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ORNITHINE DECARBOXYLASE ACTIVITY AS A PROGNOSTIC MARKER FOR COLORECTAL CANCER

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Abstract: Ornithine decarboxylase (ODC) is a key enzyme in the biosynthesis of polyamines, which are essential for cell proliferation. ODC activity was measured in 47 colorectal cancer patients, 5 patients with adenoma of colorectum and 4 healthy volunteers. Mean ODC activities of cancer tissue, non-cancerous mucosa from cancer-bearing colorectum, adenoma tissue, and normal mucosa from healthy volunteers were 435±392, 154±173, 295±202, 103±60 pmol CO2/h/mg protein, respectively. ODC activity of cancer tissue or adenoma tissue was significantly higher than that of the others. Among colorectal cancer patients, ODC activity in cancer tissue was correlated with T factors, lymph node metastasis and stages. Patients with tumors that had high ODC activity (≧350 pmol CO2/h/mg protein) showed a poor 10-year survival rate. These results suggest that ODC activity may be a useful marker for patients' prognosis after surgery.

Key words: ornithine decarboxylase activity, colorectal cancer, biological marker

INTRODUCTION

Ornithine decarboxylase (ODC) is a key enzyme in the biosynthesis of polyamines, which are essential for cell proliferation. Since the ODC activity is elevated during the promotion stage in carcinogenesis of animal models[1)], the ODC activity can be used as a biomarker of potential malignancy. Recent studies have demonstrated that ODC activity in cancer tissue is high in carcinoma of the stom-

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ach[2-4], lung[5], breast[6,7], head and neck[8], skin[9], and colorectum[10-14]. However, high ODC activity was not always correlated with poor prognosis in patients bearing one of these cancers[14]. Here, we show that the high ODC activity of tumors correlates with lymphatic as well as distant metastasis and also with 10-year survival of patients after surgery.

SUBJECTS AND METHODS

Four normal colorectal specimens were obtained from healthy volunteers including five men and one woman with informed consent. Seven specimens of adenoma of colorectum (three mild atypia and four severe atypia) were obtained from 5 male patients. Forty-eight specimens of cancer tissue as well as 80 non-cancerous adjacent specimens ware obtained from 47 patients including 29 males and 18 females with a mean age of 63 years (range 32-82). All patients had undergone elective tumor resection with lymph node dissection at Fukushima Medical University Hospital, between 1991 and 1994. The median postoperative follow-up time was greater than 10 years.

Among 47 patients with colorectal cancers, there were 6 Tis, 4 T1, 7 T2, 14 T3 and 17 T4. According to TNM staging criteria[15], patients were subdivided into stage 0(7), stage I(10), stage II(10), stage III(15) and stage IV(6), respectively. Lymph node metastases were present in 22 patients (N1 ; 15, N2 ; 7), while 6 patients had distant metastases. Histopathologically, these tumors were subdivided into G1(14), G2(29) and G3(5), respectively. None of the patients received preoperative chemotherapy or radiotherapy. Postoperative follow-up data were obtained by periodic examinations at the out patient clinic, or from mailed questionnaires.

ODC activity assay

All specimens were obtained by the endoscopic biopsy, and were immediately frozen at −80°C. ODC activity assay was performed within two weeks. ODC activity was assayed by modified Furihata's method[16]. Frozen specimens were homogenized in 50 mM sodium phosphate buffer 3 ml (pH=7.2) containing 0.1 mM pyridoxal phosphate and 0.1 mM EDTA, using Polytron R Model K with PTA10S generator. The homogenates were centrifuged at 30,000 G for 15 min at 2°C, and the supernatants, 0.2 ml, used as enzyme extracts Supernatant remnants were used to determine the protein content by micro-assay using UVDEC-660R. Enzymes were assayed in the out side of a double test tube with rubber cap, and released 14CO2 was trapped in Protozol (Scintilamine R -OH) 200 μl, in the inside of test tube. Enzyme extracts were incubated at 37°C for 60 min after addition of DL-[1-14C] ornithine hydrochloride 80 μl ; 0.5 μCi (58.4 mCi/mmol, NEC-469), 50 mM sodium phosphate buffer (pH=7.2) containing 0.2 mM pyridoxal phosphate, 50 μM EDTA and 0.5 mM dithiotreitol 720 μl (Total 1.0 ml). The enzyme reaction was terminated by addition of 2 M citric acid 0.4 ml to the outside of the test tube, and incubation was continued

for another 30 min at 37°C. Protosol was then transferred to the scintillator (Scintisol R EX-H : 10 ml), and its radioactivity was determined in a scintillation counter (LSC-3500, Aloka). The results were expressed as pmol CO2/h/mg protein.

Statistical analysis

The relationship between ODC activity and various clinicopathological variables was examined individually using Mann-Whitney's U test or one way Analysis of Variance with Fishier's exact test when appropriate. The primary statistical outcome in the study of prognosis was overall survival measured from the data of resection. Overall survival was calculated according to the method of Kaplan-Meier. The difference in survival among the clinicopathological variables, or between low and high ODC activity was tested using Willcoxson-Graham tests. The relative importance of the prognostic factors extracted by the univariate analysis, was assessed in a multivariate analysis by the Cox proportional hazards regression model. The differences were considered significant when the p-value was less than 0.05. The program used for these analyses was the StatView 4.5J package (Abacus Concepts Inc., Berkeley, CA).

RESULTS

ODC activities in normal mucosa, adenoma and non-cancerous mucosa from cancer-bearing colorectum were 103±60, 295±202, 154±173 pmol CO2/h/mg protein, respectively (Table 1). ODC activity in cancer tissue was 435±392 pmol CO2/h/mg protein. Significant difference was noted between cancer tissue and non-cancerous or normal colon tissue. The same was true for adenoma over non-cancerous or normal colon tissue, although the levels tended to be low as compared to those of cancer tissue. Thus, cancer tissue had significantly higher ODC activity than the normal appearing mucosa from cancer bearing patients or from healthy volunteers.

ODC activity in cancer tissue and clinicopathological features were compared and summarized in Table 2. Mean ODC activities increased in association with elevation of T factors (Tis, T1, T2, T3, T4) giving values of 175±84, 164±77, 339±272, 558±429, 528±446 pmol CO2/h/mg protein, respectively. ODC activities in T3 and T4 were significantly higher than those of Tis, T1 and T2. This was the case for N status (N0, N1, and N2) giving values of 276±227, 437±286, 975±596 pmol

Table 1. ODC activity in normal mucosa, adenoma and cancer tissue

Group	Patients	Cases		Samples	ODC activity	significance
1	Colorectal cancer	47	48	cancer	435±392	$P<0.05$ vs 2, 4
2	Colorectal cancer	47	80	non cancerous mucosa	154±173	
3	Adenoma	5	7	3 mild, 4 severe atypia	295±202	$P<0.05$ vs 2, 4
4	normal	4	6	normal rectal mucosa	103±60	

Table 2. Correlation between ODC activity and clinicopathological features

Clinicopathological findings		Cases	Mean ODC activity	P value
Age				0.7604 (over 60 vs under 60)
	32-82	Mean 63	-	
Sex				0.4837
	Male	29	456±381	
	Female	18	414±424	
Tumor location				0.1940 (Colon vs Rectum)
	Right sided colon	16	352±254	
	Left sided colon	11	432±511	
	Rectum	21	499±414	
Primary tumor				0.0035 (Tis, 1, 2 vs T3, 4)
	Tis	6	175±84	
	T1	4	164±77	
	T2	7	339±272	
	T3	14	558±429	
	T4	17	528±446	
Lymph node metastasis				0.0020 (N0 vs N1, 2)
	N0	26	276±227	
	N1	15	437±286	
	N2	7	975±596	
Distant metastasis				0.1429
	M0	42	391±342	
	M1	6	743±593	
Histological classification				0.0883 (G1, 2 vs G3)
	G1	14	318±323	
	G2	29	432±352	
	G3	5	779±633	
	G4	0	-	
Residual tumor				0.0721 (R0 vs R2)
	R0	40	385±349	
	R1	0	-	
	R2	8	684±515	
Stage				0.0402 (ANOVA)
	0	7	175±84	
	I	10	351±301	
	II	10	326±267	
	III	15	535±432	
	IV	6	743±593	

CO2/h/mg protein, respectively. ODC activities in N1 and N2 were significantly higher than that of N0. Mean ODC activities in cancer tissue of patients with or without distant metastasis were not significantly different. Mean ODC activity tended to increase in association with the degree of adenocarcinoma differentiation (G1, G2 and G3) or in patients with residual tumor, however it was not statistically significant. Mean ODC activity increased in association with elevation of TNM stages (0, I, II, III, IV) giving values of 175±84, 351±301, 326±267, 535±432, 743±593 pmol CO2/h/mg protein, respectively, reaching a statistically significant difference with the ANOVA test.

Table 3. Effect of cut off values of ODC activity on 5 year overall survival after colorectal resection

Cutoff	Patients with the lower value	Survival over 5 year	%	Patients with the higher value	Survival over 5 year	%	Significance
100	2	1	50	46	31	67.4	0.5682
150	12	9	75	36	23	63.9	0.5079
200	17	12	70.6	31	20	64.5	0.6625
250	22	16	72.7	26	16	61.5	0.381
300	26	20	76.9	22	12	54.5	0.0883
350	29	23	79.3	19	9	47.4	0.0159
450	31	24	77.4	17	8	47.1	0.0297
500	34	26	76.5	14	6	42.9	0.0179
550	36	27	75	12	5	41.7	0.0197
600	37	27	73	11	5	45.5	0.0817
650	38	28	73.7	10	4	40	0.036
800	39	28	71.8	9	4	44.4	0.1015
850	40	29	72.5	8	3	37.5	0.0405
900	41	29	69	6	3	50	0.3158
1,050	46	32	69.6	2	0	0	0.0069
1,700	47	32	68.1	1	0	0	0.0718

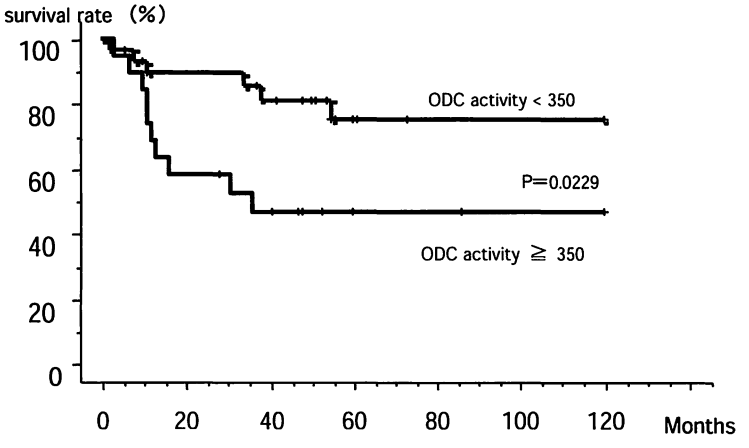

Fig. 1. Ten year overall survival rates of patients showing high or low ODC activity (≧350 or <350 pmol CO2/h/mg protein) of cancer tissue.
The patients with high ODC activity showed significantly lower survival rates than those with low ODC (47.4% vs 79.3%, $p<0.03$)

Patient overall survival was compared with ODC activity by changing cutoff points between the high and low ODC activity groups. Table 3 shows the relationship of overall survival to the different threshold points of ODC activity in patients with colorectal cancer. Several cutoff points between 350 and 550 pmol CO2/h/mg protein proved to be significantly associated with overall survival. Since the most significant association was observed at the cutoff value of 350 pmol CO2/h/mg protein ($p=0.0159$), this value was applied to draw 10 year over all survival curves between the low and high value groups (Fig. 1). The patients with high ODC activity had significantly lower 10-year survival rates than those with low ODC

Table 4. Clinicopathological findings and long-term survival (univariate analysis)

Clinicopathological findings		Cases	5 year survivial rate	*P* value
Sex				0.939
	Male	29	65.5	
	Female	18	66.7	
Tumor location				0.433
	Colon	27	70.4	
	Rectum	21	61.9	
Primary Tumor				0.001
	Tis, T1, T2	17	100.0	
	T3, T4	31	48.4	
Regional Lymph Nodes				0.001
	N0	25	88.0	
	N1, N2	22	45.5	
Distant Metastasis				0.0001
	M0	42	76.2	
	M1	6	0.0	
Histological Grading				0.009
	G1, G2	43	72.1	
	G3	5	20.0	
Residual Tumor				0.0001
	R0	40	80.0	
	R2	8	0.0	
ODC activity				0.02
	<350	29	79.3	
	≧350	19	47.4	

Table 5. Clinicopathological findings and long-term survival (multivariate analysis)

Clinicopathological findings	*P* value	Hazard ratio	95% CI
ODC activity*	0.9745	0.977	0.239−3.996
Histological classification	0.7979	1.212	0.278−5.281
Distant Metastasis	0.0905	8.941	0.708−112.949
Residual Tumor	0.0004	0.003	−1.193−0.075

*: Cutoff 350 pmol CO2/h/mg protein

activity (47.4% vs 79.3%, $p<0.03$). Univariate analysis demonstrates a significant association of tumor stage, lymph node metastasis, distant metastasis, histological grading, tumor residual and ODC activity with five year survival (Table 4). Multivariate analysis did not demonstrate a significant prognostic factor (Table 5).

DISCUSSION

Recently, polyamines have attracted the attention of many investigators, because of the relation of cell growth to polyamine synthesis. Among the enzymes involved in polyamine synthesis, special attention has been paid to ornithine decarboxylase (ODC) activity, which has been shown to be first metabolizing, rate-limiting and sensitive to various stimuli[17-20]. ODC activity is generally elevated in tumor tissue compared with normal tissue in both experimental and human cancers[3-9,21,22]. Also, there are a few reports concerning ODC activity in human colorectal cancer[10-14].

The major findings of our study were the following; 1) An approximately four fold higher ODC activity in cancer tissue than the normal mucosa from non-colorectal cancer patients, 2) Increased ODC activity in adenoma, 3) ODC activity in cancer tissue was correlated with T factors, lymph node metastasis and stages, and 4) Patients with tumors that had high ODC activity (≧350 pmol CO2/h/mg protein) showed poor 10-year survival rates. Our results indicated that colorectal cancer tissue had an approximately four fold higher ODC activity than the normal mucosa from non-colorectal cancer patients, and even the normal appearing mucosa in those with colorectal cancer had 1.5 fold higher ODC activity than that of patients without colorectal cancer. This data confirms earlier findings by Berdinskikh *et al.*[3] that these differences in ODC activity may be related to differences in proliferative activity.

When ODC activity in cancer tissue was compared with clinicopathological findings, it was significantly higher in patients with deep tumor invasion, lymph node metastasis, or low grade differentiation. It has been reported that there is a negative correlation between ODC activity and progression or stage of colorectal cancer[14]. On the other hand, a positive correlation between ODC activity and TNM classification was reported[13]. The difference in tissue samples (ie. biopsy or resected specimens), may partly explain this discrepancy. As ODC has a short half life on the order of 10 minutes, we compared ODC activity in endoscopic specimens with that in surgical samples from the same 5 patients. ODC activity in biopsy specimens was approximately two to ten fold higher than that in operative specimens (data not shown). Porter *et al.*[13] reported similar results with a comparison of biopsy and surgical specimens, and recommended that ODC activity be measured using biopsy samples. The time required for surgical tissue resection may be sufficient to account for substantial loss in enzyme activity. In this study, we measured ODC activity using biopsy samples[13]. More detailed investigations are

also recommended to ascertain the problems including heterogeneity of the tumor and contamination of the inflammation or fibrous tissue.

In the present study, higher ODC activity correlates with poor prognosis, suggesting various inhibitors against ODC activity or inducers for catabolic enzyme (the spermidine/spermine-N(1)-acetyltransferase; SSAT) of polyamine may have some role on chemoprevention as well as on colon cancer progression[23-28]. Thus, careful follow-up and intense postoperative therapy are required for patients with colorectal cancer showing high ODC activity.

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