascular endothelial growth factor and dendritic cell infiltration. Oncology 68: 471-478.
Yoshida M, Mizukoshi T, Hirayama K, Miyano H (2007). Comprehensive analytical method for the determination of hydrophilic metabolites by high-performance liquid chromatography and mass spectrometry. J. Agric. Food Chem. 53: 551-560.
Zhang Q, Takahashi M, Noguchi Y (2006). Plasma amino acid profiles applied for diagnosis of advanced liver fibrosis in patient with chronic hepatitis C infection. Hepatol. Res. 34: 170-177.