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ORIGINAL ARTICLE

Expression of ERCC1 and class III β-tubulin in non-small cell lung cancer patients treated with a combination of cisplatin/docetaxel and concurrent thoracic irradiation

Koichi Azuma · Tetsuro Sasada · Akihiko Kawahara · Satoshi Hattori · Takashi Kinoshita · Sinzo Takamori · Masao Ichiki · Youhei Imamura · Jiro Ikeda · Masayoshi Kage · Michihiko Kuwano · Hisamichi Aizawa

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Abstract
Introduction The expression of excision repair cross-complementation group 1 (ERCC1) is reported to be correlated with resistance to platinum-based drugs. Class III β-tubulin is reported to be correlated with resistance to taxanes.
Methods In the present study, we evaluated whether ERCC1 and class III β-tubulin expression could be used to predict progression-free and/or overall survival in 34 patients with locally advanced non-small cell lung cancer (NSCLC) receiving concurrent chemoradiation therapy with cisplatin and docetaxel, and immunohistochemistry was used to examine the expression of these two proteins in tumor samples obtained from the patients.
Results Immunostaining for ERCC1 and class III β-tubulin was positive in 16 and 12 patients, respectively. A significant correlation was observed between ERCC1 expression and response to chemotherapy ($P = 0.012$), and between class III β-tubulin expression and histology ($P = 0.029$). Patients negative for ERCC1 had a significantly longer median progression-free (62.5 vs. 36 weeks, $P = 0.009$), but not overall (171 vs. 50.5 weeks, $P = 0.208$), survival than those positive for ERCC1. Expression of class III β-tubulin was not correlated with progression-free or overall survival ($P = 0.563$ and $P = 0.265$, respectively). Multivariate analysis adjusting for possible confounding factors showed that negative ERCC1 expression (hazard ratio = 3.972, $P = 0.009$) was a significantly favorable factor for progression-free survival.
Conclusions This retrospective study indicates that immunostaining for ERCC1 may be useful for predicting survival in NSCLC patients receiving concurrent chemoradiotherapy with cisplatin and docetaxel, and can provide information critical for planning personalized chemotherapy.

Keywords ERCC1 · Class III β-tubulin · NSCLC · Chemoradiation

Abbreviations

NSCLC	Non-small cell lung cancer
ERCC1	Excision repair cross-complementation group 1
p-stage	Pathological stage
RECIST	Response evaluation criteria in solid tumors

K. Azuma (✉) · T. Kinoshita · M. Ichiki · Y. Imamura · J. Ikeda · H. Aizawa
Division of Respirology, Neurology, and Rheumatology, Department of Internal Medicine, Kurume University School of Medicine, 67 Asahi-machi, Kurume, Fukuoka 830-0011, Japan
e-mail: azuma@med.kurume-u.ac.jp

A. Kawahara · M. Kage
Department of Pathology, Kurume University School of Medicine, Kurume, Japan

S. Takamori
Department of Surgery, Kurume University School of Medicine, Kurume, Japan

K. Azuma · A. Kawahara · M. Kage · M. Kuwano
Research Center for Innovative Cancer Therapy, Kurume University School of Medicine, Kurume, Japan

S. Hattori
Biostatistics Center, Kurume University, Kurume, Japan

T. Sasada
Department of Surgery, Kitano Hospital, Tazuke-Kofukai Medical Research Institute, Osaka, Japan

M. Ichiki
Department of Respiratory Medicine, National Hospital Organization, Kyushu Medical Center, Chuo-ku, Fukuoka, Japan

Introduction

Lung cancer continues to be the leading cause of cancer death worldwide. Non-small cell lung cancer (NSCLC) is the most common type of lung cancer, accounting for approximately 80% of cases. The majority of NSCLC patients with locally advanced disease develop distant metastasis. In spite of intensive research into the treatment of NSCLC, the prognosis of affected patients still remains poor. To improve this situation, therapeutic strategies involving combinations of chemotherapy and radiotherapy have been selected. One such combination is concurrent chemoradiotherapy with cisplatin and docetaxel, which is reported to yield a higher response rate (79%) and overall survival rate (76%) at one year [1]. Failure of chemotherapy is attributable mainly to the acquisition of drug resistance by cancer cells. Therefore, it is very important to understand the molecular mechanisms of resistance to chemotherapeutic drugs. Resistance to platinum-based drugs is one of the major obstacles to treatment, and is related to poor prognosis in NSCLC patients. ERCC1 is a component of the nucleotide excision repair (NER) pathway, which is essential for the repair of platinum-DNA adducts and is associated with cellular resistance to platinum compounds. Overexpression of ERCC1 has a tendency to be associated with resistance to platinum compounds. Two laboratories have shown that NSCLC patients with a low level of ERCC1 mRNA expression survive for significantly longer after treatment with cisplatin and gemcitabine [2, 3]. In addition, Olaussen et al. [4] have demonstrated a survival benefit of adjuvant cisplatin-based chemotherapy in patients with completely resected NSCLC, whose tumors were negative for ERCC1 expression. Previously, we also showed that ERCC1 expression estimated by immunohistochemistry was an independent prognostic factor for progression-free and overall survival in relapsed NSCLC patients receiving platinum-based chemotherapy [5]. Taken together, patients with a low level of ERCC1 exhibit prolonged survival after treatment with platinum-based chemotherapy, compared with those showing a high level of ERCC1. Similar data have been reported for patients with ovarian, gastric, and colorectal cancer [6–8].

Taxanes, such as docetaxel and paclitaxel, are also widely used anticancer agents in the treatment of NSCLC, either singly or in combination with other platinum compounds such as cisplatin and carboplatin. Taxanes bind to β-tubulin, which is one of the major components of microtubules, and exert their growth-inhibitory effects through the inhibition of microtubule dynamics, resulting in growth arrest of tumor cells at the G2-M phase [9–11]. In particular, overexpression of class III β-tubulin has been frequently demonstrated in drug-resistant cancer cells. Several studies have shown that overexpression of class III β-tubulin is correlated with poor prognosis in NSCLC, ovarian cancer, breast cancer, and pancreas cancer [12–14].

Analysis of the molecular factors predicting the chemosensitivity and prognosis of patients receiving chemotherapies is considered important for understanding the tumor biology of NSCLC and devising better therapeutic strategies. Treatment with inadequate chemotherapy regimens causes various undesirable side effects without benefits, whereas administration of effective drugs selected according to the predicted chemosensitivities of the cancers could help improve the survival and/or quality of life of patients. In this study, we used immunohistochemistry to examine the expression of ERCC1 and class III β-tubulin in tumor samples from patients with locally advanced NSCLC receiving concurrent chemoradiotherapy with cisplatin and docetaxel. We analyzed the relationships between ERCC1 and class III β-tubulin expression in tumors and survival time to determine whether or not the expression of these molecules could be used to predict progression-free and/or overall survival in this cohort of patients.

Materials and methods

Patients and treatment

Between March 1999 and December 2004, 51 patients with NSCLC received concurrent chemoradiotherapy with cisplatin and docetacel at a single institution (Kurume University Hospital, Kurume, Japan). Histological specimens were available for 34 of these patients (bronchoscopic biopsy 27 patients; surgical resection 7 patients). The stage was classified as IIB in two patients, IIIA in 16, and IIIB in 16. Patients with malignant pleural effusion, pericardial effusion, or pleural dissemination were excluded. Details on the patients' clinical characteristics, including age, gender, histology, smoking status, performance status, and treatment response, were obtained from chart review by an independent reviewer unaware of the results of the immunohistochemical analysis. The tumors were diagnosed as adenocarcinoma in 16 patients, squamous cell carcinoma in 17, and unclassified in one, on the basis of the World Health Organization (WHO) criteria. Details of the demographics, treatment, and follow-up characteristics are shown in Table 1.

All patients received concurrent chemoradiotherapy with cisplatin (70 mg/m^2) and docetaxel (25 mg/m^2), given on days 1 and 8. Patients received at least two cycles of chemotherapy with an interval of 3–4 weeks. After these cycles, cisplatin (80 mg/m^2) and docetaxel (60 mg/m^2) were given on day 1 at an interval of 3–4 weeks. Thoracic radiotherapy was administered from day 8 of chemotherapy using a linear accelerator in 2-Gy single daily fractions for

Table 1 Patients' characteristics

Characteristics	Number
Age (years)	
Median	66.5
Range	46–77
Gender	
Male	28
Female	6
Histology	
Adenocarcinoma	16
Squamous cell carcinoma	17
Unclassified	1
Smoking status	
Never smoker	7
<50 pack-year	13
≧50 pack-year	14
Performance status	
0	25
1	5
2	4
Stage	
IIB	2
IIIA	16
IIIB	16
Cycles	
2	15
3	12
4	7
Treatment response	
Complete response(CR)	2
Partial response (PR)	19
Stable disease (SD)	7
Progressive disease (PD)	6

five consecutive days each week. A total radiation dose of 60 Gy was planned. Patients showing a good response after completion of 40 Gy of radiotherapy underwent surgical resection of their tumors.

Tumor response was evaluated after chemotherapy according to the RECIST (Response Evaluation Criteria for Solid Tumors). All patients underwent chest plain X-ray examination, computed tomography scans of the chest and upper abdomen, bone scans, and brain magnetic resonance imaging (MRI) before chemotherapy and at least every 6 weeks during chemotherapy. Complete response (CR) was defined as the disappearance of all clinically detectable tumor lesions, lasting for at least 4 weeks. Partial response (PR) was defined as a decrease of at least 30% in the sum of the longest dimensions of the target lesions for at least 4 weeks, with no appearance of new lesions. Progressive disease (PD) was defined as an increase of at least 20% in the sum of the longest dimensions of the target lesions or the emergence of new lesions. Stable disease (SD) was defined as a decrease in tumor lesions that was insufficient to qualify as PR and an increase that was insufficient to qualify as PD [15].

Immunohistochemical evaluation of paraffin-embedded tumor tissues

Tissue samples were fixed in 10% neutral-buffered formalin and embedded in paraffin. Paraffin sections were then cut at a thickness of 4 μm, mounted on coated glass slides, and labeled with antibodies against class III β-tubulin (TUJI I; Covance, Berkeley, CA) or ERCC1 (clone 8F1; Neomarkers, Fremont, CA). After the sections had been deparaffinized in xylene and dehydrated in a graded ethanol series, endogenous peroxidase activity was blocked with H_2O_2 in methanol. For ERCC1 staining, antigens were retrieved by heating in citric acid (pH 6.0) for 10 min. Sections were incubated with blocking solution (10% Block Ace; Yukijirushi, Tokyo, Japan) and then reacted with anti-ERCC1 monoclonal antibody. After excess antibody had been washed out with PBS, the samples were incubated with horseradish peroxidase (HRP)-labeled goat anti-mouse or anti-rabbit antibody (Nichirei, Tokyo, Japan) for 60 min. The reaction was visualized using 3,3' diaminobenzidine (DAB) as a substrate (DAKO, Glostrup, Denmark), and the slides were counterstained with hematoxylin. For class III β-tubulin staining, immunohistochemistry with anti-class III β-tubulin antibody was performed using the avidin–biotin–peroxidase complex method (Nichirei, Tokyo, Japan) with DAB as the chromogen. Each slide was heat-treated using Target Retrieval Solution, pH 9.0 (DAKO, Glostrup, Denmark) for 30 min, and incubated with the antibody at 4°C overnight. ERCC1 nuclear expression was classified into four categories: score 0, no staining at all <10% of tumor cells; score 1+, faint/barely perceptible partial nuclear expression in >10% of tumor cells; score 2+, weak to moderate staining of the entire nucleus in >10% of tumor cells; and score 3+, strong staining of the entire nucleus in >10% of tumor cells. The extent of immunohistological staining for ERCC1 was defined as fellows: scores of 2+ or 3+ were regarded as positive, and scores of 0 or 1+ were regarded as negative. Class III β-tubulin cytoplasmic expression was classified into five categories: score 0, nostaining at all; score 1+, faint/barely perceptible partial cytoplasmic expression in <10% of tumor cells; score 2+, weak to moderate staining of the entire cytoplasm in >10% of tumor cells; score 3+, moderate staining of the entire cytoplasm in >10% of tumor cells; and score 4+, strong staining of the entire cytoplasm in >10% of tumor cells [16]. The extent of immunohistological staining for Class

III β-tubulin was defined as fellows: scores of 3+ or 4+ were regarded as positive, and scores of 0 or 1+ or 2+ were regarded as negative. All IHC studies were evaluated by two IHC-experienced observers who were unaware of the conditions of the patients (A. K and M. Kage).

Statistical methods

Fisher's exact test was used to analyze the significance of associations between ERCC1 and class III β-tubulin expression and other patient characteristics and the overall response (CR and PR by RECIST). Progression-free survival was defined as the time between the onset of chemotherapy and the date when disease progression began. Patients without progression were regarded as censored at the date of the last follow-up. Overall survival was defined as the time between the onset of chemotherapy and the date of death due to any cause. Patients were regarded as censored if they were alive on the date of the last follow-up. Curves for progression-free and overall survival were estimated by the Kaplan–Meier method, and differences in survival functions were compared by the log-rank test.

The primary objective of this study was to evaluate the influence of ERCC1 and class III β-tubulin on progression-free and overall survival. To this end, Cox proportional hazards models with ERCC1 and class III β-tubulin as explanatory variables were fitted. In addition to ERCC1 and class III β-tubulin, patient characteristics that correlated significantly with ERCC1 or class III β-tubulin and those that were found to have a significant influence on progression-free or overall survival by the log-rank test were included in the Cox models, as they were potential confounding factors for evaluation of ERCC1 and class III β-tubulin. All tests were two-sided, and differences at $P < 0.05$ were considered statistically significant. All the statistical analyses were conducted using JMP version 7 software and SAS version 9.1 (SAS Institute Inc., Cary, NC).

Results

Immunohistochemical assessment of ERCC1 and class III β-tubulin expression

Expression of ERCC1 and class III β-tubulin proteins was assessed by immunohistochemistry (Fig. 1). Expression of the ERCC1 protein was detected in the nuclei of cancer cells, and expression of the class III β-tubulin protein was evident in the cytoplasm. Table 2 shows the relationships between the expression of ERCC1 or class III β-tubulin and other clinicopathologic factors. ERCC1 nuclear expression was classified into four categories. Eleven patients had score 0, 7 had score 1+, 7 had score 2+, and 9 had score 3+ (median 1). Scores of 2+ and 3+ were regarded as positive. ERCC1 expression was positive in 16 patients and negative in 18. A significant correlation was observed between ERCC1 expression and response to chemotherapy ($P = 0.012$), but not other factors, including age ($P = 0.302$), gender ($P = 1.000$), histology ($P = 0.100$), smoking status ($P = 0.681$), performance status ($P = 0.249$), stage ($P = 0.491$), or class III β-tubulin expression ($P = 1.000$). Class III β-tubulin cytoplasmic expression was classified into five categories. Eleven patients had score 0, 5 had score 1+, 6 had score 2, 9 had score 3+, and 3 had score 4+ (median 2). Scores of 3+ and 4+ were regarded as positive. Expression of class III β-tubulin was positive in 12 patients and negative in 22. A significant correlation was observed between ERCC1 expression and histology ($P = 0.029$), but not other factors, including age ($P = 0.720$), gender ($P = 0.154$), smoking status ($P = 0.211$), performance status ($P = 1.000$), stage ($P = 0.628$), response to chemotherapy ($P = 0.138$) or ERCC1 expression ($P = 1.000$).

Chemoradiotherapy and response

Chemotherapy regimens for NSCLC patients consisted of cisplatin and docetaxel. The median number of chemotherapy cycles was 3 (range 2–4). Seven patients received 4 cycles of chemotherapy, 12 received 3 cycles, and 15 received 2 cycles. Five patients underwent surgery after completion of chemoradiotherapy (total 40 Gy). Overall response (CR + PR) was observed in 21 (62%) of the patients, whereas 7 (20%) had SD and 6 (18%) had PD. The response rates for patients who were ERCC1-positive and -negative were 37.5% (6 of 16 patients) and 83% (15 of 18 patients), respectively. The relationship between the overall response and ERCC1 status was statistically significant ($P = 0.012$). The response rates for patients who were class III β-tubulin-positive and -negative were 41% (5 of 12 patients) and 72% (16 of 22 patients), respectively, and were not significantly different ($P = 0.138$).

Association between patient characteristics and progression-free and overall survival

The median follow-up time was 104 (range 8–328) weeks, and the median progression-free and overall survival times were 48 (range 8–260) and 104 (range 8–328) weeks, respectively. A cut-off value was selected for each clinical and pathological factor according to the median value of continuous variables. As a preliminary analysis, univariate Cox analysis was carried out to identify the factors that were significantly associated with progression-free and overall survival (Table 3). Univariate analysis showed that performance status ($P = 0.003$), stage ($P = 0.005$) and

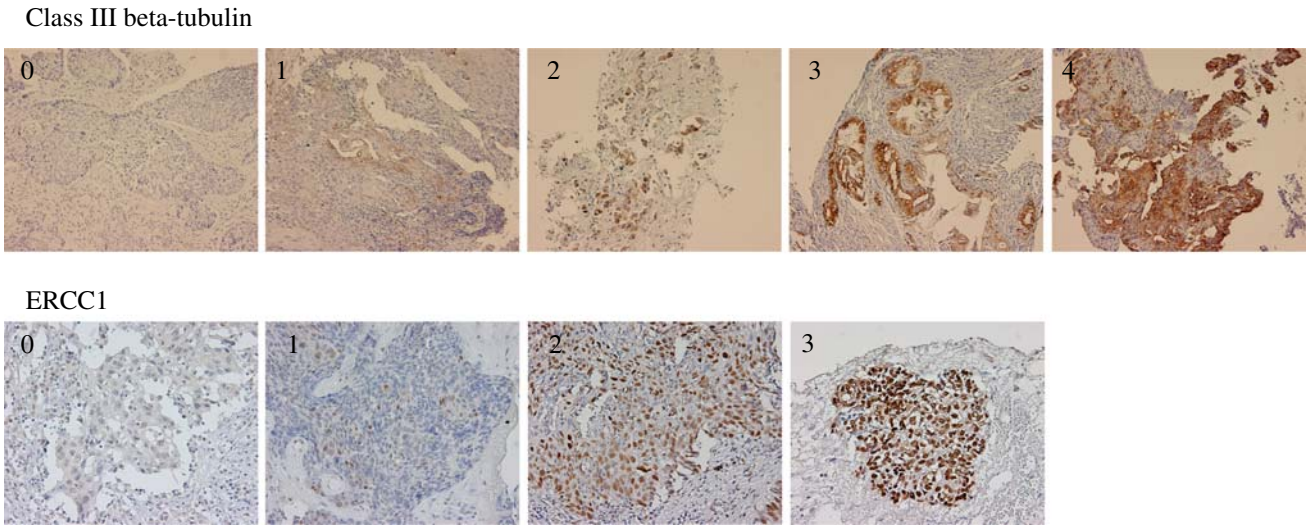

Fig. 1 Immunohistochemical staining of ERCC1 and class III β-tubulin proteins in lung cancer tissues. Examples of the expression of each protein are shown. Expression of ERCC1 protein was detected in the nuclei of cancer cells, and expression of class III β-tubulin protein was present in the cytoplasm. Quantitative estimation of immunohistochemical staining was judged as described in "Materials and methods". Original magnification ×400

Table 2 Relationship between ERCC1, β-tubulin status and various characteristics

Characteristics	Number	ERCC1 (−)	ERCC1 (+)	*P* value[a]	β-Tubulin (−)	β-Tubulin (+)	*P* value[a]
Age (years)							
High (67)	17	7	10	*P* = 0.302	10	7	*P* = 0.720
Low (<67)	17	11	6		12	5	
Gender							
Male	28	15	13	*P* = 1.000	20	8	*P* = 0.154
Female	6	3	3		2	4	
Histology							
Adenocarcinoma	16	11	5	*P* = 0.100	7	9	*P* = 0.029
Others	18	7	11		15	3	
Smoking status							
Never-smoker	7	3	4	*P* = 0.681	3	4	*P* = 0.211
Smoker	27	15	12		19	8	
Performance status							
0	25	15	10	*P* = 0.249	16	9	*P* = 1.000
1 or 2	9	3	6		6	3	
Stage							
IIB	2	2	0	*P* = 0.491	2	0	*P* = 0.628
IIIA	16	9	7		9	7	
IIIB	16	7	9		11	5	
Treatment response							
CR or PR	21	15	6	*P* = 0.012	16	5	*P* = 0.138
SD or PD	13	3	10		6	7	
Overexpression of ERCC1							
–	18				12	6	*P* = 1.000
+	16				10	6	
Overexpression of β-tubulin							
–	22	12	10	*P* = 1.000			
+	12	6	6				

[a] By Fisher's exact test

CR complete response, *PR* partial response, *SD* stable disease, *PD* progressive disease

Table 3 Factors associated with progression-free and overall survival

Factor	Number	Progression-free-survival				Overall survival			
		Median PFS (weeks)	Univariate analysis	Multivariate analysis	*P* value[b]	Median OS (weeks)	Univariate analysis	Multivariate analysis	*P* value[b]
			Log-rank[a]	Hazard ratio (95% CI)			Log-rank[a]	Hazard ratio (95% CI)	
Age (years)									
High (≧67)	17	61	$P = 0.487$			328	$P = 0.050$	0.355 (0.130–0.971)	$P = 0.044$
Low (<67)	17	36				57			
Gender									
Male	28	44.5	$P = 0.709$			94	$P = 0.596$		
Female	6	62				136			
Histology									
Adenocarcinoma	16	44	$P = 0.402$	4.085 (1.366–12.220)	$P = 0.012$	80	$P = 0.832$	1.864 (0.553–6.288)	$P = 0.316$
Others	18	55				152			
Smoking status									
Never-smoker	7	64	$P = 0.606$			172	$P = 0.683$		
Smoker	27	41				80			
*P*erformance status									
0	25	61	$P = 0.003$	5.738 (2.135–15.430)	$P < 0.001$	152	$P = 0.154$		
1 or 2	9	22				35			
Stage									
IIB, IIIA	18	62.5	$P = 0.030$	3.383 (1.493–9.860)	$P = 0.005$	272	$P = 0.002$	3.550 (1.346–9.364)	$P = 0.011$
IIIB	16	33				50.5			
Overexpression of ERCC1									
−	18	62.5	$P = 0.009$	3.972 (1.405–11.231)	$P = 0.009$	171	$P = 0.208$	2.406 (0.856–6.759))	$P = 0.096$
+	16	36				50.5			
Overexpression of β-tubulin									
−	22	54.5	$P = 0.563$	0.839 (0.340–2.072))	$P = 0.704$	152	$P = 0.265$	1.371 (0.446–4.213)	$P = 0.581$
+	12	38				62			

PFS progression-free survival, *OS* overall survival, *CI* confidence interval

[a] Univariate analysis by log-rank test

[b] Multivariate analysis by Cox proportional hazards model

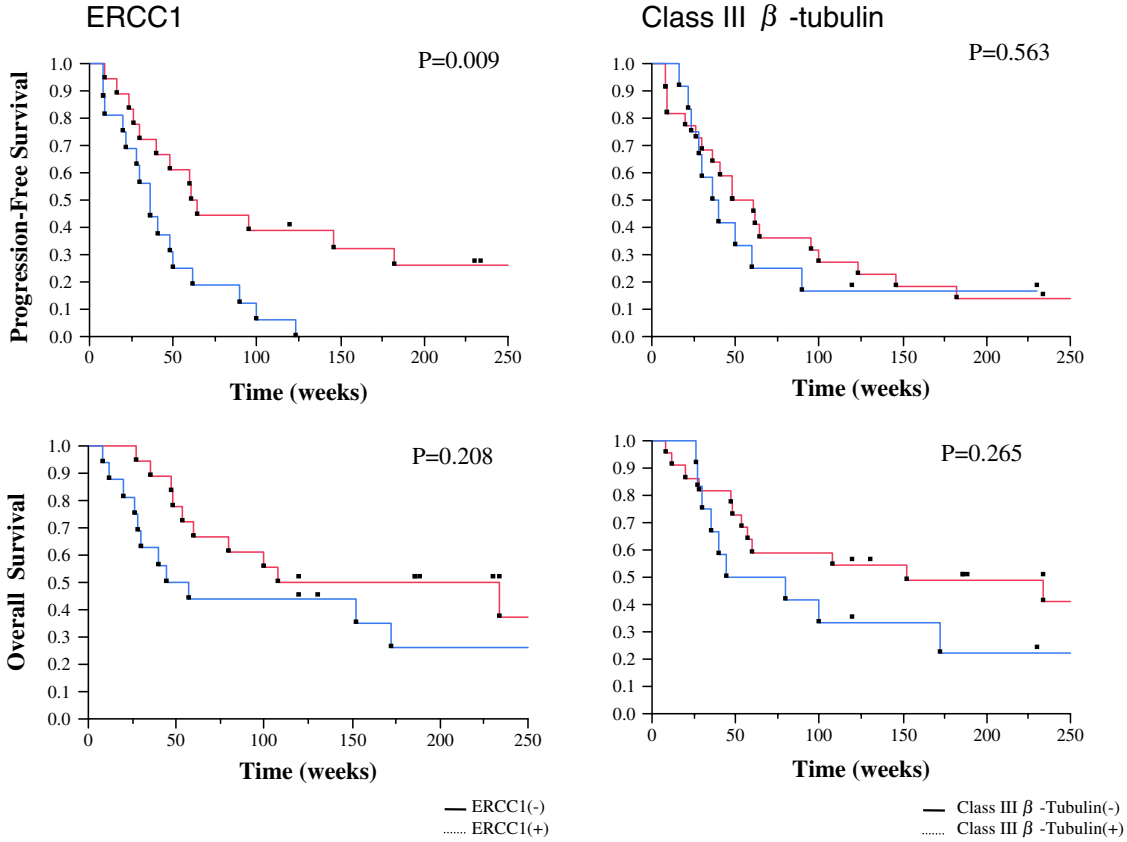

Fig. 2 Kaplan–Meier survival analysis in patients positive and negative for ERCC1 or class III β-tubulin expression. Differences in progression-free survival and overall survival between subgroups were analyzed by the log-rank test

ERCC1 expression ($P = 0.009$) were predictive of progression-free survival. For overall survival, age ($P = 0.050$) and stage ($P = 0.002$) were predictive. None of the other factors examined were statistically correlated with progression-free or overall survival. Figure 2 shows the Kaplan–Meier survival curves for patients positive and negative for ERCC1 and class III β-tubulin expression. Patients negative for ERCC1 expression had a significantly longer median progression-free (62.5 vs. 36 weeks, $P = 0.009$), but not overall (171 vs. 50.5 weeks, $P = 0.208$), survival, than those positive for ERCC1 expression. Expression of class III β-tubulin was not correlated with progression-free or overall survival ($P = 0.563$ and $P = 0.265$, respectively).

The effects of ERCC1 and class III β-tubulin expression were evaluated by applying the Cox regression models adjusting for possible confounding factors. As shown in Table 2, histology was significantly correlated with class III β-tubulin expression. As shown in Table 3, the log-rank test indicated that performance status, stage and age significantly influenced progression-free and overall survival, respectively. Therefore, histology, performance status and stage were adjusted for progression-free survival, and histology, stage and age for overall survival. For progression-free survival, ERCC1 expression showed a significant correlation [$P = 0.009$; hazard ratio, 3.972 (95% CI 1.405–11.231)], while class III β-tubulin did not ($P = 0.704$). For overall survival, neither ERCC1 nor class III β-tubulin expression was significantly correlated ($P = 0.096$ and $P = 0.581$, respectively) (Table 3).

Discussion

In the present study, we demonstrated that patients negative for ERCC1 had a significantly higher response rate and longer median progression-free survival than those positive for ERCC1 after concurrent chemoradiotherapy with cisplatin and docetaxel. Furthermore, multivariate analysis revealed that ERCC1 expression was an independent factor associated with progression-free survival. ERCC1 is a component of the nucleotide excision repair pathway, which is essential for the repair of platinum-DNA adducts and is associated with cellular resistance to platinum compounds. Lord et al. [2] and Ceppi et al. [3] reported that a low level of ERCC1 RNA is a predictive factor for longer survival in patients with advanced NSCLC treated with cisplatin and

gemcitabine. In a recent randomized and customized ERCC1 mRNA trial involving 444 patients, where patients with a low level of ERCC1 mRNA received platinum-based chemotherapy and those with a high level received non-platinum-based chemotherapy, 53 patients (39.3%) attained an objective response in the control arm and 107 (50.7%) in the customized arm [16]. Simon et al. [17] also conducted a prospective phase II clinical study of NSCLC patients. Patients were assigned to receive combination chemotherapy with two agents that were selected based on ERCC1 and RRM1 gene expression, and the disease response rate was 44%. These results indicate that the use of gene expression analysis to choose the optimal therapy is a promising strategy for improvement of prognosis in NSCLC patients.

In agreement with these results, our study demonstrated that ERCC1 expression was an independent predictive factor of progression-free survival in patients receiving concurrent chemoradiation therapy with cisplatin and docetaxel. Our findings suggest that platinum-based chemotherapy is more effective against ERCC1-negative NSCLC tumors. However, it is also possible that patients with ERCC1-negative tumors might have a better prognosis irrespective of chemotherapy. However, in view of studies showing that NSCLC patients with low ERCC1 expression have a shorter survival after resection than those with high ERCC1 expression [18], we favor the interpretation that ERCC1 expression determines the efficacy of platinum-based chemotherapy in NSCLC patients.

Several studies have shown that overexpression of class III β-tubulin is frequently observed in drug-resistant cancer cells and correlated with poor prognosis in NSCLC, ovarian cancer, breast cancer, and pancreas cancer [9–14]. Seve et al. [9] reported that the class III β-tubulin level was independently correlated with response rate and progression-free and overall survival in NSCLC patients. Furthermore, we reported that immunostaining for ERCC1 and class III β-tubulin may be useful for predicting survival in NSCLC patients receiving carboplatin and paclitaxel for recurrent tumors after curative resection [19]. However, in this study, expression of class III β-tubulin was not correlated with progression-free or overall survival. One reason for this discrepancy may have been that the number of patients was too small to exhibit differences, and thus the definitive role of class III β-tubulin expression for prognostication of NSCLC patients remains to be addressed in a further study.

Despite efforts to identify factors that can predict response to chemotherapy in patients with advanced NSCLC, no suitable markers have been established. Analysis of the molecular factors predicting the chemosensitivity and prognosis of patients receiving chemoradiotherapy is considered important for understanding the tumor biology of NSCLC and devising better therapeutic strategies. Recent progress in molecular biology has encouraged further research into human malignancies, including lung cancer. The development of gene expression profiles that can predict responses to commonly used cytotoxic agents will provide opportunities to better use these drugs for molecular-based personalized chemotherapies. In the present study, we used immunohistochemistry to test the expression of ERCC1 and class III β-tubulin in tumor samples, and analyzed whether these factors were correlated with the survival of patients receiving concurrent chemoradiotherapy with cisplatin and docetacel. We demonstrated that patients negative for ERCC1 had a significantly longer progression-free survival and higher response rate than those positive for ERCC1 after treatment. A similar tendency was observed for overall survival, although this was not statistically significant (significance level 5%). It should be noted that this study was retrospective, and that the sample number was a little small. Therefore, a larger-scale prospective randomized control study employing homogeneous standard regimens is recommended to verify the role of ERCC1 and/or class III β-tubulin expression.

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Conflict of interest statement None of the authors has a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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