Expression Levels of Thymidine Phosphorylase (TP) and Dihydropyrimidine Dehydrogenase (DPD) in Patients with Gastrointestinal Cancer

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Abstract. Background: Thymidine phosphorylase (TP) is a key enzyme involved in pyrimidine nucleoside metabolism. Dihydropyrimidine dehydrogenase (DPD) is the major catabolic enzyme of 5-fluorouracil (5-FU). These are important enzymes in the pyrimidine salvage pathway and are considered to be key enzymes for determining the prognosis of patients with gastrointestinal cancer. In the present study, TP and DPD were quantified and evaluated in gastric and colorectal cancer. Patients and Methods: In 111 cases of malignancy, including 30 gastric cancers and 81 colorectal cancers, the expression levels of both TP and DPD in fresh-frozen samples from either tumor or adjacent normal tissue were quantified using enzyme-linked immunosorbent assay (ELISA). The relationships between TP or DPD expression levels in tumor tissues or adjacent normal tissues and clinicopathological factors were evaluated. Results: The TP expression levels in gastric or colorectal tumor tissues were found to be significantly higher than those in the adjacent normal tissue. Although the DPD expression levels in gastric tumor tissue were significantly higher than those in adjacent normal tissue, the DPD expression levels in colorectal tumor tissue were nearly identical to those in the adjacent normal tissue. The DPD expression levels in gastric tumor tissues were significantly higher than those in colorectal tumor tissues. The TP expression levels correlated significantly with the DPD expression levels in tumor or adjacent normal tissues. The DPD expression levels in tumor tissues significantly correlated with those in adjacent normal tissue. Conclusion: The difference in DPD expressions between gastric and colorectal cancer tissues may reflect the organ specificity of the carcinomas and a difference in chemotherapeutic sensitivity to 5-FU or its analogs. The correlation between TP and DPD expression levels suggests the existence of a common regulatory pathway.

5-Fluorouracil (5-FU) or its analogs have been widely used in the treatment of gastrointestinal carcinomas. Their clinical effectiveness differs according to patient characteristics or carcinoma location, which is thought to be based on the difference in sensitivity of carcinoma cells to 5-FU (1).

Thymidine phosphorylase (TP) is a key enzyme involved in pyrimidine nucleoside metabolism. TP is also identical to platelet-derived endothelial cell growth factors and is implicated in angiogenesis (2-4). TP is widely expressed in carcinomas such as breast, gastric, colorectal, pancreatic, hepatic, pulmonary, esophageal, urinary bladder, kidney and uterine (5-11). Elevated TP expression is also reported to predict poor prognosis in colorectal cancer (12, 13), gastric cancer (14), esophageal cancer (15), carcinoma of the bladder (9), renal cell carcinoma (10, 16) and non-small cell lung cancer (17).

Dihydropyrimidine dehydrogenase (DPD) is the major catabolic enzyme of 5-FU, which converts uracil and thymidine to dihydrouracil and dihydrothymidine. DPD is widely distributed in various organs, such as the liver, peripheral blood mononuclear cells, small intestinal mucosa, spleen (18) and several cancer cells, such as gastric (19-21), colorectal (12, 20-22), breast, (23, 24), hepatic (25), renal (10), urinary bladder (6), endometrial (26) and pulmonary (27). DPD levels are thought to predict the toxicity of or sensitivity to 5-FU (19, 20). Both enzymes are important in the pyrimidine salvage pathway and are considered to be key enzymes affecting the prognosis of patients with various
cancers. In the present study, the expression of these enzymes in gastrointestinal cancers was quantified and evaluated.

Patients and Methods

Patients and samples. From July 2002 to December 2003, 111 patients with primary cancer, including stomach and colorectal, who underwent surgery at the Department of Surgery 1, University Hospital of Occupational and Environmental Health, Japan, were recruited for this study. Tissue samples were collected and stored at <−80°C until analysis. The clinical data of these patients is summarized in Table I. Informed consent was obtained from all patients prior to the study. Before collection of the tissue samples, no patients had received chemotherapy or radiotherapy. Moreover, no patients were under therapy with drugs that influence cytokine secretion, such as steroids or interferon.

TP and DPD expression levels. The TP expression levels were measured by enzyme-linked immunosorbent assay (ELISA) (5) and expressed as U/mg protein, where one U is an amount equivalent to one μg of 5-FU produced in an hour. The DPD expression levels were measured by a sandwich ELISA (6) using two monoclonal antibodies specific to human DPD and also expressed as U/mg protein, where one U is equivalent to the amount of DPD catabolizing one pmol of 5-FU/min.

Clinicopathological assessment. The tumors were staged by two pathologists, who had no prior knowledge of the results of the assays, according to the tumor-node-metastasis (TNM) classifications. Clinicopathological factors such as age, gender, nodal involvement, depth of invasion, vessel invasion, histopathological type and staging were analyzed for association with TP or DPD expression levels. The blood levels of CEA and CA19-9 were also analyzed for association with TP or DPD expression levels.

Statistical analysis. The data are expressed as the mean±SD, and were statistically analyzed using the Student’s t-test and regression theory, as appropriate. The relationships between parameters were assessed statistically with Pearson’s correlation coefficient test using Stat View-J. Statistical significance was established at the p<0.05 level.

Results

The TP expression levels in both the stomach and colorectum were found to be significantly higher than those in the adjacent normal tissue (Table II). Although the DPD expression levels in gastric tumor tissues were significantly higher than those in adjacent normal tissues, the DPD expression levels in colorectal tumor tissues were not higher than those in adjacent normal tissues (Table II). In tumor tissues, although the TP expression levels in the stomach were not higher than those in the colorectum, the DPD expression levels in the stomach were significantly higher than those in the colorectum (Table II). In adjacent normal tissues, the TP and DPD expression levels in the stomach were not higher than those in the colorectum (Table II).

In tumor tissues, the TP and DPD expression levels in patients with peritoneal dissemination were significantly higher than in those without peritoneal dissemination (p=0.0214 and p=0.0465, respectively). The other clinicopathological factors were not correlated in each case (data not shown). In tumor tissues, the TP expression levels

Table I. Characteristics of patients.

<table>
<thead>
<tr>
<th>No. of patients</th>
<th>111</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (M/F)</td>
<td>58/53</td>
</tr>
<tr>
<td>Age (yr) (mean±S.D.)</td>
<td>66.5±11.1</td>
</tr>
</tbody>
</table>

Site of carcinoma

<table>
<thead>
<tr>
<th>Site</th>
<th>Stomach</th>
<th>Colorectum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (M/F)</td>
<td>30</td>
<td>81</td>
</tr>
<tr>
<td>Age (yr) (mean±S.D.)</td>
<td>70.0±12.4</td>
<td>66.9±10.7</td>
</tr>
<tr>
<td>TNM stage I/II/III/IV</td>
<td>2/5/10/6/7</td>
<td>42/39</td>
</tr>
<tr>
<td>Site. colon /rectum</td>
<td>5/33/18/14/11</td>
<td></td>
</tr>
</tbody>
</table>

S.D.: Standard deviation

Table II. Relationship of TP or DPD levels between tumor and adjacent normal tissues.

<table>
<thead>
<tr>
<th>Site</th>
<th>TP (U/mg protein)</th>
<th>DPD (U/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site TT ANTR</td>
<td>p-value</td>
<td></td>
</tr>
<tr>
<td>Stomach</td>
<td>78.4±41.6</td>
<td>40.8±24.4</td>
</tr>
<tr>
<td>Colorectum</td>
<td>80.8±58.1</td>
<td>43.2±21.1</td>
</tr>
<tr>
<td>Total</td>
<td>80.1±54.0</td>
<td>42.5±21.9</td>
</tr>
<tr>
<td>Stomach</td>
<td>53.6±33.4</td>
<td>34.5±24.1</td>
</tr>
<tr>
<td>Colorectum</td>
<td>35.8±23.3</td>
<td>34.4±11.3</td>
</tr>
<tr>
<td>Total</td>
<td>40.6±27.5</td>
<td>34.4±15.5</td>
</tr>
</tbody>
</table>

TP: thymidine phosphorylase; DPD: dihydropyrimidine dehydrogenase. Each enzyme level is shown as the mean±standard deviation. TT: tumor tissues; ANTR: adjacent normal tissues.

The groups were compared using the Student’s t-test and regression theory, as appropriate. *p=0.0020.
in the stomach and colorectum were significantly correlated with the DPD expression levels ($p<0.0001 \ r=0.579$ and $p<0.0001 \ r=0.534$, respectively) (Figures 1A, 3A). Moreover, in the adjacent normal tissues, the TP expression levels in the stomach and colorectum were also significantly correlated with the DPD expression levels ($p=0.0486 \ r=0.382$ and $p=0.0406 \ r=0.234$, respectively) (Figures 1B, 3B). In both the stomach and colorectum, the DPD expression levels in the tumor tissue significantly correlated with those in the adjacent normal tissue ($p=0.0428 \ r=0.391$ and $p=0.0298 \ r=0.247$, respectively) (Figures 2B, 4B). On the other hand, in both the stomach and colorectum, the TP expression levels in tumor tissue were not correlated with those in the adjacent normal tissue ($p=0.1266$ and $p=0.2900$, respectively) (Figures 2A, 4A).

In gastrointestinal cancers, including gastric and colorectal cancer, the TP expression levels in tumor and adjacent normal tissues were significantly correlated with the DPD expression levels ($p<0.0001 \ r=0.506$ and $p=0.0029 \ r=0.288$, respectively) (Figures 5A, 5B). In gastrointestinal cancers, although the TP expression levels in the tumor tissue were not correlated with those in the adjacent normal tissue ($p=0.1072$) (Figure 6A), the DPD expression levels in tumor tissue were significantly correlated with the DPD expression levels in adjacent normal tissue ($p=0.0017 \ r=0.303$) (Figure 6B).
Discussion

Table II indicates that the TP expression levels in gastric and colorectal tumor tissues were significantly higher than those in adjacent normal tissues. These data reconfirmed previous studies (19-22, 28). Although, the DPD expression levels in gastric tumor tissue were significantly higher than those in the adjacent normal tissue, no statistical differences were observed in colorectal cancer between the tumor tissue and adjacent normal tissue (Table II). These differences may reflect the organ specificity of the carcinomas and a difference in chemotherapeutic sensitivity to 5-FU or its analogs.

At present, the regulatory mechanism of TP expression remains unclear. Several reports indicate that TP expression is accelerated by hypoxia, hypoglycemic condition (29), tumor necrosis factor (TNF)-α, interleukin (IL)-1, interferon (IFN)-γ (30), or anticancer drugs such as cyclophosphamide, paclitaxel and docetaxel (31). TP expression is also positively associated with TNF-α, IL-1 (30), vascular endothelial growth factor (VEGF) (32) and hypoxia-inducible factor (HIF)-2α (33). Zhu et al. indicated that the Sp1 transcription factor contributes to the tumor necrosis factor-induced expression of TP in human colon carcinoma cells (34).
The regulation of DPD expression also remains unclear. DPD is thought to be regulated at both transcription and translation (35). Since an NF-κB binding site is present in the promoter region of the DPD gene (36), it is possible that inhibition of NF-κB activity by 5-FU has an inhibitory effect on DPD mRNA expression.

Our data indicated that the TP expression levels are significantly correlated with the DPD expression levels in tumor tissue and adjacent normal tissue (Figures 1A, 1B, 3A, 3B, 5A, 5B). Previous studies using ELISA for analysis of protein levels (6, 7) and PCR for mRNA levels (37) reported the same results for colorectal carcinoma.

These results suggest a common regulating pathway for TP and DPD expressions. However, additional detailed studies are required to clarify such a common regulating mechanism.

Several previous studies have investigated whether TP and DPD expression levels or the TP/DPD ratio could predict cancer patient prognosis (9, 10, 13, 14, 16, 17). A high TP expression has been shown to predict poor prognosis in colorectal cancer (13), gastric cancer (14), esophageal cancer (15), carcinoma of the bladder (9), renal cell carcinoma (10, 16) and non-small cell lung cancer (17). On the other hand, other reports indicated no
correlation between TP expression levels and prognosis in patients with colorectal cancer (7) and gastric cancer (19). In 100 patients with colorectal cancer, who underwent intravenous adjuvant 5-FU chemotherapy, prognosis was also not correlated with tumor-TP, DPD, or TP/DPD values (7). Although the TP and DPD expression levels had no impact on overall survival after surgery for gastric cancer, the survival rate was significantly better in patients with a high TP/DPD ratio than in those with a low TP/DPD ratio in a subgroup of patients who were administered 5'-DFUR postoperatively (19). These discrepancies might arise from differences in the quantitative method and the recruited patients.

Tumor-associated monocytic cells (TAMs) are a major component of the stroma responsible for tumor formation. Toi et al. previously reported that the monocytic TP state could categorize CD68+ TAMs, who exhibited extensive accumulation of CD68+ TAMs, into two subgroups with strikingly contrasting prognoses: a good prognostic monocytic TP− group and a poor prognostic monocytic TP+ group (38). Moreover, in uterine endometrial cancer, positive staining for both cancer cells and tumor stromal cells expressing TP was noted in 41% of cases. Most tumor stromal cells expressing TP were shown to co-express CD68, which is a marker for macrophages. When stromal macrophages/fibroblasts exhibited high TP expression, a significant decrease in disease-free and overall survival was observed (11). These data indicated the importance of the tumor stromal cells expressing TP. Further evaluation of the cells expressing TP in patients with gastrointestinal cancer is necessary.

In conclusion, the difference in DPD expression between gastric and colorectal cancer tissue may reflect the organ specificity of the carcinomas and a difference in their chemotherapeutic sensitivity to 5-FU or its analogs. The correlation between TP and DPD expression levels suggests the existence of a common regulatory pathway, but more detailed studies are required to clarify this.

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References


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