Colorectal Liver Metastasis Thymidylate Synthase Staining Correlates with Response to Hepatic Arterial Floxuridine

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ABSTRACT

We assessed whether intensity of colorectal liver metastasis staining with the thymidylate synthase (TS) antibody TS106 predicted response to hepatic arterial infusion (HAI) of floxuridine chemotherapy.

Liver metastasis biopsies were taken during laparotomy for hepatic arterial cannulation and stained using the TS106 monoclonal antibody. Staining intensity was designated at histological examination by two independent assessors as either “high” or “low.” Patients were treated by HAI, and liver metastasis response was assessed by comparison of computed tomography scan tumor volume before and after 4 months of treatment.

A significant correlation (Fisher’s exact test, $P = 0.01$) was noted between partial response to HAI and TS106 staining intensity in patients with colorectal liver metastases. Seventy-five percent of patients with evidence of a partial response had low TS staining compared with 29% of non-responders. There was a significant difference (Fisher’s exact test, $P = 0.01$) in the proportion of low (9 of 16) compared with high (3 of 20) TS staining tumors in which a partial response occurred. There was no significant difference (logrank test, $P = 0.4$) in survival from hepatic cannulation and HAI treatment of high (median, 322 days; interquartile range, 236–411) compared with low (median, 335 days; interquartile range, 301–547) TS staining patients.

This study demonstrates an inverse correlation between TS immunohistochemical staining intensity in colorectal liver metastases and response to HAI. The results suggest that a prospective assessment of TS staining intensity in colorectal liver metastases would be useful to determine whether this method can be used to define patients who will benefit from HAI chemotherapy.

INTRODUCTION

HAI³ of floxuridine achieves a 40% partial or complete tumor response rate associated with significant survival benefit (1) in patients with colorectal liver metastases. HAI requires a laparotomy for hepatic arterial cannulation, and it would be preferable if patients with unresponsive liver metastases could be excluded, to avoid the risks (2) and costs of laparotomy (3) in patients who will not benefit.

TS catalyzes the conversion of dUMP to dTMP and is the rate-limiting enzyme in synthesis of pyrimidine nucleotides, which are required for DNA synthesis. Fluorinated pyrimidines including floxuridine and fluorouracil are intracellularly metabolized to fdUMP, which binds covalently to TS with a folate cofactor to block the dUMP binding site and inhibit dTMP synthesis (4). In vitro studies suggest that tumor resistance to fluorinated pyrimidines is related to insufficient cytotoxic-induced TS inhibition. This may be due either to a reduction in the enzyme’s affinity for fdUMP caused by structural variation in the TS protein (5), or to gene amplification in floxuridine- (6) or fluorouracil (7, 8)-resistant cells leading to TS protein overexpression. Therefore, tumor floxuridine resistance could be influenced by TS (9), and intratumoral TS levels may predict response to regional floxuridine (10).

Measurement of intracellular TS by estimation of its ability to release tritium from the conversion of dUMP to dTMP (tritium release assay) or fdUMP binding capacity (ligand binding assay; Ref. 11) are limited by the need for large (>50 mg), fresh or fresh-frozen tissue samples. However, immunohistochemical staining using an anti-TS monoclonal antibody (12) can be used to estimate TS in smaller quantities of fresh or paraffin-embedded tissue. In addition, errors from inclusion of normal tissue, which has a lower TS level than tumor (13), can be reduced by histological identification and exclusion of normal tissue from assessment. Immunohistochemical estimation of TS protein expression has been shown to correlate with TS mRNA levels (14) assessed by the PCR technique (15, 16).

In this study, we assessed whether estimation of TS staining intensity in colorectal liver metastases by immunohistochemical staining could be used as a predictor of tumor response to hepatic arterial floxuridine.

PATIENTS AND METHODS

All patients had histologically confirmed primary colorectal carcinoma with liver metastases. A liver metastasis biopsy was taken during laparotomy for hepatic arterial cannulation, frozen in isopentane and stored at −70°C for subsequent analysis. HAI was commenced on the 5th day after the cannulation laparotomy. Patients were treated with a regimen of hepatic

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3 The abbreviations used are: HAI, hepatic arterial infusion; TS, thymidylate synthase; fdUMP, 5-fluoro-dUMP; CT, computed tomography; iqr, interquartile range.
arterial floxuridine (0.2 mg/kg/day) for 14 days, followed by a 14-day saline infusion rest period, and the cycle then repeated, as described previously (1).

Chemotherapy response, as defined by Union Internationale Contre le Cancer criteria (17) for partial response (>50% reduction in tumor volume), and stable (<25% increase in tumor volume) or progressive (>25% increase in tumor volume) disease, modified for liver metastasis assessment (18), was determined by comparison of liver metastasis volume on pretreatment CT scans with that at 4 months from onset of treatment. Liver metastasis volume was measured as described previously (19). In brief, liver metastasis area was measured on each CT slice using a Kontron image analysis system (Imaging Associates, London, United Kingdom), and the volume for each slice was calculated by multiplying the area by the CT slice thickness. The volumes for all slices were then summed to obtain a total liver metastasis volume for each patient.

TS Immunohistochemical Staining. The TS106 immunohistochemical technique has been described (14). TS staining was categorized independently by two assessors (M. M. D., P. G. J.) who, without knowledge of clinical outcomes, used standard criteria (20) to define both TS106 staining intensity based on a visual grading scale (0–3) and heterogeneity of staining (focal, <25% of tumor staining positive; diffuse, >25% of tumor staining positive). Intensity levels 0–1 were grouped together as low intensity, and levels 2–3 were grouped as high intensity staining (Fig. 1). The two assessors concurred in 33 cases but in 3 patients where their assessments disagreed, TS staining intensity was decided by consensus between the assessors.

Statistical Analysis. Comparison of TS staining intensity between HAI responders and nonresponders was by Fisher’s exact test (21), and survival difference by high or low liver metastasis TS staining intensity was by logrank test (22) of Kaplan-Meier survival curves (23).

RESULTS

Thirty-six patients (M:F, 24:12; median age, 62 years; iqr, 52–65 years) were studied. The median liver metastasis volume fell from 415 ml (iqr, 107–770) at baseline to 189 ml (iqr, 102–829) after 4 months of HAI. There were no complete and 12 partial responders, and 19 patients with stable disease.

Eleven of 24 previously untreated patients had a partial response to HAI (Table 1), in contrast to 1 of 12 patients who had previously not responded to a minimum of three courses of continuous infusion systemic fluorouracil/folinic acid chemotherapy (24).

Diffuse TS staining was noted in all except one biopsy where focal staining occurred. The staining within liver metastases was predominantly cytoplasmic and was identified in the epithelial, but not stromal cells. In two biopsies, positive nuclear staining for TS was present in addition to cytoplasmic staining, whereas in two biopsies, nuclear staining for TS occurred in the absence of cytoplasmic staining.

There was a significant correlation between partial response (Fisher’s exact test, \( P = 0.01 \)), but not stable disease (\( P = 0.24 \)), and TS106 staining (Table 2). Nine of 12 (75%) patients who had a partial response compared with 7 of 24 (29%) nonresponders showed low TS staining. There was a significant difference (Fisher’s exact test, \( P = 0.01 \)) in the proportion of low (9 of 16) compared with high (3 of 20) TS staining tumors in which a partial response occurred. There was no significant increase (Fisher’s exact test, \( P = 0.28 \)) in the prevalence of high TS staining among patients previously exposed (8 of 12) compared with those not exposed (12 of 24) to fluorouracil (Table 1). There was also no significant difference in survival from hepatic cannulation for HAI treatment (logrank

Table 1 TS (TS106) staining intensity and tumor response to HAI by previous systemic fluorouracil treatment

<table>
<thead>
<tr>
<th>HAI response</th>
<th>No TS intensity</th>
<th>Yes TS intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>High (2, 3)</td>
<td>Low (0, 1)</td>
</tr>
<tr>
<td>Partial</td>
<td>3</td>
<td>8</td>
</tr>
<tr>
<td>No partial</td>
<td>9</td>
<td>4</td>
</tr>
<tr>
<td>Stable</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td>Progressive</td>
<td>6</td>
<td>3</td>
</tr>
</tbody>
</table>

Fig. 1 Immunoperoxidase staining of colorectal liver metastasis biopsies using TS106 monoclonal antibody (magnification, \( \times 100 \)), demonstrating low intensity (A) and high intensity (B) tumor cell staining for TS (seen as pigment deposition in epithelial cells).
DISCUSSION

Previous studies of colorectal cancer (14, 16, 25) have demonstrated a correlation between intratumoral TS level and response to systemic fluorouracil chemotherapy. The partial response and disease stabilization rates for untreated patients (46% and 63%, respectively) and systemic fluorouracil-resistant patients (8% and 17%) treated with HAI in the present study are consistent with those previously reported (1, 26). This is the first study of a correlation between response to regional fluorouridine and pretreatment liver metastasis TS staining, and the findings are similar to those reported using TS mRNA assessment of regional fluorouridine response (27).

The lack of response in the 44% of patients with low TS staining intensity (Table 2), suggests that regional fluorouridine resistance could not be attributed entirely to increased TS. Possible additional explanations include alterations in fluorouridine activation, with reduced conversion to fdUMP (28) and reduced affinity of TS for fdUMP (5). In addition, colorectal liver metastases are hypovascular, and variation in tumor blood flow may have reduced tumor fluorouridine delivery and response (29) despite low TS levels. The absence of a significant correlation between stable disease and TS106 staining (Table 2) suggests that TS staining may not predict lesser degrees of response.

HAI exerted a cytotoxic effect despite high TS levels in some patients (Table 2) who had partial responses (15% of high TS intensity patients) or stable disease (45% of high TS intensity patients). Thus, high TS staining did not exclude response to hepatic arterial fluorouridine. Possible reasons are the increased tumor cytotoxic dose achieved by regional fluorouridine compared with systemic fluorouracil infusion (30) and the higher rate of fluorouridine compared with fluorouracil conversion to fdUMP (31).

These regional fluorouridine advantages were insufficient to produce a partial response in any high TS staining patient previously exposed to systemic fluorouracil (Table 1). In addition, 75% of fluorouracil-exposed patients who were predicted by low TS staining intensity to achieve a partial response to HAI failed to do so (Table 1). Thus, previous fluorouracil exposure seemed to increase tumor cell resistance to regional fluorouridine by both TS up-regulation, as well as other (5, 28) mechanisms.

There was a small (3.9%), but nonsignificant, survival difference by TS staining intensity, and the number of patients in the analysis was too small to allow sufficient power to assess the statistical significance of such a modest difference. Possible reasons for the absence of a larger and statistically significant difference are that survival in HAI patients depended on additional factors to liver metastasis response (for example, development of extrahepatic disease; Ref. 1) and that TS staining did not identify all patients obtaining some benefit from HAI treatment (Table 2).

This study demonstrates a positive correlation between TS immunohistochemical staining of colorectal liver metastases and response to HAI. The results suggest that a prospective assessment of TS staining intensity in colorectal liver metastases would be useful to determine whether this method could be used to define those patients in whom the inconvenience and costs of a hepatic arterial cannulation laparotomy and insertion of an implantable chemotherapy pump can be justified by additional benefit (4).

Table 2  There was a significant correlation between TS (TS106) staining intensity and partial response of colorectal liver metastases to hepatic arterial fluorouridine

<table>
<thead>
<tr>
<th>Response</th>
<th>n (patients)</th>
<th>TS106 staining intensity (patients)</th>
<th>P (Fisher’s exact test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Partial response</td>
<td>12</td>
<td>3 9</td>
<td>0.01</td>
</tr>
<tr>
<td>No partial response</td>
<td>24</td>
<td>17 7</td>
<td>0.24</td>
</tr>
<tr>
<td>Stable disease</td>
<td>19</td>
<td>9 10</td>
<td></td>
</tr>
<tr>
<td>Progressive disease</td>
<td>17</td>
<td>11 6</td>
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</tr>
</tbody>
</table>

REFERENCES


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