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ORIGINAL ARTICLE

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Gene expression of 5-fluorouracil metabolic enzymes in primary colorectal cancer and corresponding liver metastasis

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Abstract *Purpose*: Expression of thymidylate synthase (TS) and the 5-fluorouracil (5-FU) metabolic enzymes, including dihydropyrimidine dehydrogenase (DPD), orotate phosphoribosyl transferase (OPRT), thymidine phosphorylase (TP), and uridine phosphorylase (UP), has been reported to be associated with the sensitivity to 5-FU-based chemotherapy in colorectal cancer. We evaluated the correlation of the expression of these genes between primary tumors and corresponding liver metastases. *Method*: The mRNA levels of TS, DPD, OPRT, TP, and UP were measured by real-time quantitative RT-PCR in samples from 23 consecutive patients with both primary colorectal adenocarcinoma and liver metastasis. *Results*: The DPD, OPRT, TP, and UP mRNA levels were significantly higher in liver metastases than in primary tumor (expression in relation to that of β-actin mRNA: 0.42 vs 0.16, $P=0.00053$; 1.4 vs 0.92, $P=0.016$; 23 vs 11, $P=0.00014$; 0.36 vs 0.25, $P=0.0026$; respectively). However, the TS mRNA level did not differ significantly between liver metastases than primary tumor (0.20 vs 0.16, $P=0.28$). No correlation was observed for any gene between primary tumor and liver metastases. In both primary tumor and liver metastasis, the TS mRNA levels correlated significantly with the OPRT mRNA level (primary $r_S=0.83$, $P=0.00000081$; liver metastasis $r_S=0.49$, $P=0.017$), while the DPD mRNA level correlated significantly with the TP mRNA level $r_S=0.81$, $P=0.0000024$; $r_S=0.63$, $P=0.0014$; respectively). *Conclusions*: The differential gene expression of 5-FU metabolic enzymes between primary colorectal cancer and corresponding liver metastases should be taken into consideration when estimating the sensitivity to 5-FU-based chemotherapy in colorectal cancer. The gene expression of TS and OPRT, which are involved in de novo pyrimidine synthesis, and that of DPD and TP, may be coregulated.

Keywords Thymidylate synthase · Dihydropyrimidine dehydrogenase · Orotate phosphoribosyl transferase · Thymidine phosphorylase · Uridine phosphorylase

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Introduction

Thymidylate synthase (TS) protein and gene expression in human colorectal cancers has been investigated as a predictor of response to chemotherapies based on 5-fluorouracil (5-FU), and as a prognostic marker [1, 2, 5, 9, 10, 20, 24, 29]. Previous studies have suggested that high TS expression in advanced colorectal cancers, determined by several methods (immunohistochemical staining, enzyme activity, and reverse transcription PCR), is followed by non-response to 5-FU and poor prognosis.

The other 5-FU metabolic enzymes have been also examined as predictors of sensitivity to 5-FU. Dihydropyrimidine dehydrogenase (DPD) is the first and rate-limiting enzyme for the catabolism of 5-FU, and its activity or mRNA level is high in various human cancers and cell lines with low sensitivity to 5-FU [3, 11, 16, 19, 33]. Ichikawa et al. [17] and Salonga et al. [33] have reported that patients with both low DPD and low TS mRNA expression in primary colorectal cancers respond to 5-FU-based chemotherapy, and their prognosis is better than patients with both high DPD and high TS expression. Other studies have indicated that the expression levels of the first metabolic enzymes of 5-FU, namely orotate phosphoribosyl transferase (OPRT) [6, 18, 30, 31], thymidine phosphorylase (TP) [6, 12, 25, 27, 28, 33, 34], and uridine phosphorylase (UP) [6, 8, 18, 25, 35], might also correlate with sensitivity to 5-FU. High TP gene expression has been shown to be followed by low

Table 1 Primers and probes

	Forward primer	Reverse primer	Probe
TS	TS-1	TS-r3	TS-P2
	GAATCACATCGAGCCACTGAAA	CAGCCCAACCCCTAAAGACTGA	TTCAGCTTCAGCGAGAACCCAGA
DPD	DPD-F11	DPD-R11	DPD-P11
	AATGATTCGAAGAGCTTTTGAAGC	GTTCCCCGGATGATTCTGG	TGCCCTCACCAAAACTTTCTCTCTTGATAAGGA
OPRT	OPRT-1107F	OPRT-1282R	OPRT-1200PF
	TCCTGGGCAGATCTAGTAAATGC	TGCTCCTCAGCCATTCTAACC	CTCCTTATTGCGGAAATGAGCTCCACC
TP	TP-700F	TP-770R	TP-722P
	CCTGCGGACGGAATCCT	GCTGTGATGAGTGGCAGGCT	CAGCCAGAGATGTGACAGCCACCGT
UP	UP-586F	UP-792R	UP-743PF
	TGACTGCCCAGGTAGAGACTATCC	AGACCTATCCCACCAGAAGTGC	TGCTCCAACGTCACTATCATCCGCAT
β-ActinACTB	ACTB-517F	ACTB-811R	ACTB-547PF
	TCACCCACACTGTGCCCATCTACGA	CAGCGGAACCGCTCATTGCCAATGG	ATGCCCTCCCCCATGCCATCCTGCGT

chemosensitivity to 5-FU in colorectal carcinoma [33]. Chung et al. have reported that OPRT, TP, and UP gene expression is downregulated in gastric cell lines with 5-FU resistance [6]. These results indicate that multiple analysis of the expression of 5-FU metabolic genes may predict sensitivity to 5-FU more precisely.

However, the relationship between the expression of these genes in primary cancers and their expression at metastatic sites has not been adequately evaluated. We have previously shown that TS gene expression is lower, and DPD gene expression is higher, in liver metastases than in primary colorectal cancers [36, 39]. As high expression of TS, DPD and TP protein or mRNA has been reported to be associated with low sensitivity to 5-FU [2, 17, 20, 24, 33], the prediction of 5-FU chemosensitivity by analysis of expression in the primary tumor may be inaccurate in patients whose TS, DPD and TP gene expression is markedly higher or lower in their liver metastases than in the primary tumor.

In the present study, the expression of TS, DPD, OPRT, TP, and UP genes in primary colorectal cancer was compared with that in the corresponding liver metastases by real-time quantitative RT-PCR.

Materials and methods

Patients and samples

We analyzed pairs of primary colorectal adenocarcinomas and corresponding liver metastatic tumors from 23 patients (13 males and 10 females, average age 62.2 years) who had undergone surgical resection of primary colorectal cancer between October 1997 and October 2001 at the Department of Digestive Surgery, Tokyo Medical and Dental University, Tokyo, Japan. This study was approved by the Institutional Review Board of the Tokyo Medical and Dental University, and written consent was obtained from all patients. Of the liver metastasis samples, 12 were obtained by surgical resection, and 11 by intraoperative core-needle biopsy at the time of resection of the primary tumor. Seven were metachronous metastases and two of the patients had received 5′-deoxy-5-fluorouridine orally as adjuvant therapy after the primary resection. One of these two patients discontinued adjuvant therapy after only 4 weeks, while the other completed a 1-year course of adjuvant therapy. Resection of the liver metastases in this patient was performed at least 6 months after completion of the adjuvant therapy regimen.

Immediately following surgery, each tissue sample was frozen in liquid nitrogen and stored at −80°C until preparation of RNA extracts. A gastrointestinal pathologist evaluated the remaining specimens. No contamination of the normal colonic mucosa or liver tissue in the tumor samples was histologically identified.

Total RNA extraction and cDNA synthesis

Our procedure has previously been described in detail [36, 38, 39]. In brief, total RNA was extracted using an RNeasy Minikit (Qiagen, Chatsworth, Calif.). The amount of total RNA was estimated by measuring absorbance, the quality was determined by electrophoresis through agarose gel in the presence of formaldehyde, and the rRNA bands were visualized. Then up to

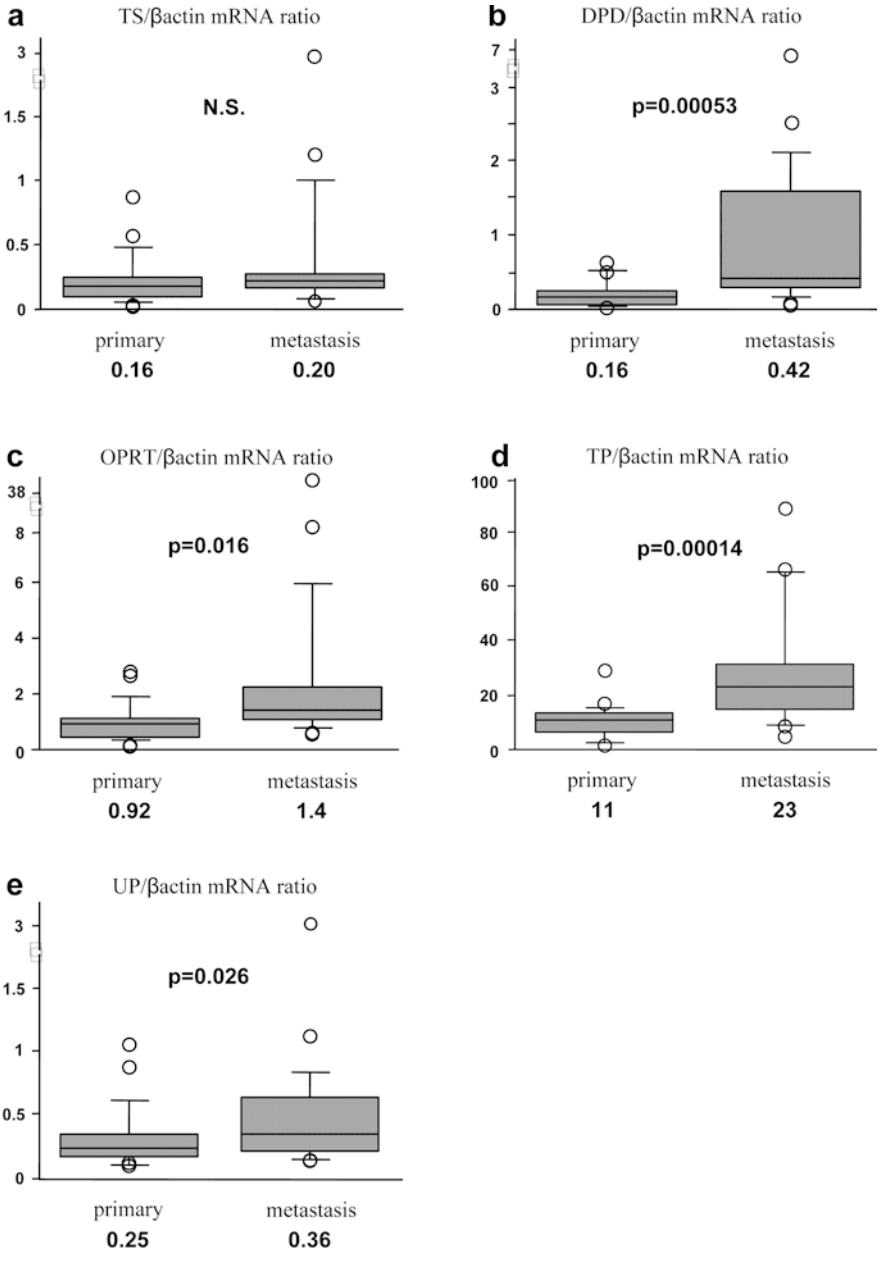

Fig. 1a–e Gene expression in primary tumors and liver metastases. The median values are shown

10 μg of the prepared RNA was reverse-transcribed to synthesize cDNA using the oligo(dT) primer, Superscript II (Life Technologies, Gaithersburg, Md.), as previously described.

Real-time quantitative RT-PCR assay

The mRNA levels of TS, DPD, OPRT, TP, and UP were evaluated by real-time quantitative RT-PCR [15, 29, 30] (TaqMan PCR) using an ABI Prism 7700 sequence detector (Perkin-Elmer Applied Biosystems, Foster City, Calif.). The β-actin gene was used as the endogenous control gene. Primers and TaqMan probes for each gene were designed based on the nucleotide sequence of human TS, DPD, OPRT, TP, UP and β-actin (Table 1). The PCR mixture contained 10 μl of each appropriately diluted cDNA sample (standard curve points and patient samples), 200 n*M* forward primer, 200 n*M* reverse primer, 100 n*M* TaqMan probe, and 12.5 μl TaqMan Universal PCR Master Mix (Perkin-Elmer Applied Biosystems), in a final volume of 25 μl. The PCR profile consisted of one incubation at 50°C for 2 min, one incubation at 95°C for 10 min, and 45 cycles of amplification for 15 s at 95°C, and 1 min at 60°C.

The amount of PCR product was determined using a standard curve of cDNA synthesized from human tumor xenograft. Each PCR run included the seven points of the standard curve (fourfold serially diluted cDNA with 100 ng/μl) and negative controls. The range of the standards was 64 to 0.00391 ng/10 μl. All samples were run in duplicate PCR experiments. The mean was then used; a few samples with more than a twofold difference in the amount of PCR product were retested. Some samples out of the range of the respective points on the standard curve were also retested using altered cDNA concentrations.

The relative amount of each gene's mRNA was expressed as the ratio of each mRNA to that of β-actin.

Statistical analysis

The mRNA levels and clinicopathological factors were compared using the Mann-Whitney *U*-test. The mRNA levels of the primary colorectal cancers and those of the liver metastases were compared

using the Wilcoxon signed-ranks test. The relationship between each gene's mRNA level in the primary cancer and that in the liver metastases, and the relationships among the mRNA levels in primary cancers or liver metastases were assessed using Spearman's rank correlation. Statistical significance was established at the $P < 0.05$ level for each analysis.

Results

Messenger RNA levels of the 5-FU metabolic enzymes were assessed in 23 pairs of primary colorectal cancers and corresponding liver metastases. The difference in the quantities between duplicate PCR products was less than 10%. No significant differences in mRNA levels were observed for any clinicopathological features such as gender, age, location of primary tumor, number of liver metastases and method of obtaining samples from liver metastases. Synchronous and metachronous liver metastasis showed the same levels of gene expressions (median values: TS 0.20 vs 0.22, $P = 0.69$; DPD 0.57 vs 0.42, $P = 0.64$; OPRT 1.6 vs 1.4, $P = 0.64$; TP 26 vs 21, $P = 0.42$; UP 0.44 vs 0.32, $P = 0.74$).

DPD, OPRT, TP and UP mRNA levels in the liver metastases were significantly higher than those in the corresponding primary tumors (DPD 0.42 vs 0.16, $P = 0.00053$; OPRT 1.4 vs 0.92, $P = 0.016$; TP 23 vs 11, $P = 0.00014$; UP 0.36 vs 0.25, $P = 0.026$; Fig. 1). The TS mRNA level did not significantly differ between the liver metastases and the primary tumors (0.20 vs 0.16, $P = 0.28$). No significant correlation between the mRNA levels of the primary tumors and those of their corresponding liver metastases was noted for any of the genes (TS $P = 0.48$, DPD $P = 0.94$, OPRT $P = 0.19$, TP $P = 0.81$, UP $P = 0.90$). There was a significant correlation between the OPRT and TS mRNA levels both in the primary tumors ($r_S = 0.83$, $P = 0.00000081$; Fig. 2a) and in the liver metastases ($r_S = 0.49$, $P = 0.017$; Fig. 2b). A similar relationship was noted between DPD and TP mRNA levels in both primary tumors ($r_S = 0.81$, $P = 0.0000024$; Fig. 3a) and liver metastases ($r_S = 0.63$, $P = 0.0014$; Fig. 3b). Other correlations among the genes were not found.

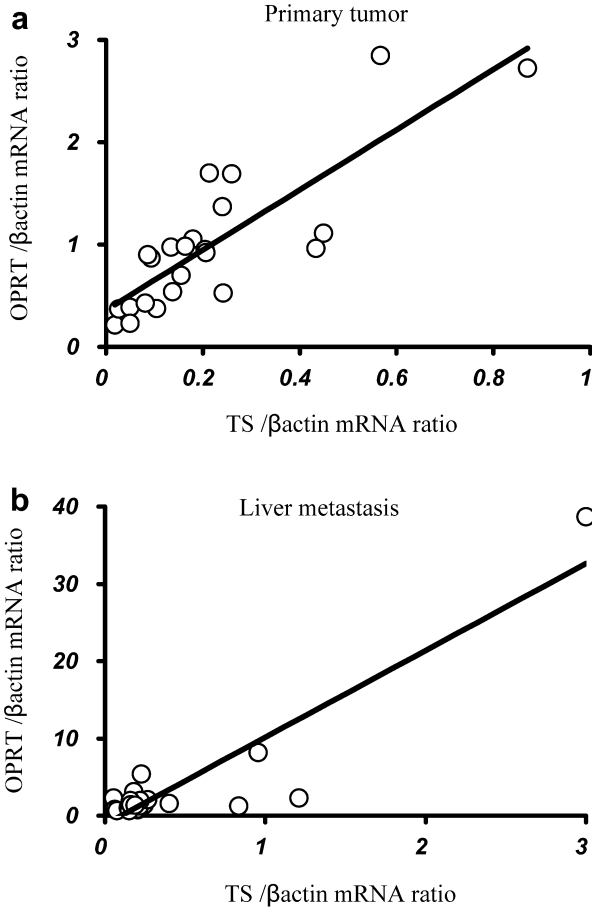

Fig. 2a, b TS and OPRT mRNA levels are significantly correlated in both primary colorectal cancers (**a** $r_S = 0.83$, $P = 0.00000081$) and liver metastases (**b** $r_S = 0.49$, $P = 0.0017$)

Discussion

We demonstrated that DPD, OPRT, TP, and UP mRNA levels in liver metastases were significantly higher than in their corresponding primary colorectal cancers, although no correlation was observed between primary tumors and liver metastases. Previously, we have shown that DPD gene expression in colorectal cancers is associated with tumor progression, and that higher DPD gene expression is present in liver metastases than in primary tumors [36]. Johnston et al. have suggested that suppression of translation of DPD mRNA is removed in tumor tissue, and proposed a general mechanism by which pyrimidine nucleotide biosynthesis and degradation are coregulated to maintain a growth advantage in the tumor [21]. Takebayashi et al. have reported that TP, which catalyses the reversible phosphorolysis of thymidine and its analogues to their respective bases 2-deoxyribose-1-phosphate, is associated with tumor progression [37]. Previous investigators have shown that the expression of the initial 5-FU-anabolizing enzymes (OPRT, UP, TP, etc.) is higher in various human cancers than in normal tissues [7, 22, 26, 32], and suggested that the increase in the expression of these enzymes may be an advantage via increased pyrimidine nucleotide biosynthesis for cell proliferation in cancer. The gene expression of thymidine kinase (TK), one of the enzymes involved in salvage DNA synthesis, correlates with malignant potential in ovarian tumors, as well as with TP gene expression [13].

In the present study, a linear relationship between TS and OPRT mRNA levels was observed in both primary colorectal cancers and liver metastases. Kasahara et al. have reported that TS gene expression correlates closely with E2F1 expression, and speculated that one mechanism by which tumor cells increase TS expression may be overexpression of E2F1, which induces S-phase-acting proteins such as TS [23]. Fujiwaki et al. have

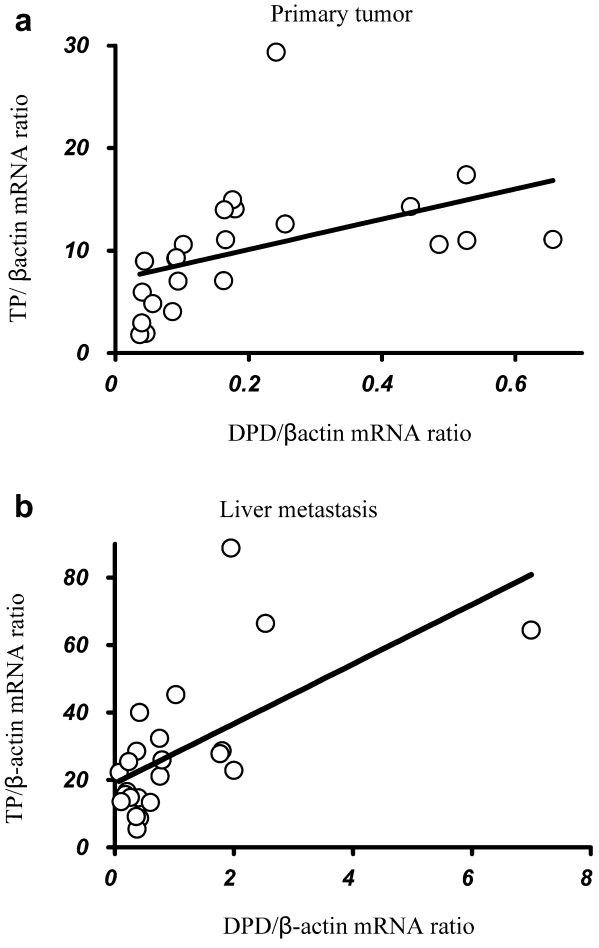

Fig. 3a, b DPD and TP mRNA levels are significantly correlated in both primary colorectal cancers (**a** $r_S = 0.81$, $P = 0.0000024$) and liver metastases (**b** $r_S = 0.63$, $P = 0.014$)

demonstrated a linear relationship in gene expression levels between TK1 and TS in ovarian cancer, suggesting that enzymes for DNA biosynthesis may be controlled by several similar mechanisms [13]. TS and OPRT, which are involved in de novo pyrimidine nucleotide biosynthesis, may be coregulated in cancer cell proliferation. DPD and TP gene expression were also positively correlated in both primary cancers and liver metastases. Collie-Duguid et al. suggested that the expression of DPD and TP protein is coregulated [7].

We measured gene expression using the TaqMan RT-PCR assay. This method is a more precise and reproducible semiquantitation of gene expression than conventional RT-PCR assays using agarose gel, because it is based on threshold values in the exponential phase of the PCR rather than end-point measurement of the amount of PCR product [4]. In addition, this PCR assay is suitable for smaller samples such as biopsy specimens and can measure a larger number of enzymes in a shorter time than other methods, including enzymatic activity or protein assays [24].

Increased OPRT gene expression in liver metastases may be associated with increased sensitivity to 5-FU, because OPRT is a 5-FU-anabolizing enzyme and is considered the main pathway of 5-FU initial phosphorylation in human cancers [14]. On the other hand, increased TS, DPD, and TP may be associated with decreased sensitivity to 5-FU. In patients with extremely low expression of OPRT mRNA or high levels of TS, DPD, or TP gene expression in liver metastasis, it may be difficult to predict 5-FU sensitivity of the metastasis via analysis of the gene expression of the primary site.

The present study showed that the expression of 5-FU metabolic genes in liver metastases does not correlate with that in the corresponding primary tumor. The difference in the expression of 5-FU metabolic genes between the primary site and liver metastases should be taken into consideration when predicting the sensitivity to 5-FU-based chemotherapy of colorectal cancer.

References

1. Allegra CJ, Parr AL, Wold LE, Mahoney MR, Sargent DJ, Johnston P, Klein P, Behan K, O'Connell MJ, Levitt R, Kugler JW, Tirona MT, Goldberg RM (2002) Investigation of the prognostic and predictive value of thymidylate synthase, p53, and Ki-67 in patients with locally advanced colon cancer. J Clin Oncol 20:1735
2. Aschele C, Debernardis D, Casazza S, Antonelli G, Tunesi G, Baldo C, Lionetto R, Maley F, Sobrero A (1999) Immunohistochemical quantitation of thymidylate synthase expression in colorectal cancer metastases predicts for clinical outcome to fluorouracil-based chemotherapy. J Clin Oncol 17:1760
3. Beck A, Etienne MC, Chéradame S, Fischel JL, Formento P, Renée N, Milano G (1994) A role for dihydropyrimidine dehydrogenase and thymidylate synthase in tumor sensitivity to fluorouracil. Eur J Cancer 30A:1517
4. Bièche I, Laurendeau I, Tozlu S, Olivi M, Vidaud D, Lidereau R, Vidaud M (1999) Quantitation of MYC gene expression in sporadic breast tumors with a real-time reverse transcription-PCR assay. Cancer Res 59:2759
5. Cascinu S, Aschele C, Barni S, Dabernardis D, Baldo C, Tunesi G, Catalano V, Staccioli MP, Brenna A, Muretto P, Catalano G (1999) Thymidylate synthase protein expression in advanced colorectal cancer: correlation with the site of metastasis and the clinical response to leucovorin-modulated bolus 5-fluorouracil. Clin Cancer Res 5:1996
6. Chung YM, Park SH, Park JK, Kim YT, Kang YK, Yoo YD (2000) Establishment and characterization of 5-fluorouracil-resistent gastric cancer cells. Cancer Lett 159:95
7. Collie-Duguid ESR, Johmston SJ, Boyce L, Smith N, Cowieson A, Cassidy J, Murray GI, Mcleod H (2001) Thymidine phosphorylase and dihydropyrimidine dehydrogenase protein expression in colorectal cancer. Int J Cancer 94:297
8. Cuq P, Rouquet C, Evrard V, Ciccolini J, Vian L, Cano JP (2001) Fluoropyrimidine sensitivity of human MCF-7 breast cancer cells stably transfected with human uridine phosphorylase. Br J Cancer 84:1677
9. Davies MM, Johnston PG, Kaur S, Allen-Mersh TG (1999) Colorectal liver metastasis thymidylate synthase staining correlates with response to hepatic arterial floxuridine. Clin Cancer Res 5:325
10. Edler D, Glimelius B, Hallstörm M, Jakobsen A, Johnston PG, Magnusson I, Ragnhammar P, Blomgren H (2002) Thymidylate synthase expression in colorectal cancer: a prognostic and predictive marker of benefit from adjuvant fluorouracil-based chemotherapy. J Clin Oncol 20:1721

11. Etienne MC, Cheradame S, Fischel JL, Formento P, Dassonville O, Renee N, Schneider M, Thyss A, Demard F, Milano G (1995) Response to fluorouracil therapy in cancer patients: the role of tumoral dihydropyrimidine dehydrogenase activity. J Clin Oncol 13:1663
12. Evrard A, Cuq P, Robert B, Vian L, Pélegrin A, Cano JP (1999) Enhancement of 5-fluorouracil cytotoxicity by human thymidine-phosphorylase expression in cancer cells: in vitro and in vivo study. Int J Cancer 80:465
13. Fujiwaki R, Hata K, Nakayama K, Moriyama M, Iwanari O, Katabuchi H, Okamura H, Sakai E, Miyazaki K (2002) Thymidine kinase in epithelial ovarian cancer: relationship with the other pyrimidine pathway enzymes. Int J Cancer 99:328
14. Fukushima M, Nomura H, Murakami Y, Shirasaka T, Aiba K (1996) Estimation of pathway of 5-fluorouracil anabolism in human cancer cells in vitro and in vivo. Jpn J Cancer Chemother 23:721
15. Heid CA, Stevenes J, Livak KJ, Williams PM (1996) Real time quantitative PCR. Genome Res 6:986
16. Huang CL, Yokomise H, Kobayashi S, Fukushima M, Hitomi S, Wada H (2000) Intratumoral expression of thymidylate synthase and dihydropyrimidine dehydrogenase in non-small cell lung cancer patients treated with 5-FU-based chemotherapy. Int J Oncol 17:47
17. Ichikawa W, Uetake H, Shirota Y, Yamada H, Nishi N, Nihei Z, Sugihara K, Hirayama R (2003) Combination of dihydropyrimidine dehydrogenase and thymidylate synthase gene expressions in primary tumors as predictive parameters for the efficacy of fluoropyrimidine-based chemotherapy for metastatic colorectal cancer. Clin Cancer Res 9:786
18. Inaba M, Mitsuhashi J, Sawada H, Miike N, Naoe Y, Daimon A, Koizumi K, Tsujimoto H, Fukushima M (1996) Reduced activity of anabolizing enzymes in 5-fluorouracil-resistant human stomach cancer cells. Jpn J Cancer Res 87:212
19. Ishikawa Y, Kubota T, Otani Y, Watanabe M, Teramoto T, Kumai K, Takechi T, Okabe H, Fukushima M, Kitajima M (2000) Dihydropyrimidine dehydrogenase and messenger RNA levels in gastric cancer: possible predictor for sensitivity to 5-fluorouracil. Jpn J Cancer Res 91:105
20. Johnston PG, Lenz HJ, Leichman, CG, Danenberg KD, Allegra CJ, Danenberg PV, Leichman L (1995) Thymidylate synthase gene and protein expression correlate and are associated with response to 5-fluorouracil in human colorectal and gastric tumors. Cancer Res 55:1407
21. Johnston SJ, Ridge SA, Cassidy J, McLeod HL (1999) Regulation of dihydropyrimidine dehydrogenase in colorectal cancer. Clin Cancer Res 5:2566
22. Kanzaki A, Takebayshi Y, Bando H, Eliason JF, Watanabe S, Miyashita H, Fukumoto M, Toi M, Uchida T (2002) Expression of uridine and thymidine phosphorylase genes in human breast carcinoma. Int J Cancer 97:631
23. Kasahara M, Takahashi Y, Nagata T, Asai S, Eguchi T, Ishii Y, Fujii M, Ishikawa K (2000) Thymidylate synthase expression correlates closely with E2F1 expression in colorectal cancer. Clin Cancer Res 6:2707
24. Leichman CG, Lenz HJ, Leichman L, Danenberg K, Baranda J, Groshen S, Boswell W, Metzger R, Tan M, Danenberg PV (1997) Quantitation of intratumoral thymidylate synthase expression predicts for disseminated colorectal cancer response and resistance to protracted-infusion fluorouracil and weekly leucovorin. J Clin Oncol 15:3223
25. Mader RM, Sieder AE, Braun J, Rizovski B, Kalipciyan M, Mueller MW, Jakesz R, Rainer H, Steger GG (1997) Transcription and activity of 5-fluorouracil converting enzymes in fluoropyrimidine resistance in colon cancer in vitro. Biochem Pharmacol 54:1233
26. Maehara Y, Moriguchi S, Emi Y, Watanabe A, Kohnoe S, Tsujitani S, Sugimachi K (1990) Comparison of pyrimidine nucleotide synthetic enzymes involved in 5-fluorouracil metabolism between human adenocarcinomas and squamous cell carcinomas. Cancer 66:156
27. Marchetti S, Chazal M, Dubreuil A, Fischel JL, Etienne MC, Milano G (2001) Impact of thymidine phosphorylase surexpression on fluoropyrimidine activity and on tumor angiogenesis. Br J Cancer 85:439
28. Metzger R, Danenberg K, Leichman CG, Salonga D, Schwartz EL, Wadler S, Lenz HJ, Groshen S, Leichman L, Danenberg PV (1998) High basal level gene expression of thymidine phosphorylase (platelet-derived endothelial cell growth factor) in colorectal tumors is associated with nonresponse to 5-fluorouracil. Clin Cancer Res 4:2371
29. Paradiso A, Simone G, Petroni S, Leone B, Vallejo C, Lacava J, Romero A, Machiavelli M, De Lena M, Allegra CJ, Johnston PG (2000) Thymidylate synthase and p53 primary tumor expression as predictive factors for advanced colorectal cancer patients. Br J Cancer 82:560
30. Peters GJ, Laurensse E, Leyva A, Lankelma J, Pinedo HM (1986) Sensitivity of human, murine, and rat cells to 5-fluorouracil and 5′-deoxy-5-fluorouridine in relation to drug-metabolizing enzymes. Cancer Res 46:20
31. Peters GJ, Braakhuis BJ, de Bruijn EA, Laurensse EJ, van Walsum M, Pinedo HM (1989) Enhanced therapeutic efficacy of 5′-deoxy-5-fluorouridine in 5-fluorouracil resistant head and neck tumors in relation to 5-fluorouracil metabolizing enzymes. Br J Cancer 59:327
32. Peters GJ, van Groeningen CJ, Laurensse EJ, Pinedo HM (1991) A comparison of 5-fluorouracil metabolism in human colorectal cancer and colon mucosa. Cancer 68:1903
33. Salonga D, Danenberg KD, Johnston M, Metzger R, Groshen S, Tsao-Wei DD, Lenz HJ, Leichman CG, Leichman L, Diasio RB, Danenberg PV (2000) Colorectal tumors responding to 5-fluorouracil have low gene expression levels of dihydropyrimidine dehydrogenase, thymidylate synthase, and thymidine phosphorylase. Clin Cancer Res 6:1322
34. Schwartz EL, Baptiste N, Wadler S, Makower D (1995) Thymidine phosphorylase mediates the sensitivity of human colon carcinoma cells to 5-fluorouracil. J Biol Chem 270:19073
35. Schwartz PM, Moir RD, Hyde CM, Turek PJ, Handschumacher RE (1985) Role of uridine phosphorylase in the anabolism of 5-fluorouracil. Biochem Pharmacol 34:3585
36. Shirota Y, Ichikawa W, Uetake H, Yamada H, Nihei Z, Sugihara K (2002) Intratumoral dihydropyrimidine dehydrogenase messenger RNA level reflects tumor progression in human colorectal cancer. Ann Surg Oncol 9:599
37. Takebayashi Y, Akiyama S, Akiba S, Yamada K, Miyadera K, Sumizawa T, Yamada Y, Murata F, Aikou T (1996) Clinicopathological and prognostic factor, thymidine phosphorylase, in human colorectal carcinoma. J Natl Cancer Inst 88:1110
38. Uetake H, Ichikawa W, Takechi T, Fukushima M, Nihei Z, Sugihara K (1999) Relationship between intratumoral dihydropyrimidine dehydrogenase activity and gene expression in human colorectal cancer. Clin Cancer Res 5:2836
39. Yamada H, Ichikawa W, Uetake H, Shirota Y, Nihei Z, Sugihara K, Hirayama R (2001) Thymidylate synthase gene expression in primary colorectal cancer and metastatic sites. Clin Colorectal Cancer 1:169