Predictive Role of Dihydropyrimidine Dehydrogenase (DPD) and Thymidylate Synthase (TS) Gene Expressions in Patients with Metastatic Colorectal Cancer Treated with Capecitabine

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Abstract

Background and Objective: Dihydropyrimidine dehydrogenase (DPD) and thymidylate synthase (TS) gene expressions in metastatic colorectal cancer have been reported to be predictive parameters for the efficacy of fluoropyrimidine-based chemotherapy. In present study, we investigated the association between both DPD and TS expressions in metastatic colorectal cancer and the response and survival in patients treated with capecitabine.

Patients and Methods: DPD and TS expressions were measured by reverse transcription-PCR in tissues obtained by ultrasound-guided needle biopsy from 37 patients who went on to receive oral treatment of capecitabine for metastatic colorectal cancer. Relative mRNA amounts of DPD or TS were expressed as the ratios of targeted gene to glyceraldehyde-3-phosphate dehydrogenase reverse transcription-PCR products.

Results: Median values of DPD mRNA expressions were 0.30 and 0.65 for responding tumors and nonresponding ones, respectively, with a statistical significance (p<0.0001). No responding tumor had a DPD mRNA expression >0.5. A total of 19 tumors had low DPD mRNA expressions of <0.5, and 63% of them showed response. There was no responding tumor with both high DPD and high TS (TS mRNA expression 1.0). However, the response rate was 75% in tumors with both low DPD and low TS. The median survival time was 16.3 months in patients with both low DPD and low TS versus 8.4 months in patients with high DPD or high TS mRNA expression.

Conclusion: In conclusion, the combination of DPD and TS mRNA expressions in metastatic tumor might be useful as predictive parameters for the efficacy of fluoropyrimidine-based chemotherapy for metastatic colorectal cancer.

Key Words: Colorectal cancer – Dihydropyrimidine dehydrogenase – Thymidylate synthase – Predictive marker.

Introduction

FOR the last four decades, 5-fluorouracil (5-FU)-based chemotherapy has been regarded as standard therapy for metastatic colorectal cancer (CRC) [1,2,3]. Capecitabine is a fluoropyrimidine-based drug (N4-pentyloxycarbonyl-5'-deoxy-5-fluorocytidine), it is the oral precursor of 5'-deoxy-5-fluorouridine (5'-DFUR), with predictable bioavailability and promising efficacy as first-line treatment in colorectal cancer [4-7]. Capecitabine is absorbed as an intact molecule through the intestinal mucosa and is then sequentially converted to cytotoxic fluorouracil by three enzymes, as follows: (i) conversion to 5'-deoxy-5-fluorouracil (5'DFUR) by carboxylesterase in the liver; (ii) 5'-DFCR is metabolized to 5'-DFUR by cytidine deaminase, an enzyme located primarily in the liver and tumor tissue; and (iii) 5'-DFUR is converted to 5-FU by thymidine phosphorylase, which appears to be expressed at higher levels in tumor cells [8]. There is strong evidence that the increased levels of thymidine phosphorylase in tumor cells selectively increase fluoropyrimidine concentrations, resulting in enhanced efficacy and reduction in systemic toxicity.

Capecitabine was compared with bolus 5-FU/LV (Mayo Clinic protocol) as first-line chemotherapy in two large randomized trials in metastatic colorectal cancer [4,6]. Although no differences in terms of median time to disease progression and overall survival were found in both trials [median time to progression 4.6 and 4.7 months, and overall survival time 12.9 and 12.8 months, respectively (integrated analysis of 1207 patients)], one study showed a significantly higher response rate for capecitabine (26% compared with 12%, p<0.0001) [4,9].

Several studies have examined the role of chemotherapeutic target enzymes such as thymidylate...
Pre-treatment evaluation and follow-up:

Predictive role of dihydropyrimidine dehydrogenase

Eligibility:

The eligibility criteria for enrollment of patients into this study were as follows: age 18 years; histologically confirmed colorectal cancer; measurable, metastatic disease; no previous chemo- or radiotherapy for metastatic disease; interval between the end of previous adjuvant radio- and/or chemotherapy and study entry at least 6 months; predicted life expectancy > 3 months; World Health Organization (WHO) performance status 2; ability to take oral medication; adequate baseline organ functions, no chronic diarrhea or unresolved bowel obstruction; no severe uncontrolled co-morbidities or medical conditions (e.g. myocardial infarction within the last 12 months); no brain metastases and signed informed consent.

Materials and Methods

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Pre-treatment evaluation and follow-up:

Pre-treatment evaluation consisted of a complete history and physical examination, complete blood cell count (CBCC), serum chemistry including electrolytes, hepatic and renal function tests, electrocardiogram (ECG), chest X-ray, and computed tomography (CT) scans of the abdomen/pelvis and chest (if indicated); all sites of measurable disease were initially documented using CT scans. If indicated, bone scans and X-rays were performed. In all patients, a pretreatment ultrasound-guided needle biopsy of metastatic tissue was performed, which yielded two to three cores, each 10 mm long. In patients with multiple metastatic lesions, a biopsy was performed from the best accessible lesion only. Each core was immediately frozen in liquid nitrogen before storage at -70°C.

During the study, patient monitoring included the assessment of clinical toxicities, CBCC, serum chemistry and physical examinations before each weekly administration of the chemotherapy. ECG, chest X-ray and measurement of the target lesion(s) by CT scan were additionally performed before each cycle and at the end of treatment. During the follow-up period patients were evaluated every 2 months after the end of study treatment, including physical examination, CBCC, serum chemistry, ECG, chest X-ray, and CT scans of the measurable lesion(s) in case of tumor response or stable disease until documented disease progression.

Chemotherapy:

Capecitabine was administered orally at a dose of 1,250 mg/m² twice daily (total daily dose 2,500 mg/m²) as an intermittent regimen in 3-week cycles (2 weeks of treatment followed by a 1-week rest period). Capecitabine was given approximately 12 hours apart and taken orally with water within 30 minutes after ingestion of food (breakfast or dinner). Patients with documented objective response or stable disease continued treatment for at least one further cycle. In case of dose-limiting toxicities, treatment was continued after dose reduction to the next lower dose level. In case of progressive disease, intolerable toxicities or a treatment delay of > 4 weeks between cycles, study treatment was discontinued. Administration of capecitabine was interrupted if diarrhea grade > 1 occurred or if grade 1 toxicity persisted for > 12 h.

Evaluation of response:

Assessments of tumor dimensions and involved sites were performed before the start of treatment and were scheduled after weeks 6, 12, 18, 24, and 30 of therapy. Follow-up assessments for disease progression and survival monitoring were performed every 3 months after the end of treatment. Tumor dimensions were assessed by use of computed tomography scans, X-rays, or magnetic resonance imaging. Tumor response classification was based on standard World Health Organization criteria [15,16]. Disappearance of all known disease at all involved sites was considered a complete response (CR). Partial response (PR) was defined as residual disease with a decrease 50% in the sum of the products of greatest perpendicular diameters
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(SP) of all indicator lesions. Progressive disease (PD) was defined as the appearance of a new lesion or an increase of 25% in the SP. Stable disease (SD) was defined as no change in SP or a change not corresponding to PR or PD.

Semiquantitative RT-PCR:

Semiquantitative RT-PCR was performed using a previously described method [17,18,19]. Briefly, total RNA for each sample was isolated using the RNeasy mini kit (Qiagen Inc., Chatsworth, CA) according to the manufacturer’s instructions. Reverse transcription using 10 µg of total RNA was performed in a total volume of 100 µg containing 250 pmol oligo (dT)18, 80 units of RNasin (Promega, Madison, WI), and 500 units Moloney murine leukemia virus reverse transcriptase (Life Technologies, Inc., Gaithersburg, MD), 50 mM Tris-HCl (pH 8.3), 75 mM KCl, 3 mM MgCl2, 10 mM DTT, and 0.5 mM deoxynucleotide triphosphates solution.

Three different concentrations of transcribed RNA were used as PCR templates. For accurate quantification, we confirmed that the PCR amplification was in the linear phase. PCRs were carried out at a final volume of 50 µl containing cDNA template, oligonucleotide primers for DPD (40 pmol of each primer), TS (10 pmol of each primer), and GAPDH (2 pmol of each primer), 1.25 units of Ex Taq (Takara, Shiga, Japan) in 10x Ex Taq buffer (Takara), and 0.2 mM deoxynucleotide triphosphates, using a thermal cycler (Takara PCR Thermal Cycler MP). The PCR profile consisted of initial 3-min degeneration at 94°C, followed by 30 cycles of 1 min of denaturation at 94°C, 1 min of annealing at 60°C, and 2 min of polymerization at 72°C and a final 10 min extension at 72°C. PCR products were separated by 2% agarose gel electrophoresis, stained with ethidium bromide, and visualized on a UV transilluminator. Gels were photographed on Type 667 films (Polaroid, Cambridge, MA), and images were scanned with an image scanner (JX-330; Sharp, Mahwah, NJ) and analyzed with an Image Master 1D (Pharmacia Biotech, Uppsala, Sweden). Relative amounts of DPD mRNA and TS mRNA were expressed as the ratios of DPD to GAPDH RT-PCR products (henceforth termed DPD mRNA expression) and TS to GAPDH RT-PCR products (henceforth termed TS mRNA expression), respectively.

Statistical analysis:

Data were entered, checked and analyzed using the SPSS Software system (version 11.0; Chicago, IL).

Results

Patient characteristics:

Thirty-seven patients were recruited into this study between February 2001 and February 2006: 24 males and 13 females aged 38 to 68 years (median, 62). Table (1) represents characteristics of patients.

DPD and TS mRNA expressions and response to treatment:

Median values of DPD and TS mRNA expressions were 0.49 (range: 0.15-1.44) and 1.02 (range: 0.21-1.59), respectively. Median values, 0.5 for DPD and 1.0 for TS, were selected for cutoff values, which separated high and low gene expression of DPD and TS.

There were 2 CRs, 10 PRs, 16 SDs, and 9 PDs, with a 32.4% response rate (95% confidence interval, 18.0-49.8%).

Patients were categorized as either responding or not responding to capecitabine. Median values of DPD mRNA expressions were 0.30 (range: 0.15-0.50) and 0.65 (range: 0.29-1.44) for responding and non responding patients, respectively, with the difference being statistically significant \( p<0.0001; \) Mann-Whitney U test; Fig. (1)). No responding patients had DPD mRNA expression of 0.5 or more (Fig. 1). A total of 19 patients had low DPD mRNA expressions <0.5 with a corresponding response rate of 63% (12 of 19).

Median values of DPD mRNA expression were 0.19, 0.35, 0.56, and 0.95 for CR, PR, NC, and PD, respectively. DPD mRNA expression inversely correlated with the proportion of responses to chemotherapy \( p<0.0001; \) Kruskal-Wallis test). On the other hand, there was no relationship between TS mRNA expression and response categories (data not shown).

A plot of DPD against TS mRNA expression showed no correlation between the expression of these genes (Spearman rank correlation coefficient 0.058; \( p=0.733 \)), with a number of low DPD patients having high TS (1.0) and high DPD patients having low TS expressions (<1.0) vice versa (Fig. 3). There were no responding patients with high DPD expression. The response rates were significantly higher \( p=0.0382; \) two-sided Fisher’s exact test) in patients with low TS expression (50% responding) than in those with high TS expression (16%). Fig. (3) additionally shows that 9 of 12 patients with both low DPD and low TS responded...
(response rate: 75%), but only 3 of 7 patients with low DPD and high TS responded (response rate: 43%). The response data are summarized in Table (2). The response rate was 25% in patients with high DPD or high TS, statistically lower than those with both low DPD and low TS ($p=0.0003$; two-sided Fisher’s exact test).

**DPD and TS mRNA expressions and survival:**

The median survival was 14.4 months (range: 8.7-28.3 months) in patients with low DPD expression, but only 7.4 months (range: 3.0-11.2 months) in patients with high expression ($p<0.0001$; log-rank test; Fig. (4)). Patients with low expression of TS mRNA survived longer than those with high TS expression {median: 8.4 months ranging from 3.3 to 17.3 months for high expression versus 12.3 months ranging from 3.1 to 28.3 months for low expression, $p=0.0069$; log-rank test; Fig. (5)}. The median survival time was 16.3 months (range: 8.7-28.3 months) in patients with both low DPD and low TS mRNA expression, which was significantly longer than the 8.4 months (range: 3.1-17.3 months) in patients with other combinations of DPD and TS mRNA expression level ($p<0.0001$; log-rank test; Fig. (6)).

### Table (1): Patient characteristics.

<p>| | |</p>
<table>
<thead>
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### Table (2): Response to treatment and expressions of DPD and TS genes.

<table>
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<th>Gene expression status</th>
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<th>No. of non-responding patients</th>
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<td>18</td>
<td></td>
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<tr>
<td>TS $&lt;1$</td>
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<td>9</td>
<td></td>
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<tr>
<td>TS $\geq 1$</td>
<td>19</td>
<td>3</td>
<td>16</td>
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<td>DPD $&lt;0.5$ and TS $&lt;1$</td>
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<td>9</td>
<td>3</td>
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<tr>
<td>DPD $\geq 0.5$ or TS $\geq 1$</td>
<td>25</td>
<td>3</td>
<td>22</td>
<td>0.0003</td>
</tr>
</tbody>
</table>

**Fig. (1):** DPD mRNA in 37 patients according to response to chemotherapy.

**Fig. (2):** DPD mRNA expression in 37 patients and response to chemotherapy. ($p<0.0001$; Kruskal-Wallis test).
Fig. (3): DPD expression plotted against TS expression in 37 patients. The grey lines indicate the no response cutoff values for each gene expression.

Fig. (4): Kaplan-Meier survival curves for patients with DPD expressions above or below the no response cutoff and for patients with TS expressions above and below the response cutoff.

Fig. (5): Kaplan-Meier survival curves for patients with TS expressions above or below the no response cutoff and for patients with TS expressions above and below the response cutoff.

Fig. (6): Kaplan-Meier survival curves for patients with both low DPD and low TS expressions and those with high DPD or high TS expressions.

Discussion

TS and DPD play key roles in fluoropyrimidine-based drugs resistance [20,21]. High intratumoral TS levels are believed to confer 5-FU resistance because of inefficient TS inhibition [21]. In tumors with high intratumoral DPD levels, 5-FU seems to be degraded to a large extent before its activation [20]. Therefore, TS inhibition may be also inefficient when DPD expression is high. In case of advanced unresectable CRC and other advanced gastrointestinal malignancies, several studies have demonstrated the predictive relevance of intratumoral TS and DPD levels for the success of palliative 5-FU treatment and the prognosis of these patients [22-25].

Our study demonstrates that there is a significant correlation among the combination of DPD and TS expression levels and both the response to capecitabine and survival in patients with metastatic colorectal cancer. These data reflect Danenberg’s report that combination of two genes, TS and DPD, in metastatic tumors was related to the antitumor effect in terms of tumor shrinkage and survival in patients treated with a 5-FU and LV regimen [25].

Apart from being a predictor of response to fluoropyrimidine-based drugs, TS expression is known to be a prognostic factor in advanced colorectal cancer (ACRC). In one review, Popat et al. [10] analyzed 20 retrospective studies which assessed the value of TS expression as prognostic factor in colorectal cancer. In general, low TS expression was associated with a better survival than high TS, not only in patients treated with adjuvant or palliative chemotherapy including 5-FU, but also in patients who did not receive any chemotherapy.
In this study, DPD mRNA expression statistically correlated with response, the proportion of responses to chemotherapy, and survival. This is one of the first reports to indicate a positive relationship between the antitumor effect of capecitabine and DPD expression in metastatic colorectal tumors. When categorizing into high and low TS tumors with a cutoff value, low TS expression was significantly related to both tumor shrinkage and extended survival. All studies on TS expression in metastatic tumors showed that responders had significantly lower TS expression than non-responders, even when evaluating TS expression by immunohistochemical methods or PCR methods [26-31].

Although the response rate of 32.4% that we obtained appears higher than the expected response rate in unselected patients, it was lower than what we had hoped for. The last updated meta-analysis on 5-FU and LV reported a response rate of 21% (95% CI 19% to 23%) in unselected patients [38]. Nevertheless, our response rate did not come near to the very high response rates (>70%) reported in retrospective studies in patients with low TS treated with 5-FU [33,34].

Immunohistochemical studies have only assessed cytoplasmic expression of TS, although the nuclear staining is also present [21]. The degree of TS nuclear expression correlated closely with TS mRNA expression, and higher nuclear TS expression in primary tumor was associated with poorer response to fluoropyrimidine-based drugs [38].

When combined DPD and TS mRNA expressions were examined, both response and survival could be predicted more precisely than on the bases of only one gene expression. There was no correlation between DPD and TS mRNA expressions. If DPD and TS co-regulated, there would be little or no additional benefit for response prediction from measuring both gene expressions. No tumor with both high DPD and high TS expression responded to capecitabine but not even all tumors with both low DPD and low TS expression responded to the therapy, having a response rate of 75%. These data suggested combined evaluation of other gene expression such as thymidine phosphorylase [23] is needed to more accurately predict response. In terms of survival, patients with tumor with both low DPD and low TS survived longer than patients with tumor having the other patterns of TS and DPD expression. Among the other three combination pattern, patients with both high DPD and high TS expressed had the worst outcome (data not shown).

In this study, we find that combined evaluation of DPD and TS gene expressions in metastatic colorectal cancer can be used to predict response and survival in patients treated with capecitabine chemotherapy. Our study is a step toward the goal of individualized cancer chemotherapy based on the fluoropyrimidine-related molecular characteristics of the tumor. However, the conclusions are drawn from a study with limited number of patients. Prospectively randomized trials are needed to confirm our results and to define the best DPD and TS cutoff points.

**References**


