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ORIGINAL ARTICLE

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Dihydropyrimidine dehydrogenase and thymidylate synthase activities in hepatocellular carcinomas and in diseased livers

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Abstract *Background/purpose*: Dihydropyrimidine dehydrogenase (DPD) and thymidylate synthase (TS) are key enzymes for predicting the efficacy of 5-FU in the treatment of malignant tumors. However, 5-FU is not commonly commonly chosen for chemotherapeutic treatment of hepatocellular carcinoma (HCC) in practice. The aim of this study was to determine the activities of both DPD and TS in HCCs and corresponding liver parenchyma and to assess the correlation between the activities of these enzymes and clinicopathological features. The possibility of using 5-FU as a first-choice chemotherapeutic agent for HCC was also evaluated. *Methods*: The study material comprised 33 pairs of hepatocellular carcinoma and noncancerous liver samples. The DPD and TS activities were quantified by a radiometric enzymatic assay and a 5-fluoro-2′-deoxyuridine-5′- monophosphate (FdUMP) ligand-binding assay, respectively. *Results*: Pathologically invasive HCCs tended to show higher DPD activity and lower TS activity with some exceptions. DPD activity was lower in the HCCs regardless of their clinical features than in the noncancerous liver parenchyma, whereas TS activity was generally lower in HCCs except for those with certain clinical features. HCCs with multiple nodules showed lower DPD activity and those with a diameter of more than 5 cm showed lower TS activity. In the noncancerous liver parenchyma, a gradual decrease in DPD activity and an increase in TS activity were associated with the age of the patient, liver damage and z-factor. Of 30 HCC samples, 10 exhibited comparatively low DPD and TS activity, and these could be considered 5-FU-sensitive HCC. *Conclusions*: DPD and TS activity may be affected by the clinicopathological status in both the HCC and the corresponding liver parenchyma. However, further investigation is necessary. Some HCC patients may be good candidates for 5-FU-based chemotherapy based on measurements of tumor levels of DPD and TS.

Keywords 5-Fluorouracil · Dihydropyrimidine dehydrogenase · Thymidylate synthase · Hepatocellular carcinoma · Diseased liver

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Introduction

5-Fluorouracil (5-FU) has been used in practice for more than 30 years, ever since Heidelberger and colleagues reported its significance as an antitumor agent [17]. It is commonly used for the treatment of gastrointestinal, breast and head and neck cancers, with reasonable results. Recently, the cytotoxic mechanisms of 5-FU have been clarified and some initial enzymes, such as dihydropyrimidine dehydrogenase (DPD) and thymidylate synthase (TS), have been recognized as key enzymes in the metabolism of 5-FU. DPD is an initial and rate-limiting enzyme for the catabolism of 5-FU [1, 3, 5, 14, 34, 40]. On the other hand, TS is an essential enzyme for DNA synthesis, and is the target enzyme of 5-FU. 5-FU is converted into 5-fluoro-2′-deoxyuridine-5′-monophosphate (FdUMP), which forms a tight complex with TS in the presence of the folate cofactor 5,10-methylene tetrahydrofolate ($CH_2H_4PteGlu$). This complex blocks DNA synthesis because the consumption of free TS leads to inhibition of the conversion of uracil to thymidine. Previous experimental and clinical studies have shown that low TS activity in cells results in low DNA replication [10, 23, 38, 39, 41, 42, 44]. Thus, the activities of DPD and TS within neoplastic cells are a

crucial determinant of whether 5-FU will be an effective cytotoxic agent against that tumor [2, 11, 36, 37].

In chemotherapy for hepatocellular carcinoma (HCC), 5-FU is preferentially used as a major anticancer agent, without or combined with other chemotherapeutic drugs. However, a major proportion of HCC patients do not satisfactorily respond to this agent, although it is effective in the treatment of some HCC patients. Therefore, the prediction of 5-FU sensitivity in each case is important to avoid unnecessary administration. Nevertheless, no detailed studies have focused on both DPD and TS to forecast the therapeutic efficiency of 5-FU against HCC. Moreover, it is surmised that HCC patients undergoing 5-FU-based chemotherapy suffer from side effects, typically due to liver dysfunction, because most HCC patients have parenchyma tissue damage and the liver is the main site of DPD activity [18].

DPD and/or TS have been evaluated in a number of studies, but in only a few has the focus been on HCC and injured liver. DPD activity in the normal liver has been reported previously [4, 31, 32] and later studies have shown that DPD activity is lower in HCC than in normal liver [15, 21]. However, the opposite tendency has also been reported [19]. TS has also been quantified in a series of metastases of colorectal cancer [25, 43, 47], but never in relation to HCC or injured liver. Overall, the enzyme activity in HCC and injured liver remains poorly understood.

Although this study is an extension of previously reported work, it is the first study in which both DPD and TS have been fully investigated in the same specimens of injured liver and HCC. Furthermore, the correlation between DPD and TS activity and the clinicopathological features of HCC, and the noncancerous liver parenchyma are discussed, providing evidence to facilitate prediction of sensitivity to 5-FU in individual HCC patients.

Materials and methods

Materials

The study material comprised 33 pairs of hepatocellular carcinoma and noncancerous liver samples obtained from patients undergoing liver surgery at Kyoto University Hospital. Sample collection was performed according to the guidelines of protocols approved by the institutional review board. Informed consent was obtained from all subjects. No treatment was carried out prior to surgery. Samples of 50 to 400 mg in weight were collected from the specimen immediately after resection inside the operating room by a special assistant standing by, and were stored in a deep freezer (−80°C) without any delay. The nearby liver parenchyma was carefully excluded when collecting the liver cancer samples. If several nodules were included in the specimen, the main nodule, which represented the main tumor, was collected. The remaining specimen was sent to the Department of Pathology for routine pathological evaluation. The patients studied included 27 men and 6 women with a mean age of 63.2 years (range 33–84 years; Table 1).

Table 1 Patient profiles

No. of patients enrolled	33
Male	27
Female	6
Age (years)	
Mean	63.2
Range	33–84
Diagnosis	
HCC	30
Other	3
Hepatitis virus infection (HBV Ag/HCV Ab)	
+/−	7
−/+	19
+/+	1
−/−	6
TNM stage	
I	2
II	13
III	9
IVA	4
IVB	0

Clinicopathological variables

The clinicopathological variables were selected according to the general rules for the clinical and pathological study of primary liver cancers: liver damage, TNM stage, histological differentiation, growth pattern, capsule formation, capsule invasion, septum formation, serosal invasion, portal vein invasion, venous invasion, bile duct invasion and intrahepatic metastasis [29]. Other factors studied included patient age, gender, hepatitis virus, serum levels of alpha-fetoprotein (AFP) and protein induced by vitamin K absence (PIVKA) II, number of tumors, size (maximum diameter), parenchyma fibrosis (z0, no or mild fibrosis; z1, moderate fibrosis or chronic hepatitis; z2, severe fibrosis or cirrhosis), and primary or recurrence status. These variables were stratified as listed in Tables 2 and 3.

DPD radiometric enzymatic assay

The DPD enzymatic assay was based on the method described by Takechi et al. [45]. Briefly, tumor tissues were sonicated in three volumes of homogenization buffer. Each homogenate was centrifuged at 105,000 *g* for 1 h at 4°C, and the supernatant (cytosol fraction) was collected. After applying 100 µl of the sample to a gel column (MicroSpin G-25 column, Pharmacia Biotech, USA), it was centrifuged at 3000 *g* for 2 min. The enzyme reaction mixture contained 10 m*M* potassium phosphate buffer (pH 8.0), 0.5 m*M* EDTA, 0.5 m*M* β-ME, 2 m*M* DTT, 5 m*M* $MgCl_2$, 20 µ*M* [6-^{14}C]5-FU, 100 µ*M* NADPH and 25 µl of the cytosol fraction in a final volume of 50 µl. The mixture was incubated at 37°C for either 10 or 30 min. The DPD activity was determined by measuring the sum of the dihydrofluorouracil and 2-fluoro-β-alanine products formed from the [6-^{14}C]5-FU. Aliquots (5 µl) of the supernatant were applied to thin layered chromatography (TLC) plates (silica gel 60 F254; Merck, Darmstadt, Germany), which were developed with a mixture of ethanol and 1 *M* ammonium acetate (5:1, v/v). Each product was visualized and quantified using an image analyzer (BAS-2000; Fujix, Tokyo, Japan).

TS FdUMP ligand-binding assay

The tumors were homogenized with three volumes of 200 m*M* Tris-HCl (pH 8.0) containing 20 m*M* β-ME, 100 m*M* NaF, and 15 m*M*

Table 2 Association between DPD/TS activities and pathological features

Variable	DPD activity		TS activity	
	No. of cases ($n=28$)	Mean ± SD (pmol/mg/min protein)	No. of cases ($n=30$)	Mean ± SD (pmol/mg/protein)
Differentiation				
Well	11	356.7 ± 178.4	12	0.036 ± 0.020
Moderately	12	237.0 ± 283.3	12	0.034 ± 0.034
Poorly	5	266.8 ± 164.8	6	0.074 ± 0.100
Growth pattern				
Expansive	19	251.9 ± 213.1	20	0.048 ± 0.062
Infiltrative	9	368.5 ± 248.6	20	0.032 ± 0.010
Capsule formation				
Positive	24	257.1 ± 209.3	25	0.044 ± 0.056
Negative	4	483.2 ± 265.6	5	0.034 ± 0.004
Capsule invasion				
Positive	16	281.4 ± 213.9	17	0.035 ± 0.030
Negative	9	213.7 ± 192.0	9	0.062 ± 0.083
Septum formation				
Positive	18	300.1 ± 215.3	20	0.036 ± 0.029
Negative	10	270.1 ± 258.4	10	0.057 ± 0.079
Serosa invasion				
Positive	7	368.8 ± 282.6	7	0.048 ± 0.057
Negative	21	262.9 ± 206.9	23	0.025 ± 0.014
Portal invasion				
Positive	12	344.6 ± 284.2	13	0.027 ± 0.012
Negative	16	247.9 ± 171.7	17	0.055 ± 0.065
Venous invasion				
Positive	5	406.0 ± 150.6	6	0.027 ± 0.004
Negative	23	264.0 ± 197.5	24	0.047 ± 0.056
Bile duct invasion				
Positive	3	214.9 ± 264.0	3	0.020 ± 0.003
Negative	25	298.3 ± 227.0	27	0.045 ± 0.053
Intrahepatic metastasis				
Positive	15	241.8 ± 238.4	17	0.045 ± 0.062
Negative	13	344.3 ± 209.3	13	0.040 ± 0.033
Primary/recurrent				
Primary	23	280.9 ± 237.0	25	0.046 ± 0.055
Recurrent	5	328.4 ± 193.4	5	0.028 ± 0.003

CMP, and were centrifuged at 105,000 *g* for 60 min. The resultant supernatant was used for the determination of TS activity according to the method of Spears et al. [42] using [6-^{3}H]FdUMP as a substrate. Both the total TS and the free TS were quantified.

Statistical analysis

The data are expressed as means ± SD. The significance of differences between the groups was tested using the unpaired Mann-Whitney *U*-test or Student's unpaired *t*-test. *P* values less than 0.05 were considered to be statistically significant for all tests.

Results

Of the 33 samples, 3 were excluded from the analysis because the final pathological diagnosis was not HCC. As a result, 30 samples of HCC and 33 noncancerous liver tissue samples were studied (Table 1).

DPD activity

Despite the standardized method of sample collection, the DPD activity of the HCC and noncancerous liver parenchyma exhibited wide variability. It was measurable in all but one sample, and varied over a range of nearly 50-fold. There was a significant difference in DPD activity between the HCC and noncancerous liver parenchyma samples ($P < 0.05$). The mean ± SD DPD activity in the HCC samples was 310.7 ± 235.1 pmol/min per milligram protein, ranging from 43.8 to 2228.0 pmol/min, whereas that for the noncancerous liver parenchyma was 532.7 ± 198.6 pmol/min, ranging from 57.3 to 1154.6 pmol/min (Fig. 1). Moreover, for every clinical feature studied, the DPD activity in the HCC was less than in the corresponding noncancerous liver parenchyma.

With respect to the tumor tissues, the DPD activity exhibited a significant difference in relation to the number of tumors. The single-nodule group showed a

Table 3 Association between DPD/TS activities and clinical features

Variable	DPD activity (mean ± SD, pmol/mg/min protein)		TS activity (mean ± SD, pmol/mg/protein)	
	HCC ($n=28$)	Non-HCC ($n=33$)	HCC ($n=30$)	Non-HCC ($n=33$)
Virus marker (HBV Ag/HCV Ab)				
+/−	368.2 ± 280.8	602.0 ± 69.6	0.031 ± 0.010	0.031 ± 0.017
−/+	233.8 ± 179.3	515.1 ± 230.6	0.052 ± 0.064	0.069 ± 0.096
+/+	278.9	199.5	0.033	0.019
−/−	401.4 ± 323.4	563.1 ± 155.0	0.025 ± 0.013	0.026 ± 0.008
Age (years)				
< 60	261.7 ± 169.9	557.9 ± 99.4	0.039 ± 0.020	0.024 ± 0.008
60–69	347.4 ± 264.0	583.7 ± 230.7*	0.034 ± 0.030	0.053 ± 0.064
≥70	162.3 ± 99.8	402.3 ± 169.8*	0.066 ± 0.095	0.081 ± 0.123*
Gender				
Male	280.2 ± 223.8	537.0 ± 205.4	0.048 ± 0.056	0.056 ± 0.083
Female	322.8 ± 258.6	513.1 ± 180.4	0.022 ± 0.010	0.032 ± 0.016
AFP (ng/ml)				
< 200	274.5 ± 232.2	528.5 ± 206.6	0.046 ± 0.056	0.058 ± 0.084
≥200	357.7 ± 212.4	548.3 ± 179.7	0.028 ± 0.010	0.027 ± 0.011
PIVKA-II (mAU/ml)				
< 40	292.6 ± 136.7	489.5 ± 165.8	0.043 ± 0.014	0.050 ± 0.081
≥40	280.5 ± 262.7	557.1 ± 215.2	0.042 ± 0.061	0.051 ± 0.076
Liver damage				
I	320.0 ± 234.5	572.7 ± 172.0	0.035 ± 0.026	0.033 ± 0.019
II	177.1 ± 169.7	352.8 ± 226.5*	0.074 ± 0.102	0.137 ± 0.154*
No. of nodules				
1	365.6 ± 239.2	573.4 ± 134.5	0.038 ± 0.030	0.043 ± 0.060
2	115.6 ± 96.2*	499.5 ± 425.4	0.030 ± 0.023	0.036 ± 0.028
≥3	239.2 ± 199.1	475.9 ± 134.8	0.058 ± 0.083	0.075 ± 0.110
Diameter (cm)				
< 5	244.5 ± 161.5	519.2 ± 143.1	0.055 ± 0.062	0.068 ± 0.096
≥5	373.3 ± 291.8	504.6 ± 202.8	0.026 ± 0.014*	0.029 ± 0.016
z-factor				
0	442.9 ± 276.9	573.2 ± 144.1	0.028 ± 0.015	0.022 ± 0.007
1	265.2 ± 217.0	530.3 ± 205.9	0.050 ± 0.062	0.057 ± 0.079*
2	229.8 ± 192.5	493.4 ± 249.8	0.032 ± 0.021	0.068 ± 0.103
TNM stage				
I	364.3 ± 26.2	575.8 ± 94.0	0.027 ± 0.001	0.025 ± 0.010
II	366.8 ± 262.4	545.0 ± 172.6	0.058 ± 0.073	0.079 ± 0.114
III	201.9 ± 147.3	503.9 ± 291.3	0.034 ± 0.020	0.038 ± 0.027
IVA	196.9 ± 259.4	513.5 ± 136.2	0.026 ± 0.010	0.035 ± 0.013

*$P < 0.05$, vs the first value of that group of values

DPD activity of 365.6 ± 239.2 pmol/min per milligram protein, whereas the activity in the multiple-nodule group was 115.6 ± 96.2 pmol/min ($P = 0.037$). Other pathological variables did not show a definite correlation (Table 2). In TNM stages I and II, the DPD activity was 366.5 ± 243.0 pmol/min, whereas in stages III and IVA, the activity was 200.4 ± 176.9 pmol/min. These differences were not statistically significant, but suggested that the advanced tumors had lower DPD activity.

On the other hand, the DPD activity in the noncancerous liver parenchyma varied in relation to the degree of liver damage, as defined by the Liver Cancer Study Group of Japan [29]. Liver damage was classified into three groups A, B and C, which are nearly identical to the Child-Pugh classification groups. The DPD activity in liver damage class A (572.7 ± 172.0 pmol/min per milligram protein) was significantly higher than that in class B (352.8 ± 226.5 pmol/min per milligram protein; $P = 0.012$). The histological liver damage was classified into three groups: z0, z1 and z2. The DPD activity decreased as the z-factor increased, although no statistical significance was observed (z0, 573.2 ± 144.1; z1, 530.3 ± 205.9; z2, 493.4 ± 249.8 pmol/min per milligram protein). In addition, the liver parenchyma samples from patients positive for hepatitis B virus surface antigen (HBs Ag) tended to show higher DPD activity than those from patients positive for hepatitis C virus antibody (HCV Ab): HBs Ag-positive, 602.0 ± 69.6; HCV Ab-positive, 515.1 ± 230.6 pmol/min per milligram protein). Age was also a significant variable. The DPD activities in the age groups 50–59, 60–69 and 70–79 years

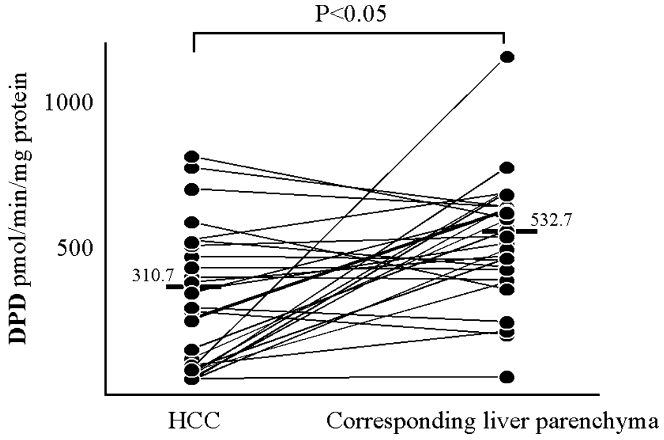

Fig. 1 DPD activities in the HCCs and the corresponding noncancerous liver samples. Values are means ± SD for all samples. The significances of the differences between the groups were determined using the Mann-Whitney *U*-test

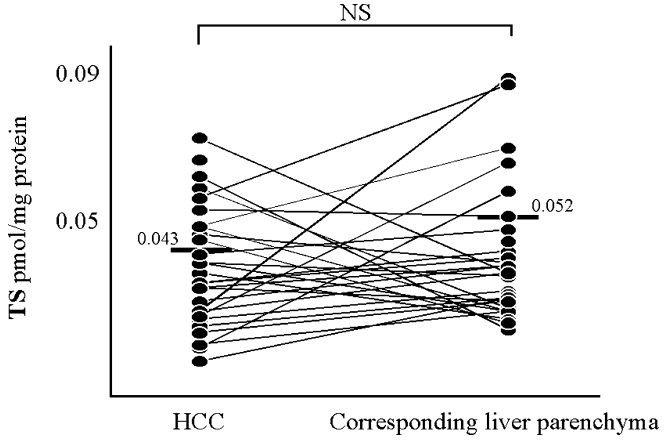

Fig. 2 TS activities in the HCCs and the corresponding noncancerous liver samples. Values are means ± SD for all samples. The significances of the differences between the groups were determined using the Mann-Whitney *U*-test (*NS* not significant)

were 261.7 ± 169.9, 347.4 ± 264.0 and 162.3 ± 99.8 pmol/min per milligram protein, respectively. There were statistically significant differences in DPD activities between the 70–79-year and the 50–59-year age groups, and between the 70–79-year and the 60–69-year age groups, with *P* values of 0.043 and 0.033, respectively. The various clinicopathological variables and the corresponding DPD activities are shown in Table 3.

Of the 30 HCCs studied, 10 exhibited a DPD activity of less than 100 pmol/min per milligram protein, which is comparable to the enzyme activity of 5-FU-responsive tumors.

TS activity

The TS activity was 0.043 ± 0.049 pmol/mg protein in the HCC, and 0.052 ± 0.075 pmol/mg protein in the noncancerous liver parenchyma (Fig. 2). Among the various clinicopathological features of HCC, only the tumor size was significantly related to TS activity. Tumors smaller than 5 cm in diameter had significantly higher TS activity (0.055 ± 0.062 pmol/mg protein) than tumors over 5 cm in diameter (0.026 ± 0.014 pmol/mg protein). None of the other pathological variables in HCCs showed a definite correlation with the TS activity. However, the TS activity tended to be lower when the tumor showed pathological invasive potential: infiltrative growth, 0.032 ± 0.010 pmol/mg protein; expansive growth, 0.048 ± 0.062 pmol/mg protein; capsule invasion (+), 0.035 ± 0.030 pmol/mg protein; capsule invasion (−), 0.062 ± 0.083 pmol/mg protein; portal vein invasion (+), 0.027 ± 0.012 pmol/mg protein; portal vein invasion (−), 0.055 ± 0.065 pmol/mg protein; hepatic vein invasion (+), 0.027 ± 0.004 pmol/mg protein; hepatic vein invasion (−), 0.047 ± 0.056 pmol/mg protein; bile duct invasion (+), 0.020 ± 0.003 pmol/mg protein; bile duct invasion (−), 0.045 ± 0.053 pmol/mg protein (see Table 2).

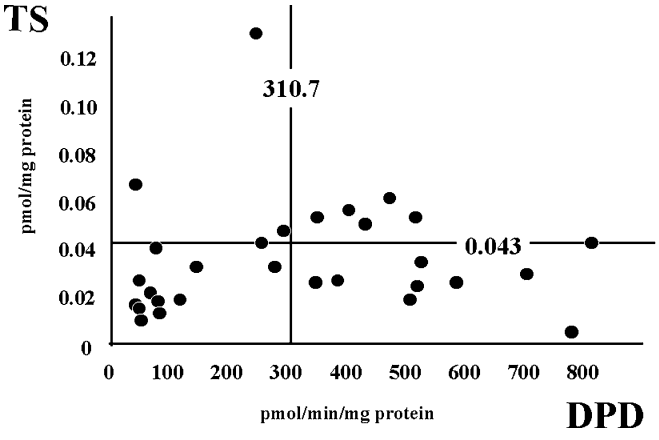

Fig. 3 Distribution of the HCC samples in relation to their DPD/TS activities. The *straight lines* show the average DPD and TS activities in the HCCs. One-third of the investigated HCCs had low DPD activity nearly equal to that of other cancers considered to be sensitive to 5-FU

On the other hand, the TS activity in the noncancerous liver parenchyma varied in relation to the liver damage. The TS activity with liver damage class A (0.033 ± 0.019 pmol/mg protein) was significantly lower than with liver damage class B (0.137 ± 0.153 pmol/mg protein; $P = 0.028$). The z-factor also showed a significant relationship (z0, 0.022 ± 0.007 pmol/mg protein; z1, 0.057 ± 0.079 pmol/mg protein; z2, 0.068 ± 0.103 pmol/mg protein; $P = 0.045$). Although not significant, samples from HBs Ag-positive patients tended to show lower TS activity than those from HCV Ab-positive patients in the liver parenchyma (HBs Ag-positive, 0.031 ± 0.017 pmol/mg protein; HCV Ab-positive, 0.069 ± 0.096 pmol/mg protein). Age also turned out to be a significant variable for TS activity. The TS activities were 0.081 ± 0.123 pmol/mg protein and 0.024 ± 0.008 pmol/mg protein in the 70–79-year and 50–60-year age

groups, respectively ($P = 0.043$), and 0.053 ± 0.064 pmol/mg protein in the 60–69-year age group. The other variables and TS activities are summarized in Table 3.

Every HCC sample with a DPD activity of less than 100 pmol/min per milligram protein exhibited a lower than average TS activity (0.043 pmol/mg protein; Fig. 3).

Discussion

Quantitating DPD and TS activity in HCC is of clinical interest because, although 5-FU may not be the first treatment of choice in HCC, some patients respond quite well to 5-FU-based chemotherapy. Moreover, since the liver is the tissue with the highest DPD content, along with peripheral blood mononuclear cells [12, 18, 30, 31, 32], the degradation rate of 5-FU should decrease when the parenchyma is injured. It is therefore relevant to understand the degradation ability of 5-FU when chronic liver disease coexists.

Our study is the first in which the activities of both DPD and TS in the same specimens of HCC and corresponding liver parenchyma have been determined. By comparing the enzyme activities with various clinicopathological variables, we aimed to find suggestive associations between these variables and the enzyme levels. Furthermore, we intended to clarify whether HCC patients with liver damage would benefit from the administration of 5-FU-based chemotherapy and whether the side effects could be avoided. Patients with DPD deficiency, which leads to severe life-threatening toxicity, have recently been reported [7, 16, 22, 46].

The methods used for the determination of the enzyme activities studied here are established techniques. Enzyme activity may be affected by preoperative chemotherapy, and hence all patients in this study received no chemotherapy prior to their surgery. Recently, the development of sensitive, reverse transcription polymerase chain reaction (RT-PCR) methods has permitted the quantification of messenger RNA (mRNA) expression in small tumor biopsy samples, and previous reports have indicated the significance of both DPD and TS mRNA quantification [4, 20, 24, 26, 27, 28, 35]. However, mRNA levels do not represent the actual activity of the enzyme. Although a correlation between mRNA levels and enzyme activity has been demonstrated in several studies, we would like to emphasize that the quantification of the active DPD and TS enzymes may provide a clearer indication for evaluating the efficacy of 5-FU-based chemotherapy. However, the biological characteristics of cells within cancer tissue are not always homogeneous in their spatial distribution. The method used here as well as RT-PCR should overcome this important issue to obtain reliable results. To resolve this problem, the cancer tissues were collected from various lesions when a heterogeneous composition was evident macroscopically.

Although the results of this study were based on a relatively small number of patients and are thus preliminary in nature, some statistically significant correlations were observed. However, they tended to lack definitive power due to the large dispersion of paired values. Nonetheless, some are thought-provoking and raise several interesting points.

Our results are in good agreement with those previously reported by Jiang et al. [21] and Guimbaud et al. [15], showing lower levels of DPD activity in HCC compared to corresponding liver parenchyma. This is consistent with reports of studies involving head and neck cancers [8, 13] and colorectal cancers [19, 33], indicating that DPD activity is lower in the tumors than in the surrounding normal tissue. Interestingly, Ikeguchi et al. observed opposite results, with higher DPD activity in HCC than in liver parenchyma [19]. Since the patient population and methodology used in the study by Ikeguchi et al. were similar, further studies are required to explain this discrepancy.

Our observation of higher DPD activities in the HCCs (310.7 ± 235.1 pmol/min per milligram protein) than in so-called 5-FU-sensitive tumors such as head and neck and colorectal cancers may explain the low response rate to 5-FU-based chemotherapy in HCC patients. Nevertheless, in the present study, the samples from10 out of 30 patients exhibited a DPD activity of less than 100 pmol/min per milligram protein, which is comparable to the enzyme activity of 5-FU-responsive tumors. Moreover, these samples exhibited lower than average TS activity. It is, therefore, conceivable that some selected HCC patients would be good candidates for 5-FU-based chemotherapy (Fig. 3). We have not had a chance to evaluate 5-FU efficacy in those HCC patients whose DPD and TS activities have been quantified. Nevertheless, we are now trying to increase the size of the patient study group to determine whether 5-FU-based chemotherapy would be beneficial in certain HCC patients.

Although DPD activity in normal liver has been previously reported by Chazal et al. [4] and Lu et al. [31, 32], we are the first to report evidence indicating that the DPD activity gradually decreases as liver function deteriorates, as evidenced by the correlation between DPD activity and the liver damage score and z-factor. This observation seems reasonable, because enzyme activity is presumably related to liver function. Accordingly, the efficiency of 5-FU chemotherapy may be affected by liver damage. A decrease in the degradation of 5-FU would increase the serum concentration of 5-FU to levels greater than expected. Therefore, we should recognize that toxic side effects may be more likely to appear in those patients with chronic liver disease. Other covariables influencing 5-FU clearance have been reported by Etienne et al. [9].

Our findings showed that invasive, aggressive HCCs tended to express lower TS activity than noninvasive HCCs. This suggests that the cell cycle in these aggressive HCCs turns over rapidly, which results in very fast

TS consumption owing to rapid DNA replication, thus resulting in low TS activity. However, this observation is not in accordance with that of Cummins et al., who found that TS activity is increased in rapidly growing hepatomas in the rat and cell lines [6]. Our study, however, is the first in which TS activity has been investigated in human liver cancers, and this activity may differ from that in rat hepatomas and cell lines.

Van der Wilt et al. [47], Spears et al. [43] and Larsson et al. [25] have previously reported the TS activity in normal liver. However, the present study is the first in which the TS activity in injured liver has been investigated. It is therefore difficult to compare the results of the present study with those of previous studies. Nonetheless, we obtained interesting and suggestive evidence. As the liver damage and z-factor increased, the TS activity rose, with the difference being significant. The precise mechanism of this increase is still unknown, but we can assume that the higher TS activity was associated with injured liver, in which DNA synthase activity is probably enhanced.

In conclusion, HCCs showed relatively low DPD activity compared to the surrounding noncancerous liver parenchyma. Furthermore, HCCs with invasive characteristics showed lower TS activity than less-invasive HCCs. These are, in part, favorable findings for 5-FU-based chemotherapy. At the same time, the present study made clear that the DPD activity in HCCs is typically higher than that in tumors known to be 5-FU-sensitive, which may be a reason for the 5-FU resistance seen in many HCCs. However, we showed that HCCs have a wide range of DPD and TS activities, which implies the necessity for individual evaluation in the assessment of drug sensitivity. Furthermore, the present study clearly showed that the hepatic parenchyma of injured liver exhibited lower DPD activity than that of undamaged tissue. Low DPD activity in the liver parenchyma suggests a greater chance of toxic side effects, so the dosage of 5-FU may need to be limited in patients with liver injury much more than in patients without.

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