Combination of S-1/Nedaplatin and Radiotherapy in Esophageal Cancer: Immunohistochemical Evaluation of Thymidylate Synthase, Dihydropyrimidine Dehydrogenase and p53

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Abstract
Results of chemoradiotherapy (CRT) in esophageal cancer are now being reported as in surgical cases, and CRT is now often selected even in surgically operable cases. A combination of a new oral dihydropyrimidine dehydrogenase inhibitory fluoropyrimidine, tegafur • gimeracil • osteracil potassium (S-1), nedaplatin (CDGP) and radiotherapy is thus used to reduce adverse reactions, maintain quality of life and improve therapeutic results. As far as concurrent CRT for esophageal cancer is concerned, if complete response (CR) is not achieved, prognosis is poor. Identifying factors to predict response and prognosis before CRT is thus crucial.

The present study determined expression levels of thymidylate synthase (TS), dihydropyrimidine dehydrogenase (DPD), and p53 in biopsy samples obtained from patients before treatment in a clinical study of CRT using S-1 and CDGP. Correlations between these findings and response and prognosis were investigated.

While 3 of 11 high TS patients displayed CR (CR rate, 27.3%), 12 of 21 low TS patients showed CR (CR rate, 57.1%). One of 5 patients with high TS and p53-positive had CR (CR rate, 20%). In contrast, 10 of 17 patients with low TS and p53-negative displayed CR (CR rate, 58.8%). For TS or p53 alone, survival tended to be longer in low TS or p53 negative patients (TS: p = 0.069; p53: p = 0.057).

From the results of multivariate analysis we consider that the combination of TS and p53 may be important prognosis factors. We divided the patients into two groups and analysed both low TS and p53 negative patients and the other patients. Survival was significantly prolonged in low TS and p53 negative patients compared to other patients (p = 0.0389).

This suggests that the combination of TS and p53 expression is a useful prognostic indicator for patients undergoing CRT with S-1 and CDGP. No correlation was found between DPD expression and survival. This suggests that DPD is not a prognostic indicator for esophageal cancer responsiveness to CRT using S-1.

Key words
Esophageal cancer, chemoradiotherapy, S-1, Nedaplatin, immunohistochemical

Introduction
Studies on chemoradiotherapy (CRT) in esophageal cancer are now being reported as in surgical cases1, and CRT is now often selected even in surgically operable cases2. In most reports, CRT is performed by concurrent administration of 5-fluorouracil (5-FU), cisplatin (CDDP), and radio-
therapy (RT), and this is considered as the standard CRT procedure for esophageal cancer. However, patient quality of life (QOL) cannot necessarily be maintained during this therapy, as 5-FU needs to be continuously infused for 24 h and CDDP administration requires a large volume of transfusion. In addition, therapeutic results have not been satisfactory. Compared to CDDP, the nephrotoxicity and gastrotoxicity of nedaplatin (CDGP: cis-diammine glycolata) are milder, and CDGP administration does not require a large volume of transfusion. Furthermore, the effectiveness of CDGP monotherapy has been favorable, at 51.7%\(^7\). We have previously performed CRT using coadministration of 5-FU and CDGP, and obtained favorable results\(^8\). The fact that 5-FU requires continuous drip infusion for 24 h has a significant impact on patient QOL.

A new oral dihydropyrimidine dehydrogenase inhibitory fluoropyrimidine, tegafur • gimeracil • osteracil potassium (S-1) is an oral anticancer drug that combines 2 modulators with the 5-FU prodrug tegafur (FT). S-1 is administered orally, not drip infused. Also, while the mechanisms of action are basically the same as those for 5-FU, efficacy against gastric\(^9\) and head-and-neck cancers\(^10\) is higher compared to 5-FU. A combination of S-1, CDGP, and RT is thus used to reduce adverse reactions, maintain QOL, and improve therapeutic results.

As far as concurrent CRT for esophageal cancer is concerned, if complete response (CR) is achieved, prognosis is relatively good\(^9\). However, CRT is meaningless if CR is not achieved, and a chance for surgically successful treatment may have been lost. Identifying factors to predict response and prognosis before CRT is thus crucial.

Thymidylate synthase (TS) is an enzyme in the nucleotide metabolic pathway that is necessary for DNA synthesis. TS, 5-fluoro-2-deoxyuridine 5-monophosphate, and 5-10-methylene-tetrahydrofolate form an irreversible ternary complex that inhibits DNA synthesis, resulting in cytotoxic effects. This is the target enzyme for 5-FU. As far as TS is concerned, regardless of the methods of determination, studies have shown that the lower TS expression, the more effective 5-FU\(^11-14\). Evaluation of 5-FU sensitivity using immunohistochemical staining can help to predict treatment response and prognosis. Previous studies have documented that therapy is more effective and survival is prolonged with 5-FU if TS expression is low rather than high\(^15-20\). However, no studies have examined CRT using S-1 in the treatment of esophageal cancer.

Dihydropyrimidine dehydrogenase (DPD) is a rate-limiting enzyme in 5-FU degradation, and level of expression is related to the antitumor effects of 5-FU. DPD is a catabolic enzyme of 5-FU and has been examined as a promising predictor for the effectiveness of 5-FU. Like TS, many studies have been conducted on gastric cancer and colon cancer\(^11,14,21\) and some studies on immunohistochemical staining have also been described\(^15\). For esophageal cancer, studies have been conducted to analyze DPD expression under combined 5-FU and CDDP administration with concurrent radiotherapy\(^22-23\). One of the modulators of S-1, 5-chloro-2, 4-dihydroxypyridine (CDHP), is a reversible inhibitor of DPD. Thus, regardless of DPD expression, more stable pharmacokinetics and increased effect of 5-FU can be achieved.

An evaluation of TS and DPD as response and prognostic factors in head-and-neck and gastric cancers found that in oral squamous cell carcinoma treated using S-1, TS, and DPD expression were lower in CR patients than in patients with stable disease\(^24\). On the other hand, in gastric cancer, survival was prolonged in DPD-positive patients compared to DPD-negative patients\(^25\). However, to our knowledge, no studies have investigated DPD expression in the treatment of esophageal cancer using S-1. Theoretically, differences in DPD expression should not affect therapeutic effect and prognosis. Baba et al. have reported that there is interindividual difference in DPD activity\(^26\). When DPD activity is so high in tumor tissue, it is not clear whether S-1 can show enough indication. While S-1 inhibits DPD, no reports have clearly demonstrated that this inhibition does not affect prognosis.

Radiation-induced apoptosis is known to involve p53,\(^27\) with p53 point mutations causing apoptosis resistance,\(^28\) and p53 representing a factor in CDDP sensitivity\(^29\). Several studies have found that mutant p53 is a significant prognostic factor in breast, lung, and colon cancers. Mutant p53 may also be a factor in predicting response and prognosis in esophageal cancer\(^29,30,31\). However, associations between p53 expression and both good response to CRT and prolonged survival have also been reported\(^32\). As uniform consensus remains elusive, immunohistological investigation of the expressions of TS, DPD, and p53 in patients with esophageal
cancer undergoing CRT comprising S-1, CDGP, and RT is clinically significant. The present study determined expression levels of TS, DPD, and p53 in biopsy samples obtained from patients before treatment in a clinical study of CRT using S-1 and CDGP. Correlations between these findings and response and prognosis were investigated.

Patients and Methods

A total of 32 patients (30 men, 2 women) with a histopathological diagnosis of squamous cell carcinoma of the esophagus were treated using CRT with S-1 (Taiho Pharmaceutical Co., Ltd. Tokyo Japan) and CDGP (Sionogi & Co., Ltd. Tokyo Japan) at St. Marianna University School of Medicine Hospital between June 2001 and June 2004. Patients eligible for this study had to be clinically judged by the criteria of the International Union Against Cancer (UICC). Pretreatment evaluation included barium esophagography, esophagoscopy, and cervical, chest and abdominal computed tomography (CT) scans. Endoscopic and abdominal ultrasound were optional. Adjacent organs were considered to be involved if the tumors extended into the lumen or caused a deformity of the airway to the tracheobronchial tree and if the tumors were attached to the organs at a contact angle 90 degree or greater in the thoracic aorta on the CT scan. T3 or lesser extent of disease was determined by endoscopic ultrasound, and endoscopy was used to evaluate gross appearance. Positive lymph nodes were defined if they were more than 1 cm in their diameter on any of the images.

Chemotherapy comprised S-1 (80–120 mg/body/day for about 2 weeks) and CDGP (total dose, 60–90 mg/m²). Two cycles of treatment were given over a 4- to 5-week interval. The dose of concurrently administered radiation ranged from 51.4 to 65.4 Gy. We did not make a clear standard for additional therapies in this clinical study. Usually, 2 to 4 cycles was added to chemotherapy of S-1/CDGP as the maintenance therapy. Recently, we have added salvage therapies (ex. surgery, EMR) actively for residual and recurrence cases after CRT, if possible.

The protocol included the immunohistochemical analysis for biopsy samples in this study. Written informed consents had been obtained from all patients who were registered for this study. The institutional review board of St. Marianna University School of Medicine have approved this study (approval number: the 463th).

Biopsy tissue specimens were obtained from 2 sites in each lesion before treatment and immunohistochemically stained for TS using anti-recombinant human TS-specific antibody (RTSSA: Taiho Pharmaceutical Co. Ltd., Saitama, Japan), for DPD using anti-human DPD polyclonal antibody (RDPDPA: Taiho Pharmaceutical Co. Ltd., Saitama, Japan), and for p53 using p53 monoclonal antibody (DO-7: Dako, Copenhagen, Denmark). TS, DPD, and p53 expression were studied and compared in clinically different conditions: depth of invasion; presence of lymph node metastases; presence of distant metastases; stage; treatment response; and survival rate.

Immunohistochemical examination

Formalin-fixed, paraffin-embedded endoscopic biopsy specimens obtained from the primary tumors of patients before treatment were examined in this study. After deparaffinization in xylene, specimens were rehydrated in graded alcohol. Sections were subjected to microwave antigen retrieval in citric acid buffer at pH 6.0 for 8×3 min. Endogenous peroxidase was blocked with 0.3% H₂O₂ in methanol for 30 min. After washing with phosphate-buffered saline (PBS), slides were incubated with 5% normal horse serum in PBS for 60 min. All sections were incubated overnight at 4°C with the primary antibodies in blocking buffer at the following concentrations: TS antibody, 1:5000; DPD antibody, 1:5000; and DO-7 antibody, 1:50. After washing 5 times in PBS, slides were incubated with biotinylated secondary anti-mouse antibodies diluted 1:250 with blocking buffer for 30 min. After washing with washing buffer 5 times, sections were incubated with avidin-biotinylated peroxidase complex reagent, and the color reaction was developed in 2% 3,3′-diaminobenzidine solution. Sections were then counterstained with Meyer’s hematoxylin, dehydrated, cleared and mounted.

Evaluation of immunostaining

Immunohistochemical staining was evaluated independently by 3 investigators, who determined the staining positivity of TS and DPD based on subjective estimates of intensity, rated 0–3. Intensity levels 0-1 were combined and considered low expression, whereas 2-3 was considered high expression. In addition, for p53 estimates of intensity, sections displaying diffusely stained cancer cell nu-
clei were defined as positive. The final grading was determined by consensus between all 3 investigators.

Statistical analysis

Correlations between clinicopathological variables and staining were examined by Fisher’s exact test and Wilcoxon rank-sum test. Correlations between treatment response and staining were examined using Fisher’s exact test. Survival curves were calculated using Kaplan-Meier methods, and significant differences were examined using the log rank test. A p-value < 0.05 was regarded as statistically significant. The Cox proportional hazard analysis by stepwise method was used to define influences on the survival rate as covariates, including immunohistochemical staining, patient characteristics factors, a TNM classification (tumor depth of invasion, lymph node metastasis, distant metastasis) or a UICC stage.

Results

Table 1 summarizes the clinical characteristics for the 32 patients. Median age was 62 years (range, 43–79 years). Performance status was PS0 in 15 patients and PS1 in 17 patients. The lesion site was the cervical esophagus (Ce) in 1 patient, upper thoracic esophagus (Ut) in 5 patients, middle thoracic esophagus (Mt) in 19 patients, lower thoracic esophagus (Lt) in 6 patients, and abdominal esophagus (Ae) in 1 patient. Histopathological examination revealed that the tumors were well differentiated in 5 patients, moderately differentiated in 18 patients, and poorly differentiated in 9 patients.

Depth of invasion was T1 in 5 patients, T2 in 7 patients, T3 in 15 patients, and T4 in 5 patients. Lymph node metastases were absent in 12 patients and present in 20 patients. Distant metastases were absent in 18 patients and present in 14 patients. There were 4 patients in stage I, 7 in stage IIA, 2 in stage IIB, 5 in stage III, 4 in stage IVA, and 10 in stage IVB. Treatment response was CR in 15 patients and non-CR in 17 patients. CR rate was 100% for Stage 1, 67% for stages II and III excluding T4, and 19% for T4 and stage IV.

Immunohistochemical staining revealed high TS expression, 11 patients; low TS expression, 21 patients. High TS expression rate, 34.4%; high DPD expression, 14 patients; low DPD expression, 18 patients. High DPD expression rate, 43.8%; and p53-positive, 9 patients; p53-negative, 23 patients (p53 positivity rate, 28.1%).

Figure 1 shows examples of immunohistochemical staining for TS, DPD, and p53. Table 2 shows correlations between clinicopathological characteristics and staining results. Advanced disease was significantly more common in p53 positive patients than in p53 negative patients (Table 2).

As to difference of expression of TS, DPD, and p53, there is no difference in the dosage quantity of anticancer drugs or radiation. CR was achieved by 3 of 11 high TS patients.
Fig. 1. Immunohistochemical staining for Thymidylate synthase (TS), Dihydropyrimidine dehydrogenase (DPD), and p53 ×200 magnification. High staining for TS is observed in the cytoplasm of cancer cells (A = intensity level: 3, B = intensity level: 2). Low TS (C = intensity level: 1). D = intensity level: 0. High staining for DPD is observed in the cytoplasm of cancer cells (E = intensity level: 3, F = intensity level: 2). Low DPD (G = intensity level: 0). Positive staining for p53 is observed in the nucleus of cancer cells (H: p53-negative (0).
(CR rate, 27.3%), compared to 12 of 21 low TS patients (CR rate, 57.1%). Likewise, CR was achieved by 6 of 14 high DPD patients (CR rate, 42.9%), compared to 9 of 18 low DPD patients (CR rate, 50.0%). Finally, CR was achieved in 3 of 9 p53 positive patients (CR rate, 33.3%), compared to 12 of 23 p53 negative patients (CR rate, 52.2%) (Table 3).

Only 1 of 5 patients with both high TS and p53 positive patients displayed CR (CR rate, 20%), compared to 10 of 17 patients with both low TS and p53 negative patients (CR rate, 58.8%) (Table 4).

For TS or p53 alone, survival tended to be longer in low TS expression, p53 negative patients than in high TS expression, p53 positive patients, but no significant differences were identified (TS: p =0.069; Fig. 2) (p53: p=0.057; Fig. 3). No significant correlation was observed between DPD and survival (Fig. 4).

Tables 5 and 6 show the results of survival analysis. When forward-backward stepwise selection analysis was performed as the covariates of patient characteristics factors, a TNM classification and the immunohistochemical staining, an opti-

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### Table 2. Correlation between TS, DPD, and p53 Expressions and Clinicopathological Characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>TS</th>
<th>DPD</th>
<th>p53</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>high</td>
<td>low</td>
<td>high</td>
</tr>
<tr>
<td>Depth of invasion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td>2</td>
<td>3</td>
<td>0.4339</td>
</tr>
<tr>
<td>T2</td>
<td>3</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>T3</td>
<td>5</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>T4</td>
<td>1</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Lymph node metastasis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>negative</td>
<td>4</td>
<td>8</td>
<td>1.0000</td>
</tr>
<tr>
<td>positive</td>
<td>7</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Distant metastasis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>negative</td>
<td>4</td>
<td>14</td>
<td>0.1422</td>
</tr>
<tr>
<td>positive</td>
<td>7</td>
<td>7</td>
<td></td>
</tr>
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<td>TNM stage</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>I</td>
<td>1</td>
<td>3</td>
<td>0.2549</td>
</tr>
<tr>
<td>II A</td>
<td>2</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>II B</td>
<td>1</td>
<td>1</td>
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</tr>
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<td>III</td>
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<td>2</td>
<td></td>
</tr>
<tr>
<td>IVB</td>
<td>5</td>
<td>5</td>
<td></td>
</tr>
</tbody>
</table>

TS: Thymidylate synthase, DPD: Dihydropyrimidine dehydrogenase

### Table 3. Correlation between TS, DPD, and p53 Expressions and Response to CRT

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Number of CR case</th>
<th>CR rate (%)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TS</td>
<td>high</td>
<td>11</td>
<td>3</td>
<td>27.3</td>
</tr>
<tr>
<td></td>
<td>low</td>
<td>21</td>
<td>12</td>
<td>57.1</td>
</tr>
<tr>
<td>DPD</td>
<td>high</td>
<td>14</td>
<td>6</td>
<td>42.9</td>
</tr>
<tr>
<td></td>
<td>low</td>
<td>18</td>
<td>9</td>
<td>50</td>
</tr>
<tr>
<td>p53</td>
<td>positive</td>
<td>9</td>
<td>3</td>
<td>33.3</td>
</tr>
<tr>
<td></td>
<td>negative</td>
<td>23</td>
<td>12</td>
<td>52.2</td>
</tr>
</tbody>
</table>

TS: Thymidylate synthase, DPD: Dihydropyrimidine dehydrogenase
CR: Chemoradiotherapy, CR: complete response
Table 4. Correlation between Combination of TS and p53 Expressions with Response to CRT

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Number of CR case</th>
<th>CR rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>high TS / p53 positive</td>
<td>5</td>
<td>1</td>
<td>20</td>
</tr>
<tr>
<td>high TS / p53 negative</td>
<td>6</td>
<td>2</td>
<td>33.3</td>
</tr>
<tr>
<td>low TS / p53 positive</td>
<td>4</td>
<td>2</td>
<td>50</td>
</tr>
<tr>
<td>low TS / p53 negative</td>
<td>17</td>
<td>10</td>
<td>58.8</td>
</tr>
</tbody>
</table>

TS: Thymidylate synthase, DPD: Dihydropyrimidine dehydrogenase
CRT: Chemoradiotherapy, CR: complete response

Fig. 2. Kaplan-Meier survival curve according to TS protein expression determined immunohistochemically (p=0.069).

Fig. 3. Kaplan-Meier survival curve according to p53 protein expression determined immunohistochemically (p=0.057).
mized model including distant metastasis, PS, p53, histological type, age as covariates was selected (Table 5). When we used as covariates of patient characteristics factors, a UICC stage and the immunohistochemical staining, in this case an optimized model including UICC stage, PS and TS as covariates was selected (Table 6).

Finally we divided the patients into two groups and analysed with both low TS and p53 negative patients and the other patients. Survival was sig-

![Kaplan-Meier survival curve](image)

**Fig. 4.** Kaplan-Meier survival curve according to DPD protein expression determined immunohistochemically (p=0.238).

**Table 5.** Result of Forward-backward Stepwise Selection Analysis Based on the Cox Proportional Hazard Model with Patient Characteristics Factors, TNM classification, and immunohistochemical staining as initial covariates

<table>
<thead>
<tr>
<th>Selected Variable</th>
<th>Coefficient</th>
<th>Probability</th>
<th>Hazard Ratio</th>
<th>95% Confidence Interval</th>
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<tbody>
<tr>
<td>distant metastasis</td>
<td>1.1983</td>
<td>0.1120</td>
<td>3.314</td>
<td>0.756 - 14.524</td>
</tr>
<tr>
<td>PS</td>
<td>1.1486</td>
<td>0.0564</td>
<td>3.154</td>
<td>0.969 - 10.264</td>
</tr>
<tr>
<td>p53</td>
<td>1.2288</td>
<td>0.0393</td>
<td>3.417</td>
<td>1.062 - 10.992</td>
</tr>
<tr>
<td>histological type</td>
<td>0.7809</td>
<td>0.1155</td>
<td>2.183</td>
<td>0.826 - 5.773</td>
</tr>
<tr>
<td>age</td>
<td>-0.0509</td>
<td>0.1365</td>
<td>0.950</td>
<td>0.889 - 1.016</td>
</tr>
</tbody>
</table>

TS: Thymidylate synthase, DPD: Dihydropyrimidine dehydrogenase
PS: Performance status

**Table 6.** Result of Forward-backward Stepwise Selection Analysis Based on the Cox Proportional Hazard Model with Patient Characteristics Factors, UICC stage, and immunohistochemical staining as initial covariates

<table>
<thead>
<tr>
<th>Selected Variable</th>
<th>Coefficient</th>
<th>Probability</th>
<th>Hazard Ratio</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>UICC stage</td>
<td>0.6550</td>
<td>0.0020</td>
<td>1.925</td>
<td>1.270 - 2.918</td>
</tr>
<tr>
<td>PS</td>
<td>1.1888</td>
<td>0.0407</td>
<td>3.283</td>
<td>1.052 - 10.248</td>
</tr>
<tr>
<td>TS</td>
<td>1.1433</td>
<td>0.0552</td>
<td>3.137</td>
<td>0.975 - 10.094</td>
</tr>
</tbody>
</table>

TS: Thymidylate synthase, DPD: Dihydropyrimidine dehydrogenase
PS: Performance status

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significantly prolonged for patients with both low TS and p53 negative patients as compared to all other patients (p=0.0389; Fig. 5).

Discussion

Prior testing of biopsy samples is needed to predict response to CRT. However, molecular clonal heterogeneity exists in esophageal squamous cell carcinomas. Biopsy samples do not always reflect the characteristics of the main tumors, due to heterogeneity. Care is thus necessary in genetic diagnosis using biopsy samples. Although no correlations were found between protein expression and either mRNA expression or enzymatic activity for either TS or DPD, immunohistochemical staining remains useful. The advantages of immunohistochemical analysis are that the technique is labor-saving, low-cost and applicable to formalin-fixed tissues, and measuring enzyme activities and mRNA levels are technologically more complex than immunohistochemical analysis. Furthermore, we analyzed 2 separate biopsy samples from the patient. Conversely, a study showed that good correlations are seen in staining results between biopsy and surgically resected specimens. In this study we considered that immunohistochemical evaluation using only a biopsy sample from before CRT would be useful, and examined how this approach might be used in clinical settings.

As far as TS expression in esophageal cancer is concerned, Tajima et al. reported that TS might be a predictive marker for CRT with 5-FU/CDDP and the reduction rate was higher in tumors negative for TS expression than in positive ones. The results of our study are similar to their findings. S-1 is effective because FdUMP forms a ternary complex with methylene tetrahydroxyfolate (CH₂FH₄) and TS to inactivate TS, thus hindering DNA synthesis. High TS concentration is disadvantageous for obtaining a high TS inhibition rate because more FdUMP and CH₂FH₄ would be necessary. This explanation is consistent with our result. If TS is too high, quantity of FdUMP becomes insufficient, and TS which cannot be a complex comes out. Therefore DNA synthesis of a tumor advances. Adversely if TS is low, an antitumor effect of 5-FU is provided, because a complex is formed without TS remaining. TS may therefore represent a predictor for the effectiveness of CRT using S-1 and CDGP in the treatment of esophageal cancer.

To our knowledge, no previous studies have used S-1 in the treatment of esophageal cancer and analyzed DPD expression. When S-1 was used in gastric cancer patients, unlike 5-FU, prognosis was more favorable in DPD-positive cases than in DPD-negative cases. Our results suggest that the level of DPD protein expression is unrelated to therapeutic effect and prognosis in esophageal cancer. And DPD thus does not represent a prognostic indicator for esophageal cancer responsiveness to CRT using S-1. There is some possibility that the results depend on the DPD-inhibitory activity of

![Kaplan-Meier survival curve](image)

**Fig. 5.** Kaplan-Meier survival curve according to combination of TS protein and p53 protein expression. Significant differences were identified between low TS/p53 negative and other patients (p=0.0389).
S-1.

While p53 plays a role in CDDP and radiation-induced apoptosis, this function is disrupted with mutant p53. The mutant p53 are refractory to apoptosis induction and less responsive to CRT. Most p53 proteins that stain immunohistochemically are mutant proteins. However, one study found no correlation between high p53 levels and mutation. As genotyping was not performed for the present study, whether wildtype or mutation was stained remains unclear. But the present results show that survival is more favorable in p53-negative cases than in p53-positive cases. Therefore, in this study stained p53 appears more likely to have represented mutant p53. When expression of mutant p53 is high, normal p53 function is suppressed, reducing the effectiveness of radiotherapy or CDGP and resulting in different prognoses.

The present study includes 16 operable cases in stage I, II, and III except T4, and 16 inoperable T4 and stage IV cases. Therefore there may be a bias in evaluating the two groups at the same time. We reviewed it only for operable cases except T4 and M1, but the small sample size was too small to evaluate, well. Median survival time (MST) of high TS cases was 334 days, low TS cases did not reach MST. MST of p-53-positive cases was 490 days and p53-negative was 746 days. For a tendency, the correlation between TS, p53, and prognosis was thus necessary by considering other variates: immunostaining; patient background factors.

Investigation of the eectiveness of radiotherapy or CDGP and RT is performed in the treatment of esophageal cancer, DPD as assessed by immunohistological staining is not a predictor for therapy responsiveness, while the combination of TS and p53 may be predictors.

Acknowledgments

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食道癌に対する S-1/Nedaplatin 放射線併用療法、および Thymidylate Synthase, Dihydropyrimidine Dehydrogenase, p53 の免疫組織学的検討

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抄 録

現在、食道癌に対する放射線化学療法（CRT）は外科的治療に匹敵する成績が報告されるようになり、手術可能症例でも CRT を選択されることが多くなってきている。我々は副作用の軽減、quality of life の維持、治療成績の向上を目的に a new oral dihydropyrimidine dehydrogenase inhibitory fluoropyrimidine, tegafur・gimeracil・osteracil potassium (S-1) と nedaplatin (CDGP) の放射線同時併用療法を施行している。しかし、complete response (CR) に至らない症例は、予後不良である。そこで CRT 前に治療効果や予後を予測することは重要であると考えられる。

今まで行われたことのない食道癌に対する S-1/CDGP-RT を行った症例の治療前の生検組織を使用し thymidylate synthase (TS), dihydropyrimidine dehydrogenase (DPD) と p53 の発現を免疫組織学的に調べ、治療効果、予後との関係を検討した。

TS 高発現の CR 率が 27.3% であったのに対し、TS 低発現では CR 率が 57.1% であった。TS 高発現と p53 陽性の組み合わせの CR 率が 20% であったのに対し、TS 低発現と p53 陰性の組み合わせでは CR 率が 88.8% であった。TS 低発現と p53 陰性は、TS 高発現、p53 陽性に比べて生存期間長い傾向であった（TS: p=0.069, p53: p=0.057）。また、多変量解析の結果 TS と p53 が、重要な予後因子となり得る可能性が示唆された。そして、TS 低発現と p53 陰性の組み合わせが、その他の症例に比べて生存期間が有意 (p=0.0389) に延長した。

この結果、S-1 と CDGP を使用した CRT において TS と p53 の組み合わせが予後因子となり得る可能性が示唆された。一方、DPD の発現の程度と生存に関連性を認めなかった。この結果からは食道癌で S-1 を使用した CRT において DPD が予後予測因子にはなり得ないと考えられる。

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