Class III β-tubulin expression in tumor cells predicts response and outcome in patients with non–small cell lung cancer receiving paclitaxel

Pascal Sève,[1,2,5] John Mackey,[5] Sylvie Isaac,[7] Olivier Trédan,[2] Pierre-Jean Souquet,[8] Maurice Pérol,[3] Raymond Lai,[6] Alain Voloch,[9] and Charles Dumontet[2,4]

[1]Service de Médecine Interne, Hospices Civils de Lyon; [2]Unité Institut National de la Sante et de la Recherche Medicale 590, Laboratoire de Cytologie Analytique, Université Claude Bernard; [3]Service de Pneumologie, Hôpital de la Croix-Rousse; [4]Laboratoire d'Immunologie des Hémopathies, Hôpital Edouard-Herriot, Lyon, France; Departments of [5]Oncology and [6]Pathology, University of Alberta, Edmonton, Alberta, Canada; [7]Laboratoire d'Anatomopathologie and [8]Service de Pneumologie, Centre Hospitalier Lyon-Sud, Pierre-Bénite, France; and [9]Polyclinique de Rillieux, Rillieux, France

Abstract

Both fundamental and clinical studies suggest that class III β-tubulin expression is associated with resistance to taxanes and constitutes a prognostic factor in several solid tumors. In this study, we assessed the prognostic and predictive value of class III β-tubulin in tumors of patients with locally advanced or metastatic non–small cell lung cancer (NSCLC) treated with paclitaxel-based or other regimens that did not include tubulin-binding agents. Expression of class III β-tubulin was examined immunohistochemically in 91 tumor samples obtained before treatment from patients with stage III and IV NSCLC, including 47 who received paclitaxel-based regimens and 44 who received regimens without tubulin-binding agents. Response to chemotherapy, progression-free survival, and overall survival were correlated with the expression of class III β-tubulin protein. The response rate was 37.5% (16 responses among 45 evaluable patients) among patients receiving paclitaxel. Patients whose tumors expressed low levels of class III β-tubulin isotype had a better response rate, longer progression-free survival, and overall survival ($P < 0.001$, 0.004, and 0.002, respectively), whereas this variable was not found to be predictive in patients receiving regimens without tubulin-binding agents. A multivariate analysis taking into account sex, age, histology, stage, and class III β-tubulin confirmed that low-level class III β-tubulin expression was independently correlated with progression-free survival ($P = 0.003$) and overall survival ($P = 0.003$). These findings suggest that the expression levels of class III β-tubulin in tumor cells is predictive of response to therapy and patient outcome in patients with NSCLC receiving paclitaxel-based chemotherapy but is not a general prognostic factor in this patient population. [Mol Cancer Ther 2005;4(12):2001–7]

Introduction

Taxanes, including paclitaxel and docetaxel, are among the most active antitumor agents in the treatment of non–small cell lung cancer (NSCLC; refs. 1, 2). 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 the growth arrest of tumor cells at the G_2-M phase (3). Because an increasing number of lung cancer patients are being treated with taxanes, development of taxane resistance is becoming a clinically important problem associated with chemotherapy. Elucidation of the clinically relevant resistance mechanisms is of pivotal importance both for the identification of patient groups with different sensitivities to these compounds and for the development of therapeutic strategies taking into account these mechanisms.

Several mechanisms have been suggested as responsible for taxane resistance (4). The first mechanism reported was the overexpression of the *mdr-1* gene, encoding for an efflux pump (P-glycoprotein) able to efflux taxanes and other cationic drugs, thereby hampering drug retention (5). Although such a mechanism is easily obtained in cell lines *in vitro*, only scattered evidence has been provided for its relevance in patients affected by solid tumors (6). Subsequently, Monzo et al. reported that somatic mutation of class I β-tubulin is observed in as much as 33% of human NSCLC and that it plays a significant role in the acquisition of paclitaxel resistance (7). However, other studies have failed to confirm the presence of tubulin point mutations in taxane-resistant patients bearing lung and ovarian tumors (8). An additional mechanism of resistance is the selective overexpression of β-tubulin isotypes. In humans, at least six distinct β-tubulin isotypes (classes I, II, III, IVa, IVb, and VI) have been reported, and their expression profile differs among tissues (6). Class III β-tubulin differs from other tubulin isotypes in its amino acid sequence

Received 7/14/05; revised 8/24/05; accepted 9/27/05.

Grant support: Fondation de France postdoctoral grant (P. Sève) and Hospices Civils de Lyon.

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Requests for reprints: Charles Dumontet, Unité Institut National de la Sante et de la Recherche Medicale 590, Laboratoire de Cytologie Analytique, Faculté de Médecine, Université Claude Bernard, 8 Avenue Rockefeller, 69373 Lyon Cedex 08, France. Phone: 33-4-78-77-72-36. E-mail: charles.dumontet@chu-lyon.fr

doi:10.1158/1535-7163.MCT-05-0244

and post-translational modifications, which include phosphorylation and polyglutamylation (9). In functional terms, the interaction of class III β-tubulin with paclitaxel is different from that of other isotypes (10, 11). Furthermore, several investigators have shown that the high levels of class III β-tubulin expression were associated with taxane resistance in human cancer cell lines [lung cancer (3), ovarian cancer (12), prostate cancer (13), breast cancer (3), and pancreatic cancer (14)]. We have shown recently that class III β-tubulin expression is correlated with patient outcome in NSCLC patients treated with vinorelbine-based regimens (15).

The relevant question is whether increased class III β-tubulin production in tumor cells can cause patients to fail taxane-based therapy. Several studies have shown that class III tubulin overexpression assessed by either immunohistochemistry or reverse transcription-PCR is a prognostic factor in patients with lung cancer (16, 17) and is associated with resistance to taxanes in ovarian (18) and breast cancer patients treated with taxane-containing regimens (19). However, the critical outstanding question is whether class III tubulin is simply a *prognostic marker* correlated with disease aggressivity independently of the type of therapy administered to patients or a *predictive marker* specifically relevant to those patients receiving compounds directed against microtubules.

To address this question, we conducted a retrospective study of patients with advanced NSCLC treated with either taxane-based regimens or gemcitabine-based regimens. Using immunohistochemistry, we assessed protein abundance of class III β-tubulin in 47 patients treated with paclitaxel and in 44 patients treated with unrelated gemcitabine-based regimens and correlated these biological results with patient outcome.

Materials and Methods

Patients and Samples

The analysis was done on samples from 47 patients with unresectable locally advanced (stage IIIB) or metastatic (stage IV) NSCLC treated with paclitaxel-based chemotherapy. Sixteen patients were part of the first preliminary study (16) and 31 patients were treated between January 2000 and December 2003 in two pneumology departments of the Hospices Civils de Lyon (Lyon, France) and in the Polyclinique de Rillieux (Rillieux, France). To assess the predictive value of class III β-tubulin expression, we analyzed 44 unselected patients treated with a first-line gemcitabine-based chemotherapy and who did not receive taxane or *Vinca* at any time during the course of their disease. There were 36 patients treated in Lyon (France) and 8 patients treated in Edmonton (Canada).

These patients had adequate tumor biopsy specimens obtained before chemotherapy. Histopathologic subtypes were determined based on the WHO classification. Clinical staging was based on the initial evaluation consisting in a clinical assessment, chest X-ray, computed tomography of the chest and abdomen, computed tomography or magnetic resonance imaging of the brain, and bone scintigraphy. The current international staging system was used for clinical disease staging (20). After obtaining informed consent in accordance with institutional guidelines, all of the patients underwent tumor biopsy and chemotherapy. All of the patients who received at least two courses of chemotherapy were evaluated for response. We used the standard response criteria (21) to evaluate response to chemotherapy. Complete response was defined as the disappearance of all signs of disease both at clinical examination and on the computed tomography scan. Partial response was defined by a reduction of >50% in the sum of products of the largest perpendicular diameters of all tumor localizations, with no new tumor lesions. Stable disease was defined by a <50% decrease or a <25% increase in tumor size. Tumor progression was defined as an increase in the size of tumor lesions by >25% or the appearance of a new lesion. Overall survival was calculated as the time between the beginning of chemotherapy and death or last follow-up. Progression-free survival was calculated as the time between the beginning of chemotherapy and the date of tumor progression or last follow-up.

Histopathologic Analysis

Immunohistochemical analyses were done on paraffin-embedded sections of pathologic samples obtained before therapy. Among the 47 paclitaxel-treated patients, samples were obtained by bronchoscopy in 29 cases, by node cervical or subclavicular biopsy in 4 cases, by mediastinoscopy in 10 cases, and by metastasis biopsy in 4 cases. The antibody used was anti–class III β-tubulin at a 1:400 dilution (clone TUJ1 produced by Anthony Frankfurter, Department of Biology, University of Virginia, Charlottesville, VA). Class III β-tubulin was stained using an automated immunohistochemical stainer (NexES, Ventana Medical Systems, Illkirch, France) using 5-μm-thick tissue sections following routine deparaffination in xylene, rehydration, and appropriate antigen retrieval for 45 minutes in EDTA (pH 8). Chromogenic detection was done with 3,3′-diaminobenzidine. Negative controls consisted in specimens incubated in the absence of primary antibodies. All of the slides were examined and scored by S.I. for the French patients and by R.L. for the 8 gemcitabine-treated Canadian patients blinded to patient characteristics and outcomes. All samples were independently reviewed by P.S. Tumor cells were stained with reference to the normally strong class III β-tubulin nuclear staining within the endothelial cells or nerves (15). Scores ranged from 0 (no staining) to 2 (at least equal to endothelial cells or nerves). Only cells with a score of 2+ were considered as positive. For correlations with patient outcome, samples were then scored as 1 (<50% positive cells) or 2 (≥50% positive cells).

Statistical Analysis

Comparisons between patients treated with taxane-based regimens and patients treated with gemcitabine-based regimens were done using the χ^2 test (or Fisher's exact test when necessary) for categorical data and nonparametric Kruskal-Wallis ANOVA for quantitative data. Bivariate correlations between immunohistochemical expression,

patient or tumor characteristics, and response to chemotherapy were examined using the χ^2 test (or Fisher's exact test, as appropriate). Survival curves were estimated by the Kaplan-Meier method, and differences in progression-free survival and overall survival between groups were determined using the log-rank test. The Cox proportional hazards model was used for multivariate analysis to adjust the observed value of the expression of microtubule components for the determination of predictive factors. $P < 0.05$ was considered as significant. All statistical analyses were done using Statistica version 5.0.

Results

Clinical Data

The clinicopathologic characteristics of the 47 patients treated with paclitaxel-based chemotherapy are listed in Table 1. Their median age at diagnosis was 60 years (range, 41–77 years). Three of the 18 stage III patients and 5 of the 29 stage IV patients were women. All the patients were treated with paclitaxel in combination with carboplatin or with *cis*-diamminedichloroplatinum (CDDP). The median follow-up of the 47 patients, measured from the onset of chemotherapy, was 336 days (range, 58–1,255 days).

The clinicopathologic characteristics of the 44 patients treated with gemcitabine-based chemotherapy and a comparison between the two groups of patients are listed in Table 1. Their median age at diagnosis was 57 years (range, 37–75 years). Four of the 13 stage IIIB patients and 8 of the 31 stage IV patients were women. All the patients were treated either with single-agent gemcitabine or with a combination of gemcitabine with CDDP or carboplatin. The median follow-up of the 47 patients, measured from the onset of chemotherapy, was 244 days (range, 10–731 days).

Chemotherapy

The taxane-based regimens were 175 mg/m^2 paclitaxel plus carboplatin dosed with an area under the curve of 5 on day 1 of a 21-day cycle (24 patients), 175 mg/m^2 paclitaxel plus 350 mg/m^2 carboplatin on day 1 of a 21-day cycle (5 patients), 200 mg/mg/m^2 paclitaxel plus 100 mg/m^2 CDDP on day 1 of a 21-day cycle (13 patients), and 175 mg/mg/m^2 paclitaxel plus 80 mg/m^2 CDDP on day 1 of a 21-day cycle (5 patients).

The gemcitabine-based regimens consisted in the combination of 1,000 mg/m^2 gemcitabine on days 1 and 8 plus 80 mg/m^2 CDDP on day 1 of a 21-day cycle (37 patients)

Table 1. Characteristics and comparison of 47 patients treated with taxane-based regimens and 44 patients treated with gemcitabine-based regimens

Characteristics	No. patients treated with taxane-based regimens (%)	No. patients treated with gemcitabine-based regimens (%)	*P*
Total no. patients	47	44	
Gender			
Male	39 (83)	32 (72.7)	0.24
Female	8 (17)	12 (27.3)	
Age, y			
Median	60	57	0.32
Range	41-77	37-75	
Histology			
Squamous cell carcinoma	17 (36.2)	15 (34.1)	0.43*
Adenocarcinoma	23 (48.9)	19 (43.2)	
Large cell carcinoma	7 (14.9)	10 (22.7)	
Stage			
IIIB	18 (38.3)	13 (29.5)	0.38
IV	29 (61.7)	31 (70.5)	
Weight loss			
<5%	23 (48.9)	16 (36.4)	0.06†
>5%	10 (21.3)	18 (40.9)	
Missing data	14 (29.8)	10 (22.7)	
Thoracic radiation for stage IIIB	9 (50)	4 (31)	0.46
Cycle of chemotherapy			
Median (range)	3 (1-6)	3 (1-9)	0.62
Chemotherapeutic regimen			
Carboplatin + paclitaxel	29		
Carboplatin + gemcitabine		5	
Cisplatin + paclitaxel	18		
Cisplatin + gemcitabine		37	
Gemcitabine alone		2	

*Comparison for squamous cell carcinoma histologic subtype.
†Patients with weight loss <5% were compared with patients with weight loss >5% without taking into account the missing data.

and 1,000 mg/m^2 gemcitabine on days 1 and 8 plus carboplatin dosed with an area under the curve of 5 on day 1 of a 21-day cycle (5 patients). Two patients were treated with single-agent 1,000 mg/m^2 i.v. gemcitabine once weekly.

Immunohistochemical Data

Results of the immunostaining of tumor samples and the comparison between the two groups of patients are summarized in Table 2. Immunostaining varied markedly among lung cancer samples in patients treated both with paclitaxel-based regimens and with gemcitabine-based regimens, but we did not observe any significant difference between the two groups of patients. An example of immunohistochemical staining with anti–class III tubulin antibody is shown in Fig. 1. All control slides, prepared without primary antibody, revealed no background immunoperoxidase staining and were used as negative controls. Class III staining was not found in the bronchial epithelium but was observed at low level in the bronchial glands and at strong levels in vessels and nerves.

Patient Outcome

Response was evaluated in 45 paclitaxel-treated patients. Sixteen patients responded (1 complete response and 15 partial responses), yielding an overall response rate of 35.6% in patients having received at least two treatment cycles. Nineteen (42.2%) patients had a stable disease at evaluation and 10 (22.2%) patients progressed on therapy. Of the 18 patients with stage IIIB disease, 9 received thoracic radiotherapy after the completion of chemotherapy. The median number of cycles received was 3 (range, 1–6). The median overall survival and progression-free survival were 336 and 209 days, respectively, in the entire patient population and 575 and 379 days, respectively, in responding patients. Forty-two patients had died at follow-up and 5 were alive.

In gemcitabine-treated patients, response was evaluated in 36 cases. Twelve patients had partial responses, yielding an overall response rate of 33.9% in patients having received at least two treatment cycles. Twelve (33.3%) patients had a stable disease at evaluation and 12 (33.3%) patients progressed on therapy. Of the 13 patients with stage IIIB disease, 4 received thoracic radiotherapy after the completion of chemotherapy. The median number of cycles received was 3 (range, 1–9). The median overall survival and progression-free survival were 244 and 159.5 days, respectively, in the entire patient population and 388.5 and 281 days, respectively, in responding patients. Forty-three patients had died at follow-up and 1 was lost to follow-up.

A comparison between the two groups of patients for age, gender, histology, stage, radiotherapy, and number of cycles did not show any significant difference. However, there was a trend for a higher proportion of patients with >5% weight loss in the group of patients receiving gemcitabine-based regimen.

Microtubule Component Expression and Response to Treatment

We correlated class III tubulin expression with response to chemotherapy (Table 3). We found a close relationship between class III tubulin expression and both response to treatment and progression during chemotherapy in patients treated with a paclitaxel-based regimen. Patients whose tumors showed low-level class III β-tubulin expression (<50% versus ≥50% of positive cells) displayed a higher response rate (61.9% versus 12.5%; $P < 0.001$) as well as a lower rate of progression on therapy (4.8% versus 37.5%; $P = 0.008$). Patient characteristics (age, gender, weight loss, histologic subtype, and stage) were not found to be correlated with response to chemotherapy (data not shown). Expression levels of class III β-tubulin were not found to be correlated with response to chemotherapy in patients treated with a gemcitabine-based regimen (33.3% in patients whose tumors showed either low-level or high-level class III β-tubulin expression).

Microtubule Component Expression and Survival

A low level of expression of class III β-tubulin protein was correlated with a longer median progression-free survival of 335 versus 105 days in paclitaxel-treated patients ($P = 0.0039$; Fig. 2A). Furthermore, a shorter overall survival was significantly correlated with a high expression level of class III β-tubulin. The median overall survival was 525 days in patients with low level of class III isotype as opposed to 206 days in patients with high level of class III isotype ($P = 0.0023$; Fig. 2B). Class III β-tubulin level was not found to be correlated with either progression-free survival or overall survival in patients receiving gemcitabine. In these latter patients, the median progression-free survival was 165 days in patients with low levels of class III β-tubulin and 140 days in patients with high levels ($P = 0.76$), whereas the median overall survival was 334 days in patients with low levels of class III β-tubulin and 222.5 days in patients with high levels ($P = 0.85$; Fig. 3A and B). Among patient characteristics, only stage IV disease was associated with shorter progression-free survival ($P = 0.017$) and shorter overall survival ($P = 0.0091$) in paclitaxel-treated patients (data not shown).

Table 2. Results of immunostaining for class III β-tubulin in tumors from 47 patients treated with taxane-based regimens and 44 patients treated with gemcitabine-based regimens and comparison between the two groups

% Cells	Patients treated with taxane (%) $n = 47$	Patients treated with gemcitabine (%) $n = 44$	P
0–24	16 (34)	10 (22.7)	0.23
25–49	6 (12.8)	7 (15.9)	0.67
50–74	6 (12.8)	6 (13.6)	0.9
75–100	19 (40.4)	21 (47.7)	0.48

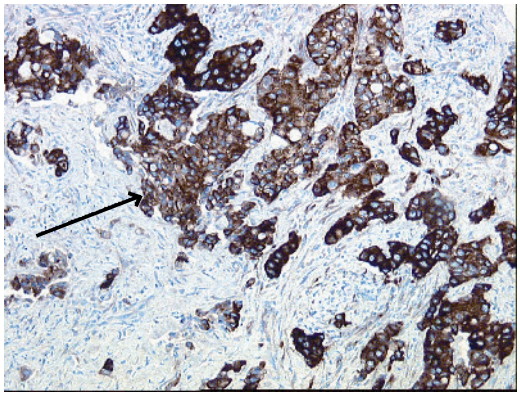

Figure 1. Adenocarcinoma of the lung strongly stained with anti – class III β-tubulin antibody surrounded with nonneoplastic lung tissue. Ninety percent of tumor cells are positive for class III β-tubulin (*arrow*).

Multivariate Analysis for Progression-Free Survival and Overall Survival

Multivariate analysis was done by using the Cox proportional hazards model to determine whether the prognostic value of class III tubulin was independent of other known prognostic factors in the taxane-treated patients (data not shown). The multivariate analysis that included sex, age, histology, stage, and class III tubulin showed that stage (stage IV versus IIIB) and class III tubulin expression (high versus low) expression were independent variables significantly correlated with a shorter progression-free survival (P = 0.0135 and 0.0026, respectively). A high-level class III level yielded a hazard ratio of 2.538 for shorter progression-free survival, with a 95% confidence interval ranging from 1.366 to 4.695. High-level class III tubulin and stage IV were independently correlated with shorter overall survival (P = 0.0033 and 0.0148, respectively). A high-level class III level yielded a hazard ratio of 2.793 for shorter overall survival, with a 95% confidence interval ranging from 1.464 from 5.319.

Discussion

This study explored the predictive value of the level of expression of a microtubular protein in tumor samples of patients with advanced NSLCL receiving a paclitaxel-based regimen. Class III β-tubulin isotype levels were found to be independently correlated with the response rate, rate of progression on therapy, progression-free survival, and overall survival. Interestingly, 12 of the 21 patients with low-level class III β-tubulin expression responded to chemotherapy, whereas only 1 progressed under paclitaxel-based regimen. Conversely, we did not find any association between class III β-tubulin expression and response to treatment or clinical outcome in patients treated with a gemcitabine-based regimen. Because of the limited sample size and the retrospective nature of our study, these results must be interpreted cautiously. The two groups are not comparable because patients receiving gemcitabine-based regimens were in poorer condition with a higher proportion of weight loss and shorter progression-free survival and overall survival. Moreover, stage IV was only found to be a prognostic factor in paclitaxel-treated patients. However, in spite of these limitations, these data suggest that a low level of class III β-tubulin expression could possess predictive value for response and outcome in advanced NSCLC patients receiving taxane-based therapy.

These results confirm our preliminary study in 19 patients receiving taxane-based regimens, which showed that progression-free survival was shorter in patients whose tumors expressed high levels of class III tubulin isotype (16). A clinical study by Mozzetti et al. on 41 advanced ovarian cancer patients treated with paclitaxel revealed a significant up-regulation of class III tubulin expression at both mRNA and protein levels in the resistant subset, defined as patients who progressed under chemotherapy (18). Paradiso et al. showed recently that class III β-tubulin immunohistochemical analysis was a relevant tumor biomarker for paclitaxel resistance in 70 advanced breast cancer patients (19). Moreover, fundamental studies showed that the high-level class III β-tubulin expression is associated with paclitaxel resistance in human cancer cell lines [lung cancer (3), ovarian cancer (12), prostate cancer (13), and breast cancer (3)] and with docetaxel resistance in human pancreatic cancer cell lines (14). The mechanistic involvement of these alterations in the determination of resistance remains open to debate. Current hypotheses are that these alterations may alter drug binding to the tubulin dimer (22) or that the microtubule contained in the tumor

Table 3. Relationship between expression of class III β-tubulin expression, response, progression on therapy, and survival in 47 NSCLC patients treated with a taxane-based regimen and 44 patients treated with a gemcitabine-based regimen

	n	Response rate (%)	*P*	Progression rate (%)	*P*	Progression-free survival (d)	*P*	Overall survival (d)	*P*
Patients treated with taxanes									
<50% Class III	22	61.9	<0.00	4.8	0.008	335	0.0039	525	0.0023
>50% Class III	25	12.5	1	37.5		105		206	
Patients treated with gemcitabine									
<50% Class III	17	33.3	1	33.3	1	165	0.76	334	0.85
>50% Class III	27	33.3		33.3		140		222.5	

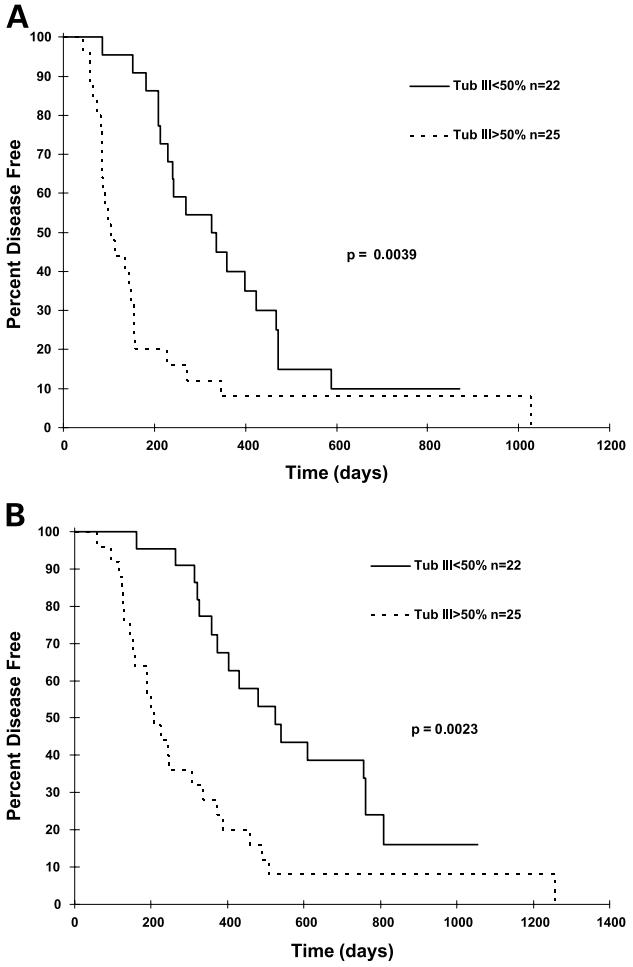

Figure 2. Progression-free survival curves (**A**) and overall survival curves (**B**) for 47 paclitaxel-treated patients with advanced NSCLC according to class III β-tubulin (*Tub III*) expression in tumors.

cells may have different dynamic properties and thus may be less sensitive to antitubulin agents (10, 23). It has been shown, in a study comparing the dynamic properties of microtubules composed of $\alpha\beta_{II}$, $\alpha\beta_{III}$, or $\alpha\beta_{IV}$ dimers, that the dynamics of $\alpha\beta_{III}$ microtubules were less sensitive to taxanes (24). Class III β-tubulin reduces the polymerization rate of microtubules, thereby overcoming microtubule polymerization by paclitaxel (25). Using an antisense approach, Kavallaris et al. showed that the reduction of class III tubulin content allowed *in vitro* sensitization to tubulin-binding agents (26). Recently, Kamath et al. showed that overexpression of class III β-tubulin induces paclitaxel resistance by reducing the ability of paclitaxel to suppress microtubule dynamics (11). Using an expression system of class III β-tubulin on the control of tetracycline regulatory element, they showed that, in the presence of paclitaxel, dynamic instability was suppressed to a significantly lesser extent in cells overexpressing class III β-tubulin than in cells overexpressing class I β-tubulin, whereas, in the absence of paclitaxel, there were no differences in any aspect of dynamic instability in the two cell lines. Thus, both these fundamental and clinical studies support a pivotal role of class III β-tubulin overexpression in paclitaxel resistance.

More than half of NSCLC are advanced-stage IIIB or IV at presentation, and patients with advanced NSCLC are candidates for systemic chemotherapy. Treatment is based on the combination of platinum-based chemotherapy and one of the new cytotoxic drugs, such as taxanes (paclitaxel and docetaxel), gemcitabine, vinorelbine, and irinotecan (27, 28). These new regimens have produced superior therapeutic results compared with CDDP alone and older CDDP-based regimens with a survival advantage of 8 to 10 months (29). Recent randomized studies indicate that there are no significant differences in efficacy among these combinations of CDDP with these new drugs, although they have shown varying profiles of toxicity (30). Identification and implementation of markers predictive of response to specific cytotoxic drugs is one way to select drug regimen based on the biological characteristics of the

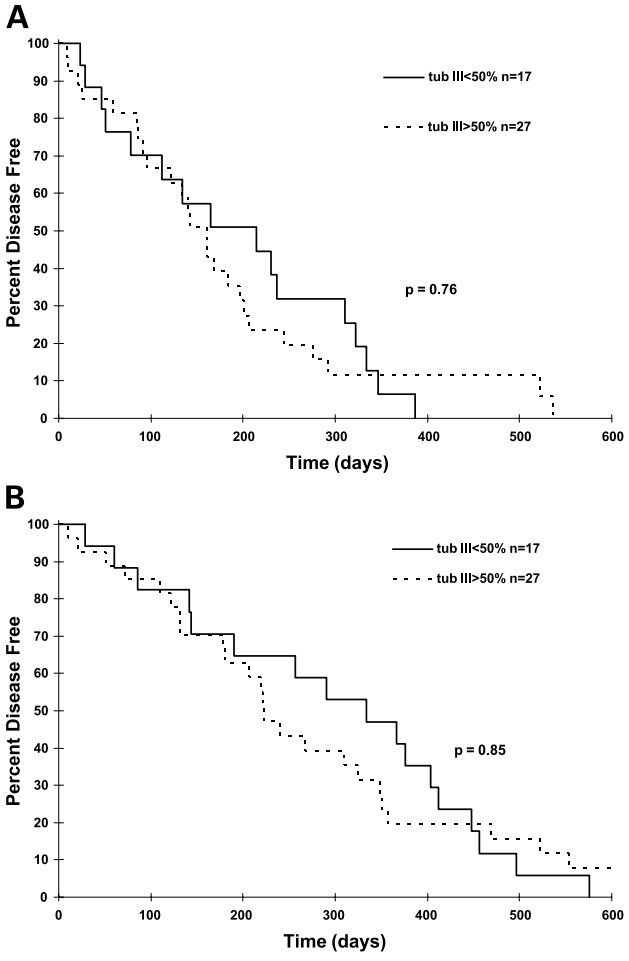

Figure 3. Progression-free survival curves (**A**) and overall survival curves (**B**) for 44 gemcitabine-treated patients with advanced NSCLC according to class III β-tubulin expression in tumors.

tumors. Using quantitative PCR to analyze expression of class III β-tubulin mRNA from tumor biopsies of 75 NSCLC patients as part of a randomized trial, Rosell et al. showed that patients with low-level RNA class III β-tubulin had, respectively, better response in the carboplatin/paclitaxel and higher time to progression in the vinorelbine/gemcitabine arm (17). These results are in keeping with that study and our previous study, which showed that protein abundance of class III β-tubulin was correlated with resistance to treatment and a poor clinical outcome in advanced NSCLC patients treated with vinorelbine-based regimen (15). In this study, we examined by immunochemistry, using the same method, expression of class II and III β-tubulin isotypes and Δ2 α-tubulin in 93 tumor samples from patients with stage III and IV NSCLC treated with a vinorelbine-based regimen. Although expression levels of microtubule components were not associated with the response rate to chemotherapy, we found a close relationship between high-level class III expression and resistance to treatment, defined as disease progression under chemotherapy. Patients whose tumors expressed high levels of class III β-tubulin isotype had shorter progression-free survival and overall survival and high Δ2 α-tubulin expression was associated with a shorter overall survival. Multivariate analysis confirmed that class III β-tubulin expression was independently correlated with progression-free survival and overall survival. In this study, patients were not compared with patients treated with an anti-tubulin-unrelated regimen. Taking together, all these data show that expression of low-level class III β-tubulin is correlated with response to treatment and better clinical outcome in advanced NSCLC patients treated with anti-tubulin agents. These data suggest that class III β-tubulin could be a predictive value, which can help tailor chemotherapy, in advanced NSCLC patients and improve patient survival. Prospective customized chemotherapy trials are now warranted to test this hypothesis.

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