The Relationship between Clinicopathological Factors and the Reduction of Pyrimidine Nucleoside Phosphorylase Activity after Preoperative Administration of 5'-Deoxy-5-fluorouridine

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Abstract. *Aim: The response to fluoropyrimidine chemotherapeutic drugs is different in individual tumors. Predictive biomarkers of antitumor effects by these drugs are unknown. 5'-Deoxy-5-fluorouridine (5'-DFUR), a fluoropyrimidine chemotherapeutic drug, is converted to 5-fluorouracil (5-FU) by pyrimidine nucleoside phosphorylase (PyNPase). It is suggested that 5'-DFUR will efficiently exert antitumor effects via PyNPase in tumor tissues. The change of PyNPase activity in tumor tissues following 5'-DFUR administration may reflect antitumor effects, and may be useful for detecting predictive factors of antitumor effects. The aim of this study was to search for predictive factors of antitumor effects by analyzing the relationship between clinicopathological factors and the change of PyNPase activity in colorectal tumor tissues after preoperative 5'-DFUR administration. Patients and Methods: PyNPase activity in colorectal tissues from 45 patients with colorectal tumors was measured using an ELISA method. Results: The reduction rate of PyNPase activity in colorectal tumor tissues after preoperative 5'-DFUR administration was correlated with significant differences in lymphatic invasion, stage, and histologic classification. It is suggested that lymphatic invasion, stage (distant metastasis), and histologic classification may be predictive factors for evaluating antitumor effects and selecting 5-FU-based chemotherapeutic drugs for patients with colorectal tumors.*

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Fluoropyrimidine chemotherapeutic drugs are widely used for postoperative adjuvant chemotherapy in patients with solid tumors, including colorectal, gastric and uterine tumors (1). 5-Fluorouracil (5-FU), a fluoropyrimidine chemotherapeutic drug, was synthesized by Duschinsky *et al*. (2). The mechanisms of antitumor actions of 5-FU have been biochemically elucidated. However, the response rate to 5-FU-based chemotherapy as a first-line treatment for advanced colorectal tumors is only 10-15%, while the response rate of 5-FU-based combination chemotherapies is 40-50% (1, 3-4). Improved strategies for using fluoropyrimidine chemotherapeutic drugs in patients with colorectal tumors have been tested; these strategies include development of new derivatives, combination chemotherapy or chemoradiotherapy, and improved methods of administration (5-8).

It is difficult to predict antitumor effects of fluoropyrimidine chemotherapeutic drugs in patients with colorectal tumors because the response to these drugs is different in individual tumors. Predictive biomarkers of antitumor effects by these drugs are unknown. Currently, these drugs have been administrated to patients with colorectal tumors, but the situations in which there would be antitumor effects remain unpredictable. It is essential that these drugs are administered with greater predictability for patients with colorectal tumors.

5'-Deoxy-5-fluorouridine (5'-DFUR), a derivative of 5-FU, was synthesized by Cook *et al*. (9). It is known that 5'-DFUR is converted to 5-FU by pyrimidine nucleoside phosphorylase

(PyNPase) (10, 11). In particular, the level of PyNPase activity is higher in tumor tissues than in normal tissues (12, 13). It is suggested that 5'-DFUR will efficiently exert antitumor effects *via* PyNPase in tumor tissues (14-16). The change of PyNPase activity in tumor tissues following 5'-DFUR administration may reflect antitumor effects, and may be useful for detecting predictive factors of antitumor effects.

In the present study, we focused on the alteration of PyNPase activity in colorectal tumor tissues following preoperative 5'-DFUR administration. The relationship between clinicopathological factors and the change of PyNPase activity in the search for predictive factors of antitumor effects by 5-FU-based chemotherapeutic drugs in patients with colorectal tumors were analyzed.

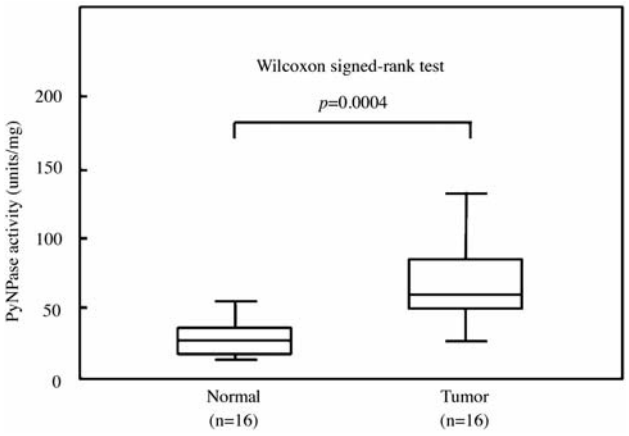

Figure 1. *PyNPase activity in normal and tumor colorectal tissues.*

Patients and Methods

Patients. Patients who underwent surgical resection because of a colorectal tumor were enrolled in this study. The age of all patients was less than 81 years. None of the patients had a serious disease or dysfunction in the liver, heart or bone marrow. Before the operation, patients were classified into either an administration group or a control group by random allocation. Ten days before the operation, 24 patients in the administration group were administered oral 5'-DFUR at 1200 mg/day. The control group consisted of 21 patients who were not administered oral 5'-DFUR before the operation.

Tissue sampling. The colorectal tumor tissues of all patients were obtained from preoperative biopsies and surgical resections. In addition to the colorectal tumor tissues, the normal tissues were obtained from surgical resections from 16 patients in the control group. The tissues obtained were stored at –80˚C until the assay of PyNPase activity.

Measurement of PyNPase activity. PyNPase activity in normal and tumor colorectal tissues was measured using an ELISA method. The change of PyNPase activity was calculated as follows: change of PyNPase activity (%)=(PyNPase activity before administration–PyNPase activity after administration)/(PyNPase activity before administration) ×100.

Statistical analyses. The Wilcoxon signed-rank test was used to analyze the differences in PyNPase activity between normal and tumor colorectal tissues. Statistical analyses between clinicopathological factors and the change of PyNPase activity were performed using the Mann-Whitney *U*-test and the Kruskal-Wallis test. A value of $p<0.05$ was considered to be statistically significant in each test.

Results

PyNPase activity in normal and tumor colorectal tissues. Both normal and tumor colorectal tissues were obtained from surgical resections from 16 patients in the control group. The mean and standard deviation of PyNPase activity in colorectal tumor tissues was 76.2±56.4 units/mg, and the mean and standard deviation of PyNPase activity in colorectal normal tissues was 29.0±19.4 units/mg. These tests demonstrated that the PyNPase activity in colorectal tumor tissues was significantly higher than in colorectal normal tissues ($p=0.0004$) (Figure 1). These results were consistent with those of previous reports (12-13).

PyNPase activity in colorectal tumor tissues after preoperative 5'-DFUR administration. Changes in PyNPase activity in colorectal tumor tissues both before the operation and at the time of the operation in the administration group and the control group are shown in Table I. An up arrow indicates an increase of PyNPase activity in colorectal tumor tissues at the time of the operation when compared to before the operation, while a down arrow indicates a decrease of PyNPase activity in colorectal tumor tissues at the time of the operation when compared to before the operation. The mean and standard deviation of PyNPase activity in colorectal tumor tissues in the administration group was 85.5±58.2 units/mg before the operation, and 65.8±54.2 units/mg at the time of the operation. The difference in PyNPase activity in colorectal tumor tissues before the operation and at the time of the operation in the administration group was statistically analyzed. These results showed that the PyNPase activity in colorectal tumor tissues at the time of the operation in the administration group had decreased significantly when compared to that before the operation ($p=0.005$) (Table I). The mean and standard deviation of PyNPase activity in colorectal tumor tissues in the control group was 54.8±30.4 units/mg before the operation, and 70.3±51.4 units/mg at the time of the operation. When comparing the PyNPase activity in colorectal tumor tissues in the control group, the values at the time of the operation had increased in 12 patients and decreased in 9 patients. The difference in PyNPase activity in colorectal tumor tissues before the operation and at the time of the operation in the control group was statistically analyzed. These results did not show a significant difference in PyNPase activity between the colorectal tumor tissues that were sampled before the operation

Table I. *PyNPase activity in colorectal tumor tissues after preoperative administration of 5'-deoxy-5-fluorouridine.*

Administration group	Before the operation (units/mg)	At the time of the operation (units/mg)	*p*-Value*	Control group	Before the operation (units/mg)	At the time of the operation (units/mg)	*p*-Value*
			0.005				N.S.(0.259)
1	50.9	31.7	↘	1	23.1	18.2	↘
2	77.6	48.5	↘	2	43.9	37.6	↘
3	36.9	31.8	↘	3	27.1	21.9	↘
4	44.4	33.8	↘	4	39.0	73.8	↗
5	56.4	47.9	↘	5	27.0	64.1	↗
6	35.2	26.6	↘	6	53.6	24.2	↘
7	26.3	54.2	↗	7	19.7	49.4	↗
8	61.4	27.8	↘	8	45.5	88.2	↗
9	71.9	27.5	↘	9	28.4	53.2	↗
10	56.1	84.1	↗	10	78.2	52.3	↘
11	84.2	24.7	↘	11	37.7	36.0	↘
12	72.5	39.5	↘	12	58.3	67.3	↗
13	50.8	37.0	↘	13	107.6	55.9	↘
14	208.9	210.3	↗	14	40.8	56.4	↗
15	101.4	36.5	↘	15	77.7	92.9	↗
16	138.5	121.5	↘	16	57.7	72.5	↗
17	95.6	47.8	↘	17	105.1	118.2	↗
18	280.1	186.5	↘	18	47.7	132.6	↗
19	142.1	173.3	↗	19	22.9	255.0	↗
20	74.4	72.3	↘	20	86.3	60.8	↘
21	111.5	75.8	↘	21	123.0	45.4	↘
22	68.6	82.4	↗				
23	60.4	7.5	↘				
24	45.8	49.1	↗				

*Wilcoxon signed-rank test comparing preoperative and operative values. N.S.: Not significant.

and those sampled at the time of the operation in the control group (p=0.259) (Table I). Therefore, it was demonstrated that PyNPase activity in colorectal tumor tissues had decreased significantly following preoperative 5'-DFUR administration.

The relationship between clinicopathological factors and the change of PyNPase activity in colorectal tumor tissues after preoperative 5'-DFUR administration. The relationship between clinicopathological factors and the change of PyNPase activity in colorectal tumor tissues after preoperative 5'-DFUR administration was examined. A reduction of PyNPase activity in colorectal tumor tissues after preoperative administration was correlated with significant differences in lymphatic invasion (p=0.046), stage I *versus* stage IV (p=0.047), stage II *versus* stage IV (p=0.018), stage III *versus* stage IV (p=0.003), stage II *versus* stage III (p=0.028), and poorly differentiated adenocarcinoma *versus* well-differentiated adenocarcinoma (p=0.041), while significant differences were not observed for lymph node metastasis (p=0.582), histologic invasion (p=0.754), and venous invasion (p=0.588) (Figure 2). The difference of clinicopathological factors between stage I, II, III and stage IV is the presence of distant metastasis: only stage IV disease is distant metastasis positive. The differences of clinicopathological factors between stage II and stage III are the presence of lymph node metastasis and histologic invasion. The reduction of PyNPase activity was not correlated with significant differences in lymph node metastasis and histologic invasion. Thus, it was shown that the reduction of PyNPase activity in colorectal tumor tissues after preoperative 5'-DFUR administration was significantly higher in patients with lymphatic invasion-negative than in those with lymphatic invasion-positive disease, in those-disease with distant metastasis negative than in those with distant metastasis-positive, and in patients with well-differentiated adenocarcinoma than in those with poorly differentiated adenocarcinoma. It is suggested that lymphatic invasion, stage (distant metastasis), and histologic classification may be predictive factors for evaluating antitumor effects and selecting 5-FU-based chemotherapeutic drugs for patients with colorectal tumors.

Discussion

The response to fluoropyrimidine chemotherapeutic drugs is different in individual tumors, so it is difficult to predict the antitumor effects of these drugs in patients with colorectal

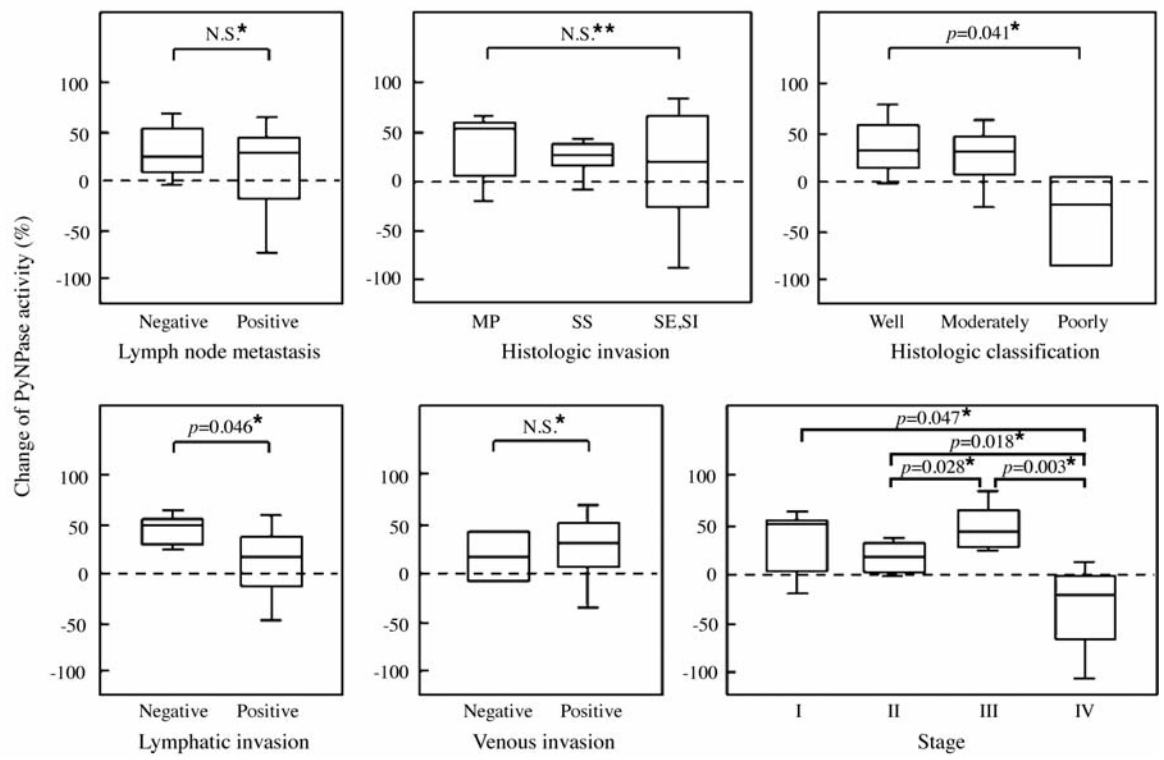

Figure 2. *The relationship between clinicopathological factors and change of PyNPase activity in colorectal tumor tissues after preoperative administration of 5'-deoxy-5-fluorouridine. MP: Muscularis propia; SS: subserosa; SE: serosa; SI: invasion to the surrounding organ; Well: well-differentiated adenocarcinoma; Moderately: moderately differentiated adenocarcinoma; Poorly: poorly differentiated adenocarcinoma. N.S.: not significant; *Mann-Whitney U-test; **Kruskal-Wallis test; Clinicopathological factors are classified according to the General Rules for Clinical and Pathological Studies on Cancer of the Colon, Rectum and Anus: Japanese Society for Cancer of the Colon and Rectum.*

tumors. Future therapies will depend on more personalized treatment, hence it is important to establish criteria for selecting suitable chemotherapeutic drugs in patients with colorectal tumors. Our results showed that the reduction of PyNPase activity in colorectal tumor tissues after preoperative 5'-DFUR administration was significantly higher in patients without lymphatic invasion than in those with, in patients without distant metastasis than in those with, and in patients with well-differentiated adenocartinoma than in those with poorly differentiated adenocartinoma. The change of PyNPase activity in tumor tissues following 5'-DFUR administration may reflect antitumor effects because 5'-DFUR will efficiently exert antitumor effects *via* PyNPase in tumor tissues (14-16). It may be useful in selecting 5-FU-based chemotherapeutic drugs for patients with lymphatic invasion-negative, distant metastasis-negative, and well-differentiated adenocartinoma.

The reduction of PyNPase activity in colorectal tumor tissues after preoperative 5'-DFUR administration was significantly higher in patients with stage III than in those with stage II. The differences of clinicopathological factors between stage II and stage III are presence of lymph node metastasis and histologic invasion. Stage II is lymph node metastasis negative, stage III is lymph node metastasis positive. Histologic invasion in stage II is equal to stage III or progress. Therefore the results may reflect the factors involved in histologic invasion.

It was reported that PyNPase is identical to platelet-derived endothelial cell growth factor (PD-ECGF) (17). There was a significant correlation between PyNPase-positive cells and the microvessel count in colorectal tumors (18). It was thought that PyNPase was involved in angiogenesis and promoted growth and malignancy of tumors (19, 20). Our results showed that the reduction of PyNPase activity in colorectal tumor tissues after preoperative 5'-DFUR administration was significantly lower in patients with lymphatic invasion- positive, distant metastasis-positive, and poorly differentiated adenocartinoma. These clinicopathological factors were involved in either angiogenesis or growth and malignancy of tumors. Therefore it is suggested that the factors induced the reduction of PyNPase activity in colorectal tumor tissues after preoperative 5'-DFUR administration are involved in suppression of angiogenesis, growth and malignancy of tumors.

It was reported that PyNPase in colorectal tumor tissues was mainly produced by tumor-associated macrophages in the

stroma (21, 22). Recently, it was discovered that there are two types of macrophages, M1 phenotype and M2 phenotype. It is thought that the M1 phenotype macrophages have the ability to destroy invading pathogens and cancer cells, and that the M2 phenotype macrophages exhibit properties that promote the growth of tumors (23). It is possible that the reduction of PyNPase activity in colorectal tumor tissues after preoperative 5'-DFUR administration may be related either increase in immunological response of M1 phenotype macrophages or suppression in immunological response of M2 phenotype macrophages. Analyzing the relationship between the reduction of PyNPase activity and function of tumor-associated macrophages in colorectal tumors may provide new insights into the role of PyNPase.

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