Analysis of cell cycle-related proteins in mediastinal lymph nodes of patients with N2-NSCLC obtained by EBUS-TBNA: relevance to chemotherapy response

S Mohamed,[1,2] K Yasufuku,[1] T Nakajima,[1] K Hiroshima,[3] R Kubo,[1] A Iyoda,[1] S Yoshida,[1] M Suzuki,[1] Y Sekine,[1] K Shibuya,[1] A Farouk,[2] T Fujisawa[1]

[1] Department of Thoracic Surgery, Graduate School of Medicine, Chiba University, Chiba, Japan; [2] Department of Pulmonology, Faculty of Medicine, Assiut University, Assiut, Egypt; [3] Department of Diagnostic Pathology, Graduate School of Medicine, Chiba University, Japan

Correspondence to: Dr K Yasufuku, Department of Thoracic Surgery, Graduate School of Medicine. Chiba University, 1-8-1 Inohana, Chuo-Ku, Chiba 260-8670, Japan; kyasufuku@faculty.chiba-u.jp

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ABSTRACT

Background: Endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) is an accurate tool for lymph node staging of non-small cell lung cancer (NSCLC). Most patients with NSCLC require systemic chemotherapy during their treatment, with relatively poor responses. If the response to chemotherapy could be predicted, ideally at the time of the initial bronchoscopic examination, the therapeutic benefit could be maximised while limiting toxicity. A study was therefore undertaken to investigate the feasibility of EBUS-TBNA for obtaining tissue samples from mediastinal lymph nodes that can be used for immunohistochemical analysis, and to stratify patients with molecular-based pN2-NSCLC into chemoresponsive and chemoresistant subgroups who might benefit from tailoring of chemotherapy.

Methods: The expression of six cell cycle-related proteins (pRb, cyclin D1, $p16^{INK4A}$, p53, $p21^{Waf1}$, Ki-67) in mediastinal lymph node specimens obtained by EBUS-TBNA was investigated by immunohistochemistry in 36 patients with pN2-NSCLC. Their predictive role(s) in the response to platinum-based chemotherapy was examined.

Results: Immunostaining was feasible in all studied specimens. Univariate analysis revealed that p53 and $p21^{Waf1}$ expressions were significantly related to the response to chemotherapy ($p = 0.002$ and $p = 0.011$, respectively). Multivariate logistic regression analysis revealed that only p53 overexpression was associated with a poor response to chemotherapy ($p = 0.021$).

Conclusions: These results suggest that EBUS-TBNA is a feasible tool for obtaining mediastinal nodal tissue samples amenable for immunohistochemical analysis. Immunostaining of p53 in EBUS-TBNA-guided specimens may be useful in predicting the response to chemotherapy in patients with N2-NSCLC and helping in the selection of patients who might benefit from certain chemotherapeutic strategies.

Lung cancer is one of the most common causes of death. While surgery is the standard approach to early stage non-small cell lung cancer (NSCLC), radiotherapy with or without chemotherapy is the main treatment option in locally advanced disease (30% of patients) and chemotherapy remains the only available treatment for those with metastatic disease (50% of patients).[1] Moreover, NSCLC is often found to be intrinsically resistant to both chemotherapy and radiotherapy at the start of treatment, but the basis of this resistance to treatment—primary or secondary—remains unknown.[2] If one could predict the response to chemotherapy based on the assessment of biological tumour markers, one could maximise the therapeutic benefit while limiting toxicity. This assessment would be ideal if performed at the time of the initial bronchoscopic examination so that it would allow patients the option of pursuing alternative regimens earlier in the course of their treatment.

Direct real-time endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) using the convex probe endobronchial ultrasound is a relatively new minimally invasive and accurate technique for preoperative staging of patients with NSCLC.[3–6] We have recently reported that EBUS-TBNA has a high sensitivity and specificity compared with computed tomography (CT) and positron emission tomography and, as a single procedure for mediastinal lymph node staging, it allows tissue diagnosis.[7] Further analysis of tissue samples obtained by EBUS-TBNA such as genetic analysis may help to direct patients with NSCLC to different molecular-based treatments.[8]

Many studies have reported the predictive value(s) of one or more cell cycle proteins for the response to chemotherapy in lung cancer,[2 9 10] but the results are still controversial.[2] As patients with stage IIIA N2-NSCLC represent heterogeneous prognostic groups, we examined the expression of the Rb pathway (pRb, cyclin D1, $p16^{INK4A}$) and p53 pathway (p53, $p21^{Waf1}$) proteins and Ki-67 labelling indices by immunohistochemistry in mediastinal lymph node specimens obtained by EBUS-TBNA from patients with pathologically-proven N2-NSCLC and investigated their predictive role(s) for the response to platinum-based chemotherapy. The main objectives of this study were (1) to investigate the feasibility of EBUS-TBNA for obtaining nodal tissue samples that can be used for immunohistochemical analysis and (2) to stratify patients with molecular-based pN2-NSCLC into chemoresponsive and chemoresistant subgroups who might benefit from tailored chemotherapy.

METHODS

Patients and tissue samples

From July 2004 to April 2006, 67 patients were diagnosed histologically with metastatic lung cancer of the hilar and/or mediastinal lymph nodes in samples obtained by EBUS-TBNA. Rapid on-site cytological examination was conducted for all patients during the procedure. Thirty-six patients in whom analysis of the histological cores revealed

a pathological diagnosis of stage IIIA N2-NSCLC were enrolled in the study. The presence of both nodal tissue and cancer tissue was confirmed in all specimens by a pathologist. The pathological diagnoses were made according to the World Health Organization classification of lung tumours.[11] The primary tumour and lymph node status were classified according to the International TNM staging system.[12] Additional inclusion criteria included (1) no past history of malignancy in the lung or elsewhere in the body; (2) no evidence of distant metastatic disease; and (3) no chemotherapy or radiotherapy before performing EBUS-TBNA. Twenty-eight of the 36 patients received platinum-based combination chemotherapy. The regimens consisted of platinum-based doublets, after which the patients underwent complete post-chemotherapy radiological restaging to evaluate the response to treatment.

EBUS-TBNA

EBUS-TBNA was performed on an outpatient basis under conscious sedation using a flexible ultrasonic puncture bronchoscope (CP-EBUS, XBF-UC260F-OL8, Olympus, Tokyo, Japan) as described previously.[3–5 7 8] Histological samples were obtained by EBUS-TBNA, as previously reported.[5 7 8] Briefly, a dedicated 22-gauge needle equipped with an internal sheath was used. After the initial puncture the internal sheath was used to clean out the internal lumen clogged with the bronchial tissue. The internal sheath was removed and negative pressure applied by a syringe. The needle was moved backwards and forwards inside the lymph node, after which the needle was retrieved and the internal sheath was used once again to push out the histological core.

Immunohistochemistry

Immunohistochemical analysis of the specimens was performed to determine the expression of pRb, cyclin D1, $p16^{INK4A}$, p53 and $p21^{Waf1}$ proteins. The Ki-67 labelling index was calculated for Ki-67 expression. All immunohistochemical assays were carried out on 10% formalin-fixed, paraffin-embedded tissue sections cut to 3–4 μm thickness and mounted on scilanised glass slides (Dako, Glostrup, Denmark). All sections were then dewaxed in xylene, rehydrated through a graded alcohol series and washed in phosphate buffered saline (PBS; 0.01 M sodium phosphate (pH 7.2), 0.15 M NaCl). This buffer was used for all subsequent washes and for the dilution of the antibodies. Antigen retrieval was achieved by heating after immersion of the tissue slides in citrate buffer (pH 6.0). Tissue sections for cyclin D1, $p16^{INK4A}$, p53 and $p21^{Waf1}$ were heated at 100°C five times in a microwave, each for 3 min, while those for Ki-67 and pRb were heated in an autoclave at 121°C for 15 min. All the tissue sections were then processed using the streptavidin-biotin technique (Histofine Kit; Nichirei, Tokyo, Japan). Mouse monoclonal antibodies (Dako, Glostrup, Denmark) specific for cyclin D1 (DSC-6), p53 (DO-7) and Ki-67 (MIB-1) were used at a dilution of 1:40 and 1:800 for cyclin D1 and p53, respectively, and prediluted for Ki-67. Monoclonal antibodies (Santa Cruz Biotechnology, Heidelberg, Germany) and (EMD Biosciences, San Diego, CA, USA) specific for $p16^{INK4A}$ (F-12; sc-1661) and $p21^{Waf1}$ (Ab-1) were used at a dilution of 1:50 and 1:20, respectively. The monoclonal antibody DO-7 reacts with both wild type and mutant p53 proteins. A rabbit polyclonal antibody (Santa Cruz Biotechnology) specific for pRb (C-15; sc-50) was used at a dilution of 1:50. All the primary antibodies were incubated overnight at 4°C. 3,3′-Diaminobenzidine was used as the final chromogen and haematoxylin as the nuclear counterstain. Positive tissue controls were included in each experiment and consisted of tissues previously shown to stain specifically for the target antigen after exposure to primary antibody.

Evaluation of the immunostaining results

All slides were evaluated without any knowledge of the clinicopathological features or chemotherapy response of the patients. Two independent observers (KH and SM) evaluated the staining pattern of the six proteins separately and scored the protein expression in each specimen by scanning the entire section and estimating the percentage of positive tumour cells. Nuclear colouration was recognised as the primary standard for demonstrating a positive reaction for pRb, $p16^{INK4A}$, p53, $p21^{Waf1}$ and Ki-67,[13 14] irrespective of staining intensity, while for cyclin D1, cytoplasmic staining was recognised as the primary standard for a positive reaction.[14] A cut-off value of >10% tumour cells with positively stained nuclei in the entire section was considered as a positive expression for pRb, $p16^{INK4A}$, p53 and $p21^{Waf1}$, while a cut-off value of >10% tumour cells with positive cytoplasmic staining was considered a positive reaction for cyclin D1.[14] Calculation of the Ki-67 labelling index was performed by counting >1000 positively-stained tumour nuclei in randomly selected high-power fields (10–100) from different representative parts of the tumour. Ki-67 labelling indices were defined as high (overexpression) if they were >20% and low if they were <20%.[15] Abnormal expression was defined as a positive expression of cyclin D1, p53 and high Ki-67 labelling indices and a negative expression (inactivation) of pRb, $p16^{INK4A}$ and $p21^{Waf1}$.

Evaluation of response to chemotherapy

Patients who received chemotherapy underwent a scheduled CT examination to measure the target tumour size. The responses to chemotherapy were evaluated using Response Evaluation Criteria in Solid Tumors (RECIST) guidelines.[16] The response rate was defined as the number of chemotherapy responders (complete response + partial response) divided by the total number of patients. The progressive disease rate was defined as the number of patients with progressive disease divided by the total number of patients.[16] The response to chemotherapy was reviewed without knowledge of the immunostaining results.

Statistical analysis

The associations between categorical immunohistochemical and clinicopathological parameters and between immunohistochemical parameters and the response to chemotherapy were analysed with the χ^2 test or Fisher exact test. The clinicopathological features were age, sex, histopathological type and number of involved mediastinal lymph node stations. To examine simultaneously the impact of more than one factor on the response to chemotherapy, multivariate logistic regression analysis was performed.[17] Statistical analysis was carried out using the SPSS V.12.0 statistical software program package. The criterion of significance chosen was $p<0.05$ and all tests were two-tailed.

RESULTS

Patient characteristics and response to chemotherapy

The clinicopathological features, immunohistochemical results and response to chemotherapy of the patients are shown in table 1. The response to chemotherapy revealed 1 complete response, 12 partial responses, 10 stable disease and 5 progressive disease. The overall clinical response rate was 46.4%.

Table 1 Characteristics of 36 patients with pN2-NSCLC

Characteristic	No (%)
Mean (SD) age (years)	66.8 (9.4)
<66.8	14 (38.9)
>66.8	22 (61.1)
Sex	
Female	5 (13.9)
Male	31 (86.1)
Histopathology	
Adenocarcinoma	19 (52.8)
SCC	17 (47.2)
Mediastinal LN stations	
Single	5 (13.9)
Multiple	31 (86.1)
Protein expressions	
pRb	
Negative	13 (36.1)
Positive	23 (63.9)
Cyclin D1	
Negative	25 (69.4)
Positive	11 (30.6)
p16	
Negative	17 (47.2)
Positive	19 (52.8)
p53	
Negative	17 (47.2)
Positive	19 (52.8)
p21	
Negative	27 (75.0)
Positive	9 (25.0)
Ki-67	
LI <20%	13 (36.1)
LI >20%	23 (63.9)
Treatment response(n = 28)	
CR	1 (3.5)
PR	12 (42.9)
SD	10 (35.7)
PD	5 (17.9)

CR, complete response; LN, lymph nodes; LI, labelling index; PD, progressive disease; PR, partial response; SCC, squamous cell carcinoma; SD, stable disease.

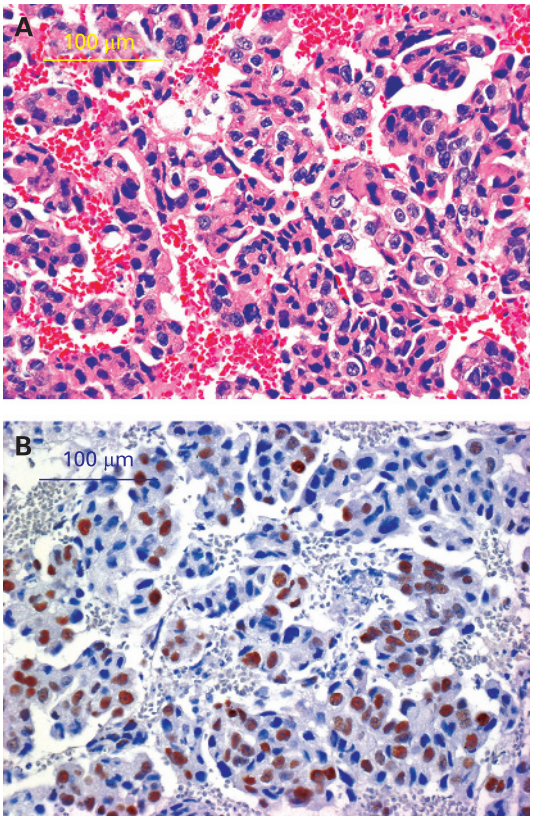

Figure 1 Representative example of mediastinal lymph node tissue sample obtained by endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) from a patient with pN2 non-small cell lung cancer (NSCLC) adenocarcinoma (original magnification ×20).
(A) Note that the main constituents are tumour cells, blood constituents and a small amount of lymphocytes and histiocytes (H&E).
(B) Immunohistochemical staining for DO-7 showing overexpression of p53 protein.

Immunostaining results

Remarkably, all the examined cases showed abnormal expression of at least one of the studied cell cycle proteins. The immunostaining results revealed altered expression of pRb, cyclin D1, $p16^{INK4A}$, p53 and $p21^{Waf1}$ in 36.1%, 30.6%, 47.2%, 52.8% and 75.0% of nodal biopsies, respectively. With regard to Ki-67, 23/36 cases (63.9%) had labelling index values >20% (table 1). Interestingly, histological cores obtained by EBUS-TBNA consisted mainly of tumour cells and blood constituents and minimal amounts of lymph node tissue (fig 1A). Expression of pRb, $p16^{INK4A}$, p53, $p21^{Waf1}$ and Ki-67 was present mainly in the nuclei of the tumour cells, whereas cyclin D1 was seen mainly in the cytoplasm (fig 1B). Some cells had additional cytoplasmic (in the case of pRb) or nuclear (in the case of cyclin D1) immunostaining.

Relationship between immunohistochemical parameters and clinicopathological features

We investigated the relationships between the immunohistochemical and clinicopathologic parameters, as well as their possible interrelationship(s). Interestingly, no statistically significant relation was found between the expressions of any two proteins within the Rb pathway nor between that of p53 and $p21^{Waf1}$. Also, no significant relation was found between any two proteins belonging to two different pathways. With regard to the clinicopathological interrelationships, we found that only histopathological type was significantly related to both age and number of involved mediastinal lymph node stations. Significantly more patients with squamous cell carcinoma were older than the mean age of 66.8 years than those with adenocarcinomas (14/22 (63.7%) vs 8/22 (36.3%); $p = 0.013$). All 17 patients with squamous cell carcinoma histopathology had multiple mediastinal lymph nodes compared with 14/19 (73.7%) of those with adenocarcinoma ($p = 0.047$, data not shown). Some relevant relationships were seen between clinicopathological characteristics and immunohistochemical features (table 2). The sex of the patients was significantly related to pRb ($p = 0.047$) and p53 ($p = 0.047$) expression; the histopathology was significantly associated with the expression of cyclin D1 ($p = 0.042$) and $p16^{INK4A}$ ($p = 0.007$); and the number of involved mediastinal lymph node stations was related to $p16^{INK4A}$ expression ($p = 0.016$).

Predictive values for chemotherapy response

We then analysed the relationship between both the clinicopathological and immunohistochemical parameters and the response to chemotherapy in the 28 patients to whom it was given. None of the clinicopathological parameters was significantly associated with the response to chemotherapy. With

Table 2 Relationships between immunohistochemical parameters and clinicopathological features

	Age (years)			Sex			Histopathology			MLN stations		
IHC parameters (%)	<66.8	>66.8	p Value*	F	M	p Value	AC	SCC	p Value	Single	Multiple	p Value
pRb												
Negative (36.1)	3	10	0.143	4	9	0.047	5	8	0.196	0	13	0.136
Positive (63.9)	11	12		1	22		14	9		5	18	
Total	14	22		5	31		19	17		5	31	
Cyclin D1												
Negative (69.4)	11	14	0.467	3	22	1.000	16	9	0.042	4	21	0.664
Positive (30.6)	3	8		2	9		3	8		1	10	
Total	14	22		5	31		19	17		5	31	
p16												
Negative (47.2)	9	8	0.102	3	14	0.650	13	4	0.007	5	12	0.016
Positive (52.8)	5	14		2	17		6	13		0	19	
Total	14	22		5	31		19	17		5	31	
p53												
Negative (47.2)	8	9	0.342	0	17	0.047	9	8	0.985	3	14	0.650
Positive (52.8)	6	13		5	14		10	9		2	17	
Total	14	22		5	31		19	17		5	31	
p21												
Negative (75.0)	11	16	1.000	4	23	1.000	16	11	0.255	4	23	1.000
Positive (25.0)	3	6		1	8		3	6		1	8	
Total	14	22		5	31		19	17		5	31	
Ki-67 LI												
LI <20% (36.1)	4	9	0.452	1	12	0.634	9	4	0.137	1	12	0.634
LI >20% (63.9)	10	13		4	19		10	13		4	19	
Total	14	22		5	31		19	17		5	31	

AC, adenocarcinoma; F, female; IHC, immunohistochemistry; LI, labelling index; M, male; MLN, mediastinal lymph nodes; SCC, squamous cell carcinoma.
*χ^2 test or Fisher exact test.

regard to the immunohistochemical parameters, univariate analysis showed that p53 and $p21^{Waf1}$ expressions were significantly related to the response to chemotherapy. Twelve of 15 non-responders to chemotherapy had p53 overexpression with response rates of 20% and 76.9% for patients with p53-positive and p53-negative expression, respectively (estimated risk 0.288, 95% confidence interval (CI) 0.104 to 0.803; p = 0.002). For $p21^{Waf1}$ expression, 14/20 non-responders had

Table 3 Relationships between the response to chemotherapy and immunohistochemical parameters (univariate analysis)

IHC parameters	Responders (CR+PR)	Non-responders (SD+PD)	Response rate (%)	Risk value (95% CI)	p Value*	PD rate (%)	p Value*
pRb							
Negative	3	5	37.5	0.692 (0.204 to 2.353)	0.686	37.5	0.123
Positive	10	10	50.0			10.0	
Total	13	15					
Cyclin D1							
Negative	10	11	47.6	0.865 (0.236 to 3.174)	1.000	14.3	0.574
Positive	3	4	42.9			28.6	
Total	13	15					
P16							
Negative	4	10	28.6	0.222 (0.045 to 1.094)	0.061	21.4	1.000
Positive	9	5	64.3			14.3	
Total	13	15					
P53							
Negative	10	3	76.9	0.288 (0.104 to 0.803)	0.002	0.0	0.044
Positive	3	12	20.0			33.3	
Total	13	15					
P21							
Negative	6	14	30.0	0.061 (0.006 to 0.613)	0.011	20.0	0.654
Positive	7	1	87.5			12.5	
Total	13	15					
Ki-67 LI							
LI <20%	5	6	45.5	1.026 (0.565 to 1.862)	0.937	18.2	1.000
LI >20%	8	9	47.0			17.6	
Total	13	15					

CI, confidence interval; CR, complete response; IHC, immunohistochemical; LI, labeling index; PD, progressive disease; PR, partial response; SD, stable disease.
*χ^2 test or Fisher exact test.

Table 4 Multivariate logistic regression analysis

Predictive factors (negative vs positive expression)	Odds ratio (95% CI)	p Value
p53	0.095 (0.013 to 0.705)	0.021
p21	0.082 (0.006 to 1.056)	0.055

$p21^{Waf1}$ inactivation with response rates of 30% and 87.5% for patients with p21-negative and p21-positive expression, respectively (estimated risk 0.061, 95% CI 0.006 to 0.613; p = 0.011). Moreover, only p53 expression was significantly related to the rate of progressive disease (p = 0.044), which is another tool for use in testing the relationship between immunohistochemical parameters and the response to chemotherapy (table 3).

Following the results of the univariate analysis, multivariate logistic regression analysis was performed for the association between p53 and $p21^{Waf1}$ expressions and response to chemotherapy (table 4). Only p53 expression was significantly associated with the response to chemotherapy (odds ratio 0.095, 95% confidence interval 0.013 to 0.705, p = 0.021).

DISCUSSION

EBUS-TBNA is a relatively new technique for the evaluation of mediastinal and hilar lymph node metastasis in patients with lung cancer.[3–7] One major advantage of EBUS-TBNA is its ability to collect samples rich in tumour cells which can be confirmed by pathology.[5 7] We have recently reported the use of EBUS-TBNA for genetic evaluation of tumour cells within the mediastinal lymph nodes of patients with NSCLC.[8] The use of EBUS-TBNA minimises contamination of non-target normal cells within the samples, which is occasionally seen in transbronchial lung biopsies and/or genetic analyses.[8] In the current study we obtained relatively small amounts of normal or reactive tissue within the samples compared with the amount of tumour cells. Aberrant expression of at least one of the six cell cycle proteins was found in all the patients studied. Moreover, the percentages of aberrant expressions of these proteins were relatively similar to those published in the literature in which larger population numbers were recruited.[13 14 18] We were also able to establish some statistically significant relationships. The histopathological type was significantly related to age, number of involved mediastinal lymph node stations, and expression of cyclin D1 and $p16^{INK4A}$. Previous reports have shown that different histopathological subtypes of NSCLC may have distinct biological behaviour patterns.[19] The sex of the patient was related to the expression of pRb and p53, and the number of lymph node stations was related to $p16^{INK4A}$ expression. These findings are in agreement with some previously published studies[14 20] but disagree with others.[18] This controversy could be explained on the basis of differences between patient populations, sample sizes, methodology and biological heterogeneity of the tumours;[18] the latter is particularly a characteristic feature of pN2-NSCLC.[14] Our results showed a lack of significant interrelationships between cell cycle markers which has also been observed by others.[14 18]

Given the low response rates to chemotherapy and the high incidence of side effects, the use of molecular marker(s) in patients with NSCLC to determine whether tumours may be resistant to a particular treatment regimen would avoid unnecessary toxicity and reduce medical costs. This assessment would ideally be performed at the time of the initial bronchoscopic examination so that it would allow a better selection of patients who may benefit from specific neoadjuvant or adjuvant chemotherapy regimens. Our results showed that only p53 expression was significantly related to the response to chemotherapy. The expression of p53 is normally not detectable by immunohistochemistry. However, the mutant p53 proteins have an extended half-life so they accumulate in tumour cells and result in the apparent overexpression of p53 on immunohistochemical examination.[21] Radiotherapy and most chemotherapeutic agents directly target DNA[2] and, in response to such therapies, p53 functions as a coordinator of the DNA repair process, cell cycle arrest and apoptosis.[22] Notably, p53 participates in the main DNA repair systems operative in cells (reviewed by Viktorsson *et al*[2]). Given the high frequency of p53 mutations in lung cancer, a role for p53 as a predictive marker for the response to treatment has been strongly suggested. In response to DNA damage at least some of the p53 mutants show less capacity to bind and initiate transcription from their target genes (eg, $p21^{WAF1}$, *Mdm2*, *Bax*, *cyclin G*), so some of the p53-mediated effects are blunted.[23] Several bodies of evidence have linked p53 with the response to treatment, observing that most chemotherapeutic agents were more effective in killing human tumours with wild type rather than mutant p53.[24 25] Indeed, given the important function of nucleotide excision repair in the repair of DNA damage induced by platinum-based chemotherapy, it has been shown that increased nucleotide excision repair activity in NSCLC cell lines or tumours is associated with increased failure in the response to chemotherapy.[26] Furthermore, resistance to cisplatin was associated with increased activity of excision repair cross-complementing group 1 (ERCC1) and, notably, this polymorphism was measurable at the mRNA level and thus could act as a predictive marker for the outcome to treatment.[27] Our results support the role of p53 as overexpression of p53 was associated with a poor response to platinum-based chemotherapy.

With regard to our previous work,[14] the discrepancy between survival and the response to chemotherapy can be explained in two ways. First, the presence of aggressive tumour features and the resulting shortened survival time does not necessarily lead to treatment resistance because, on the one hand, chemotherapy and radiotherapy may target different pathways and, on the other, a shorter survival time is not always linked to treatment resistance.[2] Second, there may be differences in protein expression between primary and metastatic sites. Primary and metastatic tumour cells may exhibit different characteristics and the former cells may undergo selection in the course of metastasis.[28] On the other hand, conserved mutations have been observed between primary and metastatic sites.[29]

Our results could have important clinical and therapeutic implications. Being a minimally invasive procedure that can be done repeatedly under local anaesthesia, EBUS-TBNA represents a very useful tool for the initial assessment and follow-up of patients with N2-NSCLC. It can help to identify patients who may benefit from induction chemotherapy or adjuvant chemoradiotherapy[30] and those for whom surgery may be beneficial. Furthermore, EBUS-TBNA may become a useful tool for molecular assessment following induction chemotherapy that may help to direct patients to different therapeutic strategies.[8 31] From the therapeutic point of view, recent studies have used manipulations to mutant p53—either functional correction or elimination—to improve existing treatments or even to highlight new anticancer strategies.[32 33]

Our study has two possible limitations—the relatively small number of patients and the fact that it is a retrospective study. Further larger prospective studies evaluating molecular markers in EBUS-TBNA-guided biopsies are therefore needed.

In conclusion, the results of this study suggest that EBUS-TBNA is a feasible tool for obtaining mediastinal nodal tissue samples amenable for immunohistochemical analysis. Immunostaining of p53 in specimens obtained by EBUS-TBNA may be useful in predicting the response to chemotherapy in patients with N2-NSCLC which may lead to a better selection of patients who might benefit from certain chemotherapeutic strategies.

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