ABSTRACT

Thymidylate synthase (TS) (EC 2.1.1.45) is an important cellular target for the fluoropyrimidines cytidylic acid (5-FU) drugs that are widely used in the treatment of solid tumors. Using the TS 106 monoclonal antibody to human TS, we have compared the immunological quantitation of TS by Western immunoblot and immunofluorescent techniques to the conventional biochemical 5-fluorodeoxyuridine monophosphate binding assay in a panel of 5-fluorouracil (5-FU)-sensitive and -resistant human cancer cell lines.

Densitometric quantitation of TS 106-labeled Western immunoblot analysis of cell lysates from two 5-FU-resistant colon carcinoma cell lines, NCI H630R1 and NCI H630R10, revealed 12.8- and 16-fold increases in TS, respectively, compared to the parent 5-FU-sensitive NCI H630 colon cell line. By biochemical analysis, the TS level was 15- and 23-fold higher, respectively, in these resistant cell lines. Similarly, immunoblot analysis of cell lysates from two 5-FU-resistant breast cancer cell lines, MCF-Ad5 and MCF-Ad10, detected a 2.3- and 6.3-fold increase in TS, respectively, compared to the parent MCF-7 cell line. By biochemical assay the TS activity was 1.8- and 7.0-fold higher in these resistant breast cell lines. Western immunoblotting analysis revealed a 35-fold range of TS protein concentrations among the 10 cell lines examined, compared to a 38-fold range demonstrated by the biochemical assay. Direct comparison of Western blotting and the biochemical assay revealed a highly significant correlation (r² = 0.93) between the two assays. Moreover, using the monoclonal antibody TS 106, the Western blotting technique was capable of detecting TS protein levels as low as 0.3 fmol in cellular lysates.

Quantitation of TS in intact cells by immunofluorescent TS labeling and flow cytometric analysis was performed using three of the cell lines, NCI H630, NCI H630R1, and MCF-Ad10. This revealed a 26-fold increase in TS in the 5-FU-resistant NCI H630R10 line compared to the parent NCI H630 line and a 3.5-fold increase in TS compared to the 5-FU-resistant MCF-Ad10 breast cell line. The 5-FU-resistant MCF-Ad10 breast cell line, in turn, displayed a 7.7-fold increase in TS, compared to the 5-FU-sensitive NCI H630 cell lines. TS immunofluorescent analysis was capable of measuring TS within individual cells.

The development of these immunological assays using an anti-TS monoclonal antibody will facilitate the quantitation of TS in cell lines and tissue samples.

INTRODUCTION

TS² (EC 2.1.1.45) catalyzes the methylation of dUMP to dTMP, an essential step in DNA biosynthesis (1, 2). TS is also a critical target for the fluoropyrimidines drugs that are widely used in the treatment of gastrointestinal tumors, breast tumors, and epithelial tumors of the upper aerodigestive tract (3–6). Both 5-FU and fluoro(deoxy)uridine are converted to fluorodeoxyuridine monophosphate; 5-FU, 5-fluorouracil; ABC, avidin-biotin complex.

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1 To whom requests for reprints should be addressed, at National Cancer Institute, NMOB 8/5101, Bethesda, MD 20892.

2 The abbreviations used are: TS, thymidylate synthase; PBS, phosphate-buffered saline; ICC, 50% inhibitory concentration; FdUMP, 5-fluorodeoxyuridine monophosphate; 5-FU, 5-fluorouracil; ABC, avidin-biotin complex.
We have recently developed several monoclonal antibodies to human TS that are highly specific and detect TS in the cytoplasm of human cells and tissues (24). A monoclonal antibody-based quantitative immunological assay to measure TS in human cells and tissues would have the advantage of being a convenient method utilizing small samples of cell lysate or thin sections of tissue. It would facilitate the study of primary and metastatic tumor samples, allow TS to be measured on a per-cell basis, and facilitate detailed correlations being made between the level of TS and various clinical and morphological parameters. Thus, the application of antibodies in the development of a quantitative TS immunological assay would help determine whether the quantity of TS within a given tumor provides information that is valuable in patient prognosis and treatment.

In the present study, we describe the quantitation of TS by immunoblotting and immunofluorescent techniques, in 5-FU-resistant and -sensitive human cancer cell lines using anti-TS antibody TS 106. The sensitivity and accuracy of these immunological techniques for measuring TS levels are evaluated by comparison to a standard biochemical assay.

MATERIALS AND METHODS

Cell Culture. The characteristics of the human colon cancer cell lines HCT 116, NCI H630, SNU-C4, and SNU-C1 have been described previously (25, 26). The resistant colon sublines NCI H630R1 and NCI H630R10 were selected for resistance to 5-FU in vitro by exposure to stepwise increases in 5-FU. The origins of the human breast cancer MCF-7 cell line and the cross-resistant 5-FU-resistant MCF-Ad5 and MCF-Ad10 lines have previously been reported (27, 28). The gastric cell line AGS was obtained from the American Type Culture Collection (Rockville, MD), and its characteristics have been previously reported (29). All cells were maintained in RPMI 1640 medium (Biofluids Inc., Rockville, MD) with dialyzed fetal calf serum (GIBCO Inc., Grand Island, NY) plus 2 mm glutamine and were grown in 75-cm² plastic culture flasks (Falcon Labware, Oxnard, CA). Dextran (clinical grade), bovine albumin fraction V, 5-FU, and acid-washed activated charcoal were purchased from Sigma Chemical Co. (St. Louis, MO). [6-3H]FdUMP (specific activity, 18 Ci/mmol) was obtained from Moravek Biochemicals (Brea, CA), and chemiluminescent Western blotting kits were obtained from Tropix (Bedford, MA). ABC immunohistological kits and phycoerythrin were purchased from Vector Laboratories (Burlingame, CA). All other chemicals were obtained from Sigma or the NIH stores (Bethesda, MD).

5-FU Sensitivity Studies. An equal number of cells (5 × 10⁶ cells/ml) from each cell line were plated onto 75 flasks (Falcon Labware) and incubated at 37°C in RPMI 1640 medium with dialyzed fetal calf serum. After 24 h, various concentrations of 5-FU were added to each flask. Phosphate-buffered saline was added to control flasks. The cells were incubated with 5-FU for 72 h at 37°C and subsequently trypsinized and counted using a Coulter counter (Coulter Electronics, Hialeah, FL). The 5-FU IC₅₀ values were determined for each cell line by using a curve of cell number versus logarithm of drug concentration.

Western Blot Analysis. Cells in the exponential phase of growth were washed twice with PBS, harvested, and resuspended in 0.1 M KH₂PO₄, pH 7.4. Cell lysis was accomplished by sonication, using three 2- to 3-s bursts from a Branson sonifier equipped with a microtip. The cellular extracts were centrifuged at 5000 x g for 30 min, and protein concentrations were determined using the Bio-Rad protein assay (30). Equivalent amounts of protein, 100 μg, from each cellular lysate were resolved by polyacrylamide gel electrophoresis using 15% acrylamide gels, according to the method of Laemmli (31). The gels were electroblotted onto nitrocellulose membrane (Scherleicher and Schuell, Keene, NH) in transfer buffer (48 mM Tris, 39 mM glycine, 0.5 mM EDTA in 20% methanol) for 2 h. Immunoblotting was carried out using the monoclonal antibody TS 106 (24) and the chemiluminescence detection method (Tropix). All blots were treated as follows. Briefly, the nitrocellulose blot was incubated with blocking buffer (0.2% Trox 1-block reagent, 0.1% Tween 20, 0.02% sodium azide, in PBS) for 1 h, washed in wash buffer (0.1% Tween 20, 0.02% sodium azide, in PBS), and incubated with the mouse monoclonal antibody TS 106 (10 μg/ml) for 2 h at room temperature. The blot was subsequently washed for 30 min in wash buffer and incubated with an alkaline phosphatase-conjugated secondary antibody (1/10,000 dilution in 0.1% Tween 20, 0.02% sodium azide, in PBS). After four additional washes in wash buffer and two in 0.1 M diethanolamine, 1 mM MgCl₂, 0.02% sodium azide buffer, the acid phosphatase- and disodium methyloxypyrophosphatase, tricyclohexyl-phenolphosphate (0.24 mM), in assay buffer, was added. The positive TS bands were identified by exposure to Kodak XAR-2 film for 15 min at room temperature.

Quantitation of TS Protein from Autoradiographs. The level of TS protein was determined by densitometric scanning of the autoradiographs, using a HP Scan Jet digital imager and an image analysis software program (NIH IMAGE version 1.38; provided by Wayne Rasband, NIMH, NIH). All densitometric readings were normalized to the colon cell line HCT 116, which was used as an internal standard on each immunoblot. The densitometry measurements were made “blinded” to the biochemical data for each cell line.

Thymidylate Synthase Binding Assay. Human cell lines in logarithmic phase of growth were washed with PBS twice, harvested, and resuspended in 1 ml of 0.1 M KH₂PO₄, pH 7.4. Cell lysis was carried out as described above. The cellular extract was centrifuged at 5000 x g for 30 min, and the supernatants were collected and assayed by previously published methods (17, 18). The assay was performed in a total volume of 200 μl containing 75 μM 5,10-methylene tetrahydrofolate, 3 pmol [3H]FdUMP, 100 μM 2-mercaptoethanol, and 50 μM KH₂PO₄, pH 7.4. Samples were incubated at 37°C for 30 min, and subsequently 1 ml of an albumin-coated charcoal slurry, pH 7.2 (prepared by mixing 10 g of acid-washed activated charcoal with 2.5 g of bovine albumin, 0.25 g of dextran, and 100 ml of ice-cold water), was added. The mixture was vortexed, allowed to stand at room temperature for 10 min, and then centrifuged for 30 min at 3000 × g. The residual radioactivity, representing enzyme-bound FdUMP, in the supernatant was counted by liquid scintigraphy (17). All assay results were standardized for cytosolic protein and expressed in picomoles per milligram of cytosolic protein.

Thymidylate Synthase Immunofluorescence. Human carcinoma cells were grown in T75 flasks (Falcon Labware) in medium containing 10% dialyzed fetal calf serum and were harvested during the exponential part of their growth phase. The cells were washed twice with PBS, transferred to Eppendorf tubes, and pelleted at 500 × g. The cells were then fixed in ethanol at 4°C for 10 min, subsequently washed in PBS/0.1% Tween for 10 min, and incubated with 100 μl of blocking serum (2% horse serum in PBS) for 30 min, to reduce nonspecific staining. The cells were then labeled with TS 106 (10 μg/ml, 100 μl) or with a nonspecific mouse IgG1, for 2 h at room temperature. After rinsing in PBS/0.1% Tween for 10 min, 100 μl of avidin biotin-conjugated antimouse secondary antibody were added for 30 min. The cells were then rinsed in PBS/0.1% Tween, followed by incubation with phycoerythrin-conjugated ABC complex (Vector) for 30 min (32, 33). After two final washes in PBS/0.1% Tween, the cells were resuspended in 100 μl of PBS. Fluorescence analysis was performed using a FACStar flow cytometer (Becton Dickinson, Mountain View, CA) interfaced with a Consort 30 data acquisition and analysis system and by direct visualization using a fluorescent light microscope (Olympus).

RESULTS

A 300-fold range of 5-FU sensitivity was detected in the cell lines examined (Table 1), with IC₅₀ values for growth inhibition ranging from 0.35 to 105 μM for the 24-h exposures to 5-FU. The human colon cancer cell lines HCT 116, NCI H630, SNU-C₁, and SNU-C₄ were the most sensitive of the lines tested, with IC₅₀ values ranging from 0.35 to 3.5 μM. In contrast, the colon cell lines NCI H630R₁ and NCI H630R₁₀, which were selected
The 5-FU IC₅₀ values were determined and the FdUMP binding and TS immunoblot densitometry were performed for each cell line as described in “Materials and Methods.” The results represent the mean ± SE of at least five separate experiments.

Table 1  5-FU IC₅₀ values and TS levels, by FdUMP binding assay and immunoblot densitometry, for human cancer cell lines

<table>
<thead>
<tr>
<th>Cell line</th>
<th>5-FU IC₅₀ (µM)</th>
<th>FdUMP binding assay (pmol/mg cytosol protein)</th>
<th>Immunoblot densitometry (arbitrary units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCI H630</td>
<td>3.0</td>
<td>0.31 ± 0.05</td>
<td>2.0 ± 1.4</td>
</tr>
<tr>
<td>NCI H630R₁</td>
<td>52.0</td>
<td>4.6 ± 0.9</td>
<td>26.8 ± 6.0</td>
</tr>
<tr>
<td>NCI H630R₁₀</td>
<td>105.0</td>
<td>6.8 ± 1.12</td>
<td>31.7 ± 9.3</td>
</tr>
<tr>
<td>MCF-7</td>
<td>1.0</td>
<td>0.27 ± 0.17</td>
<td>1.8 ± 0.3</td>
</tr>
<tr>
<td>MCF-Ad₅</td>
<td>15.0</td>
<td>0.49 ± 0.05</td>
<td>3.1 ± 0.6</td>
</tr>
<tr>
<td>MCF-Ad₁₀</td>
<td>25.0</td>
<td>2.1 ± 0.12</td>
<td>8.2 ± 2.9</td>
</tr>
<tr>
<td>HCT 116</td>
<td>1.0</td>
<td>0.22 ± 0.02</td>
<td>1.2 ± 0.4</td>
</tr>
<tr>
<td>SNU-C₄</td>
<td>2.5</td>
<td>0.55 ± 0.14</td>
<td>2.4 ± 1.6</td>
</tr>
<tr>
<td>SNU-C₁</td>
<td>0.35</td>
<td>0.18 ± 0.05</td>
<td>0.9 ± 0.02</td>
</tr>
<tr>
<td>AGS</td>
<td>18.0</td>
<td>1.1 ± 0.3</td>
<td>8.9 ± 2.1</td>
</tr>
</tbody>
</table>

Fig. 1. Relationship of the TS level (pmol/mg cytosol protein) measured by the FdUMP binding assay to the IC₅₀ for growth inhibition, in 10 malignant human cell lines exposed for 72 h to 5-FU. These results are the mean ± SE of at least five separate experiments.

in vitro for 5-FU resistance, had IC₅₀ values of 52 µM and 105 µM, respectively (Table 1). The breast cancer cell lines MCF-Ad5 and MCF-Ad10, which were selected for Adriamycin resistance, were also relatively cross-resistant to 5-FU, with IC₅₀ values of 15 µM and 25 µM, respectively, compared with the parent MCF-7 cell line (IC₅₀ = 1 µM).

The TS activity detected by the biochemical assay ranged from 0.18 to 6.8 pmol/mg cytosolic protein (38-fold) in the cell lines examined (Fig. 1). In the 5-FU-resistant NCI H630 colon and MCF-7 breast cell lines, a significantly higher level of TS activity was detected by the biochemical assay compared with the resistant NCI H630R and H630R₁₀ colon cell lines (Table 1). Furthermore, in the resistant NCI H630R₁₀ cell line, the TS levels measured by immunoblot analysis were 12.8- and 16-fold higher, respectively, than those in the parent H630 cell line (Table 1). In an effort to demonstrate that immunoblot detection of TS is an accurate method of quantitating human TS, a direct comparison of the Western blotting densitometry measurements and the biochemical FdUMP binding measurements was performed. Among the 10 cell lines examined, a highly significant correlation (r² = 0.93, P = 0.0001) was found between these two assays (Fig. 3).

The sensitivity of TS Western blot analysis was also studied by addition of decreasing amounts of cytosolic protein from the NCI H630R₁₀ cell line into each lane and electrophoresis under denaturing conditions. Using the chemiluminescent Western blotting technique, it was possible to detect a specific M₉, 36,000 signal that was easily quantifiable, above background, down to 0.3 fmol of TS (Fig. 4).

TS Immunofluorescence. We have previously demonstrated, using the monoclonal antibody TS 106 and immunocytochemical techniques, that it is possible to detect TS in the cytoplasm of intact cells that have been fixed in ethanol (24). In an effort to demonstrate that TS immunolabeling in intact cells corresponds to TS activity detected by the FdUMP binding and a 15% polyacrylamide gel and electrophoresed under denaturing conditions. In experiments using a chemiluminescent detection system, the monoclonal antibody TS 106 specifically detected a peptide of M₉, 36,000 in each of the cellular extracts and displayed negligible cross-reactivity with other peptides. This M₉, 36,000 protein was easily visible in all the cell lines examined; however, in those cell lines that were 5-FU resistant a marked increase in the intensity of TS staining was visible (Fig. 2). Compared to the parent MCF-7 breast cancer cell line, densitometric analysis revealed a 2.3- and 6.3-fold increase in TS activity in the MCF-Ad5 and MCF-Ad10 cell lines, respectively (Table 1). Furthermore, in the resistant NCI H630R₁₀ cell line, the TS levels measured by immunoblot analysis were 12.8- and 16-fold higher, respectively, than those in the parent H630R₁₀ cell line (Table 1). This A/r 36,000 protein was easily visible in all the cell lines examined; however, in those cell lines that were 5-FU resistant a marked increase in the intensity of TS staining was visible (Fig. 2). Compared to the parent MCF-7 breast cancer cell line, densitometric analysis revealed a 2.3- and 6.3-fold increase in TS activity in the MCF-Ad5 and MCF-Ad10 cell lines, respectively. Furthermore, in the resistant NCI H630R₁₀ cell line, the TS levels measured by immunoblot analysis were 12.8- and 16-fold higher, respectively, than those in the parent H630 cell line (Table 1). In an effort to demonstrate that immunoblot detection of TS is an accurate method of quantitating human TS, a direct comparison of the Western blotting densitometry measurements and the biochemical FdUMP binding measurements was performed. Among the 10 cell lines examined, a highly significant correlation (r² = 0.93, P = 0.0001) was found between these two assays (Fig. 3).

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Western blotting analyses in cell lysates, we analyzed intracellular TS levels in intact cells using immunofluorescence labeling. Three cell lines (NCI H630, MCF-Ad10, and NCI H630R10) that reflect the range of values detected by both the Western blotting and radiolabeled FdUMP binding assays were analyzed. On direct fluorescence microscopic examination, the fluorescence signal appeared to be predominantly located in the cytoplasm of cells (Fig. 5). The signal appeared specific for TS, inasmuch as it was not observed with nonspecific mouse IgG, and a marked reduction in the TS fluorescence occurred when the antibody was preabsorbed with purified recombinant human TS.

The fluorescence intensity was also analyzed using a flow cytometer, which measures the TS fluorescence intensity in individual cells. We found a wide range of fluorescence intensity (50-fold) within the cellular population of each of the individual cell lines. The mean TS fluorescence intensity was highest in the 5-FU-resistant NCI H630R10 colon carcinoma cell line. In this cell line the range of TS staining varied from 6.0 to 4517 fluorescence units, with a mean TS fluorescence intensity of 351.7 units. In addition, TS immunofluorescence appeared to define two separate populations of cells within this cell line, one with a lower TS staining intensity (range, 6–18; mean, 9.2 fluorescence units) and the other with a much greater TS staining intensity (range, 25–4517; mean, 351.7 fluorescence units) (Fig. 6). In comparison to the 5-FU-resistant NCI H630R10 cell line, the 5-FU-sensitive parent line, NCI H630, displayed a much lower mean TS fluorescence intensity (range, 5.4–160; mean, 12.9 units). In the MCF-Ad10 breast cancer cell line the TS fluorescent intensity ranged from 24 to 373.72 units, with a mean intensity of 99.01 units. Thus, the mean TS immunofluorescence intensity in the 5-FU-resistant NCI H630R10 cell line was 26-fold higher compared to the parent NCI H630 line and 3.5-fold higher compared to the 5-FU-resistant MCF-Ad10 breast cell line. The 5-FU-resistant MCF-Ad10 breast cell line, in turn, displayed a 7.7-fold higher TS fluorescence intensity, compared to the 5-FU-sensitive NCI H630 cell line (Fig. 6). These differences correlated closely with the relative differences obtained in both the biochemical and Western blot analyses. These data suggest that the signal generated using fluorescence intensity represents a reliable quantitation of TS and is consistent with the level of TS detected by conventional biochemical assays.

Fig. 4. Western blot analysis of human TS from NCI H630R10, using TS 106. Cytosolic extracts from cells in the exponential phase of growth were prepared as described in “Materials and Methods.” Decreasing amounts of protein were loaded into each well, electrophoresed on 15% sodium dodecyl sulfate-polyacrylamide gels, transferred, and incubated with TS 106. Western blot was performed using the chemiluminescence method. The specific amount of TS added to each lane was determined by serial dilutions of total cytosolic protein, in which total TS per milligram of cytosol was quantitated using the FdUMP binding assay.

DISCUSSION

Thymidylate synthase is an important cellular enzyme that plays a critical role in DNA nucleotide precursor synthesis and represents an important therapeutic target for the fluoropyrimidine antineoplastic agents (3–6). In the present study, we have described the use of the monoclonal antibody TS 106 to detect and quantitate TS protein by Western blotting analysis and immunofluorescence cell scanning, compared with results obtained using a standard biochemical assay. Moreover, the TS enzyme was generally increased in 5-FU-resistant cell lines. This association was apparent using quantitative immunanalysis and was confirmed using biochemical methods.

Among the 5-FU-sensitive and -resistant cell lines studied, we have shown that an association exists between the level of TS detected by biochemical and Western immunoblot analysis using TS 106 ($r = 0.93$) (Fig. 2). In addition, we have demonstrated that Western immunoblotting is sensitive and capable of detecting TS down to 0.3 fmol (Fig. 3). Thus, the immunological detection of TS appears to be an accurate method for quantitating TS and appears to have improved sensitivity, compared with the biochemical assay. The sensitivity of the immunoassay appears to be approximately 20-fold greater than that reported for the FdUMP binding assay (23). Thus, the immunoassays may be applied to cells and tissues where the TS activity is low or where only small amounts of tissue are available, such as samples obtained by needle biopsy or aspiration. The ability of immunoblotting analysis to demonstrate significant differences in TS levels between 5-FU-sensitive and -resistant cell lines suggests that the immunological quantitation of this enzyme may be useful for the identification of patient subgroups with tumors that may respond poorly to 5-FU-based chemotherapeutic regimens. TS quantitation may also be used to determine the role of TS in the development of tumor resistance.
To demonstrate that the immunological quantitation was applicable to TS measurement in intact cells, as well as cell lysates, we compared immunofluorescence labeling using TS 106 with Western blot analysis and the biochemical assay. Immunofluorescent TS labeling of cells revealed a distinct cytoplasmic fluorescence pattern. The fluorescence intensity appeared to increase in proportion to the level of TS within the various cell lines examined (Fig. 5). When the cellular fluorescence intensity was measured by FACStar flow cytometric analysis, a wide range of TS staining was apparent within the cellular population of each individual cell line. Both the mean TS fluorescence staining intensity and the range of TS fluorescence levels appeared to be greater in the 5-FU-resistant cell lines. Moreover, the 26- and 7.7-fold increases in TS levels detected by immunofluorescence in the resistant colon and breast cell lines, respectively, paralleled increased TS levels detected by Western immunoblotting and the FdUMP binding assays (Fig. 6). Thus, by using this immunofluorescence technique it is possible to quantitate TS in individual tumor cells and to measure the range of TS expression found within tumor cell populations.

In biochemical assays performed using intact cells or cell-free systems, cellular specificity is not maintained. The biochemical assays do not discriminate between areas of the tumor with differing levels of TS, nor do they measure TS on a cell-to-cell basis, and, because tissues and cell preparations are a composite of a heterogeneous population, any measurement of TS enzyme is confounded by the degree of contamination by cells other than those of interest. In contrast, these sensitive immunologically based assays may facilitate accurate quantitation of TS expression on a cell-by-cell basis, rather than an average expressed by a heterogeneous population of cells. Detailed correlations between the level of TS and various clinical and morphological parameters may be possible using these immunologically based assays.

The biochemical significance of the TS enzyme is related to its role in the cell cycle and its clinical importance as a mechanism of drug resistance. The level of TS is very low in resting nondividing cells but increases about 20-fold as cells enter late-G1-early-S phase of the cell cycle (11–13). To date, studies of cell cycle kinetics have relied on the measurement of S phase fractions, using tritiated thymidine labeling techniques. These studies have improved our understanding of neoplastic transformation and are important predictors of disease-free interval and survival in diseases such as breast cancer and colon cancer (34–37). However, for routine clinical application, these methods are tedious and require in vivo administration of radioactively labeled compound, as well as multiple serial tissue
biopsies. The ability to quantitate changes in TS in relation to the cell cycle by immunofluorescent flow cytometry techniques may serve as a surrogate in the determination of S phase fractions and may prove useful in studies of cellular division and proliferation. This information may provide insights into the biological behavior of solid tumors and provide a more precise approach to predicting their clinical outcome.

The clinical importance of TS in the development of tumor resistance has been suggested by studies that have demonstrated that the acute induction of TS proteins, as well as stable amplification of TS-specific genes, may be associated with 5-FU resistance (14–16, 18–20). Our findings are consistent with previous reports demonstrating that the level of TS enzyme measured by both biochemical and immunological assays is relatively higher in 5-FU-insensitive cell lines. The clinical significance of acute TS induction is suggested by a previous study of serial tumor biopsy samples obtained from patients with advanced breast carcinoma. This study demonstrated a 2.6-fold increase in TS 24 h following fluorouracil therapy (17). While the underlying mechanisms involved in this enhanced TS expression have not yet been fully defined, these in vivo and in vitro studies suggest that the ability of a tumor to overexpress TS acutely in response to cytotoxic agents may play a role in the development of tumor resistance. Since these studies suggest that the level of TS protein may correlate with response to cytotoxic therapy and, ultimately, to prognosis and survival, the availability of sensitive quantitative immunological methods for detecting TS may permit a determination of the prognostic importance of TS levels in patient tumor samples.

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REFERENCES

TS QUANTITATION WITH THE MONOCLONAL ANTIBODY TS 106

Immunological Quantitation of Thymidylate Synthase Using the Monoclonal Antibody TS 106 in 5-Fluorouracil-sensitive and -resistant Human Cancer Cell Lines

Patrick G. Johnston, James C. Drake, Jane Trepel, et al.


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