FOLIA HISTOCHEMICA ET CYTOBIOLOGICA
Vol. 50, No. 3, 2012
pp. 392–397

ORIGINAL STUDY

VIA MEDICA

Expression of p130cas, E-cadherin and β-catenin and their correlation with clinicopathological parameters in non-small cell lung cancer: p130cas over-expression predicts poor prognosis

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Abstract: p130cas (p130 Crk-associated substrate) is a scaffolding protein and plays an important role in regulating focal adhesion and driving cell migration. Also, the destruction of the E-cadherin/β-catenin adhesive complex is one of the changes that characterizes the invasive phenotype of tumors**.** The aim of this study is to evaluate the role of p130cas, E-cadherin, and β-catenin expression in patients with non-small cell lung cancer (NSCLC). We examined the expression of p130cas, E-cadherin, and β-catenin in 105 lung cancer tissues and paired adjacent normal lung tissues using immunohistochemistry. The overexpression of p130cas was observed in 61.9% (65/105) of lung cancer samples. The overexpression of p130cas was correlated with abnormal expression of E-cadherin and β-catenin ($p = 0.002$ and $p = 0.006$, respectively). Chi-square test showed that the overexpression of p130cas correlated positively with lymph node metastasis and high TNM stage. The Log-Rank test revealed that the mean survival time of patients with p130cas overexpression (36.31 ± 5.66 months) was markedly shorter than that of those with p130cas normal expression (60.57 ± 6.95 months). Multivariable analysis indicated p130cas overexpression ($p < 0.001$) to be an independent significant prognostic factor for NSCLC patients' survival. These results indicate that p130cas may impact a variety of clinicopathological features of NSCLC and may influence the prognosis of lung cancer patients. *(Folia Histochemica et Cytobiologica 2012, Vol. 50, No. 3, 392–397)*

Key words: p130cas**,** E-cadherin, β-catenin**,** prognosis, non-small cell lung cancer

Introduction

Lung cancer is a leading cause of cancer death worldwide [1]. Non-small cell lung cancer (NSCLC) accounts for nearly 85% of all cases of lung cancer [2]. Unfortunately, most patients with NSCLC are diagnosed in an advanced stage with local or distant metastases [3]. Therefore, identification of biomarkers for predicting metastasis and prognosis is urgently needed for the treatment of NSCLC.

p130cas (p130 Crk-associated substrate) is the product of BCAR1 (breast cancer anti-estrogen resistance 1) gene, which is the premier member of the CAS (Crk-associated substrate) scaffold protein family [4]. p130cas was first described as a heavily tyrosine-phosphorylated component of v-Src and v-Crk transformed cells [5–7]. p130cas itself has no intrinsic kinase activity, but instead functions as a signal assembler [4, 8, 9]. Recent studies have demonstrated that p130cas regulates focal adhesion turnover and plays

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an important role in driving cell migration [10–12]. Although its expression in tissues and cell lines has been investigated in multiple malignancies [13–16], the relationship between p130cas expression and lung cancer patients' clinicopathological parameters, particularly prognosis, remains largely unknown.

E-cadherin and β-catenin, which form an adhesion complex, are specifically involved in epithelial cell-to-cell adhesion. During the development of cancer, the destruction of the E-cadherin/β-catenin complex is one of the changes that characterizes the invasion phenotype [17, 18]. Some recent studies have raised the possibility that p130cas might influence E-cadherin/β-catenin expression. A 2008 clinicopathological study of E-cadherin and p130cas in hepatocellular carcinoma identified a negative correlation between the expression of these two proteins, while another report demonstrated that p130cas negatively regulated E-cadherin/β-catenin membrane localization and promoted E-cadherin degradation in breast cancer cell lines [19]. However, to the best of our knowledge, there has been no published information discussing the correlations between p130cas expression and E-cadherin/β-catenin expression in NSCLC.

The aim of this study was to analyze p130cas expression in NSCLC, and to evaluate whether the presence of p130cas correlates with the expression of E-cadherin/β-catenin and with clinicopathological features.

Material and methods

Material. We selected 105 cases of lung squamous cell carcinoma and lung adenocarcinoma, with corresponding normal lung tissues, collected and diagnosed at the First Affiliated Hospital of China Medical University (Shenyang, China) between October 2004 and July 2006. The samples were from 63 male and 42 female patients with an average age of 60.4 years. According to the World Health Organization (WHO) lung tumor histological classification criteria (2004) [20], the samples were classified as lung squamous cell carcinoma (43 cases) or lung adenocarcinoma (62 cases). Thirty-six cases were highly (G1) or moderately (G2) differentiated, and 69 cases were poorly (G3) differentiated. In addition, there were lymph node metastases in 64 cases, but not in the other 41. Tumor staging was performed according to the seventh edition of TNM for Lung Cancer of the International Union against Cancer (UICC) [21]. There were 46 cases of stage I–II, and 59 cases of stage IIIa–IIIb. Patient survival was counted from the day of the operation to the end of follow-up or to the day the patient died from recurrence or metastasis. All patients had no radiotherapy or chemotherapy before the operation, and were given the standard treatment following the surgery. All samples were fixed in formalin, embedded in paraffin, and stained with hematoxylin and eosin for pathological analysis, to make the diagnosis.

Immunohistochemistry and evaluation of immunostaining. Surgically excised tumor specimens were fixed with 10% neutral formalin and embedded in paraffin, and 4-μm thick continuous sections were prepared. Normal bronchial epithelium present in the tumor slides was used as an internal positive control. Immunostaining was performed by the streptavidin-peroxidase (S-P) method. The sections were incubated with the monoclonal antibody ab31831 (1:250; Abcam, Cambridge, MA, USA) to detect p130cas, the monoclonal antibody sc-8426 (1:150; Santa Cruz Biotechnology, Santa Cruz, CA, USA) to detect E-cadherin, or the monoclonal antibody 610154 (1:200; BD Transduction Laboratories, Lexington, KY, USA) to detect β-catenin, at 4°C overnight. Biotinylated goat anti-mouse serum IgG (1:500; Santa Cruz Biotechnology, Santa Cruz, CA, USA) was used as a secondary antibody. After washing, the sections were incubated with streptavidin–biotin conjugated with horseradish peroxidase (Ultrasensitive, MaiXin, Fuzhou, China), and the peroxidase reaction was developed with 3,3-diaminobenzidine tetrahydrochloride (MaiXin, Fuzhou, China). Counterstaining was done lightly with hematoxylin, and the sections were dehydrated in alcohol before being mounted.

Two investigators, who were unaware of the clinical data, examined all the tumor slides randomly. Five views were examined per slide, and 100 cells were observed per view, at magnification × 400. The cytoplasmic p130cas labeling score was defined by multiplying the percentage of positive cells by the staining intensity. The percentage of positive cells was scored as follows: 0 = 0–25%; 1+ = 26–50%; 2+ = 51–75%; and 3+ = 76–100%.

The staining intensity was scored as follows: 0 = negative; 1 = weak; 2 = moderate; and 3 = strong. When the p130cas score was more than 3 points, the case was defined as positive; when it was equal to or less than 3 points, the case was defined as negative. The E-cadherin and b-catenin scores were determined by the percentage of membranous positive cells per slide, as in our previous study [22, 23]. When more than 90% of the tumor cells were stained for E-cadherin or b-catenin in the cell membrane, the case was defined as normal membranous expression. When fewer than 90% of the tumor cells were stained for membranous expression, the case was defined as loss of expression.

Statistical analysis. All statistical analyses were performed using SPSS for Windows 17.0. Results from immunohistochemistry were analyzed by the Chi-square test and Spearman correlation test. For survival analysis, the Log-Rank test was used. Survival curves were computed according to the Kaplan–Meier method. The Cox regression model was used to test the prognostic value. A p-value of less than 0.05 was considered statistically significant.

Results

Expression pattern of p130cas, E-cadherin and β-catenin

p130cas was overexpressed in the cytoplasm of lung cancer cells. The positive expression rate of p130cas in normal bronchial epithelium (Figure 1A) was lower than in cancer tissue (Figures 1D, G) (9.5% and 61.9%, respectively; $p < 0.001$). The membranous expression of E-cadherin was downregulated in lung cancer samples. The normal expression rate of E-cadherin was higher in normal bronchial epithelium (Figure 1B) than in cancer tissue (Figures 1E, H) (100% and 33.3%, respectively; $p < 0.001$). The membranous expression of β-catenin was decreased significantly in lung cancer samples. The normal membranous expression rate of β-catenin in normal bronchial epithelium (Figure 1C) was higher than in cancer tissue (Figures 1F, I) (86.7% and 17.1%, respectively; $p < 0.001$).

p130cas overexpression correlated with abnormal expression of E-cadherin and β-catenin in non-small cell lung cancer

In 105 NSCLC samples, the correlation between p130cas overexpression and loss of E-cadherin and β-catenin expression are shown in Table 1. The overexpression of p130cas was correlated with abnormal expression of E-cadherin and β-catenin ($p = 0.004$, $r = 0.301$ for E-cadherin and $p = 0.006$, $r = 0.268$ for β-catenin, respectively).

Relationship between p130cas and clinicopathological parameters of non-small cell lung cancer

In 105 NSCLC samples, the overexpression of p130cas correlated positively with lymph node metastasis and high TNM stage ($p = 0.001$, $r = 0.337$ for lymph node metastasis and $p = 0.027$, $r = 0.268$ for TNM stage, respectively). However, there were no statistically

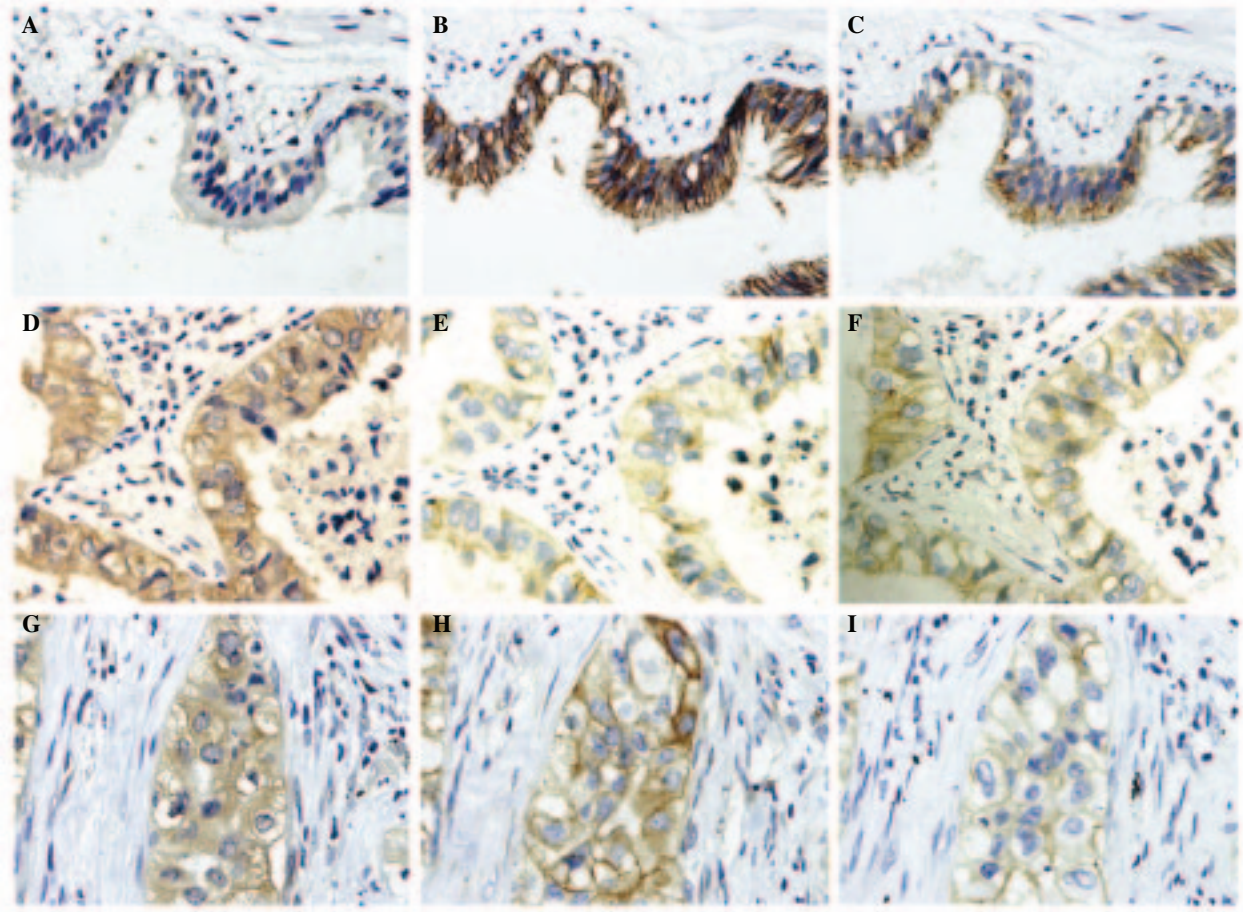

Figure 1. Immunohistochemistry reveals the localization of p130cas, E-cadherin and β-catenin. In normal bronchial epithelium, p130cas was negatively or weakly stained in the cytoplasm (**A**); E-cadherin stained strongly at the cell membrane (**B**); β-catenin stained strongly at the cell membrane (**C**); When p130cas was found moderately or strongly stained in lung adenocarcinoma (**D**) and squamous cell carcinoma (**G**); E-cadherin was also absent or weakly stained at the membrane in lung adenocarcinoma (**E**) and squamous cell carcinoma (**H**); β-catenin was absent or weakly stained at the membrane in lung adenocarcinoma (**F**) and squamous cell carcinoma (**I**). Magnification × 200

significant differences between overexpression of p130cas and sex, age, histology type or differentiation (Table 2). The Log-Rank test revealed the difference in the survival time of patients between overexpression of p130cas and normal expression of p130cas ($p < 0.001$, Figure 2). The mean survival time of patients with p130cas overexpression (36.31 ± 5.66 months) was markedly shorter than that of patients with p130cas normal expression (60.57 ± 6.95 months). Subsequently, all the clinicopathological parameters were entered into the Cox model and tested by univariate survival analysis (the enter method) and multivariate survival analysis (the forward stepwise logistic regression method) (Table 3). The univariate survival analysis indicated that TNM stage, histology type and overexpression of p130cas were significantly associated with poor prognosis in NSCLC patients ($p = 0.005$ for TNM stage, $p = 0.044$ for histology type and $p = 0.001$ for p130cas overexpression, respectively). Based on the multivariate survival analysis, only p130cas overexpression played a significant role in the clinical outcome ($p < 0.001$). p130cas overexpression was thus considered as an independent and effective predictor of prognosis in NSCLC patients.

Discussion

p130cas, introduced by Sakai in 1990 and originally identified as a highly phosphorylated protein in cells transformed by the v-Src and v-Crk oncogenes, is a scaffolding protein at focal adhesion known to be overexpressed in cancer cells and to be associated with solid tumor metastasis [5–7, 9]. In the present study, we demonstrated the p130cas is expressed in the cytoplasm and overexpressed in lung cancer tissue (61.9%, 65/105), which is significantly higher than that in paired adjacent normal lung tissue (9.5%, 10/105; $p < 0.001$).

Table 1. Correlations between p130cas overexpression and abnormal expression of E-cadherin and β-catenin

		p130cas expression		χ^2	r	p
		Normal	**Increased**			
E-cadherin expression	Normal	20	15	8.077	0.301	**0.004**
	Abnormal	20	50			
β-catenin expression	Normal	12	6	7.520	0.268	**0.006**
	Abnormal	28	59			

Table 2. Correlation of p130cas overexpression with clinicopathological parameters in NSCLC

Item	**Number**	**p130cas overexpression**	χ^2	r	p
Gender					
Male	63	42	1.514	–0.120	0.218
Female	42	23			
Age (years)					
< 60	47	30	0.134	–0.36	0.715
≥ 60	58	35			
Histology type					
Squamous cell carcinoma	43	27	0.024	–0.015	0.876
Adenocarcinoma	62	38			
Lymph node metastasis					
Negative	41	17	11.918	0.337	**0.001**
Positive	64	48			
Differentiation					
High	36	19	1.935	–0.136	0.164
Moderate or poor	69	46			
TNM classification					
I–II	46	23	4.920	0.216	**0.027**
IIIa–IIIb	59	42			

The results of this study are consistent with most existing studies showing that p130cas is expressed in the cytoplasm and function at the focal adhesion. Nevertheless, Deng et al. revealed that p130cas was stained in cytoplasm and/or nuclear and positively expressed in 98.8% (79/80) of NSCLC samples and 12.5% (10/80) of normal adjacent lung tissues [14]. The distinction of subcellular distribution and positive rate of p130cas in lung carcinoma between that study and ours might contribute to the difference of primary antibody selection and evaluating standard. Therefore, the precise expression pattern of p130cas in NSCLC is still being investigated.

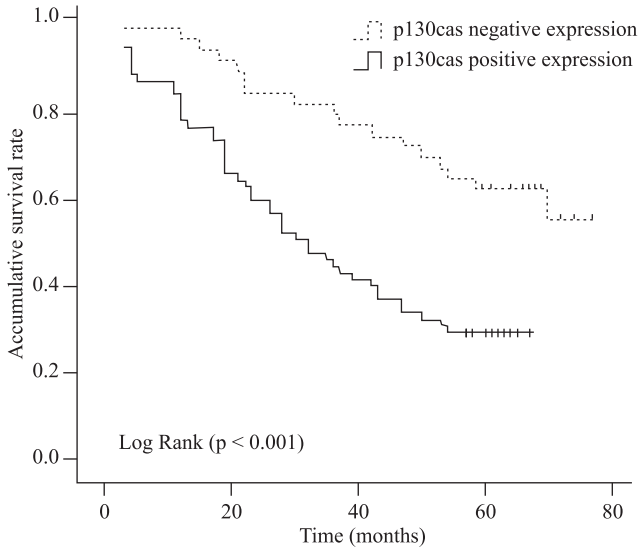

Figure 2. Survival curve of patients with p130cas overexpression. Kaplan–Meier curves demonstrate that overexpression of p130cas was positively correlated with the overall survival of patients with NSCLC ($p < 0.001$)

E-cadherin and β-catenin as a complex are specifically involved in the regulation of epithelial cell-to-cell adhesion and have a close relationship with the invasion ability of cancer. The E-cadherin/β-catenin complex is often destroyed during the process of carcinogenesis [17]. In our study, we showed that overexpression of p130cas was significantly correlated with loss of membranous E-cadherin/β-catenin expression. Previous studies have demonstrated that p130cas was functioned at the focal adhesion, and recent reports have revealed that p130cas was involved in negative regulation of E-cadherin membrane localization in breast cancer cell lines and correlated with loss of E-cadherin/β-catenin expression in hepatocellular carcinoma [4, 15, 19, 24]. These studies and our results suggest that p130cas may play a critical role in cell-to-cell adhesion through its interaction with E-cadherin/β-catenin complex.

Subsequently, we tested the associations between p130cas and clinicopathological parameters. We found that the overexpression of p130cas was correlated positively with lymph node metastasis and high TNM stage in NSCLC samples. More importantly, we also examined the relationship between p130cas protein expression and the prognosis of NSCLC patients. The results revealed that p130cas overexpression significantly correlated with poor survival of NSCLC patients. Cox regression model demonstrated that p130cas overexpression can be an independent factor that impacts lung adenocarcinoma patients' prognosis. The expression of p130cas has been

Table 3.

A. Overall survival in Cox regression analysis in NSCLC

B. Independent predictor of overall survival in Cox regression in NSCLC

Factors	Regression coefficient	Wald chi-square test	p	Risk ratio
A				
Gender	0.197	0.507	0.476	1.218
Age	0.019	0.005	0.943	1.020
Differentiation	-0.459	2.446	0.118	0.632
TNM classification	1.445	7.729	**0.005**	4.240
Histological type	0.564	4.072	**0.044**	1.757
Lymph node metastasis	–0.954	3.307	0.069	0.385
p130cas	1.090	11.231	**0.001**	2.975
E-cadherin	–0.045	0.021	0.885	0.956
β-catenin	0.334	0.545	0.461	1.396
B				
p130cas	1.035	11.988	**0.001**	2.816

extensively studied in breast cancer and has been linked with proliferation, metastasis, poor prognosis and tamoxifen resistance. Similarly, overexpression of p130cas has been observed in hepatocellular carcinoma, prostate carcinoma and ovarian carcinoma, which were correlated with high tumor stage, metastasis and inverse clinical outcome.

However, to the best of our knowledge, there have been no reports discussing the relationship between p130cas expression and clinicopathological parameters in NSCLC. Our findings are consistent with these reports and suggest a positive correlation of overexpression of p130cas with lymph node metastasis and poor prognosis in NSCLC. It should be noted that this study has examined p130cas expression only in a relatively small sample size. Therefore, larger-scale studies are needed to further confirm the function of p130cas in NSCLC.

In conclusion, p130cas is overexpressed in NSCLC samples. The overexpression of p130cas is correlated with the loss of membranous E-cadherin/β-catenin expression. Furthermore, overexpression of p130cas is linked to positive lymph node metastasis and high TNM stage in NSCLC samples. More importantly, p130cas overexpression indicates a poor prognosis in NSCLC patients.

Acknowledgements

This work was supported by grants from the National Natural Science Foundation of China (No. 81000942 to Yuan Miao and No. 30900562 to Yang Liu). All the lung tissue samples were obtained from the First Affiliated Hospital of China Medical University. The study was conducted according to the regulations of the institutional review boards at China Medical University.

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Submitted: 12 January, 2012
Accepted after reviews: 13 May, 2012