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Relationships between the expression of thymidylate synthase, dihydropyrimidine dehydrogenase, and orotate phosphoribosyltransferase and cell proliferative activity and 5-fluorouracil sensitivity in colorectal carcinoma

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Abstract

Background. The site of action of the 5-fluorouracil (5-FU) antitumor effect has been explicated in recent years. Many studies have investigated enzymes involved in 5-FU metabolism in attempts to predict this effect, and a correlation of enzyme activity with the 5-FU drug sensitivity test has been reported. The aim of this study was to identify the biochemical response determinants of 5-FU. Additionally, we aimed to clarify the association between cell proliferative activity and the response to 5-FU of colorectal cancer.

Methods. Our research subjects were 54 patients with colorectal carcinoma who had undergone operations between August 1999 and July 2001 in our department. Assays of thymidylate synthase (TS), dihydropyrimidine dehydrogenase (DPD), and orotate phosphoribosyltransferase (OPRT) activities in colorectal carcinoma tissue and assays of 5-FU sensitivity by the collagen gel droplet embedded culture drug sensitivity test (CD-DST) were conducted to investigate the relationships between each enzyme activity and 5-FU sensitivity. In addition, the proliferative activity of cancer cells was evaluated with Ki-67 antibody, and the relationship of this activity to each enzyme activity and 5-FU sensitivity were investigated.

Results. 5-FU sensitivity was high in the low-TS-activity group and in the high-OPRT-activity group. Cancers with high cell proliferative activity showed good sensitivity to 5-FU, and TS and OPRT activities were high in such cancers.

Conclusion. The results suggest that OPRT activity can predict sensitivity to 5-FU, and high OPRT activity may cause good 5-FU sensitivity in cancers with high cell proliferative activity.

Key words Orotate phosphoribosyltransferase · Collagen gel droplet embedded culture drug sensitivity test · Ki-67 · 5-Fluorouracil · Colon cancer

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Introduction

5-Fluorouracil (5-FU) has been among the most frequently used drugs in the treatment of advanced/recurrent gastrointestinal cancer since its introduction to clinical practice more than 40 years ago. The response rate to this drug as a single agent is usually less than 20%,[1] but the introduction of biochemical modulation (BCM) therapy has resulted in 5-FU playing a more central role in the treatment of advanced colorectal carcinoma. However, even such combination therapy has a response rate of only about 40%. Given that more than 50% of patients do not respond under present treatment conditions, the development of new clinical drugs and more effective combination therapy is essential. In addition, the development of highly specific and responsive sensitivity tests for anticancer agents is important to be able to predict the response to an anticancer agent before using it in treatment.

The site of action of the antitumor effect of 5-FU has been explicated in recent years. Many studies have investigated whether the activity levels of enzymes involved in 5-FU metabolism can predict this effect, and a correlation of enzyme activity level with the 5-FU sensitivity test has been reported. Most of these reports have been concerned with thymidylate synthase (TS), the target of BCM therapy, and dihydropyrimidine dehydrogenase (DPD), the rate-limiting enzyme in the catabolism of 5-FU. Fukushima et al.[2] have maintained that the phosphorylation of 5-FU is one of the rate-limiting steps in the manifestation of the antitumor effect of 5-FU, and that orotate phosphoribosyl-transferase (OPRT) is the enzyme of the main pathway of 5-FU phosphorylation in human cancer cells. Although basic research into the relationship between OPRT and the main pathway of 5-FU phosphorylation has been described, reports of clinical investigations have been few.[3,4] We have measured OPRT activity, as well as the activities of TS and DPD, in tissue samples of colorectal carcinoma to investigate the relationship between the activities of each of these enzymes and 5-FU sensitivity. We used the collagen gel droplet embedded culture drug sensitivity test (CD-DST)[5] to investi-

gate the relationship between each of these enzyme activities and 5-FU sensitivity.

This anticancer agent – 5-FU – is generally agreed to be more effective against highly malignant and rapidly proliferating tumors, but a mechanism for this action has not been described. Therefore, we evaluated cell proliferative activity by using Ki-67 as an index of cancer malignancy, to validate this action by the 5-FU sensitivity test in the investigation of its relationship with each enzyme activity. Ki-67 antibody was discovered as an autoantibody in leukemia patients and recognizes only proliferative cells. Ki-67 antigen exists in the cell nucleus from the late G_1 phase to the M phase of the cell cycle, and cells in the proliferation process can be recognized via the use of immunostaining to identify this antigen. In addition, the dynamics of cancer cell proliferation can be reflected by the labeling index (Ki-67 LI), i.e., the proportion of Ki-67-positive cells in more than 100 examined cancer cells.[6]

Patients and methods

Patients

Fifty-four colorectal carcinoma patients who had undergone operations without prior chemotherapy between August 1999 and July 2001 in our department were selected for this trial. Patient anonymity was ensured at our institution, and patient identification codes were used to protect patient privacy information. Table 1 presents the characteristics of these patients.

Measurements of enzyme activity

Tissue was taken from the tumor site of the resected sample, and immediately stored in a refrigerator.

TS activity

Previous investigations of TS mainly measured protein levels; however, the present investigation measured TS enzyme activity by means of a tritium release method.[7]

The tissue sample was homogenized in 50 mmol/l Tris-HCl (pH 7.3) that contained 2 mmol/l dithiothreitol. After centrifugation (105 000 *g*, 1 h, 4°C), the supernatant was collected and incubated at 37°C with methylene tetrahydrofolic acid and [^{3}H]-dUMP as the substrate. Aliquots of the reaction mixture were removed after 10, 20, and 30 min of incubation, and the reaction was stopped immediately by adding 10% active carbon suspension containing 4% trichloroacetic acid. After centrifugation, ^3H_2O formed in the supernatant was quantified with a liquid scintillation counter. The reaction rate was obtained based on the relationship between reaction time and the amount of ^3H_2O formed. From this reaction rate and the protein concentration determined separately, TS activity (pmol/min per mg protein) was calculated.

DPD activity

DPD enzyme activity was measured in a sample of the stored tissue by the radio isotope-high performance liquid chromatography (RI-HPLC) method.[8] The tissue sample was homogenized in 20 mmol/l phosphate buffer (pH 8.0)

Table 1. Characteristics of patients

Sex	Male	31 (57.4%)	Depth of tumor invasion	sm	1 (1.9%)
	Female	23 (42.6%)		mp	3 (5.6%)
				ss/a1	40 (74.1%)
Age (years)	40–84	Mean, 62.7 years		se/a2	9 (16.7%)
				si/ai	1 (1.9%)
Tumor location	Colon	37 (68.5%)			
	C	4 (7.4%)	Vascular invasion	ly (−)	6 (11.1%)
	A	8 (14.8%)		ly (+)	48 (88.9%)
	T	8 (14.8%)			
	D	15 (9.3%)		v (−)	40 (74.1%)
	S	12 (22.2%)		v (+)	14 (25.9%)
	Rectum	17 (31.5%)			
	Rs	6 (11.1%)	Lymph node metastasis	n (−)	28 (51.9%)
	Ra	4 (7.4%)		n (+)	26 (48.1%)
	Rb	7 (13.0%)			
			Clinical staging	Stage I	3 (5.6%)
Histological classification	Well	18 (33.3%)		II	19 (35.2%)
	Mod	30 (55.6%)		IIIa	14 (25.9%)
	Por	4 (7.4%)		IIIb	5 (9.3%)
	Muc	2 (3.7%)		IV	13 (24.1%)

Clinicopathological factors and clinical stages are according to the *General rules for clinical and pathological studies on cancer of the colon, rectum and anus* (6th edition)[36]
C, Cecum; A, ascending colon; T, transverse colon; D, descending colon; S, sigmoid colon; Rs, rectosigmoid; Ra, rectum above the peritoneal reflection; Rb, rectum below the peritoneal reflection; well, well-differentiated adenocarcinoma; mod, moderately differentiated adenocarcinoma; por, poorly differentiated adenocarcinoma; muc, mucinous adenocarcinoma; ly, lymphatic invasion; v, venous invasion

that contained 1 mmol/l ethylenediamine tetraacetic acid (EDTA)·2K and 1 mmol/l 2-mercaptoethanol. After centrifugation (105000*g*, 1 h, 4°C), the supernatant was collected and incubated at 37°C in the presence of 6.25 mmol/l Nicotinamide Adenine Dinucleotide Phosphate (NADPH) and 125 μmol/l[^{3}H]-5-FU (25 μCi/ml). Aliquots of the reaction mixture were removed after 10, 20, and 30 min of incubation, and the reaction was stopped immediately by adding an equal volume of 5% $HClO_4$. The aliquots were diluted 1:2 with a mobile phase consisting of 20 mmol/l NaH_2PO_4 (pH 3.5) and centrifuged, and the supernatant obtained was analyzed according to the RI-HPLC conditions shown below. The reaction rate was obtained based on the relationship between reaction time and the concentrations of 5-FU and its metabolites, 5-fluorodihydrouracil (5-FDHU), 2-fluoro-β-ureidopropionate (FUPA), and α-fluoro-β-alanine (FBAL). From this reaction rate and the protein concentration determined separately, DPD activity (pmol/min per mg protein) was calculated.

HPLC conditions. The column was a YMC-Pack Pro C18 (AS-301-3, 4.6 × 100 mm; YMC, Kyoto, Japan) and it was at room temperature. The guard column was a Guard-Pak Puresil C_{18} (Waters, Milford, MA, USA). The mobile phase consisted of 20 mmol/l phosphate buffer (pH 3.5) and the Flow rate was 0.5 ml/min.

RI detection. The scintillation cocktail used was Pico Fluour40 (PerkinElmer Life Sciences, Boston, MA, USA). The scintillation flow rate was 3.0 ml/min, and the injection volume was 40 μl.

OPRT activity

OPRT enzyme activities in samples of the refrigerated tissue were measured by the paper disk method.[9] The tissue sample was homogenized in 50 mmol/l Tris-HCl (pH 7.5) containing 1.5 mmol/l $MgCl_2$ and 2 mmol/l dithiothreitol. After centrifugation (105000*g*, 1 h, 4°C), the supernatant was collected and incubated at 37°C with [^{3}H]-5FU as the substrate. Aliquots of the reaction mixture were removed after 5, 10, and 15 min of incubation, and the reaction was stopped immediately by placing them in a 100°C water bath. After centrifugation, the supernatant was placed on an ion exchange filter paper made from Diethylaminoethyl (DEAE)-cellulose, and washing operation was repeated to remove unreacted [^{3}H]-5FU. The filter paper was placed in a scintillation vial, followed by the addition of scintillation cocktail; the radioactivity of the [^{3}H]-fluorouridine monophosphate (FUMP) formed was quantified to determine the concentration of FUMP. The reaction rate was obtained based on the relationship between the reaction time and the concentration of the FUMP formed. From this reaction rate and the protein concentration determined separately, OPRT activity (pmol/min per mg protein) was calculated.

The enzyme activity assay was conducted at the Analysis Center, Pharmaceutical Research Institute, Kyowa Hakko Kogyo (Shizuoka, Japan).

Measurement of 5-FU sensitivity by the CD-DST method[5]

Tissue taken from the tumor site of the resected sample was aseptically cut into pieces and treated with the cell distribution enzyme for 1–2 h, and tumor cells were recovered. After a preliminary incubation of 24 h, the recovered tumor cells were mixed with collagen solution to a cell density of 1×10^5 cells/ml. Subsequently, three drops of the collagen-tumor cell mixture were put into a well and incubated for 1 h to prepare the collagen gel drop. Three milliliters of DF medium (Sanko-Junyaku, Tokyo, Japan), containing 10% fetal bovine serum, was superposed in each well, 5-FU was added, and the plate was cultured for 7 days. The contact condition of 5-FU was 20.0 mg/μl·3 h, which was about two fold higher than that in blood after a massive intermittent clinical injection (600 mg/m^2).[2] A proliferative rate of 0.8-fold or more in wells not containing anticancer agent was defined as the evaluable case. For the antitumor effect, the selection of cancer cell colonies was based only on the difference in proliferative form. The percent inhibition rate (IR) was expressed as $(C - T)/C \times 100$, where T is the value in the antitumor agent group and C is the value in the control group. An IR of 45% or more was defined as a case of high sensitivity. This sensitivity test was conducted at BML (Saitama, Japan).

Evaluation of cell proliferative activity with Ki-67[6]

Paraffin sections (4-μm thick) were prepared from tissue taken from the tumor site of the resected sample, and Ki-67 was stained with MIB-1 antibody by means of the labeled streptavidin-biotin (LSAB) method. Four to five randomly selected foci were observed microscopically, and the mean positive cell ratio of more than 100 tumor cells was calculated as the Ki-67 labeling index (Ki-67 LI). Because the value of the Ki-67 LI in normal deep crypt was 50 to 75, LI values of 75 or more and less than 75 were defined as the high-value and low-value groups, respectively.

Statistical analysis

The Mann-Whitney test was used to evaluate the relationship between each enzyme activity and clinical pathologic factors and the relationship between the Ki-67 LI and each enzyme activity or 5-FU sensitivity. To evaluate the relationship between each enzyme activity and 5-FU sensitivity, the level of each enzyme activity was divided into high-activity and low-activity groups, and differences were analyzed with the Pearson χ^2 statistic. The maximally selected χ^2 method of Miller and Siegmund[10] and Halpern[11] was used to determine a cutoff value for each enzyme activity. For each observed enzyme activity value, patients were classified as falling below or equal to that value, or above that value. The Pearson χ^2 test statistic was used to compare the IR of the two resulting groups of patients (below or equal to the value versus above the value). The enzyme activity value that yielded the largest χ^2 test statistic (the maximal χ^2 statistic) was selected as the optimal cutoff point.

Table 2. Enzyme activity assay results

		TS activity (pmol/min per mg protein)		DPD activity (pmol/min per mg protein)		OPRT activity (nmol/min per mg protein)	
Mean		7.72 ± 6.55		41.9 ± 31.6		0.387 ± 0.168	
Histologic type	Well	8.78 ± 9.37		35.2 ± 19.5		0.398 ± 0.142	
	Mod	7.45 ± 5.21	NS	42.3 ± 29.9	NS	0.418 ± 0.174	$P = 0.0279$
	Por	5.05 ± 1.93		145		0.208 ± 0.148	$P = 0.0299$
	Muc	8.65 ± 1.63		28.5 ± 0.71		0.244 ± 0.023	
Depth of tumor invasion	sm/mp	4.35 ± 2.16		77		0.389 ± 0.111	
	ss/a1	8.36 ± 7.53	NS	42.7 ± 34.4	NS	0.398 ± 0.180	NS
	se/a2	6.98 ± 2.91		35.2 ± 19.5		0.347 ± 0.137	
Vascular invasion	ly (−)	6.67 ± 3.53	NS	34.8 ± 10.6	NS	0.453 ± 0.148	NS
	ly (+)	7.88 ± 6.89		42.7 ± 33.1		0.380 ± 0.169	
	v (−)	7.71 ± 7.08	NS	42.2 ± 34.9	NS	0.400 ± 0.176	NS
	v (+)	7.76 ± 5.12		41.2 ± 24.5		0.350 ± 0.141	
Lymph node metastasis	n (−)	8.18 ± 8.27	NS	42.8 ± 31.7	NS	0.407 ± 0.168	NS
	n (+)	7.25 ± 4.19		40.7 ± 32.3		0.366 ± 0.168	
Clinical staging	I	4.20 ± 2.62		77		0.389 ± 0.111	
	II	8.82 ± 9.12		33.6 ± 20.0		0.403 ± 0.153	
	IIIa	6.62 ± 4.80	NS	45.8 ± 44.5	NS	0.380 ± 0.191	NS
	IIIb	7.78 ± 6.54		52.0 ± 24.7		0.291 ± 0.170	
	IV	8.11 ± 4.83		44.0 ± 37.1		0.411 ± 0.188	

TS, Thymidylate synthase; DPD, dihydropyrimidine dehydrogenase; OPRT, orotate phosphoribosyltransferase; NS, not significant

Results

Investigation of 5-FU metabolic enzyme activity

Table 2 shows the enzyme activity assay results. The range of TS activities was 0.3 to 37.5 pmol/min per mg protein (mean activity, 7.72 ± 6.55 pmol/min per mg protein). The range of DPD activities was 8.0 to 145 pmol/min per mg protein (mean activity, 41.9 ± 31.6 pmol/min per mg protein), and the range of OPRT activities was 0.072 to 0.777 nmol/min per mg protein (mean activity, 0.387 ± 0.168 nmol/min per mg protein).

TS and DPD demonstrated no clear relationship with the histologic type (Table 2). On the other hand, the OPRT activity level was lower in poorly differentiated adenocarcinoma and mucinous adenocarcinoma than in well-differentiated adenocarcinoma and moderately differentiated adenocarcinoma. With respect to depth of tumor invasion, vascular invasion, and lymph node metastasis, no clear relationship was found for any enzyme. For clinical staging also, no clear relationship was found for any enzyme.

Investigation of the 5-FU sensitivity test

The sensitivity test was conducted by the CD-DST method for 37 cases, and in 29 cases (78.4%) results were successful. The causes of failure included contamination (2 cases), small numbers of viable cells (1 case), and low growth rates (5 cases). The mean IR was 36.07% ± 13.89%, and 7 (24.1%) of the 29 cases were of high sensitivity (IR ≥45%).

Relationship of 5-FU sensitivity with activities of enzymes involved in 5-FU metabolism

Figure 1 shows the relationships of 5-FU sensitivity with enzymes involved in 5-FU metabolism. To investigate the influence of each enzyme activity on the sensitivity, the cutoff value of each enzyme was calculated with the maximal χ^2 statistic.

TS activity. The cutoff value calculated by the maximal χ^2 statistic was 7.3 pmol/min per mg protein, and high-sensitivity cases tended to be in the low-TS activity group ($P = 0.0571$; Fig. 1).

DPD activity. No significant relationship was found between DPD activity and 5-FU sensitivity. A peak χ^2 value was not shown by the maximal χ^2 statistic.

OPRT activity. The cutoff value determined by the maximal χ^2 statistic was 0.295 nmol/min per mg protein. No case of high sensitivity was found at OPRT activities of ≤0.295 nmol/min per mg protein ($P = 0.0302$; Fig. 1).

Investigation of cell proliferative activity with Ki-67

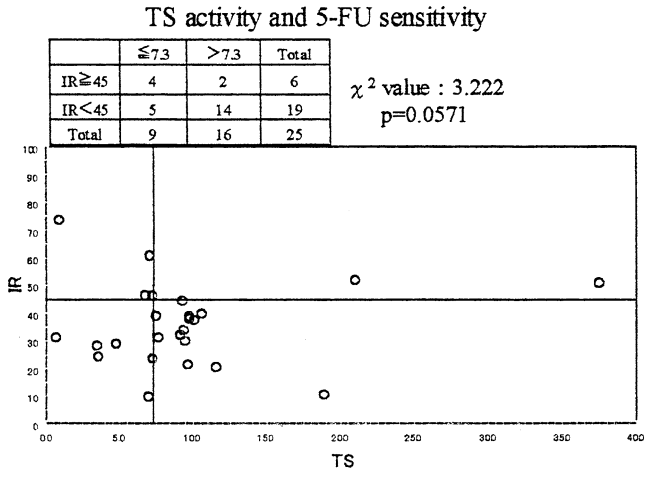
Figure 2 shows the relationship of cell proliferative activity with the 5-FU sensitivity test. IR was significantly higher in the high-LI group than in the low-LI group (39.0 ± 14.3 vs 28.4 ± 9.9; $P = 0.0429$).

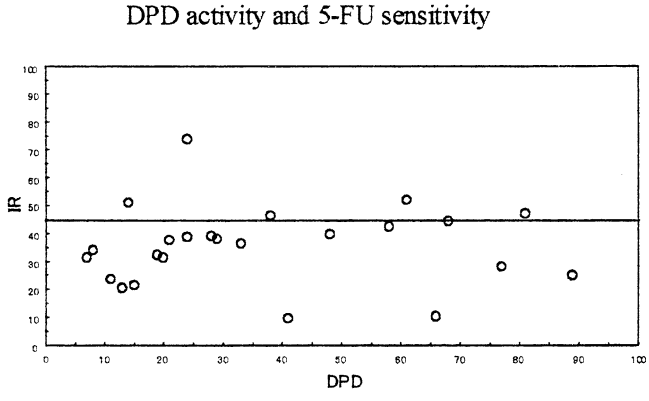
Relationship of cell proliferative activity with activities of enzymes involved in 5-FU metabolism

Table 3 shows the relationship of cell proliferative activity with the activities of enzymes involved in 5-FU metabolism.

TS activity. TS activity in the high-LI group was significantly higher than that in the low-LI group ($P = 0.0016$).

DPD activity. No difference was found between the high-LI and low-LI groups.





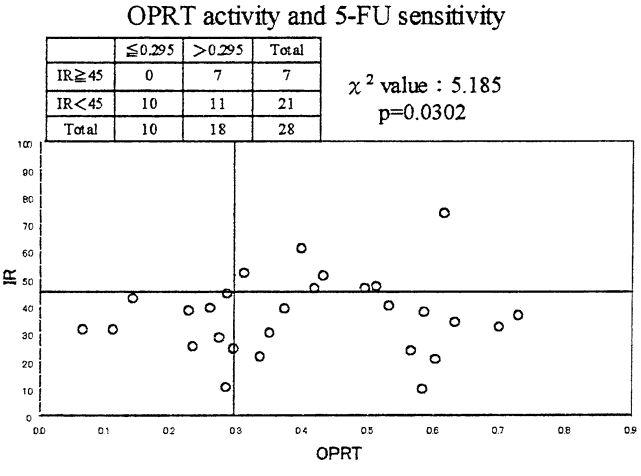


Fig. 1. Relationship of 5-fluorouracil (*5-FU*) sensitivity with enzymes involved in 5-FU metabolism. *IR*, Inhibition rate; *IR* ≧ *45*, high sensitivity; *IR* < *45*, low sensitivity. The cutoff value of thymidylate synthase (*TS*) activity calculated by the maximal χ^2 statistic was 7.3 pmol/min per mg protein. For dihydropyrimidine dehydrogenase (*DPD*) activity, a peak χ^2 value was not shown by the maximal χ^2 statistic. The cutoff value of orotate phosphoribosyltransferase (*OPRT*) activity determined by the maximal χ^2 statistic was 0.295 nmol/min per mg protein. The *tables* show the Pearson χ^2 test statistic of the two resulting groups of patients (below or equal to the value vs above the value)

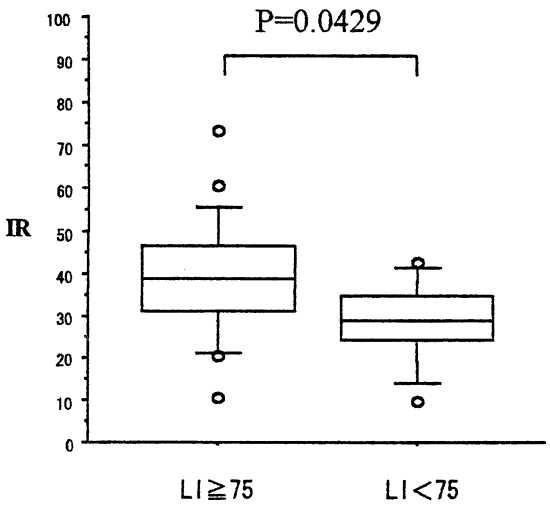


Fig. 2. Ki-67 labeling index (*LI*) and 5-FU sensitivity. *IR*, Inhibition rate; *LI* ≧ *75*, high-LI group; *LI* < *75*, low-LI group. IR was significantly higher in the high-LI group than in the low-LI group (39.0 ± 14.3 vs 28.4 ± 9.9; $P = 0.0429$)

Table 3. 5-Fluorouracil (FU) metabolic enzyme activities and cell proliferative activity

	LI < 75	LI ≧ 75	*P*
TS activity[a]	4.58 ± 2.04	9.66 ± 7.61	0.0016
DPD activity[b]	45.6 ± 35.8	36.0 ± 21.8	0.5714
OPRT activity[c]	0.323 ± 0.125	0.428 ± 0.170	0.0357

LI, Labeling index
[a] TS activity: pmol/min per mg protein
[b] DPD activity: pmol/min per mg protein
[c] OPRT activity: nmol/min per mg protein

OPRT activity. OPRT activity in the high-LI group was significantly higher than that in the low-LI group ($P = 0.0357$).

Discussion

5-FU is phosphorylated in cells and changed to an active metabolite that inhibits DNA synthesis and induces RNA dysfunction.[12] The mechanism of 5-FU action suggests that the antitumor effect could be determined by the levels of target enzyme TS and degradation enzyme DPD, and many data have been obtained to support this supposition.[13–15]

In many previous reports, TS protein levels were measured to assess the expression of TS, but more recently, TS enzyme activities have been measured. By employing enzyme assays using tritium labeling, the present investigation discovered the tendency for cases in the high-TS-activity group to have lower 5-FU sensitivities than those in the low-TS-activity group. Peters et al.[15] concluded that the duration of TS inhibition was important for determining the response to 5-FU, as well as for determining total TS activity and TS inhibition rate, and the expression of the *TS* gene and the TS protein should be measured directly, as other indices are.[15]

In a recent study, a correlation was found between TS immunostaining and the degree of expression of *TS* mRNA[16] and between the magnitude of *TS* mRNA expression and the level of the TS protein.[17] Most studies of advanced cancer have reported worse prognoses and decreased responses to chemotherapy with higher levels of TS expression.[16–21] However, there have also been a few reports that found no correlation between TS expression and the response rate.[22,23] In an Eastern Cooperative Oncology Group (ECOG) study, patients with a high degree of TS expression responded to 5-FU/leucovorin (LV) therapy.[24] In a study of adjuvant chemotherapy, the response to chemotherapy in patients with positive TS expression was clear, as it was in the ECOG study,[24] unlike the results in studies of advanced cancer.[25–27] Contradictory results have been reported in investigations of the relationship between TS expression and response. A variety of factors related to TS inhibition by 5-FU are being considered.

DPD, the inactivation enzyme of 5-FU, has drawn attention for its prediction of the side effects of 5-FU.[28] Because DPD activity levels in established cancer cells have been found to be inversely correlated with the cytotoxic effect of 5-FU,[29] the relationship between DPD activity levels in tumors and 5-FU's antitumor effect has commanded great interest. Ishikawa et al.[30] reported that 5-FU sensitivity was low at tumor sites with high DPD activity, and that it was high at tumor sites with low DPD activity. Uetake et al.[31] reported that DPD activity in tumors and *DPD* mRNA expression were positively correlated, and Ichikawa et al.[32] reported that *DPD* mRNA expression was high in tumors without 5-FU sensitivity. In addition, these investigators reported that the combined expression of *TS* and *DPD* mRNAs may more accurately predict the response to 5-FU.[32] In a recent follow-up to the study by Leichman et al.[19] that analyzed the expression of DPD and thymidine phosphorylase (TP), an assimilatory enzyme of 5-FU metabolism in addition to TS, the expressions of these three enzymes were low in patients responding to 5-FU, whereas at least one of these enzymes was highly expressed in nonresponding patients.[33]

Although TS, the enzyme targeted by 5-FU, and DPD, the rate-limiting enzyme, have been considered as independent factors, Hamaji and colleagues[34] have documented an inverse relationship between TS levels and the difference in DPD activity between colorectal cancer and the neighboring normal mucosa, and Johnston and colleagues[35] have reported that: "The down-regulation of DPD in tumor is in direct contrast with the over-expression of enzymes of the pyrimidine salvage pathway, which is observed in colorectal tumor compared with normal mucosa. "This may suggest a general mechanism by which pyrimidine nucleotide biosynthesis and degradation are coregulated to maintain a growth advantage in the tumor.[35]

In our investigation, no clear relationship was found between DPD activity and 5-FU sensitivity. Some cases demonstrated low 5-FU sensitivity despite having low DPD activity, so it is not clear whether only DPD activity could predict response to 5-FU treatment.

In the present investigation, the number of cases with high 5-FU sensitivity was significantly higher in the high-OPRT-activity group (OPRT activity >0.295 nmol/min per mg protein) than in the low-OPRT-activity group (OPRT activity ≤ 0.295 nmol/min per mg protein). Therefore, we suggest that OPRT activity level is one factor that can predict 5-FU sensitivity.

In addition, we evaluated cell proliferative activity with Ki-67, and the relationships of cell proliferative activity to the antitumor effect of 5-FU and to TS, DPD, and OPRT activities were investigated.

It is generally agreed that an anticancer agent is more effective against rapidly proliferating, highly malignant tumors, and in this investigation, the high-Ki-67 LI group showed a significantly higher sensitivity than the low-Ki-67 LI group. Because the number of cases of high sensitivity was higher in the low-TS-activity group, we suggest that TS activity is related to cell proliferative activity, i.e., cancer malignancy, and that OPRT activity is more intimately related to 5-FU sensitivity.

In recent years, chemotherapy for colorectal carcinoma has progressed to a new level because of the introduction of methods of anticancer agent administration that are based on biochemical modulation (BCM) therapies and the introduction of new anticancer agents, including irinotecan (CPT-11). Consequently, the options for treatment have increased. The choice of treatment depends on the particular cancer and the anticancer agent expected to have the highest therapeutic efficacy in light of the patient's sensitivity to the anticancer agent and the particular needs at the time. Therefore, the identification of factors that influence sensitivity will become more important as a substitute for the anticancer agent sensitivity test.

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