RESEARCH ARTICLE

Correlation of Expression Levels of Copper Transporter 1 and Thymidylate Synthase with Treatment Outcomes in Patients with Advanced Non-small Cell Lung Cancer Treated with S-1/Carboplatin Doublet Chemotherapy

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Abstract

Background: Copper transporter 1 (CTR1) is a critical determinant of the uptake and cytotoxic effect of the platinum drugs carboplatin and cisplatin. Thymidylate synthase (TS) is an enzyme involved in DNA synthesis and is associated with resistance of tumor cells to 5-fluorouracil. We investigated the correlation between CTR1 and TS expression levels and treatment outcomes in patients with advanced non-small-cell lung cancer (NSCLC) treated with S-1/carboplatin doublet chemotherapy. **Methods:** Twenty-nine patients were enrolled in this study. Tumor expression of CTR1 and TS was measured immunohistochemically and analyzed for correlation with tumor response, progression-free survival (PFS), and overall survival (OS). **Results:** Tumor response was significantly better in patients with CTR1^{High} tumors than in patients with CTR1^{Low} tumors (64% vs. 18%, $P = 0.02$). Patients with TS^{Low} tumors had a significantly longer OS (median 21.2 vs. 8.5 months, $P = 0.02$), but not PFS, than patients with TS^{High} tumors. When CTR1 and TS co-expression was analyzed, patients with either CTR1^{High} or TS^{Low} tumors showed a significantly better tumor response (50% vs. 0%, $P = 0.01$), longer PFS (median 4.2 vs. 2.1 months, $P = 0.03$), and longer OS (median 21.2 vs. 8.5 months, $P = 0.01$) than patients with both CTR1^{Low} and TS^{High} tumors. **Conclusions:** Our study suggests that combined CTR1/TS expression status has the potential to be an important predictor of good treatment outcomes in patients with advanced NSCLC treated with S-1/carboplatin doublet chemotherapy.

Keywords: Copper transporter 1- thymidylate synthase-non-small cell lung cancer- S-1- carboplatin

Introduction

Lung cancer is one of the most common cancers worldwide and is a major cause of cancer-related death (Torre et al., 2015). Non-small cell lung cancer (NSCLC) accounts for approximately 80% of all lung cancer cases. Platinum-based doublet chemotherapy is the standard first-line treatment for advanced NSCLC and shows response rates of approximately 20–40% and a median survival of 8–10 months (Grossi et al., 2007).

S-1 is an oral fluoropyrimidine agent that consists of tegafur, 5-chloro-2,4-dihydroxypyridine (CDHP), and potassium oxonate (Shirasaka et al., 1996; Malet-Martino and Martino, 2002). Tegafur is a prodrug of fluorouracil (5-FU), and CDHP is an inhibitor of dihydropyrimidine dehydrogenase, an enzyme involved in the degradation of 5-FU. Thus, CDHP allows 5-FU to be maintained at higher levels in the plasma and tumor tissue than when

5-FU is intravenously infused. S-1 is active against a wide range of solid tumors, including NSCLC. Although many treatment options are available for patients with advanced NSCLC, S-1–containing regimens offer several potential clinical benefits, such as oral administration and well-tolerated toxicity (Takeda, 2013).

Carboplatin (CBDCA) is a cisplatin (CDDP) analog that has lower nonhematological toxicity than CDDP. Clinical trials have shown that combination therapy with S-1 and CBDCA is feasible and well tolerated in patients with advanced NSCLC and has efficacy similar to that of other platinum doublet regimens (Kaira et al., 2007; Tamura et al., 2009; Yoshioka et al., 2013; Kuyama et al., 2017). However, as with other treatment regimens, individual patients vary in their response to S-1/CBDCA doublet chemotherapy. Therefore, biomarkers that identify subgroups of patients who will benefit from S-1/CBDCA doublet chemotherapy would be useful for guiding

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treatment decisions.

Cellular uptake of anticancer drugs is mediated by facilitated transport systems, suggesting that expression of transport-related molecules may predict the response to chemotherapy. Copper transporter 1 (CTR1; SLC31A1) controls the intracellular accumulation and cytotoxic effects of CBDCA and CDDP (Holzer et al., 2004; Song et al., 2004; Holzer et al., 2006). Thymidylate synthase (TS) catalyzes methylation of deoxyuridine monophosphate to deoxythymidine monophosphate (Malet-Martino and Martino, 2002; Pinedo and Peters, 1988). 5-FU is metabolized to 5-fluorodeoxyuridine monophosphate (FdUMP) by thymidine kinase, thymidine phosphorylase, and other enzymes. In turn, FdUMP forms a covalent ternary complex with TS and reduced folic acid, inhibiting TS activity. Multiple investigators have reported that high TS expression is associated with resistance to 5-FU (Beck et al., 1994; Fukushima et al., 2001; Miyoshi et al., 2007).

Based on these findings, we considered that the expression of CTR1 and TS might influence treatment outcomes of S-1/CBDCA-treated NSCLC patients. Here, we investigated the relationship between CTR1 and TS expression levels and tumor response, progression-free survival (PFS), and overall survival (OS) in patients with advanced NSCLC treated with S-1/CBDCA doublet chemotherapy.

Materials and Methods

Patients and chemotherapy

This was a retrospective observational study. A total of 31 consecutive patients with advanced NSCLC were treated with S-1/CBDCA doublet chemotherapy between March 2011 and March 2015 at the Japanese Red Cross Kobe Hospital and the Kobe City Medical Center General Hospital. Inclusion criteria were as follows: histological diagnosis of NSCLC; measurable patient prognosis; clinical stage of III or IV; adequate hematologic, hepatic, and renal function; treatment with S-1/CBDCA doublet chemotherapy; and sufficient availability of tissue samples in paraffin blocks. Two patients were excluded because the tissue samples were too small for immunohistochemical analysis, and consequently, the remaining 29 patients were included in this study.

All patients received S-1/CBDCA doublet chemotherapy as first- or subsequent-line treatment. The S-1 dose was based on body surface area (BSA) as follows: BSA <1.25 m², 80 mg/day; BSA 1.25 m² to <1.50 m², 100 mg/day; and BSA ≥1.5 m², 120 mg/day. Patients received S-1 in two divided doses on days 1–14. CBDCA (area under the curve = 5) was administered intravenously on day 1 and then again every 3−4 weeks. This treatment cycle was repeated until disease progression, development of unacceptable toxicity, or patient refusal. The median number of cycles administered was 3 (range 1–6). After the failure of S-1/ CBDCA chemotherapy, docetaxel- or gemcitabine-containing regimens were given as standard therapies based on the Japanese Lung Cancer Clinical Practice Guidelines. A subset of patients received palliative radiotherapy to reduce the uncontrolled pain that is associated with bone metastasis or to control brain metastasis. There were no patients who received surgery. This study was approved by the ethics committees of Kobe University Graduate School of Health Sciences, Japanese Red Cross Kobe Hospital, and Institute of Biomedical Research and Innovation (reference numbers 470, 38, and 15-15, respectively). All patients provided written informed consent for chemotherapy and for the use of their tissue samples for this study.

Formalin-fixed, paraffin-embedded tissue blocks were available for diagnostic purposes. Sections (3-µm-thick) were cut and mounted on aminopropyltriethoxysilane-coated slides, and hematoxylin and eosin staining was performed to assess histopathological features. Clinical tumor size (T), lymph node metastasis (N), and distant metastasis (M) were categorized according to the tumor-node-metastasis (TNM) classification of the International Union against Cancer (Sobin et al., 2009). Histological classification was determined using the criteria of the World Health Organization (Travis et al., 2015).

Assessment of treatment outcomes

Computerized tomography scanning was performed before treatment, every 3 months during treatment until completion, and every 3–6 months thereafter until disease progression. Tumor response to chemotherapy was assessed according to the criteria of the response evaluation criteria for solid tumors (RECIST) (Eisenhauer et al., 2009) and was classified as complete response (CR), partial response (PR), stable disease (SD), or progressive disease (PD). CR was defined as disappearance of all target lesions, PR as ≥30% decrease in the sum of target lesion diameters, SD as <20% increase or <30% decrease in the sum of diameter of target lesions, and PD as ≥20% increase in the sum of target lesion diameters and at least a 5 mm absolute increase or appearance of new lesions. Patients who achieved CR or PR were considered to have good tumor responses, whereas SD or PD were considered poor tumor responses.

PFS was measured from the date of chemotherapy initiation to either the date of disease progression or the date of death from any cause. OS was measured from the date of chemotherapy initiation to the date of death or last follow-up.

Immunohistochemistry

Sections were deparaffinized in xylene, rehydrated through a graded series of ethanol, and rinsed with tap water. CTR1 and TS antigen retrieval was performed using protease-induced and heat-induced methods, respectively. Protease-induced retrieval was performed by incubating sections with pronase solution (Nichirei Bioscience, Tokyo, Japan) for 3 min at room temperature (RT). For heat-induced retrieval, sections were placed in 10 mM Tris base containing 1 mM ethylenediaminetetraacetic acid (pH 9.0) and incubated in a pressure cooker for 8 min at 120°C.

The sections were rinsed with 10 mM phosphate-buffered saline (PBS, pH 7.2), and nonspecific binding sites were blocked with 0.25% casein solution (Dako, Glostrup, Denmark) for 5 min at RT. Sections were then incubated

overnight at RT with rabbit polyclonal anti-CTR1 (1:100, Abnova, Taipei, Taiwan) or mouse monoclonal anti-TS (1:40, Santa Cruz Biotechnology, Dallas, TX, USA) primary antibodies.

After rinsing with PBS, sections for CTR1 staining were incubated with anti-rabbit horseradish peroxidase (HRP) polymer (Histofine Simple Stain MAX-PO; Nichirei Bioscience) for 1 h at RT. Sections for TS staining were incubated with MACH3 mouse probe for 30 min, rinsed, and then incubated with MACH3 mouse micro-polymer HRP (MACH3 Mouse HRP-Polymer Detection; Biocare Medical, Concord, CA, USA) for 30 min at RT. Sections were then rinsed again with PBS, and the reaction products were developed with a diaminobenzidine solution (Dako). Finally, sections were lightly counterstained with Mayer's hematoxylin, dehydrated in an ethanol gradient, treated with xylene, and coverslipped. Positive controls were sections of serous ovarian cancer for CTR1 and gastric cancer for TS. Negative controls were NSCLC tumor sections processed as above except the primary antibody was replaced with PBS containing 1% bovine serum albumin.

Assessment of CTR1 and TS levels

The immunostained sections were reviewed by three investigators (MK, AI, and SK) who were blinded to the clinical characteristics and outcome data. Expression levels of CTR1 and TS were semiquantitatively assessed by scoring the staining intensity (0, no staining; 1, weakly positive; 2, moderately positive; and 3, strongly positive) and the percentage of positively stained tumor cells (0, none; 1, 1–10%; 2, 11–49%; 3, ≥50%). A composite score (0–6) was obtained for each sample by adding together the intensity and percentage scores. Differences in scores were adjudicated by consensus among the three investigators.

Receiver operating characteristic curve analysis showed that the best sensitivity and specificity cut-off values for CTR1 and TS were 4.0. Tumors were then classified into low (CTR1^{Low}, TS^{Low}) and high (CTR1^{High}, TS^{High}) expression groups.

Statistical analysis

All statistical analyses were performed using SPSS 23.0 software (SPSS Inc., Chicago, IL, USA). Correlations between CTR1 and TS expression levels or clinicopathological parameters and tumor response were examined using Fisher's exact test. Kaplan–Meier curves were constructed to estimate PFS and OS, and statistical significance was analyzed using the log-rank test. A $P < 0.05$ was considered to be statistically significant.

Results

Patient characteristics

The clinicopathological characteristics of the 29 patients are summarized in Table 1. The group comprised 19 men and 10 women with a median age of 68 years (range 48–84 years). Seven patients were diagnosed with adenocarcinoma (one lepidic, 2 acinar, 2 papillary, and 2 solid), 20 with squamous

Table 1. Clinicopathological Characteristics of Patients

Variables		n
All patients		29
Age	<65	8
	≥65	21
Sex	Male	19
	Female	10
Tumor histology	Adenocarcinoma	7
	Non-adenocarcinoma	22
T stage	T1/T2	12
	T3/T4	17
N stage	N0/N1	11
	N2/N3	18
Clinical stage	III	7
	IV	22
Performance status	0/1	26
	2	3
Smoking status	Never	4
	Ever	25
Line	1st	14
	2nd and later	15

cell carcinoma (8 keratinizing and 12 non-keratinizing), and two with pleomorphic carcinoma. The clinical tumor stages were T1 in 5 patients, T2 in 7 patients, T3 in 6 patients, and T4 in 11 patients. Sixty-two percent of the patients had advanced lymph node stage N2 or N3. Two patients had stage IIIA disease, 5 patients had stage IIIB, and 22 patients had stage IV. All patients had epidermal growth factor receptor wild type tumors. Ninety percent of the patients had an Eastern Cooperative Oncology Group performance status of 0–1. All patients received S-1/CBDCA doublet chemotherapy: first line in 14
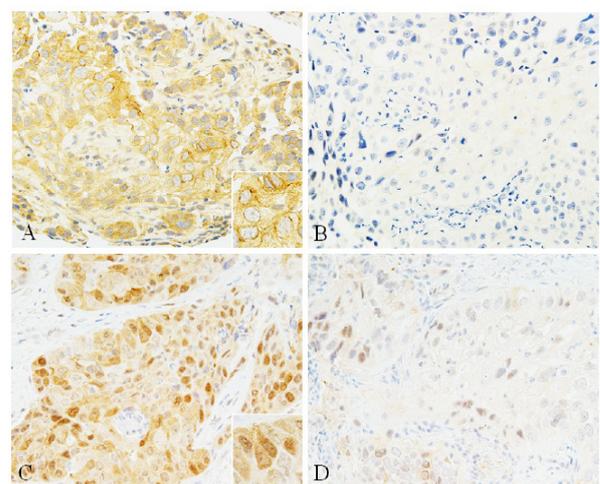

Figure 1. Representative Immunostaining Patterns of CTR1 and TS in NSCLC Tissue Samples. (A) High CTR1 expression, (B) low CTR1 expression, (C) high TS expression, (D) low TS expression. Insets show high-power views of positive cells. A number of tumor cells showed strong positive staining for CTR1 in the cell membrane (A) and for TS in the nucleus and cytoplasm (C).

Table 2. Correlations between Clinicopathological Parameters or Expression Levels of CTR1 and TS and Tumor Response in NSCLC Patients Treated with S-1/carboplatin Doublet Chemotherapy

Variables		n[a]	Good response (%)	Poor response (%)	*P* value
Age	<65	8	5 (63)	3 (78)	0.08
	≥65	20	5 (25)	15 (75)	
Sex	Male	18	8 (44)	10 (56)	0.41
	Female	10	2 (20)	8 (80)	
Tumor histology	Adenocarcinoma	6	2 (33)	4 (67)	1.00
	Non-adenocarcinoma	22	8 (36)	14 (64)	
Clinical stage	III	7	4 (57)	3 (43)	0.19
	IV	21	6 (29)	15 (71)	
Performance status	0/1	26	10 (38)	16 (62)	0.53
	2	2	0 (0)	2 (100)	
Smoking status	Never	4	0 (0)	4 (100)	0.27
	Ever	24	10 (42)	14 (58)	
CTR1	High	11	7 (64)	4 (36)	0.02[b]
	Low	17	3 (18)	14 (82)	
TS	High	15	3 (20)	12 (80)	0.06
	Low	13	7 (54)	6 (46)	
CTR1/TS	Either CTR1^{High} or TS^{Low}	20	10 (50)	10 (50)	0.01[b]
	Both CTR1^{Low} and TS^{High}	8	0 (0)	8 (100)	

[a]Tumor response was evaluable in 28 of the 29 patients; [b]Statistically significant; CTR1, copper transporter 1; NSCLC, non-small cell lung cancer; TS, thymidylate synthase

patients, second line in 9 patients, and third or later line in 6 patients.

Correlation of CTR1 and TS levels with tumor response

Representative staining patterns of CTR1 and TS in NSCLC tissues are shown in Figure 1. CTR1 was detected in the cell membrane, whereas TS was observed in the nucleus and cytoplasm. Of the 29 patients, 11 (38%) and 18 (62%) patients had CTR1^{High} and CTR1^{Low} tumors, respectively, and 16 (55%) and 13 (45%) patients had TS^{High} and TS^{Low} tumors, respectively.

Twenty-eight of the 29 patients were evaluable for

Table 3. Correlations between Clinicopathological Parameters or Expression Levels of CTR1 and TS and PFS or OS in NSCLC Patients Treated with S-1/carboplatin Doublet Chemotherapy

	Variables	n	Median PFS (95% CI)[a]	*P* value	Median OS (95% CI)[a]	*P* value
Age	<65	8	5.3 (3.3−7.4)	0.47	22.6 (0.0−55.1)	0.68
	≥65	21	3.4 (2.0−4.7)		12.2 (2.5−22.0)	
Sex	Male	19	4.0 (2.7−5.3)	0.87	16.0 (4.2−27.7)	0.88
	Female	10	3.9 (1.0−6.9)		10.7 (7.8−13.7)	
Tumor histology	Adenocarcinoma	7	4.2 (0.7−7.6)	0.98	12.2 (8.4−16.1)	0.99
	Non-adenocarcinoma	22	3.9 (2.8−5.1)		17.3 (1.7−32.9)	
Clinical stage	III	7	5.3 (2.3−8.4)	0.25	22.6 (0.0−51.6)	0.35
	IV	22	3.4 (2.0−4.7)		10.7 (5.3−16.2)	
Performance status	0/1	26	4.0 (3.0−5.0)	0.16	16.0 (3.2−28.7)	0.02[b]
	2	3	2.1 (0.2−4.0)		3.2 (0.0−6.8)	
Smoking status	Never	4	1.9 (0.7−3.1)	0.37	10.7 (3.4−18.1)	0.92
	Ever	25	4.1 (3.8−4.5)		12.2 (0.0−25.2)	
CTR1	High	11	5.3 (2.0−16.0)	0.14	22.6 (0.0−58.1)	0.30
	Low	18	3.2 (2.1−4.4)		10.7 (4.5−17.0)	
TS	High	16	3.2 (1.0−5.5)	0.11	8.5 (3.7−13.2)	0.02[b]
	Low	13	4.2 (3.8−16.9)		21.2 (15.0−27.3)	
CTR1/TS	Either CTR1^{High} or TS^{Low}	20	4.2 (2.5−5.8)	0.03[b]	21.2 (14.0−28.4)	0.01[b]
	Both CTR1^{Low} and TS^{High}	9	2.1 (2.0−2.1)		8.5 (8.2−8.7)	

[a]In months; [b]Statistically significant; CI, confidence interval; CTR1, copper transporter 1; NSCLC, non-small cell lung cancer; OS, overall survival; PFS, progression-free survival; TS, thymidylate synthase
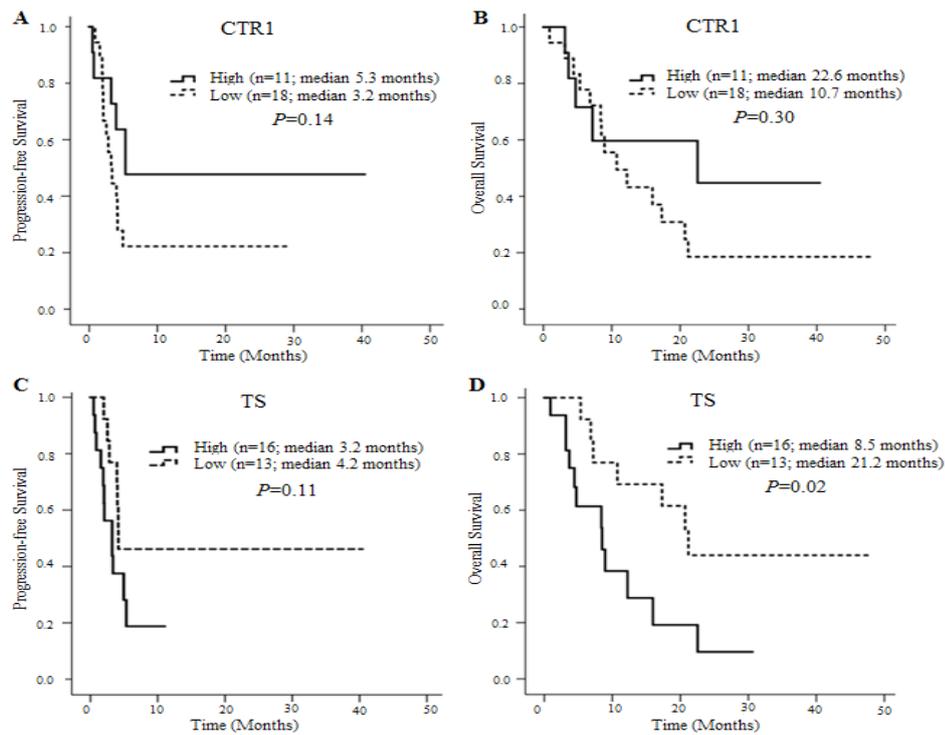
Figure 2. Kaplan–Meier Survival Curves for PFS and OS of Patients Stratified by CTR1 or TS Expression. (A) PFS Stratified by CTR1 Expression level (5.3 vs. 3.2 months, P = 0.14). (B) OS stratified by CTR1 expression level (22.6 vs. 10.7 months, P = 0.30). (C) PFS stratified by TS expression level (median 3.2 vs. 4.2 months, P = 0.11). (D) OS stratified by TS expression level (median 8.5 vs. 21.2 months, P = 0.02).

tumor response. The overall response rate was 36% and the disease control rate was 75%. One (4%) patient achieved CR, 9 patients (32%) achieved PR, 11 patients (39%) had SD, and 7 (25%) had PD. Table 2 shows the correlations between CTR1 and TS levels or clinicopathological parameters and tumor response. CTR1[High] was significantly correlated with a good tumor response. Indeed, 7 of the 11 (64%) patients with CTR1[High] tumors but only 3 of the 17 (18%) patients with CTR1[Low] tumors showed a good tumor response (P = 0.02). However, no correlations were noted between tumor response and any other variables, including TS level.

When CTR1 and TS co-expression was analyzed, a good tumor response was detected in 10 of the 20 (50%) patients with either CTR1[High] or TS[Low] tumors but none

of the 8 patients with both CTR1[Low] and TS[High] tumors (P = 0.01).

Correlation of CTR1 and TS levels with PFS and OS

Figures 2 and 3 show Kaplan–Meier survival curves for PFS and OS of patients stratified by tumor expression of CTR1 and TS. All patients were evaluable for PFS and OS, and the median follow-up time was 9.0 months. The median PFS and OS were 4.0 months (range 0.5–40.4) and 9.2 months (range 0.9–47.8), respectively. Patients with TS[Low] tumors had a significantly longer OS (median 21.2 vs. 8.5 months, P = 0.02) but not PFS (median 4.2 vs. 3.2 months, P = 0.11) than patients with TS[High] tumors (Table 3). A performance status of 0/1 also correlated with a longer OS (P = 0.02) but not PFS (P = 0.16).
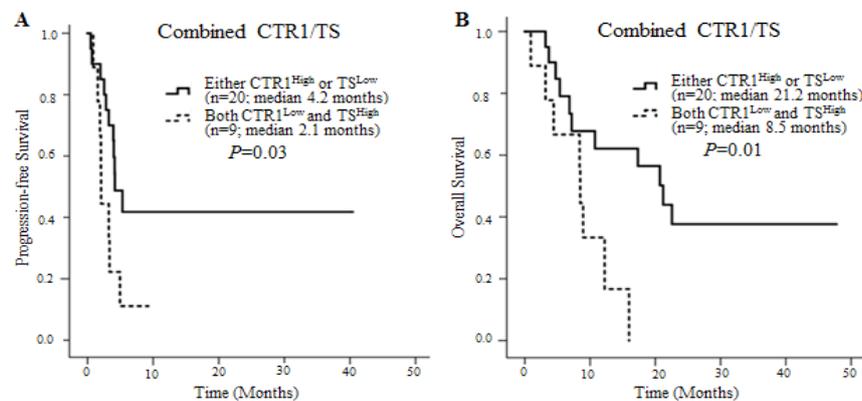

Figure 3. Kaplan–Meier Survival Curves for PFS and OS of Patients Stratified by Co-expression of CTR1 and TS. (A) PFS stratified by CTR1 and TS co-expression level (median 4.2 vs. 2.1 months, P = 0.03). (B) OS stratified by CTR1 and TS co-expression level (median 21.2 vs. 8.5 months, P = 0.01).

However, no other variables, including CTR1 level, were significantly correlated with PFS and OS.

When CTR1 and TS co-expression was analyzed, patients with either CTR1^{High} or T^{SLow} tumors had a significantly longer PFS (median 4.2 vs. 2.1 months, $P = 0.03$) and OS (median 21.2 vs. 8.5 months, $P = 0.01$) than patients with both CTR1^{Low} and TS^{High} tumors (Table 3).

Discussion

S-1/CBDCA doublet chemotherapy is feasible and well tolerated in patients with advanced NSCLC (Kaira et al., 2007; Tamura et al., 2009; Yoshioka et al., 2013; Kuyama et al., 2017), although individual patients vary in their responses to the chemotherapy. Therefore, biomarkers with predictive and prognostic value for S-1/CBDCA doublet chemotherapy would be of considerable help in selecting the optimal treatment regimen for each patient. CTR1 has been shown to control the accumulation and cytotoxic effect of CBDCA and CDDP in tumor cells (Holzer et al., 2004; Song et al., 2004; Holzer et al., 2006). TS is involved in DNA synthesis, and TS^{High} is reportedly associated with resistance to 5-FU (Beck et al., 1994; Fukushima et al., 2001; Miyoshi et al., 2007). In this study, we investigated the predictive value of CTR1 and TS expression levels for treatment outcomes of advanced NSCLC patients treated with S-1/CBDCA doublet chemotherapy.

We found that a good tumor response was significantly more common in patients with CTR1^{High} tumors than in patients with CTR1^{Low} tumors ($P = 0.02$), but we failed to detect an association between CTR1 level and either PFS or OS. This result contrasts with the finding of Chen (2012), who found that CTR1^{High} is associated with a longer PFS and OS in NSCLC patients treated with first-line chemotherapy consisting of a platinum (CBDCA or CDDP) plus a non-platinum (gemcitabine or taxane) agent; however, they also noted a positive association between CTR1^{High} and tumor response. In addition, Kim (2014) reported that NSCLC patients whose tumors showed undetectable CTR1 levels also had reduced tissue concentrations of platinum and showed poor responses to neoadjuvant chemotherapy with platinum (CBDCA or CDDP) plus taxane or other drugs. Taken together, these and our results suggest that the level of CTR1 in NSCLC tumors may have value in predicting tumor response to platinum-containing chemotherapy.

S-1 is an oral fluoropyrimidine that combines the 5-FU prodrug tegafur as the effector drug with two modulators, CDHP and potassium oxonate (Shirasaka et al., 1996). Several in vitro and in vivo studies with tumor cell lines have implicated TS^{High} as a mechanism of resistance to 5-FU (Beck et al., 1994; Fukushima et al., 2001; Miyoshi et al., 2007), suggesting that TS expression may influence the effectiveness of S-1–containing chemotherapy. In our study, TS levels, unlike CTR1 levels, did not correlate with tumor response. However, patients with TS^{Low} tumors had a significantly longer OS ($P = 0.02$), but not PFS, than did patients with TS^{High} tumors. Takeda (2011) found that TS^{Low} predicted better tumor response in patients with advanced NSCLC treated with S-1/CBDCA doublet chemotherapy, which contrasts with our findings;

however, they, like us, observed a significant association between TS^{Low} and longer OS. The conflicts between the results of our and other studies might be due to a variety of factors, including differences in the sample size, clinical stage, treatment line, chemotherapy regimen, and immunostaining method.

Tumor response to chemotherapy is a complex process involving many factors. Thus, it seems reasonable that a combination of biomarkers might be more accurate and/or reliable than single markers in predicting the effectiveness of chemotherapy in NSCLC. Indeed, when we performed the analyses using CTR1 and TS co-expression levels, patients with either CTR1^{High} or TS^{Low} tumors showed a significantly better tumor response ($P = 0.01$), longer PFS ($P = 0.03$), and longer OS ($P = 0.01$) than did patients with both CTR1^{Low} and TS^{High} tumors. These results indicate that combining CTR1 and TS can significantly increase the predictive power for tumor response, PFS, and OS, compared with CTR1 or TS alone. Thus, S-1/CBDCA doublet chemotherapy might be particularly beneficial for advanced NSCLC patients with either CTR1^{High} or TS^{Low} tumors.

Our study has several limitations: (1) the line of S-1/CBDCA chemotherapy was not constant (i.e., first, second, and later line), (2) the patients were at different disease stages, (3) the study was retrospective, and (4) the sample size was small, which resulted in low statistical power and prevented multivariate analysis. Therefore, our results are still exploratory, and they will need to be confirmed in prospective studies of large patient cohorts at the same stage of disease and receiving the same line of treatment.

In conclusion, this study suggests that combined CTR1/TS expression status has the potential to be an important predictor of good treatment outcomes in patients with advanced NSCLC treated with S-1/CBDCA doublet chemotherapy.

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