Thymidylate Synthase Gene and Protein Expression Correlate and Are Associated with Response to 5-Fluorouracil in Human Colorectal and Gastric Tumors

Patrick G. Johnston, Heinz-Josef Lenz, Cynthia G. Leichman, Kathleen D. Danenberg, Carmen J. Allegra, Peter V. Danenberg, and Lawrence Leichman

NCI-Navy Medical Oncology Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20889-5105 (P. G. J., C. J. A. L., and Norris Comprehensive Cancer Center, University of Southern California School of Medicine, Los Angeles, California 90033 [H.-J. L., C. G. L., K. D. D., P. V. D., L. L.])

Abstract

Thymidylate synthase (TS) is the target enzyme for 5-fluorouracil (5-FU). We have correlated TS protein and gene expression with the response in patients with colorectal (n = 9) and gastric cancer (n = 12) treated with infusional 5-FU plus leucovorin (LV) or infusional 5-FU/LV and cisplatin, respectively. TS protein expression was analyzed by Western blot using TS106 monoclonal antibody and densitometry scanning. TS gene expression was measured by PCR analysis using β-actin as an internal standard and expressed as a TS:β-actin mRNA ratio. A close linear relationship was noted between TS protein expression and TS gene expression (r² = 0.60) for the 21 tumor samples analyzed. TS immunohistochemical staining on 15 of the 21 samples revealed that the TS staining intensity correlated closely with TS protein and mRNA expression. In two biopsy samples, TS protein levels and TS gene expression did not correlate; however, one of these exhibited a focal TS staining pattern.

Both the TS protein level and TS gene expression were significantly associated with response to 5-FU-based therapy. Patients with responsive disease had a mean TS protein level of 0.17 ± 0.03 arbitrary units (range, 0.05 to 0.38), whereas in patients whose tumors did not respond, the mean TS protein level was significantly higher 0.60 ± 0.09 (range, 0.06 to 1.01; P < 0.01). A similar pattern was noted with TS gene expression (r² = 0.60) for the 21 tumor samples analyzed. TS immunohistochemical staining on 15 of the 21 samples revealed that the TS staining intensity correlated closely with TS protein and mRNA expression. In two biopsy samples, TS protein levels and TS gene expression did not correlate; however, one of these exhibited a focal TS staining pattern.

Introduction

TS (EC 2.1.1.45) catalyzes the methylation of dUMP to dTMP, an essential step in DNA biosynthesis (1). TS is also a critical target for the fluoropyrimidines, an important group of antineoplastic drugs that are widely used in the treatment of gastrointestinal tumors, breast tumors, and epithelial tumors of the upper aerodigestive tract (2, 3). Both 5-FU and fluorodeoxyuridine are converted in tumor cells toFdUMP, which forms a tight-binding covalent complex with TS in the presence of the folate cofactor 5-10 methylene tetrahydrofolate (CH₂H₄PteGlu).

The clinical importance of TS in the development of tumor resistance has been suggested by studies demonstrating that the acute induction of TS protein as well as the stable amplification of TS-specific genes may be associated with 5-FU resistance (7–10). Other studies have demonstrated in vivo as well as in vitro that TS enzyme levels in neoplastic cells rise acutely when cells are exposed to cytotoxic agents such as 5-FU (11, 12). These tumors display an increase in TS after exposure to 5-FU, resulting in the maintenance of free enzyme. These studies suggest that the degree of FdUMP binding and the ability of a tumor to acutely overexpress TS in response to cytotoxic agents may play a role in the development of fluorouracil resistance. The quantitation of TS is critical to understanding the mechanisms involved in antimetabolite resistance in patients.

In view of the potential role of TS as a determinant of response to fluoropyrimidine-based chemotherapy, significant efforts have been made to quantitate TS enzyme levels in tumor tissue from individual patients. The quantitation of TS has traditionally been performed using biochemical assays; however, these assays have major limitations, including the need for relatively large amounts of tissue (>50 mg) (13, 14). Thus, studies examining the clinical importance of TS have been difficult. Recently, sensitive methods for quantitating the level of TS protein and TS gene expression have been developed. TS monoclonal antibodies have facilitated the development of immunological assays that can detect femtomolar amounts of TS protein using small tissue biopsy samples (15). Moreover, the development of a sensitive RT-PCR method has permitted the quantitation of TS mRNA expression in small (<10 mg) tumor biopsy samples (16). Investigators at the National Cancer Institute (10) and at the USC (16) have independently applied these quantitative techniques in human cancer cell lines.

Using these recently developed assays for the quantitation of TS within human tumors, we conducted a study to compare the level of TS protein with TS mRNA expression from the same human colorectal and gastric specimens to determine their ability to predict response to 5-FU-based therapy.

Materials and Methods

Patient Population

Gastric Cancer. In August 1987, clinical investigators at USC initiated a pilot protocol for patients with primary gastric cancer in which two courses of protracted infusion 5-FU 200 mg/m²/day for 3 weeks with weekly bolus LV 20 mg/m² and cisplatin 100 mg/m² was given as neoadjuvant chemotherapy prior to surgery (17). In February 1990, the Institutional Review Board at USC approved all patients entering this trial to sign a separate consent for an extra endoscopic biopsy to determine TS expression in fresh-frozen tissue obtained prior to chemotherapy and at the time of operation after chemotherapy was completed. Only biopsies for which gastric cancer was confirmed histologically were analyzed for TS expression. For patients with primary gastric cancer, responses were evaluated by upper gastrointestinal endoscopy and CAT scan of abdomen, pelvis, and thorax (Table 1).

Colorectal Cancer. In March 1993, USC investigators began a protocol to determine if TS gene expression as determined by RT-PCR is associated with response to protracted infusion 5-FU of 200 mg/m²/day for 4 consecutive
Table 1 Demographics from patients with primary gastric cancer

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age/sex</th>
<th>Ethnic</th>
<th>Histology</th>
<th>Biopsy site</th>
<th>TS score</th>
<th>TS:β-actin (× 10³)</th>
<th>IHC</th>
<th>Response</th>
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<tbody>
<tr>
<td>L101</td>
<td>72/M</td>
<td>Cauc</td>
<td>Intestinal</td>
<td>Lymph node</td>
<td>1.010</td>
<td>35.9</td>
<td>D3⁺</td>
<td>No response</td>
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<td>L53</td>
<td>50/M</td>
<td>Asian</td>
<td>Diffuse</td>
<td>Stomach</td>
<td>0.060</td>
<td>18.9</td>
<td>F2⁺</td>
<td>No response</td>
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<td>5-6</td>
<td>56/F</td>
<td>Black</td>
<td>Intestinal</td>
<td>Stomach</td>
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<td>18.7</td>
<td>F2⁺</td>
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<td>Diffuse</td>
<td>Lymph node</td>
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<td>17.4</td>
<td>F2⁺</td>
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<td>Intestinal</td>
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<td>13.2</td>
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<td>Hosp</td>
<td>Intestinal</td>
<td>Stomach</td>
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<td>12.9</td>
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<td>59/M</td>
<td>Asian</td>
<td>Intestinal</td>
<td>Stomach</td>
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<td>3.3</td>
<td>F1⁺</td>
<td>Response</td>
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<tr>
<td>L102</td>
<td>72/M</td>
<td>Cauc</td>
<td>Intestinal</td>
<td>Stomach</td>
<td>0.180</td>
<td>1.9</td>
<td>D1⁺</td>
<td>Response</td>
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<td>L129</td>
<td>75/M</td>
<td>Hosp</td>
<td>Diffuse</td>
<td>Stomach</td>
<td>0.090</td>
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<td>Response</td>
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<td>L127</td>
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<td>Cauc</td>
<td>Signet cell</td>
<td>Stomach</td>
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<td>Response</td>
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<td>L115</td>
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<td>Cauc</td>
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<td>Stomach</td>
<td>0.210</td>
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Table 2 Demographics from patients with disseminated colorectal cancer

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age/sex</th>
<th>Lesion</th>
<th>Previous 5-FU</th>
<th>TS score</th>
<th>TS:β-actin (× 10³)</th>
<th>IHC</th>
<th>Response</th>
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<td>L201</td>
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<td>D2⁺</td>
<td>No response</td>
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<td>68/F</td>
<td>Liver</td>
<td>Yes</td>
<td>0.710</td>
<td>11.6</td>
<td>No response</td>
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<td>L157</td>
<td>55/M</td>
<td>Pelvis</td>
<td>Yes</td>
<td>0.430</td>
<td>9.8</td>
<td>D2⁺</td>
<td>No response</td>
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<td>L196</td>
<td>43/M</td>
<td>Peritoneal</td>
<td>Yes</td>
<td>0.170</td>
<td>8.0</td>
<td>D2⁺</td>
<td>No response</td>
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<tr>
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<td>69/M</td>
<td>Liver</td>
<td>Yes</td>
<td>0.300</td>
<td>2.7</td>
<td>D2⁺</td>
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<td>69/F</td>
<td>Liver</td>
<td>Yes</td>
<td>0.050</td>
<td>1.6</td>
<td>F1⁺</td>
<td>Response</td>
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<td>57/F</td>
<td>Peritoneal</td>
<td>Yes</td>
<td>0.230</td>
<td>1.0</td>
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<td>L137</td>
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<td>0.160</td>
<td>0.5</td>
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<td>Yes</td>
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**Notes:**
- IHC, immunohistochemistry.
- D, Diffuse (>25% of tumor staining); F, Focal (<25% of tumor staining).
- Darker staining; 3+, moderate TS staining; 2+, weak TS staining; 1+, negative TS staining.
- Treatment: 5-FU is administered as an iv bolus. Patients were evaluable for response after two weeks with weekly bolus LV 20 mg/m². Patients entered in this trial were required to have biimensionally measurable disease for response determination. The same lesion used for response measurement was assayed for TS expression. Previous therapy with 5-FU was allowed if the drug had been administered as an iv bolus. Patients were evaluable for response after two cycles of therapy (8 weeks). A coxial system of tumor tissue procurement was used for the patients with disseminated colorectal cancer. This system localizes the tumor by physical palpation or CAT scanner before introducing a stilette through which a fine needle aspirate is taken. The stilette stays in place for the 5–10 minutes it takes for the cytologist to analyze the specimen. If tumor is confirmed, a core needle biopsy through the stilette will then provide adequate tissue for TS quantitation. All patients with colorectal cancer reported in this study were evaluated for response by CAT scan (Table 2).

**Preparation of Tissue Lysates for Western Blotting.** Tissue samples were placed in 100 μl of lysis buffer [300 mM NaCl, 50 mM Tris (pH 7.4) and 1% Triton X-100] with protease inhibitors (0.1 mM phenylmethylsulfonyl fluoride, 3.3 μg/ml aprotinin, and 10 μg/ml of leupeptin, 0.1% chymotrypsin), and homogenized immediately using a Tekmar tissue homogenizer (Cincinnati, OH). The lysates were centrifuged at 14,000 rpm for 15 mm, and the supernatant was separated from the tissue. Protein was measured by the method of Bio-Rad (18).

**Western Blot Analysis.** An equal amount of protein (200 μg) from the tissue lysates were resolved by polyacrylamide gel electrophoresis using 12.5% acrylamide, according to the method of Laemmli (19). The gels were transferred onto a nitrocellulose membrane in transfer buffer (48 mM Tris, 39 mM glycine, and 0.5m EDTA in 20% methanol) for 2 h. The nitrocellulose blots were incubated at room temperature with blocking solution (blotto: 5% nonfat dry milk, 1% Tween 20, 3.3 mM CaCl₂, 3.3 mM MgCl₂, 20 mM Tris (pH 7.5), 150 mM NaCl). After washing, the blots were incubated with mouse MOPC-21 blocking serum was removed by gently blotting the excess serum, and the blots were washed with PBS-Tween and two washes with blotto, secondary antibody (goat anti-mouse horseradish peroxidase; Bio-Rad; 1:2000 in blotto) was applied for 90 min. After four washes with PBS-Tween and two washes with blotto, secondary antibody (goat anti-mouse horseradish peroxidase; Bio-Rad; 1:2000 in blotto) was applied for 1 h. After another four washes with PBS-Tween, the chemiluminescent substrate (luminol, plus enhancer, according to the ECL method of Amersham) was applied for 1 min. Blots were then air-dried, covered by a plastic foil, and exposed to film (Kodak, X-Omat AR) for 5 mm. Densitometry scanning of the film was performed using a Hewlett Packard Scan Jet Plus and analyzed using an image analysis software program (NIH IMAGE v.1.38; provided by Wayne Rasband, National Institute of Mental Health, NIH). A TS score in au was developed by densitometry scanning. Investigators were blinded to the clinical and pathological data.

**TS PCR mRNA Expression**

**PCR Quantitation of TS mRNA.** The isolation of RNA was based on the method of Chomczynski and Sacchi (20). RNA from tumor samples as small as 10 mg were isolated and converted to cDNA using random hexamers. A PCR-based method was used to quantitate the TS gene expression level according to our previous study (16). Gene expression of the β-actin gene was used as an internal standard. In each sample, the linear range of amplification for each cDNA was established. Relative gene expressions were calculated by determining the ratio between the amount of the radiolabeled PCR product within the linear amplification range of the TS gene and the β-actin gene. The method was accurate to less than 2-fold difference in expression levels. The primers listed below were synthesized for the PCR using an Applied Biosys tem model 391 PCR-Mate DNA synthesizer by the phosphoramidite method. PCR condition, T7 RNA polymerase transcription, and the quantitation are described by Horikoshi et al. (16). Each 5' primer had the T7 polynucleotide sequence TAA TAC GAC TCA TTA attached to its 5' end. The primers used were: TS60: GATGTGCGCAATCATGTACGTGAG (bases 697–720 of the TS coding sequence; Ref. 21); TS61: T7- GGAGA/′GGTGGTCG-CACCTGAAGAGTGG (bases 469–492 of the TS coding sequence; ref. 21); BA 67: T7- GGAGA′GGGAAAATCGTGCGTGACAU (bases 2104–2127 of the β-actin genomic sequence, located in exon 3; Ref. 22); and BA 68: GATGGAGATGACATCGTTTCTGT (bases 2409–2432 of the β-actin genomic sequence, located in exon 4; Ref. 22).

**TS Immunohistochemical Labeling.** The avidin-biotin complex immunohistochemical technique was used for detection of TS in the tissue specimens (23). Paraffin-embedded tissue sections 6 μm thick were deparaffinized in xylene, rehydrated through graded alcohols, and placed in PBS. Endogenous peroxidase activity was quenched with 3% hydrogen peroxide in PBS for 10 min. After rinsing for 5 min in tap water, followed by 5 min in PBS, sections were preincubated with 5% blocking serum (horse serum). After 30 min, the blocking serum was removed by gently blotting the excess serum, and the monoclonal antibody TS 106 (10 μg/ml) was applied to the tissue for 45 min. A tissue section from the same tumor was incubated with mouse MOPC-21.
Fig. 1. Western blot analysis of human TS from colorectal and gastric biopsy samples using the TS 106 monoclonal antibody. Lanes 1-9, TS protein analysis in biopsy samples from individual patients. Serial dilutions of NCI H630 and MCF-7 cell lysates were used as internal controls.

IgGI (Sigma Chemical Co., St. Louis, MO) for a similar period as a negative control for each tumor section. After rinsing the tissue sections twice in PBS for 10 min, 100 μl of biotin-conjugated goat anti-mouse secondary antibody was added for 30 min. The tissue sections were again rinsed in PBS for 10 min and incubated with avidin-biotin complex (Vector Laboratories) for 30 min. After a final rinse in PBS for 10 min, the tissues were incubated with diaminobenzidine substrate (0.02% diaminobenzidine and 0.005% hydrogen peroxide) for 15 min. The tissues were then rinsed in distilled H2O and counterstained in hematoxylin 100% (Gill 11 formula) for 3 min. The tissue sections were subsequently dehydrated in ethanol, cleared in xylene, and mounted with glass coverslips using permount. A positive control consisting of a formalin-fixed paraffin-embedded colon cancer cell line NCI H630 was included in each batch of tissues stained.

Tissue Evaluation. The slides were examined and scored without knowledge of clinical data, PCR, or Western analysis. TS expression was quantitated using a visual grading system based on the intensity of staining (0–3) and on whether the staining pattern was focal or diffuse.

Statistical Analysis. Spearman rank correlation analysis was used to assess the correlation between TS protein and TS:β-actin ratio. The Wilcoxon rank sum test was used to evaluate the relationship between TS protein and TS gene levels among responders and nonresponders. All P values are two sided.

Results

Protein and Gene Expression. TS protein and TS gene expression were compared within the same biopsy sample to determine if the level of TS protein correlated with the TS mRNA expression (Fig. 1 and 2). The TS protein densitometry score ranged from 0.05 to 1.01 au (20-fold) in the 21 biopsy samples. The TS:β-actin ratios ranged from 0.5 to 35.9 × 10⁻³ (70-fold, Tables 1 and 2). Within these biopsies, a close linear relationship was noted between the level of TS protein and TS gene expression [r² = 0.6 (P = 0.009); Fig. 3]. We also performed TS immunohistochemistry on paraffin-embedded tissue sections, which were available from 15 of these 21 biopsy samples; in the 6 remaining specimens, insufficient tissue remained for immunohistochemistry (Fig. 4). The TS staining intensity correlated closely with the TS densitometry score and TS:β-actin ratios.

Fig. 2. RT-PCR product formed as a function of serial dilutions of (A) TS cDNA and (B) β-actin cDNA. Aliquots of a cDNA dilution from HT-29 cells were PCR amplified for β-actin and for TS as outlined in “Materials and Methods.” The RNA was electrophoresed on a 6% polyacrylamide gel, and the dried gels were exposed to a Kodak XAR5 film. The fragments were excised and counted in a liquid scintillation counter. The insets show the linear region of the curves.
levels and TS gene expression did not correlate in two biopsy samples (L53 and L196); however, in one of these (L53), the pattern of TS expression exhibited a focal rather than a diffuse staining pattern (Tables 1 and 2). In samples L220 and L196, no tumor immunostaining was detected, despite a moderate amount of TS protein and TS mRNA expression detected by Western blotting.

Overall, both TS protein level and TS gene expression were significantly associated with response to 5-FU-based therapy. Those patients with responsive disease had a mean TS protein level of 0.17 ± 0.03 au (range, 0.05 to 0.38), whereas in patients whose tumors were unresponsive to chemotherapy, the mean TS protein level was significantly higher (0.60 ± 0.09 au; range, 0.06 to 1.01; P < 0.01; Fig. 5). A similar pattern was noted with TS:β-actin gene expression. In patients with responsive disease, the mean TS:β-actin gene ratio was 1.36 ± 0.3 (range, 0.5–3.3 × 10^-3). By comparison, in biopsy samples from patients with unresponsive disease, the mean TS gene ratio was 15.4 ± 2.6 × 10^-3 (range, 2.7–35.9; P < 0.01; Fig. 6). Thus, both TS gene and TS protein levels are significantly higher in tumor biopsy samples from patients with tumors that are unresponsive to these 5-FU-based therapies. The data from patients with primary gastric and metastatic colorectal cancers are presented separately below.

**Patients with Primary Gastric Cancer.** Biopsies were obtained from 12 patients with primary gastric cancer; 10 patients had biopsies taken preoperatively, and the remaining 2 had posttreatment biopsies only (Table 1). Six patients with TS protein scores ranging from 0.09 to 0.38 au (mean, 0.18 ± 0.04) and TS:β-actin ratios from 0.9 to 3.3 × 10^-3 (mean 1.75 ± 0.34) demonstrated a response to chemotherapy either by CT scan, gastrointestinal endoscopy, or both. Patients who did not respond to chemotherapy had significantly higher TS protein scores (0.06 to 1.01 au; mean, 0.68 ± 0.15) and TS:β-actin ratios between 12.9 to 35.9 × 10^-3 (mean 19.5 ± 3.45 × 10^-3). In one gastric tumor sample (L53) in which the TS:β-actin ratio and the TS protein score were discordant, a focal nature to the TS staining intensity was noted by immunohistochemical staining. From one patient (L101), we were able to quantitate the TS protein score and relative TS gene expression in a supraclavicular lymph node. Of interest, the primary tumor from the same patient (L102) had a low level of TS gene and protein expression and responded to the chemotherapy, whereas the lymph node metastasis which had the highest level of TS protein and gene expression progressed despite chemotherapy.

![Fig. 3. Correlation of TS:β-actin ratio to the TS protein score in biopsy samples of colorectal and gastric cancer.](image-url)

![Fig. 4. Immunohistochemical staining in a gastric biopsy tumor specimen with high TS protein and gene expression.](image-url)
Patients with Disseminated Colorectal Cancer. Table 2 describes the measurable lesions of the nine patients with disseminated colorectal cancer. The four responding patients had TS protein scores ranging from 0.05 to 0.23 au (mean, 0.16 ± 0.04 au); three of these patients had TS:β-actin ratios from 0.5–1.6 × 10⁻³ (mean, 0.8 ± 0.34); one responding patient did not have a detectable TS:β-actin ratio. In contrast, tumors from patients who had progressive disease and did not demonstrate a response to 5-FU/LV chemotherapy had protein scores ranging from 0.17–0.71 au (mean, 0.46 ± 0.10 au) and TS:β-actin ratios from 2.7–19.6 × 10⁻³ (mean, 10.3 ± 2.7 Table 2).

Discussion

Thymidylate synthase is an important therapeutic target for the fluoropyrimidines. In this study, we have compared TS protein and TS gene expression in biopsy specimens from patients with metastatic colorectal and primary gastric cancers treated with 5-FU-based chemotherapy regimens. We have demonstrated that the level of TS protein and gene expression varies widely, up to 70-fold, between different tumors. In tumor biopsy samples, a close correlation was noted between the level of TS protein detected by Western immunoblot and immunohistochemical analysis and the level of TS mRNA expression measured by RT-PCR analysis. In several samples where expression of TS protein and TS mRNA did not correlate, there was evidence of heterogeneity of TS protein expression in the tissue section. It is possible that the observed differences in TS gene and TS protein expression were due to measurement of TS protein from tissue sites distant to those analyzed for TS mRNA expression.

This study is the first to compare TS protein and gene expression and to examine the heterogeneity of TS expression in the same tumor biopsy sample. Until recently, the measurement of TS has been performed using radioenzymatic assays (13). These assays are generally performed on cytosolic extracts using relatively large tissue biopsy samples. They do not discriminate between areas of tumor with differing morphologies or measure TS on a cell-by-cell basis because cellular specificity is lost. Since tissues are a composite of a heterogeneous population, any measurement of TS is confounded by the degree of contamination by cells other than those of interest. Moreover, biochemical assays are limited to prospective studies requiring fresh or fresh-frozen tissue. A previous study from this laboratory used the FdUMP-binding assay to quantitate TS levels in human breast tumor samples. This required greater than 50 mg of tumor to reliably detect the enzyme, and, in one-third of the biopsy samples, TS protein was undetectable (14). The development of quantitative immunological and PCR methods for the detection of TS represents an advance because both techniques are highly sensitive and can be applied to clinical specimens in both a prospective and retrospective fashion (15,16). These techniques have facilitated the measurement of TS protein and TS mRNA from the same tissue sample and allowed comparisons with morphological features to be made.

In this study, pretreatment TS protein level and TS gene expression predicted for response to 5-FU/LV (metastatic colorectal cancer) or 5-FU/LV/cisplatin therapy (primary gastric cancer). Those tumors with high levels of TS protein and TS gene expression tended to be unresponsive to therapy, whereas tumors with low TS protein and gene expression had more responsive disease. In one patient with low TS protein and gene expression levels, no response to therapy was noted. Other determinants of 5-FU responsiveness, such as the ability to convert 5-FU to its active metabolites or incorporation into RNA, may have played a role in the unresponsiveness of this tumor.

Although these clinical correlations are based on small numbers of patients and are preliminary in nature, they suggest that the ability to predict response and outcome based on TS expression in the primary tumor, prior to or early in the course of treatment, may provide the opportunity to select patients who are most likely to benefit from 5-FU-based therapy and the possibility to offer alternative regimens to those who will not. Thus, patients might be selected for 5-FU regimens based upon sensitivity of the tumor as assessed by their pretreatment TS levels. However, before clinical decisions are made on the basis of pretreatment TS protein or gene expression, many more patients will need to be analyzed.

This study has demonstrated that the level of expression of TS protein is closely correlated to the level of TS mRNA expression within individual tumors. Lower levels of TS protein or gene expression appear to predict for tumor sensitivity. Conversely, higher levels of either may predict for relative tumor resistance. These data indicate that either TS protein or TS gene expression may be used in future clinical trials to prospectively determine the influence of TS quantitation on the response of fluoropyrimidine-based therapy. The association of TS immunostaining with protein levels and gene expression

![Fig. 5.](#) The correlation of TS protein level and response in patients with colorectal and gastric cancer treated with 5-FU/LV-based chemotherapy (P < 0.01). Dotted line, the mean TS protein level in responders and nonresponders.

![Fig. 6.](#) The correlation of TS:β-actin gene ratio and response in patients with colorectal and gastric cancer treated with 5-FU/LV based chemotherapy (P < 0.01). Dotted line, the mean TS:β-actin gene ratio level in responders and nonresponders.
will need further confirmation because this technique could have an important role in the future design of preoperative and postoperative adjuvant strategies for patients with fluoropyrimidine-sensitive tumors. Finally, the ability to quantitate TS levels in patient samples pre- and posttherapy will aid in determining the role of TS in drug resistance and may lead to improved therapeutic strategies at inhibiting this critical enzyme.

References
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