Expression of thymidylate synthase predicts clinical outcomes of S-1-based chemotherapy in squamous cell lung cancer

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Abstract. Non-small cell lung cancer (NSCLC) patients with squamous cell carcinoma (SCC) histology have limited chemotherapeutic options. Treatment with S-1 combined with carboplatin (CBDCA) has been shown to provide a significant survival benefit in SCC patients compared with treatment with combined CBDCA and paclitaxel. The aim of the present study was to investigate the association between the expression of molecular markers related to the pharmacological action of S-1, including thymidylate synthase (TS), orotate phosphoribosyltransferase (OPRT) and dihydropyrimidine dehydrogenase (DPD), and the clinical efficacy of S-1-based chemotherapy in SCC patients. The immunohistochemical expression of TS, OPRT and DPD were retrospectively analyzed in tumor biopsy and resection specimens from patients with advanced SCC (n=32). Immunohistochemical H-scores were calculated and their association with S-1/CBDCA response was evaluated. Median progression-free survival time was significantly longer in patients with low TS H-scores than in those with high TS H-scores (162.5 vs. 97 days; P=0.004); by contrast, overall survival time was not observed to differ significantly between these groups (P=0.185). In the multivariate analysis, low TS expression was a significant positive factor for progression-free survival rate (hazard ratio, 0.40; P=0.021). A low TS H-score was also associated with an increased response to S-1-based chemotherapy compared with a high TS H-score (P=0.002). This indicates that SCC patients with low TS expression can benefit significantly from S-1-based chemotherapy, and that H-score measurement of intratumoral TS expression may represent a useful predictive biomarker for response to S-1-based chemotherapy by patients with SCC-type NSCLC.

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Introduction

In recent years, histology-based chemotherapy selection for advanced non-small cell lung cancer (NSCLC) has been advocated. Specifically, the chemotherapeutic agent pemetrexed (PEM) is predominantly restricted to treating patients with non-squamous cell carcinoma (non-SCC) based on phase III trials (1). Thus, there are less treatment options available for SCC compared with for adenocarcinoma (AC). The molecular basis underlying histology-specific chemotherapy selection, and the predictive value of chemotherapy sensitivity/resistance biomarkers in SCC remain unclear.

The clinical use of S-1, a chemotherapeutic agent composed of tegafur, gimeracil, and oteracil potassium, for NSCLC has been investigated in clinical trials (2). In the multicenter randomized phase III Lung Cancer Evaluation of TS-1 (LETS) study, Okamoto *et al* (3) reported that

S-1/carboplatin (CBDCA) was not inferior to CBDCA/pacli-taxel as a first-line treatment in terms of overall survival (OS) time in patients with advanced NSCLC (3). In the updated survival time data based on NSCLC histology, SCC patients in the S-1/CBDCA group had a longer median OS time than those in the CBDCA/paclitaxel group (4). According to this analysis, S-1-based chemotherapy is now considered as the major therapeutic option for lung SCC therapy among the limited available options for chemotherapy regimens.

Several enzymes participate in the metabolic pathways of 5-fluorouracil (5-FU) and folate, including thymidylate synthase (TS), a target enzyme of 5-FU; dihydropyrimidine dehydrogenase (DPD), which catalyzes 5-FU degradation; and orotate phosphoribosyltransferase (OPRT), which activates 5-FU and produces 5-fluoroudine monophosphate. TS, DPD and OPRT expression levels have been shown to be associated with 5-FU sensitivity in solid tumors (5). A previous study (6) has demonstrated that low TS and DPD expression levels are predictive biomarkers for an improved response to S-1/CBDCA in NSCLC patients, including an increased survival time. TS and OPRT expression were significantly reduced in tissue samples from NSCLC patients with AC compared with those without, whereas DPD expression was higher in AC samples (7). A low TS expression level in lung SCC tissue is associated with better response to 5-FU-based chemotherapy (8). In addition, the response to S-1-based chemotherapy was higher in head and neck SCC patients with low TS activity than in those with high TS activity (9,10). Thus, the evaluation of TS, OPRT and DPD expression levels in histological subtypes may aid in predicting the clinical response to chemotherapy, including S-1, in SCC patients who have restricted chemotherapeutic options. However, the clinical relevance of TS, OPRT and DPD has not been established for lung SCC patients treated with S-1 alone or S-1 combination chemotherapy. The aim of the present study was to evaluate the predictive value of immunohistochemically detected TS, DPD and OPRT expression for the response to S-1/CBDCA chemotherapy in patients with lung SCC.

Materials and methods

Patients. The inclusion criteria for the present retrospective study were as follows: i) Pathologically confirmed SCC; ii) diagnosed with unresectable stage IIIA, IIIB or IV, or postoperative recurrence without preoperative chemotherapy, or radiation; and iii) an Eastern Cooperative Oncology Group Performance status between 0 and 2. A total of 37 patients with relapsed or advanced SCC who received CBDCA (Nippon Kayaku Co., Ltd., Tokyo, Japan) treatment at an area under the curve of 5 on day 1, and S-1 (Taiho Pharmaceutical Co., Ltd., Tokyo, Japan) at 80 mg/m^2 on days 1-14 at Juntendo University Hospitals (Tokyo, Japan) between April 2011 and July 2014, were retrospectively analyzed. Tumor response was examined using computed tomography and evaluated according to the Response Evaluation Criteria in Solid Tumors (version 1.1) (11). Comprehensive consent was obtained from the patients, and the study protocol was approved by the Ethics Committee of Juntendo University School of Medicine (no. 2013068).

Tissue samples. A total of 28 biopsy specimens and 9 resection specimens (relapsed SCC, 6 specimens; incompletely resected

SCC, 3 specimens) were fixed in 10% formalin for 48 h and embedded in paraffin for evaluation by pathologists. Among the biopsy specimens, 5 small specimens did not have sufficient tissue available in paraffin blocks for immunohistochemical assessment. The remaining 32 samples were investigated by immunohistochemical analysis in the present study.

Immunohistochemistry and scoring of protein expression. Tissue sections (thickness, 4 μm) were deparaffinized in xylene and then rehydrated. Antigen retrieval was conducted by microwaving at 750 W for 10 min in 10 mM citric acid buffer (Ph 6.0) for TS and OPRT, and by boiling at 97˚C for 40 min in 1 mM EDTA/10 mM Tris buffer (pH 9.0) for DPD. Endogenous peroxidase activity was deactivated by a 5-min incubation in 0.3% H_2O_2/methanol. Following washing in phosphate-buff-ered saline, the sections were incubated at room temperature with primary polyclonal antibodies against TS (dilution, 1:100; provided by Taiho Pharmaceutical Co., Ltd.) and OPRT (dilu-tion, 1:100; cat. no. 28135; Immuno-Biological Laboratories Co., Ltd., Minneapolis, MN, USA) for 1 h, and against DPD (dilution, 1:50; cat. no. 10411; Immuno-Biological Laboratories Co., Ltd.) overnight. Ready-to-use peroxidase-based EnVi-sion™ + (cat. no. K5027; Dako; Agilent Technologies, Inc., Santa Clara, CA, USA) was applied as a secondary antibody for 30 min at room temperature. Peroxidase activity was visual-ized with diaminobenzidine tetrahydrochloride solution (Dako; Agilent Technologies, Inc.). Sections were counterstained with Mayer's hematoxylin.

All immunostained sections were evaluated separately by three observers (Y.H., S.T., and K.S.) without knowledge of the patients' clinical data. Sections with discrepant results were re-evaluated by the pathologists until a consensus was reached. TS, OPRT and DPD cytoplasmic staining were scored in a semiquantitative manner reflecting the staining intensity and percentage of area with stained cells at each intensity, as previ-ously described (12). Staining intensity (I) was classified as 0 (no staining), +1 (weak staining), +2 (intermediate staining) or +3 (strong staining). The percentage of positively stained cells (PC) was graded as 0 (0%), 0.1 (1-9%), 0.5 (10-49%) or 1.0 (≥50%). H-scores were obtained by calculating the mean I x PC value as follows: Mean value of I x PC = Σ(I x PC) among all fields/total number of fields evaluated.

Statistical analysis. TS, OPRT and DPD expression were compared between groups using the Spearman rank-correla-tion coefficient. The selection of clinically important cut-off scores for TS, OPRT and DPD expression was based on median values. OS and progression-free survival (PFS) times were assessed from the first day of chemotherapy administration to the date of mortality due to any cause, and the date of objective disease progression, respectively. Patients without documented mortality at the time of the final analysis were evaluated on the last date they were known to be alive or the date of their last objective tumor assessment. The Kaplan-Meier method was used to estimate the probability of survival as a function of time, and differences in the survival of patient subgroups were evaluated using the log-rank test. The multivariate logistic regression models and Cox proportional hazards regres-sion models were used to assess the predictive value of TS, OPRT and DPD for PFS time in lung SCC patients treated

Table I. Characteristics of 32 patients with lung squamous cell carcinoma.

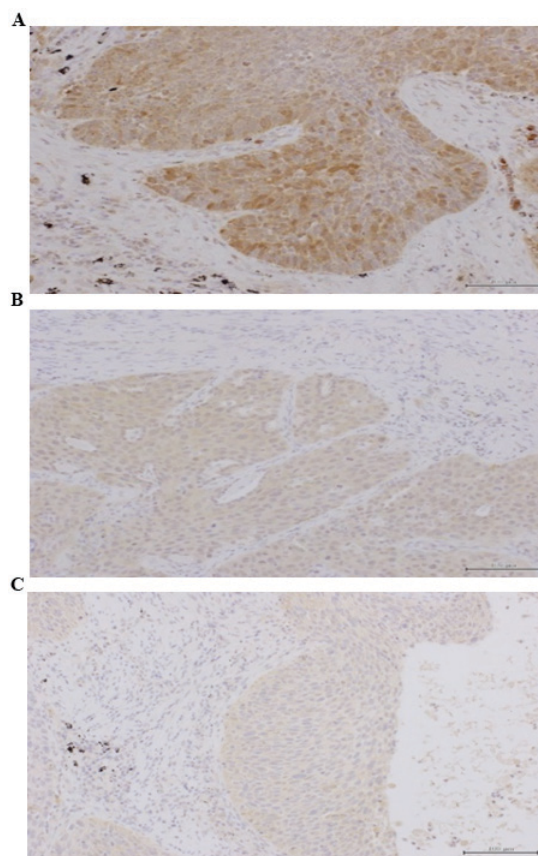| Characteristics	Value
Age, years	
Median	69.5
Range	52-82
Gender, n (%)	
Male	27 (84)
Female	5 (16)
Performance status, n (%)	
0	17 (53)
1	11 (34)
2	4 (13)
Stage, n (%)	
IIIA	6 (19)
IIIB	6 (19)
IV	14 (43)
Relapsed	6 (19)
Smoking status, n (%)	
Non-smoker	1 (3)
Smoker	31 (97)
Specimens, n (%)	
Biopsy	23 (72)
Resection	9 (28)
Histological differentiation, n (%)	
Differentiated	21 (66)
Undifferentiated	11 (34)
Treatment response, n (%)	
Complete response	1 (3)
Partial response	16 (50)
Stable disease	12 (38)
Progressive disease	3 (9)

Figure 1. Immunohistochemical staining of human SCC tissues; representative sections of SCCs with high expression levels of (A) thymidylate synthase, (B) orotate phosphoribosyltransferase and (C) dihydropyrimidine dehydrogenase are shown. Scale bars, 100 μm. SCC, squamous cell carcinoma.

with S-1/CBDCA as a first-line chemotherapy. P<0.05 was considered to indicate a statistically significant difference. All statistical analyses were performed using JMP software (version 11.0.0; SAS Institute Inc., Cary, NC, USA).

Results

Patient characteristics and S-1/CBDCA response. The characteristics of the patients are summarized in Table I. A total of 32 patients were administered S-1/CBDCA as first-line chemotherapy. This included 27 male and 5 female patients. The patients ranged in age from 52 to 82 years (median, 69.5 years). There were 31 patients who smoked, and 1 patient who had never smoked. Histological differentiation was classed as differentiated in 21 patients and undifferentiated in 11 patients. The clinical stage was diagnosed as stage IIIA in 6 patients, stage IIIB in 6 patients, and stage IV in 14 patients. Relapse occurred in 6 patients who had undergone surgery without preoperative chemotherapy or radiation. The median

number of chemotherapy cycles was 4 (range, 1-6 cycles). Of the 32 patients, 2 received 6 cycles, 1 received 5 cycles, 17 received 4 cycles, 6 received 3 cycles, 3 received 2 cycles, and 3 received 1 cycle. Complete response (CR) was observed in 1 patient (3%), partial response (PR) in 13 patients (41%), stable disease (SD) in 15 patients (47%) and progressive disease (PD) in 3 patients (9%).

Immunohistochemical expression of TS, OPRT and DPD in tumor tissues. Intratumoral TS, OPRT and DPD expression levels (H-scores) ranged from 1.0 to 3.6 (median, 2.0), 0.4 to 2.5 (median, 1.0), and 0.5 to 2.7 (median, 1.1), respectively. Representative tumor sections with high TS, OPRT and DPD expression levels are shown in Fig. 1. TS H-score was correlated with DPD H-score (R=0.509; P=0.023) and OPRT H-score (R=0.343; P=0.042). Intratumoral TS, OPRT and DPD H-scores did not significantly differ between tumor biopsy and resection specimens (P=0.205, P=0.642 and P=0.267, respectively). Tumor biopsy and resection specimen H-scores, respectively, were 2.12 [95% confidence interval (CI), 1.80-2.46] and 1.74 (95% CI, 1.31-2.18) for TS, 1.15 (95% CI, 0.93-1.37) and 1.11 (95% CI, 0.94-1.28) for OPRT, and 1.19 (95% CI, 1.01-1.37) and 0.95 (95% CI, 0.71-1.19) for DPD.

Association of TS, OPRT and DPD expression levels with patient characteristics. TS, OPRT and DPD H-scores were not significantly associated with patient demographics, including

Table II. Associations between H-scores of TS, OPRT and DPD and various characteristics in 32 patients with lung squamous cell carcinoma.

		TS		OPRT		DPD	
Variable	n	H-score, mean ± SD	P-value	H-score, mean ± SD	P-value	H-score, mean ± SD	P-value
Age, years			0.234		0.246		0.595
<75	22	2.14±0.78		1.13±0.35		1.18±0.45	
≥75	10	1.76±0.55		1.17±0.63		1.03±0.21	
Gender			0.513		0.476		0.524
Male	27	1.99±0.76		1.16±0.48		1.10±0.37	
Female	5	2.16±0.61		1.04±0.09		1.30±0.47	
Performance status			0.17		0.623		0.268
0/1	28	1.94±0.67		1.15±0.48		1.05±0.19	
2	4	2.58±1.02		1.05±0.10		1.68±0.83	
Stage			0.295		0.624		0.704
IIIA/IIIB	12	2.28±0.84		1.23±0.63		1.25±0.52	
IV	14	1.93±0.69		1.10±0.34		1.09±0.31	
Relapsed	6	1.70±0.46		1.02±0.18		1.02±0.27	
Pathology			0.412		0.6		0.204
Differentiated	21	1.99±0.84		1.14±0.38		1.15±0.39	
Undifferentiated	11	2.07±0.50		1.14±0.57		1.08±0.39	

H-score, immunohistochemistry score; TS, thymidylate synthase; OPRT, orotate phosphoribosyltransferase; DPD, dihydropyrimidine dehydrogenase; SD, standard deviation.

age, gender, performance status, stage or differentiation (Table II).

Predictive relevance of TS, OPRT and DPD expression levels for response to S-1/CBDCA. To evaluate the relationship between TS, OPRT and DPD H-scores and S-1/CBDCA response, tumors were categorized as either responding (CR or PR) or non-responding (SD or PD) (Fig. 2). TS H-scores were significantly lower in responding tumors than in non-responding tumors (P=0.002). High-TS tumors were defined as tumors with a TS H-score >2.0. The proportion of low-TS tumors responding to therapy was 71.4% (n=10/14) and the proportion of high-TS tumors which did not respond was 83.3% (n=15/18). OPRT H-scores was not significantly associated with tumor response to S-1/CBDCA therapy (P=0.849). Furthermore, DPD H-scores demonstrated an association with tumor response to S-1/CBDCA therapy; however this was insignificant (P=0.086).

Association of TS, OPRT and DPD expression and patient characteristics with PFS and OS. The median PFS and OS times were 137 (range, 25-455) days and 348 (range 41-929) days, respectively. A cut-off value was selected for each clinicopathological factor according to the median value. Univariate Cox analysis identified factors that significantly affected PFS (Table III) and OS (Table IV) times. TS expression was a prognostic factor for PFS (P=0.008) but not OS (P=0.185) time. DPD and OPRT expression were not significant prognostic factors for PFS time (P=0.772 and P=0.828, respectively) or

OS time (P=0.313 and P=0.650, respectively). Performance status was significantly correlated with PFS (P=0.033) and OS (P=0.0001) time.

Multivariate analysis by Cox proportional hazards model was performed to evaluate the influence of TS expression and performance status on PFS time after adjusting for possible confounding factors. TS expression was the only significant factor associated with PFS time [hazard ratio (HR), 0.40; 95% CI, 0.18-0.87; P=0.021].

Kaplan-Meier survival curves of PFS and OS time for patients in the high and low TS, DPD and OPRT expression groups are presented in Fig. 3. Patients in the low TS group had a significantly longer median PFS time compared with that of patients in the high TS group (162.5 vs. 97 days; P=0.004). OS time was not significantly different between TS expression groups (370 vs. 309.5 days; P=0.177). DPD and OPRT H-scores were not significantly associated with median PFS time (P=0.772 and P=0.828, respectively) or OS time (P=0.313 and P=0.650, respectively). Notably, 1 patient with a CR from S-1/CBDCA treatment presented with the lowest TS H-score of 1.0. By contrast, two patients with PD during treatment presented with the highest TS H-scores of 3.6.

Discussion

The present study investigated the association between immunohistochemical TS, OPRT and DPD expression levels and clinical outcomes for SCC patients treated with combination

Table III. Logistic analysis for response in terms of progression-free survival in 32 patients with lung squamous cell carcinoma.

Variable	n	Univariate HR (95% CI)	P-value[a]	Multivariate HR (95% CI)	P-value[b]
Age, years					
<75	22	1	-	-	-
≥75	10	1.17 (0.52-2.50)	0.687	-	-
Gender					
Male	27	1	-	-	-
Female	5	1.94 (0.62-5.14)	0.230	-	-
Performance status					
0/1	28	1	-	1	-
2	4	4.16 (1.14-12.34)	0.0327	2.96 (0.80-9.04)	0.0975
Stage					
IIIA/IIIB	12	1	-	-	-
IV	14	0.73 (0.32-1.65)	0.443	-	-
Relapsed	6	0.77 (0.27-2.01)	0.609	-	-
Pathology					
Differentiated	21	1	-	-	-
Undifferentiated	11	1.61 (0.71-3.50)	0.244	-	-
TS H-score					
>2.0	14	1	-	1	-
≤2.0	18	0.35 (0.17-0.75)	0.0076	0.40 (0.18-0.87)	0.0213
OPRT H-score					
>1.0	14	1	-	-	-
≤1.0	18	0.83 (0.40-1.78)	0.828	-	-
DPD H-score					
>1.0	13	1	-	-	-
≤1.0	17	1.12 (0.53-2.38)	0.772	-	-

[a]Univariate analysis by log-rank test; [b]Multivariate analysis by Cox proportional hazards model. HR, hazard ratio; CI, confidence interval; H-score, immunohistochemistry score; TS, thymidylate synthase; OPRT, orotate phosphoribosyltransferase; DPD, dihydropyrimidine dehydrogenase.

S-1/CBDCA therapy. Patients with a low TS expression level had a significantly longer median PFS time, although not a significantly longer OS time, when compared with patients with high TS expression.

The majority of advanced NSCLC cases are diagnosed by examination of small biopsy specimens, as it is difficult to obtain resection specimens except from relapsed patients who previously underwent surgical resection. Therefore, small tumor biopsy specimens are often used to identify potential biomarkers. In the use of such samples, the important issues are tumor cell content, representativeness of the sample, and mode of biomarker assessment. In the present study, H-scores of biopsy specimens and resection specimens were observed to be equally evaluable. A previous study also demonstrated that TS expression scores in biopsy specimens largely reflect TS expression in the corresponding resection specimens; the authors recommended a cut-off value of 10% moderately to strongly stained tumor cells in order to obtain the highest agreement between biopsy and resection specimens in NSCLC,

particularly in SCC (13). By contrast, cut-off criterions for TS positivity in NSCLC range from 29.6-72.5% in previously published studies (14,15). The variability in TS expression cut-offs may be attributed to the heterogeneous histology of invasive AC subtypes and the lack of a standardized immunohistochemistry scoring system. Therefore, the present study quantified H-score by calculating the intensity and percentage of stained tumor cells, as described in a previous report (12). Several groups have used immunohistochemistry and reverse transcription-polymerase chain reaction (RT-PCR) to detect TS expression (15,16). Shimizu et al (16) reported a significant correlation between the two detection methods. TS gene copy number by silver in situ hybridization was found to be significantly correlated with the immunohistochemical expression of TS (17). However, the contamination of whole tumor tissue with non-neoplastic cells cannot be avoided in mRNA extraction from tissue, requiring time-consuming microdissection to ensure that only neoplastic tissue is obtained. Immunohistochemistry is preferred over RT-PCR, as well-visualized
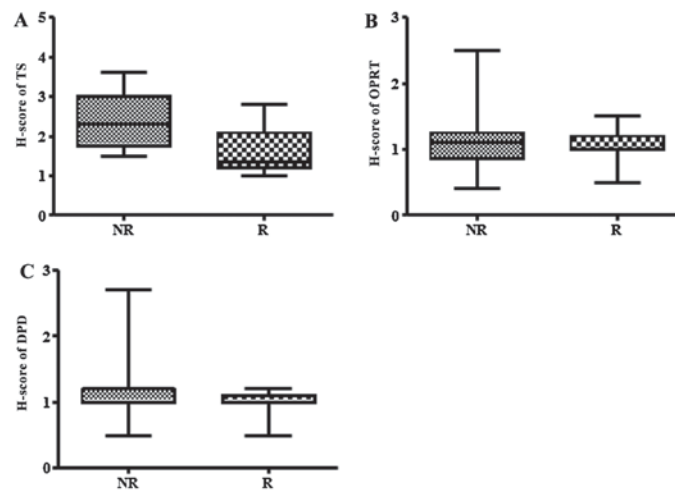
Figure 2. Association between TS, OPRT and DPD H-scores and the response to S-1/carboplatin in patients with squamous cell carcinoma. Expression levels (H-scores) of (A) TS, (B) OPRT and (C) DPD were determined in R tumors (including complete or partial response) and NR tumors (including stable or progressive disease). Horizontal lines indicate the median H-score values. Differences in TS, OPRT and DPD H-scores between R and NR tumors were analyzed using the Mann-Whitney *U* test. TS, thymidylate synthase; OPRT, orotate phosphoribosyltransferase; DPD, dihydropyrimidine dehydrogenase; R, responding; NR, non-responding.
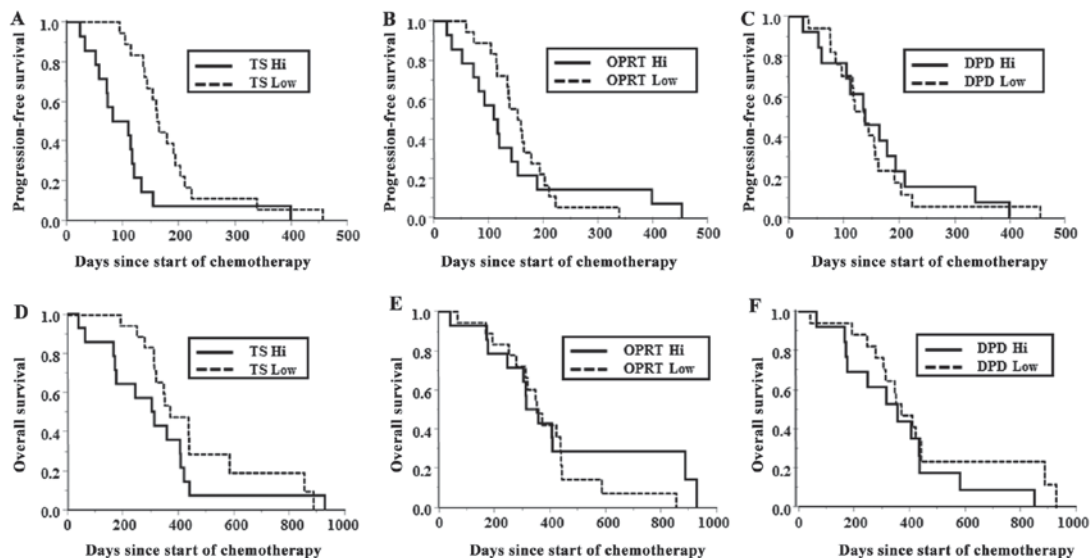

Figure 3. Kaplan-Meier survival analyses of PFS time in patients with high and low H-scores for (A) TS, (B) OPRT and (C) DPD, and of OS time in patients with high and low H-scores for (D) TS, (E) OPRT and (F) DPD. The selection of high and low cut-off levels was based on median values. Differences in PFS and OS times between subgroups were analyzed using the log-rank test. PFS, progression-free survival; TS, thymidylate synthase; OPRT, orotate phosphoribosyltransferase; DPD, dihydropyrimidine dehydrogenase; OS, overall survival.

immunohistochemical staining can allow neoplastic tissues to be distinguished from non-neoplastic tissues.

Several studies have analyzed TS expression in patients with non-squamous cell lung cancer treated with PEM-based chemotherapy. Low gene copy number and low protein levels of TS in biopsy specimens have been associated with an improved response to PEM-based chemotherapy in lung AC (18-20). In the present study, the superior response to S-1-based chemotherapy in SCCs with low TS expression is consistent with TS being a target enzyme and a predictive biomarker of chemotherapy response for PEM. Takeda *et al* (6) reported that low TS and DPD immunohistochemical expression levels, according to cut-off criteria for high or low expression, were associated with improved response and longer survival time

in 22 patients with advanced NSCLC treated with S-1-based chemotherapy. However, only 1 of the 22 patients with NSCLC treated with S-1/CBDCA in this study had histological SCC. In the present study, the immunohistochemical expression levels of TS, OPRT and DPD were quantified in 32 SCC patients, and low TS expression levels were demonstrated to be strongly associated with longer PFS time in patients with lung SCC treated with S-1/CBDCA.

Several studies have suggested that DPD and OPRT are also predictors of S-1 response in NSCLC (6,21-23). DPD activity levels have been shown to be higher in NSCLC tissues than in gastric, colorectal and breast cancer tissues (21). S-1 contains gimeracil, which acts as a DPD inhibitor; gimeracil is a stronger DPD inhibitor than uracil when it is used

Table IV. Univariate logistic analysis for response in terms of overall survival in 32 patients with lung squamous cell carcinoma.

Variable	n	HR (95% CI)	P-value[a]
Age, years			
<75	22	1	-
≥75	10	0.81 (0.34-1.79)	0.610
Gender			
Male	27	1	-
Female	5	0.95 (0.27-2.53)	0.920
Performance status			
0/1	28	1	-
2	4	34.15 (6.30-255.06)	0.0001
Stage			
IIIA/IIIB	12	1	-
IV	14	0.50 (0.21-1.18)	0.500
Relapsed	6	0.39 (0.12-1.12)	0.082
Pathology			
Differentiated	21	1	-
Undifferentiated	11	1.04 (0.44-2.27)	0.919
TS H-score			
>2.0	14	1	-
≤2.0	18	0.60 (0.28-1.29)	0.185
OPRT H-score			
>1.0	14	1	-
≤1.0	18	1.20 (0.55-2.76)	0.650
DPD H-score			
>1.1	13	1	-
≤1.1	17	0.66 (0.29-1.49)	0.313

[a]Univariate analysis by log-rank test. HR, hazard ratio; CI, confidence interval; H-score, immunohistochemistry score; TS, thymidylate synthase; OPRT, orotate phosphoribosyltransferase; DPD, dihydropyrimidine dehydrogenase.

in combination with tegafur. Therefore, NSCLC exhibits a different response to S-1 when compared with other solid tumors (21-23), and a high DPD expression level predicts resistance to S-1-based chemotherapy (6). Patients with AC with low intratumoral DPD mRNA expression who received 5-FU subsequent to surgery had a significantly better prognosis than those who received surgery alone (24). The present study demonstrated that low DPD expression levels, compared with high DPD levels, were only weakly associated with an improved response to S-1/CBDCA (P<0.1).

OPRT, a key enzyme that catalyzes the first step in nucleic acid-mediated 5-FU phosphorylation, is hypothesized to have significant associations with the antitumor activity of 5-FU. Ichikawa et al (22) reported that low TS expression and high OPRT expression are predictors of S-1 response in gastric cancer. Nakano et al (25) found that, in surgically resected NSCLC specimens, TS and OPRT immunohistochemical expression are higher in SCC than in AC. High OPRT expression in SCC tissues may account for the increased response and longer median OS time in patients treated with S-1/CBDCA compared with those treated with CBDCA/paclitaxel combination therapy (4,26). However, in the H-score analysis of the

present study, OPRT expression level was not associated with treatment response or PFS and OS times in patients with lung SCC treated with S-1/CBDCA.

The present study demonstrated that TS, OPRT and DPD H-scores were not associated with OS in SCC patients. However, low TS expression is significantly associated with a higher rate of response and longer PFS in SCC patients treated with S-1/CBDCA. Hence, the evaluation of TS expression level may be a sensitive biomarker to predict the response to S-1-based chemotherapy of patients with advanced SCC, and may be a more useful biomarker for decision-making regarding further S-1 maintenance therapy subsequent to S-1-based induction therapy and adjuvant S-1-based chemotherapy following curative resection in future clinical studies.

The sample size in the present study was limited, and the majority of the available information regarding the predictive value of TS has been derived from retrospective studies. Large-scale prospective clinical trials using appropriate biomarker evaluation methodology are required to validate the prospective utility of TS in clinical decision-making. However, the present study has demonstrated that patients

with lung SCC with low tumor TS expression can significantly benefit from S-1-based chemotherapy, and indicates that TS expression level is an independent predictive biomarker of response to S-1-based chemotherapy in patients with lung SCC.

Acknowledgements

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