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Correlation between Clinical Pathologic Factors and Activity of 5-FU-Metabolizing Enzymes in Colorectal Cancer

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Abstract

Introduction: Orotate phosphoribosyl transferase (OPRT), dihydropyrimidine dehydrogenase (DPD), and thymidylate synthase (TS) are initial key enzymes in the 5-fluorouracil (5-FU) metabolic pathway. The expression levels and activities of these three enzymes play important roles in the response of cancer patients to 5-FU-based chemotherapy.

Purpose: The purpose of this study was to investigate the relationship between the activities of 5-FU metabolic enzymes and clinicopathologic factors in colorectal cancer.

Methods: We measured the activities of OPRT, DPD, and TS in colorectal cancer tissues. We also investigated the correlations between the activities of these three enzymes and clinicopathologic factors (histological type, depth of tumor invasion, extent of lymph node metastasis, Dukes' stage, lymphatic invasion, and vascular invasion). We examined 100 patients with surgically resected colorectal cancer.

Results: Poorly differentiated adenocarcinoma showed significantly higher DPD activities than did moderately differentiated or well-differentiated adenocarcinoma. In patients with lymph-node metastasis, OPRT activity was significantly lower than in patients without lymph-node metastasis. No significant relation was found between TS activity and histological type, depth of tumor invasion, extent of lymph node metastasis, Dukes' stage, lymphatic invasion, or vascular invasion.

Conclusion: The response to 5-FU may be poor in patients with lymph-node metastasis, because of low OPRT activity, and in patients with poorly differentiated adenocarcinoma, because of high DPD activity.

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Key words: orotate phosphoribosyl transferase, dihydropyrimidine dehydrogenase, thymidylate synthase, colon cancer

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Table 1 Patient characteristics

No. of patients		100
Age		67.5 (38 ～ 90)
Gender	male/female	67/33
location	C/A/T/D/S/R	6/20/7/4/19/44
Classification of tumor	Well/mod/por/muc	41/48/9/2
Depth of tumor invasion	T2/T3/T4	11/83/6
Lymph node metastasis	positive/negative	39/61
Duke's stage	B/C/D	36/40/23
Lyphatic invasion	Ly (−)/Ly(+)	9/91
Vascular invasion	V (−)/V (+)	13/87

well: well-differentiated adenocarcinoma, mod: moderate differentiated adenocarcinoma, por: poorly differentiated adenocarcinoma, muc: mucinous adenocarcinoma

Introduction

Chemotherapy for colorectal cancer has progressed in recent years, and the prognosis of such cancer has improved. However, many patients show a poor response to anticancer agents and have severe adverse toxic effects. Molecular profiling of tumors may identify patients who are more likely to benefit from chemotherapy. Such profiling would enable clinicians to tailor treatment to each patient and tumor profile. Although a limited number of predictive markers have been identified, they may improve the response rate to chemotherapy.

5-Fluorouracil (5-FU) is one of the drugs most often used against colorectal cancer[1]. If we can predict the effect of 5-FU before administration, patients can receive considerable benefit. Thymidylate synthase (TS), a target enzyme of 5-FU, affects 5-FU sensitivity[2]. A number of studies have demonstrated that patients with a low tumoral TS expression level have higher response rates to 5-FU than do patients with tumors having a high TS expression level[3,4]. It has also been shown that dihydropyrimidine dehydrogenase (DPD), a rate-limiting enzyme for the degradation of 5-FU, determines 5-FU sensitivity[5,6]. Furthermore it has been shown that orotate phosphoribosyl transferase (OPRT), an enzyme in the main pathway of 5-FU phosphorylation, also determines 5-FU sensitivity[7]. These three enzymes may be strong predictive markers of 5-FU sensitivity.

If possible, it is desirable to measure the activities of these three enzymes or carry out a chemosensitivity test before administering 5-FU. However, it is impossible, as standard treatment, to measure the enzymatic activities or carry out a chemosensitivity test. Therefore, if we can predict the activities of these three enzymes on the basis of clinicopathologic factors, patients can receive a considerable benefit.

In this study, we measured the activities of OPRT, DPD, and TS in 100 patients with colorectal cancer and evaluated their relationships with clinicopathological factors.

Materials and Methods

Surgical specimens were obtained from patients 100 colorectal cancer, comprising 67 men and 33 women aged 38 to 90 years (**Table 1**). The specimens were frozen at −80℃ until the OPRT, DPD and TS activities were assayed. Written informed consent was obtained from all patients, and the study was approved by the ethics committee of our university hospital.

Measurement of OPRT Activity

A tissue sample (300 mg) was obtained from the resected tumor. The sample was immediately frozen and stored at −80℃ until the assay of OPRT activity. OPRT activity was determined with a 5-FU phosphorylation assay, as previously described[8]. The reaction rate was determined from the quantity of 5-fluorouridine monophosphate (FUMP) produced through 5-FU phosphorylation by phosphoribosyl

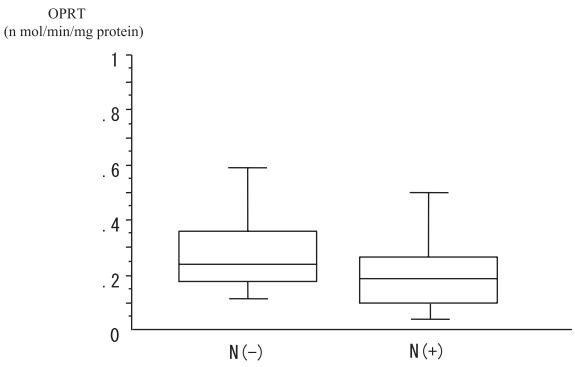

Fig. 1 OPRT and lymph node metastasis
The OPRT activity was lower in cases of lymph node metastasis than in cases of no lymph node metastasis. The upper and lower limits of the boxes, and the lines across the boxes, indicate the 75th and 25th percentiles and medians, respectively.

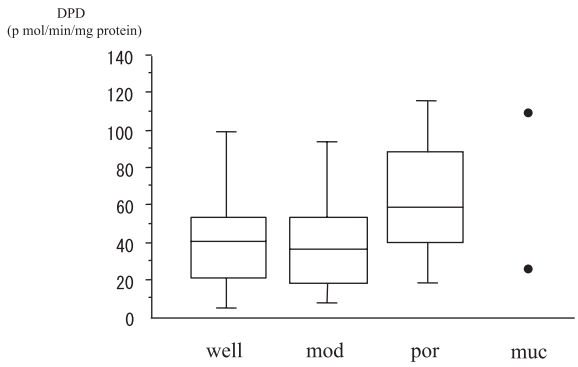

Fig. 2 DPD and Pathological type
Poorly differentiated adenocarcinomas showed a significantly higher DPD activity than moderately differentiated or well-differentiated adenocarcinomas. The upper and lower limits of the boxes, and the lines across the boxes, indicate the 75th and 25th percentiles and medians, respectively.

pyrophosphate (PRPP), and OPRT activity per milligram of protein was calculated.

Measurement of DPD Activity

A tissue sample (300 mg) was taken from the resected tumor. The sample was immediately frozen and stored (−80℃) until the assay of DPD activity by radioassay. DPD activity was calculated by measuring the total production of metabolites of [^{14}C]-5-FU, which was added to the homogenized tissue samples, i.e., dihydrofluorouracil (DHFU), 2-fluoro-b-ureidopropionate (F-b-UPA) and a-fluoro-b-alanine (F-b-Ala). 5-FU and the metabolites were separated by the thin-layer chromatography[9].

Measurement of TS Activity

A tissue sample (300 mg) was taken from the resected tumor, and it was immediately frozen (−80℃). TS (TS-free) activity was measured with a 5-fluorodeoxyuridine monophosphate (FdUMP) ligand-binding assay[10].

Statistical Analysis

The Mann-Whitney U-test was used to compare the means between the groups. P-values less than 0.05 were considered to indicate statistical significance.

Results

No significant correlation was found between OPRT activity and histological type, depth of tumor, or Dukes' stage. However, the OPRT activity was lower in cases of lymph node metastasis than in cases of no lymph node metastasis **(Fig. 1)**.

No significant correlation was found between DPD activity, depth of tumor or Dukes' stage. Poorly differentiated adenocarcinoma and mucinous adenocarcinoma showed a significantly higher DPD activity than did moderately differentiated or well-differentiated adenocarcinoma **(Fig. 2)**. DPD activity tended to be higher in cases with lymph-node metastasis than in cases without lymph-node metastasis; however, the difference was not significant.

No significant relationship was found between TS activity and histological type, lymph node metastasis, depth of tumor, Dukes stage, vascular invasion, or lymphatic invasion **(Table 2)**.

Discussion

5-FU is the most widely used anticancer drug for colon cancer, and its efficacy varies with the activities of metabolic enzymes in cancer tissues[3–7]. Following uptake into cells, 5-FU is metabolized into

Table 2 Enzyme activity assay results

		OPRT n mol/min/mg protein		DPD p mol/min/mg protein		TS p mol/min/mg protein	
location	colon	0.234 ± 0.124	N.S.	44.2 ± 28.3	N.S.	9.7 ± 12.0	N.S.
	rectum	0.252 ± 0.188		37.4 ± 25.8		6.3 ± 6.2	
Histologic type	well	0.257 ± 0.173	N.S.	38.2 ± 26.0	p<0.05	9.4 ± 12.0	N.S.
	mod	0.239 ± 0.153		38.7 ± 25.6		7.1 ± 8.7	
	poor	0.193 ± 0.076		63.1 ± 27.6		8.4 ± 6.3	
	muc	0.229 ± 0.086		67.0 ± 59.4		8.2	
Lymph node metastasis	n (−)	0.280 ± 0.163	p<0.05	35.7 ± 24.0	N.S.	8.8 ± 12.0	N.S.
	n (+)	0.216 ± 0.145		44.9 ± 28.9		7.7 ± 8.4	
Depth of tumor invasion	T2	0.270 ± 0.160	N.S.	24.6 ± 12.3	N.S.	9.2 ± 5.9	N.S.
	T3	0.237 ± 0.138		43.8 ± 28.1		8.1 ± 10.7	
	T4	0.262 ± 0.328		36.2 ± 28.2		7.6 ± 4.7	
Dukes stage	B	0.258 ± 0.126	N.S.	37.4 ± 24.7	N.S.	9.1 ± 12.6	N.S.
	C	0.242 ± 0.175		46.8 ± 28.2		8.4 ± 8.8	
	D	0.218 ± 0.162		37.8 ± 29.0		6.4 ± 7.0	
Vascular invasion	v (−)	0.238 ± 0.133	N.S.	32.8 ± 17.9	N.S.	8.1 ± 3.8	N.S.
	v (+)	0.242 ± 0.159		42.5 ± 28.3		8.2 ± 10.6	
Lyphatic invasion	ly (−)	0.219 ± 0.120	N.S.	41.4 ± 36.6	N.S.	5.2 ± 2.9	N.S.
	ly (+)	0.244 ± 0.158		41.2 ± 26.5		8.5 ± 10.4	

well: well-differentiated adenocarcinoma, mod: moderate differentiated adenocarcinoma, por: poorly differentiated adenocarcinoma, muc: mucinous adenocarcinoma. Values are reported as mean ± SD.

two different active forms: 5-fluorouridine triphosphate (FUTP) and FdUMP. These active forms induce anticancer effects by inhibiting RNA function and DNA synthesis, respectively. FdUMP inhibits DNA synthesis by binding with the nucleotide synthetic enzyme TS. Johnston et al. and Salonga et al. have reported that 5-FU is more sensitive in cases of advanced colon cancer with low TS gene expression levels[3,11]. OPRT plays an extremely important role in cell proliferation. On the other hand, OPRT converts 5-FU to its active nucleotide, FUMP[12]. RNA function is inhibited when 5-FU is phosphorylated by OPRT. DPD inactivates FU immediately.

Cases with high OPRT activity have a better prognosis than do cases with low OPRT activity[13], and a positive correlation between OPRT activity and the antitumor effects of 5-FU has been reported[2]. Cases with high DPD activity have a poorer prognosis than do cases with low DPD activity,and DPD activity negatively correlates with 5-FU sensitivity[14–16]. TS is similar to DPD. In other words, OPRT, DPD, TS are not only prognostic factors for colorectal cancer but are also predictive factors of sensitivity to 5-FU. Therefore, the measurement of the activities of OPRT, TS, and DPD is useful for planning the treatment of colorectal cancer[7,16]. However an association of these enzyme activities with clinicopathological background factors of cancers has not been examined in detail.

This is the largest study that has measured OPRT, DPD, and TS activities in colorectal cancer. In this study, we found that OPRT activity is significantly lower in cases with lymph-node metastasis than in cases without lymph-node metastasis. Moreover, the DPD activity is higher in poorly differentiated adenocarcinoma than in well-differentiated or moderately differentiated adenocarcinoma. In mucinous adenocarcinoma, OPRT activity tended to be high, and one of two cases showed high DPD activity. However, the number of cases of mucinous adenocarcinoma was small, and the differences did not reach the level of significance. Mucinous carcinoma tends to be hyposensitive to 5-FU, and low OPRT activity and high DPD activity are possible reasons.

The prognosis of colorectal cancer depends on

pathological TMN staging and nodal status. In cases with lymphatic metastasis, the OPRT activity in the tumor may be lower. The prognosis of cases with lymph node metastasis may be poor because of low OPRT activity, indicating low sensitivity to 5-FU, as well as lymph-node metastasis. Ochiai has reported that OPRT actitivity may be a prognostic factor and that patients with OPRT activity of at least 0.147 nmol/min mg have a better prognosis[13]. OPRT may also be a predictive factor for sensitivity to 5-FU; thus, when we perform chemotherapy, we should select agents other than 5-FU, such as irinotecan, or a combination therapy.

Similarly, the prognosis of poorly differentiated adenocarcinoma, in which the DPD activity is high in many cases, is poor because of low sensitivity to 5-FU. Thus, we should select combination therapy or S-1, which is combined with a DPD inhibitor.

Conclusion

The response to 5-FU may be poor in patients with lymph-node metastasis, because of low OPRT activity , and in patients with poorly differentiated adenocarcinoma, because of high DPD activity. One reason for the poor prognosis of these cases is low sensitivity to 5-FU.

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