The relationship between survival and the expression of dihydropyrimidine dehydrogenase in patients with colorectal cancer

TSUKASA HOTTA[1], KATSUNARI TAKIFUJI[1], KATSUTOSHI TANIGUCHI[2], MASAKI SAHARA[2], SHOZO YOKOYAMA[1], KENJI MATSUDA[1], TAKASHI HIGASHIGUCHI[1], TOSHIJI TOMINAGA[1], YOSHIMASA OKU[1] and HIROKI YAMAUE[1]

[1]Second Department of Surgery, Wakayama Medical University, School of Medicine; [2]Department of Surgery, Wakayama Rosai Hospital, Wakayama, Japan

Received February 6, 2006; Accepted March 30, 2006

Abstract. Dihydropyrimidine dehydrogenase (DPD) is considered to be a key enzyme affecting the prognosis for patients with colorectal cancer. We investigated whether a correlation exists between the expression of DPD and survival in patients with colorectal cancer. The present study was designed to quantify the DPD level using an enzyme-linked immunosorbent assay in tumors and normal tissue specimens obtained from 22 colorectal cancer patients. There were no significant differences in the preoperative features, neither in the intra- and post-operative findings of patients between the high DPD and low DPD groups in tumor tissue. In patients showing an expression of DPD in tumor tissue, the overall survival in the low DPD group tended to be longer than that in the high DPD group (P=0.076). In contrast, in patients showing an expression of DPD in normal tissue, no significant difference was observed in the overall survival between the high DPD and low DPD groups (P=0.358). In patients showing an expression of DPD in tumor tissue, the disease-free survival in the low DPD group was longer than that in the high DPD group (P=0.046), whereas in patients showing an expression of DPD in normal tissue, no significant difference was seen in the disease-free survival between the high DPD and low DPD groups (P=0.473). There tended to be a correlation between the DPD expression in tumor tissue and that in adjacent normal tissue (R=0.390, P=0.073). Based on these findings, we demonstrated the importance of DPD expression in tumor tissue as a prognostic factor in patients with colorectal cancer.

Correspondence to: Dr Hiroki Yamaue, Second Department of Surgery, Wakayama Medical University, School of Medicine, 811-1 Kimiidera, Wakayama 641-8510, Japan
E-mail: yamaue-h@wakayama-med.ac.jp

Key words: dihydropyrimidine dehydrogenase, colorectal cancer

Introduction

Anticancer combination chemotherapy using 5-fluorouracil (5-FU) and its analogue is considered to be a standard regimen for advanced gastrointestinal cancers, including colorectal cancer (1-3).

Dihydropyrimidine dehydrogenase (DPD) is considered to catalyze 5-FU to inactive molecules (2,4,5). Previous studies have demonstrated significant variations in the DPD levels in tumors from different human tumor cell lines, and clinical samples, and an elevated intratumoral DPD activity has been implicated in a low antitumor activity of 5-FU due to increased 5-FU activation (1-3,5-13). DPD inhibitors have been demonstrated to enhance the 5-FU antitumor activity through the inhibition of tumoral DPD activity in human tumor cell lines (6). Therefore, DPD is considered to be a key enzyme in overcoming refractory tumors, such as advanced colorectal cancer.

In previous studies, we also demonstrated the variations of DPD expression in gastric cancer, colorectal cancer, metastatic liver cancer, and hepatocellular carcinoma (14-16). In this study, we clarified the relationship between the DPD expression in both tumor and adjacent normal tissue and the survival in patients with colorectal cancer, while also demonstrating the importance of DPD expression in tumor tissue as a prognostic factor in patients with colorectal cancer.

Materials and methods

Patients. Twenty-two patients with colorectal cancer who underwent surgery at Wakayama Rosai Hospital, an affiliated institution of Wakayama Medical University Hospital, were enrolled in this study between 2001 and 2006. The data on these patients was retrospectively collected and all patients were followed-up from 2001 through 2006 in this study.

The ages of the patients ranged from 49 to 85 (mean ± standard deviation: 66±10) years. The female to male ratios of the patients were 7:15. The clinical stages according to the classification of malignant tumors by UICC (17) were: 6 with Stage II, 12 with Stage III, and 4 with Stage IV.

Table I. Preoperative features of patients.

Variables	Low DPD group (n=11)	High DPD group (n=11)	P-value
Age (years)	63±9	69±11	0.194
Gender (n)			>0.999
Male	8	7	
Female	3	4	
Liver cirrhosis (n)			0.476
+	0	2	
-	11	9	
Diabetes mellitus (n)			>0.999
+	2	2	
-	9	9	
Fasting blood glucose (mg/dl)	99±22	98±16	0.851
Total protein (g/dl)	6.7±0.5	6.9±0.8	0.618
Albumin (g/dl)	3.8±0.3	3.7±0.4	0.555
Cholinesterase (U/l)	144±37	125±36	0.250
Total bilirubin (mg/dl)	0.6±0.2	0.6±0.4	0.771
Aspartate aminotransferase (U/l)	23±7	32±21	0.237
Alanine aminotransferase (U/l)	17±6	27±21	0.125
Lactate dehydrogenase (U/l)	186±39	204±54	0.362
Alkaline phosphatase (U/l)	236±71	257±79	0.516
γ-glutamyltranspeptidase (U/l)	28±14	34±29	0.538
Triglyceride (mg/dl)	113±115	94±37	0.605
Total choresterol (mg/dl)	191±28	179±56	0.549
Blood urea nitrogen (mg/dl)	13±3	13±4	0.919
Creatinine (mg/dl)	0.8±0.2	0.8±0.2	0.904
Amylase (IU/l)	107±31	91±14	0.129
White blood cell count (/μl)	5900±1500	6400±1800	0.535
Red blood cell count (10^4/μl)	412±52	392±57	0.409
Hemoglobin (g/dl)	12.1±1.8	11.4±2	0.433
Hematocrit (%)	36±4	34±6	0.405
Platelet (10^4/μl)	29.9±12.7	25.8±6.8	0.358
Serum CEA (ng/ml)	22.6±41.4	8.7±6.6	0.284
Serum CA19-9 (U/ml)	20±31.1	37.9±66.9	0.434

Data are expressed as the mean ± standard deviation.

A resection of the primary tumor in the rectum and colon was performed with a lymphadenectomy from along the rectal or large intestinal wall to around the main feeding artery. A primary liver resection was performed when metastases were recognized in the liver during the resection of the primary tumor.

Three courses of *l*-leucovorin (LV; folinic acid, citrovorum factor)/5-fluorouracil (5-FU) adjuvant chemotherapy were performed (18). One course of *l*-LV/5-FU chemotherapy consisted of weekly administration of *l*-LV and 5-FU for 6 weeks, at 2-week intervals. During the chemotherapy, *l*-LV (Wyeth Co., Tokyo, Japan) 250 mg/m^2 was drip intravenous infused for two hours and 5-FU (Kyowa Hakko, Tokyo, Japan) 600 mg/m^2 was intravenously administered one hour after the start of the *l*-LV administration. Fifteen patients agreed to undergo adjuvant chemotherapy, but 7 patients declined due to the high medical cost, or based on either their own or their family's wishes.

Tumor specimens were obtained for diagnosis or therapeutic indications, and informed consent was obtained from each patient for the use of such samples to measure the levels of dihydropyrimidine dehydrogenase (DPD).

We evaluated the following points: i) DPD expression and the preoperative features, ii) DPD expression and the intra- and post-operative findings, iii) DPD expression and the overall survival, iv) DPD expression and the disease-free

survival, and v) any correlations between the DPD expression in tumor tissue and adjacent normal tissue.

Sample preparation, and expression of DPD. Freshly excised tumor tissue specimens were processed and the DPD level was quantified with an enzyme-linked immunosorbent assay (ELISA) as previously described (4,5,7-9,14-16), in collaboration with the Nippon Roche Research Center (Kanagawa, Japan).

Each sample was homogenized in a 10-fold volume of 10 mM Tris-HCl buffer (pH 7.4) containing 15 mM NaCl, 1.5 mM $MgCl_2$, and 50 μM potassium phosphate, and then centrifuged at 10,000 x g for 15 min. The supernatant was stored at -80˚C until use. The protein concentration of the supernatant extracted from the tumor tissue specimens was determined using a DC protein assay kit (Bio-Rad Laboratories, Hercules, CA). The supernatant was dispensed onto microplates, coated with 10 μg/ml of an anti-DPD MoAb 4B9, and incubated at 37˚C for 1.5 h. As the standard solution for DPD, serially diluted HT-3 tumor homogenate was used. After washing, the plates were incubated at 37˚C for 1 h with 1 μg/ml of secondary antibody, anti-DPD MoAb 3A5. The plates were then further reacted with peroxidase-conjugated anti-mouse IgG antiserum and substrate solution.

The DPD levels in these samples obtained by ELISA were expressed as U/mg protein, with 1 U equivalent to the amount of DPD protein that catabolizes 1 pmole of 5-FU per min.

Statistical analysis. A statistical analysis was performed with the Stat View-J software package version 5.0 using the Windows XP operating system. Significant differences in the preoperative features of the patients and the intra- and post-operative findings were determined by Student's t-test, and either the Chi-square test or Fisher's exact test. The overall survival rate and disease-free survival rate were estimated by the Kaplan-Meier method, and a univariate analysis of significance for each factor was evaluated by the log-rank test. Any correlation between DPD expression in tumor tissue and in the adjacent normal tissue was evaluated using Pearson's correlation coefficient. A p-value of <0.05 was considered to be statistically significant.

Results

Preoperative features of patients. The median value of the DPD expression in the tumor tissue was 36.8 U/mg protein (range: 10.5-79.9 U/mg protein). The median value of 36.8 U/mg protein for DPD expression in the tumor tissue was selected for a cutoff value separating a high and low DPD level in the tumor tissue.

There were no significant differences in the preoperative features of the patients between the high DPD and low DPD groups based on the tumor tissue specimens (Table I).

Intra- and post-operative findings. There were no significant differences in the intra- and post-operative findings between the high DPD and low DPD groups measured in the tumor tissue (Table II).

Overall survival and DPD expression. In patients with a DPD expression measured in the tumor tissue, the overall survival in the low DPD group tended to be longer than that in the high DPD group (P=0.076) (Fig. 1A).

The median value of the DPD expression in the adjacent normal tissue was 48.8 U/mg protein (range: 23.4-91.2 U/mg protein). The median value of 48.8 U/mg protein for DPD expression in the normal tissue was selected for a cutoff value separating the high and low DPD levels in the normal tissue.

In patients showing an expression of DPD in normal tissue, no significant difference was seen in the overall survival between the high DPD and low DPD groups (P=0.358) (Fig. 1B).

Disease-free survival and DPD expression. In patients showing an expression of DPD in tumor tissue, the disease-free survival in the low DPD group was longer than that in the high DPD group (P=0.046) (Fig. 2A).

In patients showing an expression of DPD in normal tissue, no significant difference was observed in the disease-free survival between the high DPD and low DPD groups (P=0.473) (Fig. 2B).

Correlation between the DPD expression in tumor tissue and that in adjacent normal tissue. There tended to be a correlation between the DPD expression in tumor tissue and that in adjacent normal tissue (R=0.390, P=0.073) (Fig. 3).

Discussion

Advanced gastrointestinal cancers, including colorectal cancer, are considered to be tumors that tend to be refractory to treatment by anticancer chemotherapy (1,2). Chemotherapy for colorectal cancer relies mainly on 5-FU and its analogue (1-3).

Dihydropyrimidine dehydrogenase (DPD), which is an initial and rate-limiting catabolic enzyme of 5-FU, has been reported to play an important role in the pharmacokinetics of 5-FU, and it has also been shown to correlate with the anti-tumor effectiveness of 5-FU in both cancer cell lines and tumors (19). A high expression level of DPD in tumor tissue is reported to result in a poor response to anticancer chemotherapy with 5-FU (6,7,20), whereas either an absence of or a low level of DPD results in hyper-uracilnemia and increased 5-FU levels in the plasma, thus causing severe toxicity.

DPD has been reported to be highly expressed in cancer in the following order: cervical, hepatic, pancreatic, esophageal, breast, gastric, prostate, bladder, renal, and colorectal cancers (7). In our previous studies, we also demonstrated variations in the expression of DPD in gastric cancer, colorectal cancer, metastatic liver cancer, and hepatocellular carcinoma (14-16). The DPD levels in liver metastasis or its adjacent liver tissues were higher than those in primary cancer or its adjacent tissue specimens (15). Furthermore, we previously proved a correlation between the DPD expression in preoperative specimens by endoscopy and in surgical specimens obtained from patients with colorectal cancer (16). In this study, we clarified the relationship between the DPD expression in both tumor and the adjacent normal tissue specimens and the survival in patients with colorectal cancer, thereby demonstrating the importance of

Table II. Intra- and post-operative findings.

Variables	Low DPDgroup (n=11)	High DPD group (n=11)	P-value
Primary lesion (n)			0.395
Colon	4	7	
Rectum	7	4	
Macroscopic type (n)			0.589
Type 1	1	0	
Type 2	8	9	
Type 3	2	2	
Maximum size of tumor (mm)	46.8±14.9	56.0±17.3	0.207
Histopathological type (n)			>0.999
Well differentiated	8	8	
Moderately differentiated	3	3	
Depth of invasion (n)			0.815
ss	7	6	
se	3	3	
si	1	2	
Lymph node metastasis (n)			0.231
N0	4	2	
N1	4	2	
N2	3	7	
Lymphatic invasion (n)			0.149
ly 0	1	0	
ly 1	3	3	
ly 2	4	8	
ly 3	3	0	
Venous invasion (n)			0.815
v 0	1	1	
v 1	5	4	
v 2	3	5	
v 3	2	1	
Liver metastasis (n)			>0.999
+	2	2	
-	9	9	
TNM stage (n)			0.607
II	4	2	
III	5	7	
IV	2	2	
Administration of anticancer agent (n)			0.362
+	9	6	
-	2	5	
Operation time (min)	192±103	174±37	0.599
Operative blood loss (ml)	300±374	210±174	0.480
Hospital stay (days)	36±25	35±14	0.869

Data are expressed as the mean ± standard deviation. Macroscopic Type 1, protuberant type; Type 2, ulcerated type with clear margin; Type 3, ulcerated type with infiltration; depth of invasion ss, tumor invasion of subserosa in the intestine with serosa or tumor invasion through muscularis propria into non-peritonealized part in the intestine without serosa; se, tumor invasion of serosa in the intestine with serosa or tumor invasion of non-peritonealized, pericolic, or perirectal tissue in the intestine without serosa; si, direct tumor invasion of other organs or structures; N0, no regional lymph node metastasis; N1, metastasis in 1 to 3 regional lymph nodes; N2, metastasis in 4 or more regional lymph nodes; ly 0, no lymphatic invasion; ly 1, minimal lymphatic invasion; ly 2, moderate lymphatic invasion; ly 3, severe lymphatic invasion; v 0, no venous invasion; v 1, minimal venous invasion; v 2, moderate venous invasion; v 3, severe venous invasion.

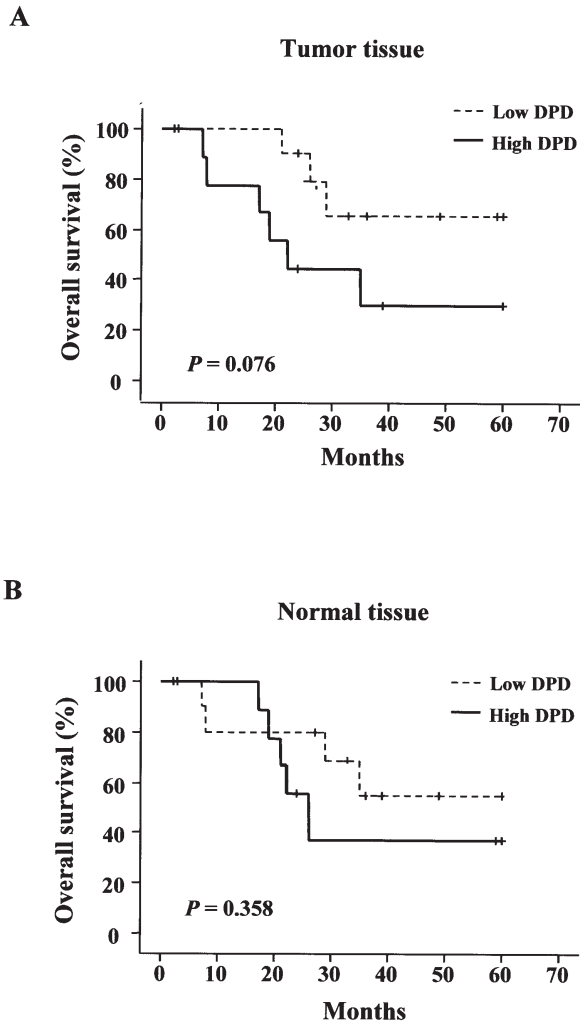

Figure 1. Overall survival and DPD expression. (A) In patients demonstrating expression of DPD in their tumor tissue, the overall survival in the low DPD group tended to be longer than that in the high DPD group (P=0.076). (B) In patients demonstrating expression of DPD in normal tissue, there was no significant difference in the overall survival between the high DPD and low DPD groups (P=0.358).

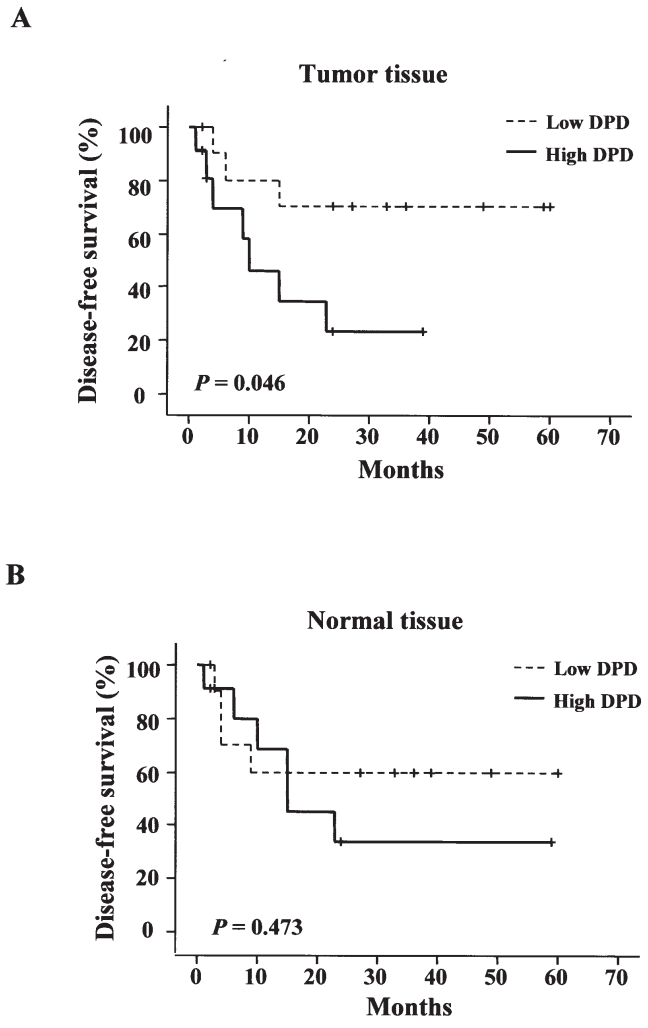

Figure 2. Disease-free survival and DPD expression. (A) In patients demonstrating expression of DPD in their tumor tissue, disease-free survival in the low DPD group was longer than that in the high DPD group (P=0.046). (B) In patients demonstrating expression of DPD in normal tissue, there was no significant difference in disease-free survival between the high DPD and low DPD groups (P=0.473).

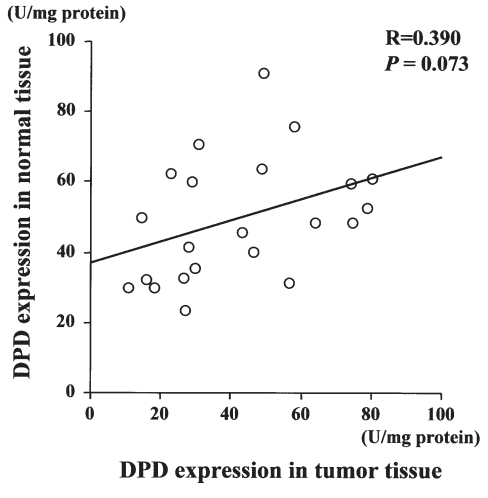

Figure 3. Correlation between the DPD expression in tumor tissue and that in adjacent normal tissue. There tended to be a correlation between the DPD expression in tumor tissue and that in adjacent normal tissue (R=0.390, P=0.073).

DPD expression in tumor tissue in patients with colorectal cancer as a prognostic factor.

This study shows that in patients with a DPD expression measured in tumor tissue, the overall survival in the low DPD group tended to be longer than that in the high DPD group, whereas in patients with a DPD expression measured in normal tissue, there was no significant difference in the overall survival between the high DPD and low DPD groups. In patients with a DPD expression measured in tumor tissue, the disease-free survival in the low DPD group was longer than that in the high DPD group, whereas in patients with a DPD expression measured in normal tissue, there was no significant difference in the disease-free survival between the high DPD and low DPD groups. We demonstrated the importance of DPD expression in tumor tissue as a prognostic factor in patients with colorectal cancer. In contrast, there tended to be a correlation between DPD expression in tumor tissue and that in adjacent normal tissue. Therefore, tumors occurring in tissue with high expression levels of DPD may have a high DPD expression.

DPD is considered to be a key enzyme in overcoming refractory tumors including colorectal cancer, and thus, it is important to assess this enzyme in tumor tissue.

We intend to perform patient-oriented chemotherapy based on the expression levels of DPD in both tumor and normal tissue specimens, and thereafter analyze the expression level of DPD using endoscopic biopsy specimens for preoperative or inoperable cancer patients, and anticancer combination chemotherapy using a DPD inhibitor. A randomized prospective study may also be useful for evaluating the therapeutic efficacy of these strategies for advanced colorectal cancers.

Acknowledgements

The authors wish to thank the Nippon Roche Research Center for its excellent technical assistance.

References

1. Kikuyama S, Inada T, Shimizu K, Miyakiya M and Ogata Y: p53, bcl-2 and thymidine phosphorylase as predictive markers of chemotherapy in patients with advanced and recurrent gastric cancer. Anticancer Res 21: 2149-2154, 2001.
2. Collie-Duguid ESR, Johnston SJ, Boyce L, Smith N, Cowleson A, Cassidy J, Murray GI and McLeod HL: Thymidine phosphorylase and dihydropyrimidine dehydrogenase protein expression in colorectal cancer. Int J Cancer 94: 297-301, 2001.
3. Osanai T, Ichikawa W, Takagi Y, Uetake H, Nihei Z and Sugihara K: Expression of pyrimidine nucleoside phosphorylase (PyNPase) in colorectal cancer. Jpn J Clin Oncol 31: 500-505, 2001.
4. Nishida M, Hino A, Mori K, Matsumoto T, Yoshikubo T and Ishitsuka H: Preparation of anti-human thymidine phosphorylase monoclonal antibodies useful for detecting the enzyme levels in tumor tissues. Biol Pharm Bull 19: 1407-1411, 1996.
5. Nishimura G, Terada I, Kobayashi T, Ninomiya I, Kitagawa H, Fushida S, Fujimura T, Kayahara M, Shimizu K, Ohta T and Miwa K: Thymidine phosphorylase and dihydropyrimidine dehydrogenase levels in primary colorectal cancer show a relationship to clinical effects of 5'-deoxy-5-fluorouridine as adjuvant chemotherapy. Oncol Rep 9: 479-482, 2002.
6. Ishikawa Y, Kubota T, Otani Y, Watanabe M, Teramoto T, Kumai K, Takechi T, Okabe H, Fukushima M and Kitajima M: Dihydropyrimidine dehydrogenase and messenger RNA level in gastric cancer: Possible predictor for sensitivity to 5-fluorouracil. Jpn J Cancer Res 91: 105-112, 2000.
7. Mori K, Hasegawa M, Nishida M, Toma H, Fukuda M, Kubota T, Nagasue N, Yamana H, Hirakawa K, Chung YS, Ikeda T, Takasaki K, Oka M, Kameyama M, Toi M, Fujii H, Kitamura M, Murai M, Sasaki H, Ozono S, Makuuchi H, Shimada Y, Onishi Y, Aoyagi S, Mizutani K, Ogawa M, Nakao A, Kinoshita H, Tono T, Imamoto H, Nakashima Y and Manabe T: Expression levels of thymidine phosphorylase and dihydropyrimidine dehydrogenase in various human tumor tissues. Int J Oncol 17: 33-38, 2000.
8. Yoshikawa T, Suzuki K, Kobayashi O, Sairenji M, Motohashi H, Tsuburaya A, Nakamura Y, Shimizu A, Yanoma S and Noguchi Y: Thymidine phosphorylase/platelet-derived endothelial cell growth factor is upregulated in advanced solid types of gastric cancer. Br J Cancer 79: 1145-1150, 1999.
9. Hiroyasu S, Shiraishi M, Samura H, Tokashiki H, Shimoji H, Isa T and Muto Y: Clinical relevance of the concentrations of both pyrimidine nucleoside phosphorylase (PyNPase) and dihydropyrimidine dehydrogenase (DPD) in colorectal cancer. Jpn J Clin Oncol 31: 65-68, 2001.
10. Konno S, Takebayashi Y, Aiba M, Akiyama S and Ogawa K: Clinicopathological and prognostic significance of thymidine phosphorylase and proliferating cell nuclear antigen in gastric carcinoma. Cancer Lett 166: 103-111, 2001.
11. Ishii R, Takiguchi N, Oda K, Koda K and Miyazaki M: Thymidine phosphorylase expression is useful in selecting adjuvant chemotherapy for stage III gastric cancer. Int J Oncol 19: 717-722, 2001.
12. Kimura H, Konishi K, Nukui T, Kaji M, Maeda K, Yabushita K, Tsuji M and Miwa A: Prognostic significance of expression of thymidine phosphorylase and vascular endothelial growth factor in human gastric carcinoma. J Surg Oncol 76: 31-36, 2001.
13. Katsumata K, Sumi T, Yamashita S, Takahashi S, Mori M, Baba T, Murohashi T, Nagashima K, Ashizawa T, Yamamoto K, Koyanagi Y and Katoh K: The significance of thymidine phosphorylase expression in colorectal cancer. Oncol Rep 8: 127-130, 2001.
14. Hotta T, Taniguchi K, Kobayashi Y, Johata K, Sahara M, Naka T, Watanabe T, Ochiai M, Tanimura H and Tsubota YT: Comparative analysis of thymidine phosphorylase and dihydropyrimidine dehydrogenase expression in gastric and colorectal cancers. Oncol Rep 11: 1045-1051, 2004.
15. Hotta T, Kobayashi Y, Taniguchi K, Johata K, Sahara M, Watanabe T, Ochiai M, Tanimura H and Tsubota YT: Thymidine phosphorylase and dihydropyrimidine dehydrogenase expression in hepatocellular carcinoma and metastatic liver cancer. Oncol Rep 12: 347-351, 2004.
16. Hotta T, Taniguchi K, Kobayashi Y, Johata K, Sahara M, Naka T, Watanabe T, Ochiai M, Tanimura H and Tsubota YT: Preoperative endoscopic analysis of thymidine phosphorylase and dihydropyrimidine dehydrogenase in gastrointestinal cancer. Oncol Rep 11: 1233-1239, 2004.
17. UICC: TNM classification of malignant tumors. 4th edition. Springer-Verlag, Berlin, Heidelberg, New York, 1997.
18. Hotta T, Takifuji K, Arii K, Yokoyama S, Matsuda K, Higashiguchi T, Tominaga T, Oku Y and Yamaue H: Toxicity during *l*-LV/5FU adjuvant chemotherapy as a modified RPMI regimen for patients with colorectal cancer. Oncol Rep 14: 433-439, 2005.
19. Fujiwara H, Terashima M, Irinoda T, Takagane A, Abe K, Kashiwaba M, Oyama K, Takahashi M, Maesawa C, Saito K, Takechi T and Fukushima M: Quantitative measurement of thymidylate synthase and dihydropyrimidine dehydrogenase mRNA level in gastric cancer by real-time RT-PCR. Jpn J Cancer Res 93: 1342-1350, 2002.
20. Ichikawa W, Uetake H, Shirota Y, Yamada H, Takahashi T, Nihei Z, Sugihara K, Sasaki Y and Hirayama R: Both gene expression for orotate phosphoribosyltransferase and its ratio to dihydropyrimidine dehydrogenase influence outcome following fluoropyrimidine-based chemotherapy for metastatic colorectal cancer. Br J Cancer 89: 1486-1492, 2003.