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The relationship between the expression of thymidylate synthase, dihydropyrimidine dehydrogenase, orotate phosphoribosyltransferase, excision repair cross-complementation group 1 and class III β-tubulin, and the therapeutic effect of S-1 or carboplatin plus paclitaxel in non-small-cell lung cancer

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Abstract. Previous studies have reported that the expressions of specific proteins may predict the efficacy of chemotherapy agents for non-small cell lung cancer (NSCLC) patients. The present study evaluated the expression of proteins hypothesized to be associated with the effect of chemotherapeutic agents in 38 NSCLC patients with pathological stage II and IIIA. The subjects received carboplatin plus paclitaxel (CP) or S-1 as adjuvant chemotherapy following complete resection. The protein expressions evaluated were those of thymidylate synthase (TS), dihydropyrimidine dehydrogenase (DPD) and orotate phsphoribosyltransferase (OPRT), which were suspected to be associated with the effect of S-1 agents, excision repair cross-complementation group 1 (ERCC1), which was suspected to be associated with the effect of platinum-based agents, and class III β-tubulin (TUBB3), which was suspected to be associated with the effect of taxane-based agents. The positive rate of TS was 55.3% (n=21/38), DPD was 57.9% (n=22/38), OPRT was 42.1% (n=16/38), ERCC1 was 47.4% (n=18/38) and TUBB3 was 44.7% (n=17/38). Among the patients who received S-1 adjuvant chemotherapy, TS-negative cases demonstrated a significantly better disease-free survival than positive cases. Thus, TS protein expression may have been a factor that predicted the effect of S-1 agent as adjuvant chemotherapy.

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Key words: non-small cell lung cancer, adjuvant chemotherapy, protein expression, thymidylate synthase, dihydropyrimidine dehydrogenase, orotate phsphoribosyltransferase, excision repair cross-complementation group 1, class III β-tubulin

Introduction

The mortality rates of patients with advanced non-small cell lung cancer (NSCLC) remain high (1). To improve this poor prognosis, several adjuvant chemotherapies have been administered in patients with completely resected NSCLC, but the improvement of the survival rate is not ideal, and patients sometimes struggle with adverse effects, like nausea, neutropenia, and fatigue (2-10). Ideally, we would be able to predict the effects of chemotherapeutic agents and regimens for patients who received chemotherapy, especially for postoperative adjuvant chemotherapy, because whether or not adjuvant chemotherapy reduces the rate of recurrence is unclear. Even with cytotoxic anticancer drugs, the predictive factors of the therapeutic effect would ideally be revealed in a manner similar to that observed for molecular targeted therapy (11-13).

Recently, the expression of some proteins has been reported as a predictor of the efficacy of cytotoxic chemotherapeutic agents. Excision repair cross-complementation group 1 (ERCC1) is a DNA repair gene in the nucleotide excision

repair pathway that is activated when platinum-based chemotherapeutic agents form DNA adducts (14). High ERCC1 expression in several cancers has been reported in association with resistance to platinum-based treatment (15-17). Class III β-tubulin (TUBB3) is a major component of the microtubules that are targeted by taxane-based agents, which exert their growth inhibitory effects through the inhibition of microtubule dynamics, resulting in the growth arrest of tumor cells at the G2-M phase (18). High expression of TUBB3 has been reported in association with resistance to taxane-based treatment in human cancers (19-21). Thymidylate synthase (TS) is an enzyme that generates deoxythymidine monophosphate, which is subsequently phosphorylated to thymidine triphosphate for use in DNA synthesis and repair. High expression of TS has been reported in association with fluorouracil (5FU)-based chemotherapy (including S-1 agent) resistance in various cancers (22-24). Dihydropyrimidine dehydrogenase (DPD) is the initial and rate-limiting enzyme in degrading 5-FU to 2-fluoro-β-alanine (25), and high expression of DPD has been reported in association with resistance to 5-FU-based chemotherapies (26-28). Orotate phosphoribosyltransferase (OPRT) is an enzyme involved in pyrimidine biosynthesis and contributes to the conversion of 5-FU into fdUMP, an active form of 5-FU. Low expression of OPRT has been reported in association with resistance to 5-FU-based chemotherapies (29,30).

In this study, we investigated the expression of several proteins in completely resected NSCLC patients who received carboplatin plus paclitaxel (CP) or S-1 regimen as adjuvant chemotherapy.

Patients and methods

Patients. A multicenter randomized feasibility study of CP vs. S-1 in patients with locally advanced completely resected NSCLC was conducted. Forty patients underwent complete resection and were diagnosed with pathological stage II or IIIA NSCLC (the 7th edition of the Tumor-Node-Metastasis classification) (31) at Nagoya City University Hospital (Nagoya, Japan) and its affiliated hospitals between January 2008 and December 2013.

Written informed consent was obtained from all patients, and the study protocol was approved by the Institutional Review Board of each participating institution (Nagoya City University Hospital No. 45-13-0020). This study was registered on the UMIN Clinical Trial database (ID:000001510). We have reported on details of this study (32). In this paper, we evaluated the relationships between the protein expression and the prognosis of patients who received adjuvant chemotherapy after complete surgical resection. The randomization was performed centrally at the Department of Oncology, Immunology and Surgery, Nagoya City University Graduate School of Medical Sciences (Nagoya, Japan).

Design of the study and treatment schedule. The patients were randomly assigned either to arm A (21 cases) receiving CP bi-weekly or to arm B (19 cases) receiving S-1. Among the 40 patients, two patients assigned to arm A could not continue the adjuvant chemotherapy because of a Grade 4 allergic reaction (anaphylactic shock) during the first cycle of paclitaxel infusion. We excluded these two patients from this additional study and investigated the 38 patients who received adjuvant chemotherapy over two courses.

The infusing dosage of paclitaxel was 120 mg m^{-2} on days 1 and 15. Carboplatin at an area under the curve (AUC 3) dose was also administered on days 1 and 15. The patients received adjuvant chemotherapy with carboplatin plus paclitaxel every four weeks for up to four cycles. Calvert's formula was used to calculate the dose of the AUC for carboplatin (33), while the creatinine clearance was determined with the Jellifie formula (34). The dosage of S-1 was established as follows: patients with a body surface area (BSA) <1.25 m^2 received 40 mg twice a day (80 mg/day), those with BSA of ≥1.25 m^2 but <1.5 m^2 received 50 mg twice a day (100 mg/day), and those with a BSA ≥1.5 m^2 received 60 mg twice a day (120 mg/day). S-1 was administered for two weeks followed by a one-week rest period for up to one year. Both arms A and B continued on the above prescription unless any evidence for relapse, other malignancies, or severe adverse events were identified.

Recurrence was diagnosed on the basis of imaging study findings. Chest and abdominal computed tomography and positron emission tomography plus head magnetic resonance imaging were performed at 6- and 12-month intervals, respectively. In addition, when patients complained of any symptoms or exhibited elevated tumor markers on blood tests, imaging studies were performed.

Protein expression by immunohistochemistry. The ERCC1 protein expression was evaluated by immunohistochemistry (IHC) using an anti-ERCC1 antibody (clone 8F1; Abcam, Cambridge, UK). We used a standard protocol for the immunostaining of the samples. The details of the method were previously described (35). Tumor nuclear staining intensity was graded on a scale of 0-3. The percentage of positive tumor nuclei was graded on a scale of 0-3. The percentage of positive tumor nuclei was evaluated, and a proportion score was attributed (0 if 0%; 0.1 if 1-9%; 0.5 if 10-49%; 1.0 if ≥ 50%), as previously described (36,37).

The antibody against TUBB3 was an anti-class III β-tubulin monoclonal antibody (clone TUJ1; Covance, Inc., Princeton, NJ, USA). Having over 50% of positive cells with a staining intensity of 2 was considered TUBB3-positive (35).

TS protein was evaluated by IHC using recombinant human TS-specific antibody (clone RTSSA; Taiho Pharmaceutical, Co., Ltd., Saitama, Japan). The slides were examined at low magnification, and the intensity of cytoplasmic staining was scored as follows: 0, no staining or faint staining; 1+, moderate staining; 2+, strong staining. We classified scores of 0 as negative and scores of 1+ and 2+ as positive for the TS antibody. We also judged cases with <10% of tumor cells with moderate or strong staining as being negative (38).

OPRT protein expression was evaluated by IHC using an anti-OPRT polyclonal antibody (Taiho Pharmaceutical, Co., Ltd.) The staining was the same as for TS (38). Scores of 0 and 1+ were classified as negative and scores of 2+ as positive for the OPRT protein. We also judged cases with <10% of tumor cells with moderate or strong staining as being negative.

DPD protein expression was evaluated by IHC using anti-DPD polyclonal antibody RDPDPA (dilution: 1:400; Taiho Pharmaceutical, Co., Ltd.) The staining was the same as

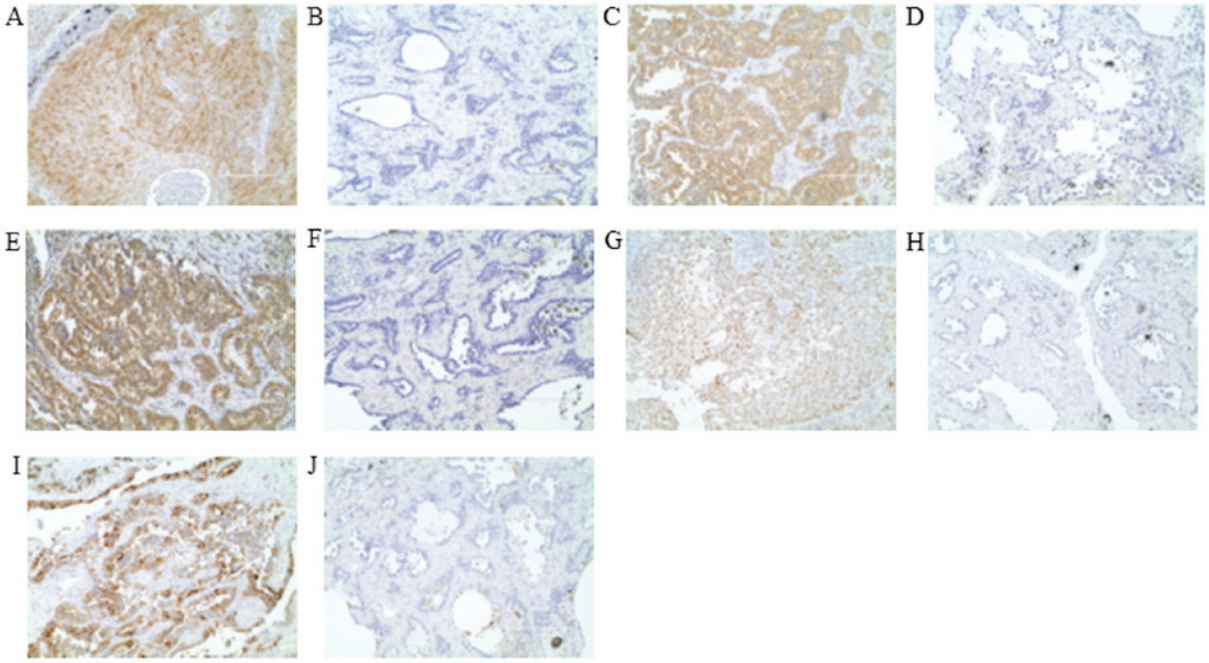

Figure 1. Representative positive and negative cases of each immunohistochemistry result observed (magnification, x200). (A) TS positive case. (B) TS negative case. (C) DPD positive case. (D) DPD negative case. (E) OPRT positive case. (F) OPRT negative case. (G) ERCC1 positive case. (H) ERCC1 negative case. (I) TUBB3 positive case. (J) TUBB3 negative case. TS, thymidylate synthase; DPD, dihydropyrimidine dehydrogenase; ORPT, orotate phsphoribosyltransferase; ERCC1, excision repair cross-complementation group 1; TUBB3, class III β-tubulin.

the previously described method (28). Scores of 0 and 1+ were classified as negative and scores of 2+ as positive for the DPD protein. We also judged cases with <10% of tumor cells with moderate or strong staining as being negative.

All immunostained sections were evaluated by separate investigator without knowledge of the patients' clinical data to evaluated H-scoring accurately. Representative positive and negative cases of each IHC are shown in Fig. 1.

Statistical analysis. The sample size was determined based on a phase II study reported by Kawamura *et al* (39) applying docetaxel plus gemcitabine as an adjuvant chemotherapy in 35 patients. The number of patients in each arm was calculated using the Fleming method and found to be 32 per arm (32). However, sufficient data for patients in the study could not be gathered within the study period.

The characteristics, disease-free survival (DFS), and the overall survival (OS) of 38 patients who received over two courses of adjuvant chemotherapy were analyzed. The five-year DFS and OS were examined by the Kaplan-Meier method, and the difference in the two arms was calculated by the log-rank test. The differences in the rate of adverse events were evaluated by the χ^2 test. All of the data were analyzed with the EZR software version 1.33 (www.jichi.ac.jp/saitama-sct/SaitamaHP.files/statmedEN.html) (40). $P \leq 0.05$ was considered to indicate a statistically significant difference.

Results

Patients' characteristics. Forty patients with stage II or IIIA NSCLC who had received surgically complete resection were enrolled. Among the 40 patients, 2 were excluded due to the cessation of adjuvant chemotherapy because of a grade 4 allergic reaction (anaphylactic shock) induced by paclitaxel. The patients' characteristics are presented in Table I. Briefly, the patients were 7 females and 31 males ranging in age from 39-75 years, with a mean age of 63.6 years. There were no significant differences in the clinicopathological characteristics between arms A and B.

Protein expression on IHC. The ERCC1 IHC staining was positive in 18/38 cases (47%) in all patients. The positive cases were 10/19 (53%) in arm A and 8/19 (42%) in arm B, and there was no significant difference in the ERCC1 protein expression among the various adjuvant chemotherapy regimens. No association between the expression of ERCC1 and clinicopathological factors was identified (data not shown).

The TUBB3 IHC staining was positive in 17/38 cases (45%) in all patients. The positive cases were 9/19 (47%) in arm A and 8/19 (42%) in arm B, and there was no significant difference in the TUBB3 protein expression among adjuvant chemotherapy regimens. No association between the expression of TUBB3 and clinicopathological factors was identified (data not shown).

The TS IHC staining was positive in 21/38 cases (55%) in all patients. The positive cases were 11/19 (58%) in arm A and 10/19 (53%) in arm B, and there was no significant difference in the TS protein expression among adjuvant chemotherapy regimens. No association between the expression of TS and clinicopathological factors was identified (data not shown).

The OPRT IHC staining was positive in 16/38 cases (42%) in all patients. The positive cases were 7/19 (37%) in arm A and 9/19 (47%) in arm B, and there was no significant difference in the OPRT protein expression among adjuvant chemotherapy regimens. No association between the expression of OPRT and clinicopathological factors was identified (data not shown).

Table I. Characteristics of the 38 patients recruited to the present study.

Characteristics	All patients (n=38)	CBDCA+PTX (n=19)	S-1 (n=19)	P-value
Observation period, months	15-98/67	19-98/67	15-87/67	0.951
Sex, n				
Male	31	14	17	0.405
Female	7	5	2	
Age, years	39-75/63.6	47-73/64.4	39-75/62.9	0.529
Histological type, n				
Adenocarcinoma	24	11	13	0.737
Squamous cell carcinoma	13	7	6	
Others	1	1	0	
Pathological stage (IIA/IIB/IIIA), n	17/11/10	9/5/5	8/6/5	0.980
ERCC1 (Positive/negative), n	18/20	10/9	8/11	0.746
TUBB3 (Positive/negative), n	17/21	9/10	8/11	0.980
TS (Positive/negative), n	21/17	11/8	10/9	0.980
OPRT (Positive/negative), n	16/22	7/12	9/10	0.743
DPD (Positive/negative), n	22/16	14/5	8/11	0.091

Data are presented as the range/median, or as the n number of patients. PTX, paclitaxel; CBDCA, carboplatin; ERCC1, excision repair cross-complementation group 1; TUBB3, class III β-tubulin; TS, thymidylate synthase; OPRT, orotate phosphoribosyltransferase; DPD, dihydropyrimidine dehydrogenase.

Table II. Correlation with overall survival plus disease free survival and clinicopathological factors.

			Overall survival		Disease free survival	
Factor	Subgroup	Total n (n=38)	5-year survival (%)	P-value	5-year survival (%)	P-value
Age, years	≤65/>65	20/18	78.8/83.3	0.182	63.6/55.6	0.898
Sex	Male/female	31/7	76.5/100	0.070	56.8/71.4	0.398
Tissue type	Adenocarcinoma/others	24/14	87.1/70.0	0.399	58.3/60.2	0.477
Pathological stage	IIA/IIB or IIIA	17/21	87.8/75.2	0.085	69.1/52.4	0.250
Chemotherapy regime	CP/S-1	19/19	78.6/83.6	0.976	50.8/68.4	0.351
ERCC1	Positive/negative	18/20	76.0/85.0	0.773	70.6/50.0	0.111
TUBB3	Positive/negative	17/21	87.5/75.6	0.696	64.7/54.8	0.869
TS	Positive/negative	21/17	74.4/88.2	0.092	50.1/70.6	0.140
OPRT	Positive/negative	16/22	86.2/77.3	0.783	66.1/54.5	0.502
DPD	Positive/negative	22/16	86.1/73.7	0.824	66.5/50.0	0.331

CP, carboplatin plus paclitaxel; ERCC1, excision repair cross-complementation group 1; TUBB3, class III β-tubulin; TS, thymidylate synthase; OPRT, orotate phosphoribosyltransferase; DPD, dihydropyrimidine dehydrogenase.

The DPD IHC staining was positive in 22/38 cases (58%) in all patients. The positive cases were 14/19 (74%) in arm A and 8/19 (42%) in arm B, and there was no significant difference in the DPD protein expression among adjuvant chemotherapy regimens. No association between the expression of DPD and clinicopathological factors was identified (data not shown).

The survival. The correlations between the OS plus DFS and the clinicopathological factors of the 38 patients are summarized in Table II. No factors, including the protein expression, were found to have significantly influenced the OS or DFS. Furthermore, there were no significant differences in the OS and DFS between the CP and S-1 adjuvant chemotherapy regimens. The 5-year OS and DFS of 38 patients was 81.0 and 59.6%, respectively (Fig. 2A and B). The Kaplan-Meier curves based on the adjuvant chemotherapy regimens are shown in Fig. 2C and D.

The correlations between the OS plus DFS and the clinicopathological factors of the 19 patients who received CP adjuvant chemotherapy are summarized in Table III. There were no factors found to have significantly influenced the

Table III. Correlation with overall survival plus disease free survival and clinicopathological factors for Carboplatin plus paclitaxel patients.

			Overall survival		Disease free survival	
Factor	Subgroup	Total n (n=19)	5-year survival (%)	P-value	5-year survival (%)	P-value
Age, years	≤65/>65	9/10	76.2/80.0	0.598	63.5/40.0	0.281
Sex	Male/female	14/5	70.7/100	0.134	47.6/60.0	0.601
Tissue type	Adenocarcinoma/others	11/8	90.9/60.0	0.325	36.4/72.9	0.172
Pathological stage	IIA/IIB or IIIA	9/10	76.2/80.0	0.473	53.3/50.0	0.902
ERCC1	Positive/negative	10/9	58.3/100	0.773	67.5/33.3	0.129
TUBB3	Positive/negative	9/10	77.8/78.7	0.527	44.4/57.1	0.502
TS	Positive/negative	11/8	71.6/87.5	0.310	53.0/50.0	0.700
OPRT	Positive/negative	7/12	68.6/83.3	0.824	68.6/41.7	0.321
DPD	Positive/negative	14/5	77.9/80.0	0.806	62.9/20.0	0.164

CP, carboplatin plus paclitaxel; ERCC1, excision repair cross-complementation group 1; TUBB3, class III β-tubulin; TS, thymidylate synthase; OPRT, orotate phosphoribosyltransferase; DPD, dihydropyrimidine dehydrogenase.

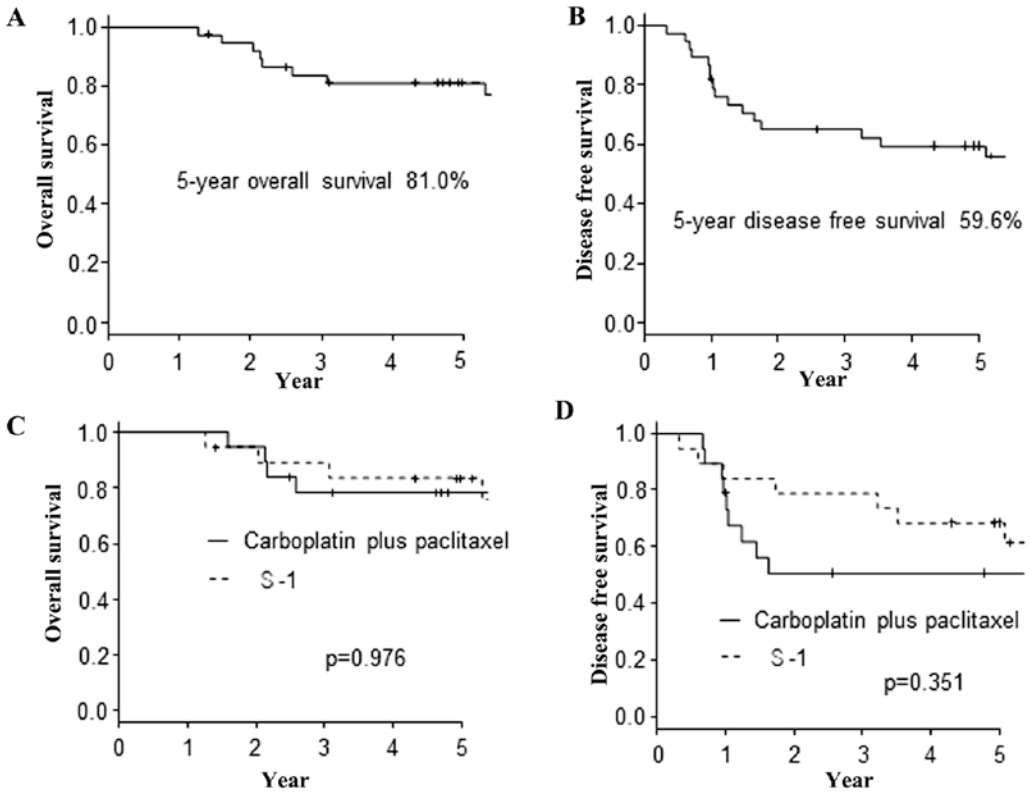

Figure 2. Kaplan-Meier analyses. (A) The 5-year overall survival of the 38 patients who received carboplatin plus paclitaxel or S-1 adjuvant chemotherapy. (B) The 5-year disease-free survival of the 38 patients who received carboplatin plus paclitaxel or S-1 adjuvant chemotherapy. (C) The overall survival divided by carboplatin plus paclitaxel or S-1 adjuvant chemotherapy. (D) The disease-free survival divided by carboplatin plus paclitaxel or S-1 adjuvant chemotherapy.

OS or DFS in the patients who received the CP regimen. The protein expressions of ERCC1 and TUBB3 did not affect the OS or DFS.

The correlations between the OS plus DFS and the clinicopathological factors of the 19 patients who received S-1 adjuvant chemotherapy are summarized at Table IV. There were no factors found to have significantly influenced the OS in the patients who received the S-1 regimens. In the analysis of the DFS, the protein expression of TS was the only significant prognostic factor. However, the protein expression of TS did not affect the OS (Fig. 3A) or DFS (Fig. 3B) in the investigation of all 38 patients. Furthermore, the protein expression of TS did not affect the OS (Fig. 3C) in the investigation of the 19 patients who received S-1 adjuvant chemotherapy. However, when we limited our investigation to the DFS of the patients who received S-1 adjuvant chemotherapy, the

Table IV. Correlation with overall survival plus disease free survival and clinicopathological factors for S-1 patients.

			Overall survival		Disease free survival	
Factor	Subgroup	Total n (n=19)	5-year survival (%)	P-value	5-year survival (%)	P-value
Age, years	≤65/>65	11/8	80.8/87.5	0.202	63.6/75.0	0.389
Sex	Male/female	17/2	81.6/100	0.316	64.7/100	0.297
Tissue type	Adenocarcinoma/others	13/6	83.9/83.3	0.839	76.9/50.0	0.570
Pathological stage	IIA/IIB or IIIA	8/11	100/70.7	0.065	87.5/54.5	0.072
ERCC1	Positive/negative	8/11	100/72.7	0.142	75.0/63.6	0.333
TUBB3	Positive/negative	8/11	100/72.7	0.241	87.5/54.5	0.346
TS	Positive/negative	10/9	77.8/88.9	0.187	50.0/88.9	0.044
OPRT	Positive/negative	9/10	100/70.0	0.496	66.7/70.0	0.783
DPD	Positive/negative	8/11	100/70.7	0.587	75.0/63.6	0.721

CP, carboplatin plus paclitaxel; ERCC1, excision repair cross-complementation group 1; TUBB3, class III β-tubulin; TS, thymidylate synthase; OPRT, orotate phosphoribosyltransferase; DPD, dihydropyrimidine dehydrogenase.

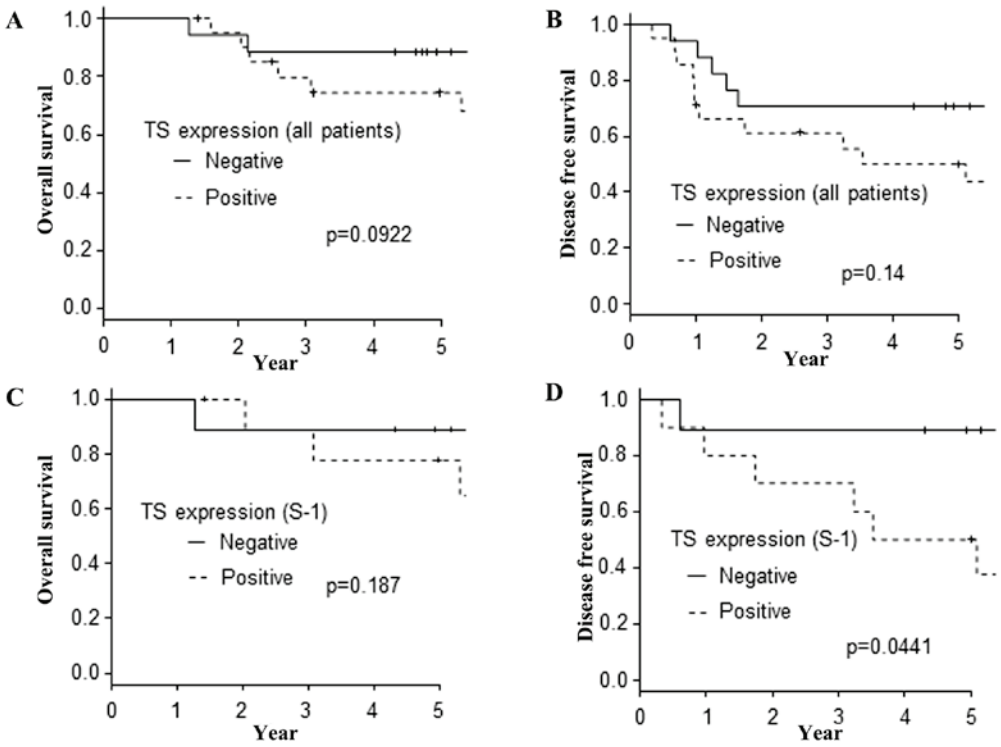

Figure 3. Kaplan-Meier analyses. (A) The overall survival of the 38 patients associated withthe protein expression of TS. (B) The disease-free survival of the 38 patients associated with the protein expression of TS. (C) The overall survival of the 19 patients who received S-1 adjuvant chemotherapy, associated with the protein expression of TS. (D) The disease-free survival of the 19 patients who received S-1 adjuvant chemotherapy, associated with the protein expression of TS. TS, thymidylate synthase.

TS-negative cases showed a longer DFS than the TS-positive cases (Fig. 3D).

Discussion

The survival of patients with advanced lung cancer is still unfavorable compared with malignant tumors of other organs (1). Recently, improved outcomes have been achieved with molecular-targeted therapy for select patients with epidermal growth factor receptor (EGFR)-activating mutations or ALK translocation (11-13). Understanding the genetic and molecular variations that affect the efficacy of chemotherapeutic agents may improve patient care by allowing physicians to optimize treatment for each patient. Even with cytotoxic anticancer drugs, it would be useful to know the factors predictive of a therapeutic effect before starting the administration of chemotherapy.

In this study, we evaluated the expression of several proteins in 38 patients with stage II and IIIA NSCLC who had received CP or S-1 as adjuvant chemotherapy. The

5-year OS and DFS of these 38 patients were 81.0 and 59.6%, respectively. These findings are comparable to those that have been reported recently (1,41). Concerning the OS analysis, the EGFR mutation status has been shown to influence the prognosis after relapse (11-13). Molecular-targeted therapeutic drugs apparently extend the OS in cases with EGFR mutations. It is therefore difficult to evaluate the effect of adjuvant chemotherapy on the OS in our small-scale study, because we don't have the data of gene mutations about all patients of this study. We should evaluate the DFS to clarify the relationship between protein expression and adjuvant chemotherapy efficacy. We should check the gene mutations (EGFR and ALK) to evaluated the effect of adjuvant chemotherapy on the OS in the future studies.

The CP regimen is considered as a standard chemotherapy regimen for recurrent and advanced lung cancer (42-46). We used the regimen of bi-weekly paclitaxel plus carboplatin to be able to complete the adjuvant chemotherapy without interruption due to side effects. As S-1 is considered more effective than UFT, long-term S-1 administration may be promising as an adjuvant chemotherapy regimen for advanced lung cancer (47). Indeed, several studies have shown that S-1 administration as adjuvant chemotherapy is associated with significant survival benefits following surgically complete resection for NSCLC (47,48). In this study, the 5-year OS and DFS were almost the same between the S-1 group and the CP group.

We investigated the protein expressions of ERCC1 and TUBB3, which are believed to be associated with the effect of platinum- and taxane-based chemotherapies, respectively. Previously, ERCC1-positive cases were reported to show more resistance to platinum-based chemotherapy than negative cases (16), but no relationship was noted between the ERCC1 expression and the prognosis, even in the patients who received the CP regimen in this study. We obtained similar findings concerning the TUBB3 expression. TUBB3-negative cases have previously been reported to show a better prognosis than positive ones. The prognostic effect of TUBB3 expression observed in this study, even in the patients who received CP regimen, was not consistent with prior published reports in the setting of advanced NSCLC (35,49,50). This discrepancy may be attributed to the small patient population in this study.

We also evaluated the protein expressions of TS, DPD, and OPRT, which are believed to be associated with the effect 5-FU-related agents, including S-1. Specifically, the overexpression of TS and DPD have been reported to be associated with resistance to S-1 (26-28). In contrast, the overexpression of OPRT was reported to be associated with a better prognosis in patients who received S-1 chemotherapy (30). In the present study, the expression of DPD and OPRT showed no association with the OS or DFS, even in the patients who received S-1 chemotherapy. The expression of TS did not have an association with the OS or DFS in the total population or with the OS in the 19 patients who received S-1. However, in the analysis of the DFS of the 19 patients who received S-1, the patients with TS overexpression showed a significantly poorer prognosis than the TS-negative patients.

One limitation associated with this study was the small patient population, as only 19 cases received S-1 and 19 cases received CP. Among the 40 patients, 2 were excluded due to the cessation of adjuvant chemotherapy because of a grade 4 allergic reaction (anaphylactic shock) induced by paclitaxel. The frequency of the anaphylactic shock (5%) was higher than previous reports. We think that the small sample size of this study will affect to the result. However, the adverse effects of S-1 were tolerable, and S-1 chemotherapy may be considered a promising adjuvant chemotherapy for patients with advanced disease who have undergone complete surgical resection. Further large-scale analyses of the relationship between TS expression and chemotherapeutic effects are desired. Moreover, we should evaluate the relationship among each protein expression in a large-scale clinical trial in the future.

We herein showed that TS is a potentially useful biomarker to help identify patients who will benefit from S-1 adjuvant chemotherapy.

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Availability of data and material

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

KO and MY designed the present study. KO, KN, TY, TK, TN, MS, SM, HH, OK and MY collected the patients' data. KO, TT, TS, RO, TW and RN analyzed the patients' data. KO was a major contributor in writing the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Written informed consent was obtained from all patients, and the study protocol was approved by the Institutional Review Board of each participating institution (Nagoya City University Hospital no. 45-13-0020). This study was carried out in accordance with the Declaration of Helsinki.

Consent for publication

Written informed consent was obtained from all patients.

Competing interests

The authors declare that they have no competing interests.

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