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Original Article

Overexpression of PTP4A1 is associated with poor overall survival in non-small cell lung cancer

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Abstract: Objective: We aimed to analyze the phosphatases of regenerating liver 1 (PTP4A1) expression and its relationship with tumor invasion, metastasis, and prognosis of non-small cell lung cancer (NSCLC). Methods: The retrospective study enrolled 150 cases who underwent radical resection for primary NSCLC during the period from January 2006 to January 2014. Baseline characteristics of patients included age, gender, smoking history, pathological type, histological grade, clinical stage, and lymphatic metastasis. Lung cancer tissues collected from 150 cases and precancerous tissues, and normal lung tissues collected from 20 cases were used for PTP4A1 detection by immunohistochemistry. Results: The expression of PTP4A1 was significantly different in different tissue samples (P = 0.025). The expression level of PTP4A1 was associated with clinical stage (P = 0.022), and lymphatic metastasis (P = 0.011). Survival analysis showed the total survival rate of overexpressed PTP4A1 group was significantly lower than that of the low expressed PTP4A1 group (P = 0.006), while the recurrence rate of overexpressed PTP4A1 was significantly higher than that of the low expressed PTP4A1 group (P = 0.014). In addition, the OS was affected by lymphatic metastasis, and disease-free survival was influenced by pathological type and lymphatic metastasis. Conclusion: The PTP4A1 expression level is a potential prognostic biomarker for NSCLC, and overexpression of PTP4A1 suggested a high risk of poor DFS during follow-up. Also, lymphatic metastasis had an adverse effect on survival time.

Keywords: PTP4A1, non-small cell lung cancer, immunohistochemistry, overall survival, disease-free survival

Introduction

Lung cancer is one of the most common causes of cancer death worldwide [1], and non-small cell lung cancer (NSCLC) approximately accounts for 80-85% of all lung cancers [2]. Despite numerous clinical attempts or even recent advances in cancer therapy, the 5-year survival rate is still less than 15% [1, 3]. Metastasis, the main reason for the death, may occurr in more than 50% newly diagnosed patients and they thereby miss the optimal opportunity for surgical resection [4, 5]. Therefore, it is essential to explore a potential prognostic biomarker to prevent cancer invasion and metastasis.

Protein tyrosine phosphatases (PTPs) are widely involved in various physiologic and pathogenic behaviors of human cells by regulating phosphorylation of some crucial signaling molecules [6]. In recent years, PTPs have been shown to have an important effect on cancer cell metastasis [7, 8], and have attracted widespread attention. PTP4A1 (also known as PRL1), a member of the PTP family, also has an oncogenic function in the invasion and metastasis of hepatocellular carcinoma [9]. Achiwa *et al*. showed that the knockout of PTP4A1 gene reduces c-src expression, which leads to a reduction in proliferation and cell adhesion [10]. However, PTP4A1 expression and its clinical/prognostic relevance in NSCLC remain unclear.

In this retrospective study, the expression of PTP4A1 in lung cancer tissues, precancerous tissues, and normal lung tissue was examined by immunohistochemistry. Also, the relationship between PTP4A1 expression and tumor

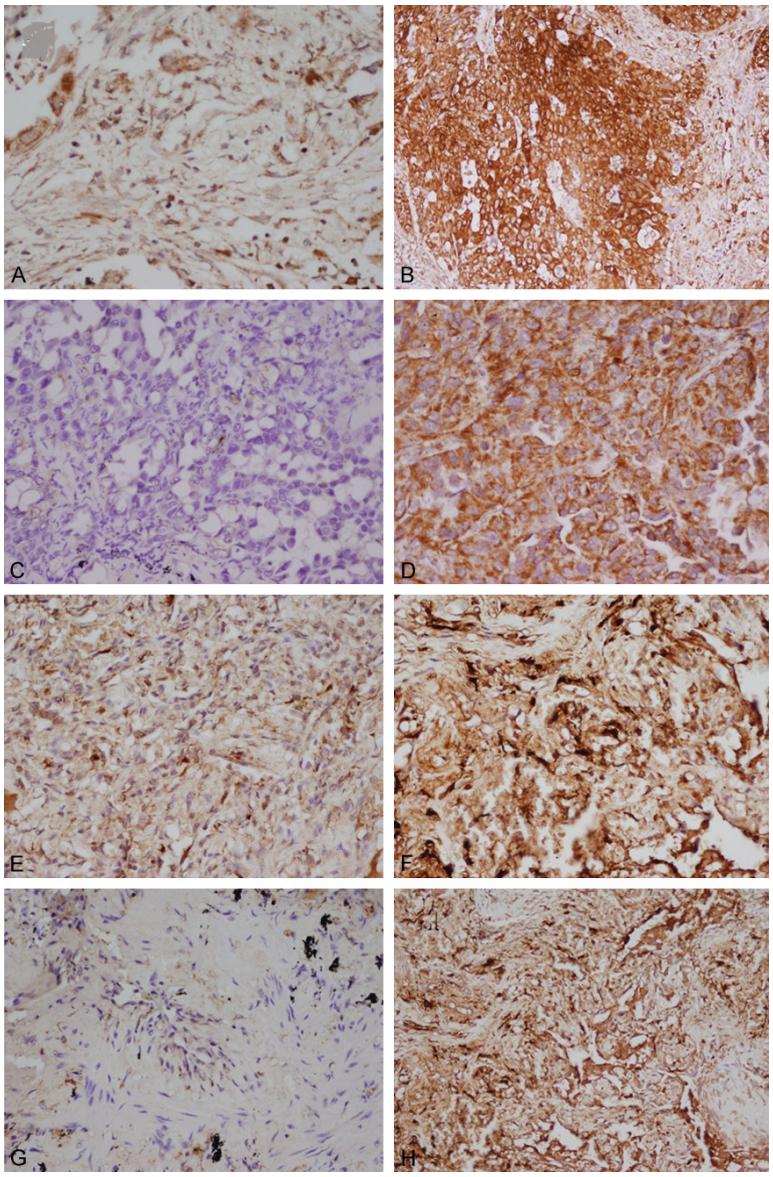

Figure 1. Microphotographs (400 ×) of immunohistochemical detection of PTP4A1 protein: A and B. low and high expression in squamous cell carcinoma tissue; C and D. low and high expressions in adenocarcinoma; E and F. low and high expression in para-carcinoma tissue: G and H. low and high expression in normal tissue.

invasion, metastasis, postoperative disease-free survival (DFS), overall survival (OS), and recurrence rates was also analyzed.

Materials and methods

Patients

The retrospective study enrolled 150 cases (115 male, 35 female, an average age of 57.96 ± 8.45 years old) who underwent radical resection for primary NSCLC during the period from January 2006 to January 2014 in the Fourth Affiliated Hospital of Hebei Medical University. Patients were enrolled if they fulfilled with the following criteria: (1) age ≥ 18 years old; (2) no history of radiotherapy, chemotherapy and sex hormone drugs before operation; (3) a defined diagnosis of NSCLC (adenocarcinoma or squamous cell carcinoma) by two experienced pathologists; (4) no tumor invasion and metastasis to other organs after receiving preoperative iconography examinations, including enhanced Computed Tomography (CT), Emission CT, positron emission tomography and computed tomography (PET/CT); (5) they underwent radical resection of the tumor. Patients with incomplete clinicopathologic data or lost to follow up information were excluded.

Lung cancer tissues were collected from the 150 patients with NSCLC by tumorectomy. Precancerous tissue (2-4 mm away from the tumor margin) and normal lung tissue (> 1 cm away from the tumor margin) specimens were collected randomly from 20 NSCLC patients, respectively. The NSCLC patients were followed up to obtain the information of survival and recurrence after surgery. The recurrence of lung cancer was diagnosed according to the histological, serological, and iconography examinations. The study was approved by an ethics review board of The Fourth Affiliated Hospital of Hebei Medical University, and the requirement to obtain informed written consent was waived.

Based on the criteria of World Health Organization (WHO) [11], the 150 tumor samples were divided into 89 lung adenocarcinoma

Table 1. Expression of PTP4A1 in different tissues of NSCLC patients

Specimens	n	Low expression	High expression	*p*-value
Lung cancer tissue	150	73	77	0.025
Precancerous tissue	20	12	8	
Normal lung tissue	20	16	4	

Lung cancer vs. Normal lung tissue, P = 0.008.

Table 2. Relationship between PTP4A1 expression and clinicopathologic parameters

Parameters	Patients, n	Low expression	High expression	*p*-value
Gender				0.709
Male	115	55	60	
Female	35	18	17	
Age, years				0.605
< 58	69	32	37	
≥ 58	81	41	40	
Smoking history				0.676
Yes	95	45	50	
No	55	28	27	
Pathological type, n				0.819
Squamous carcinoma	61	29	32	
Adenocarcinoma	89	44	45	
Histologic grade[1], n				0.075
G I	7	6	1	
G II	96	48	48	
G III	47	19	28	
Clinical stage[2]				0.022
I+II	120	64	56	
III+IV	30	9	21	
Lymphatic metastasis				0.011
Yes	50	17	33	
No	100	56	44	

[1]G I = well differentiated, G II = moderately differentiated, and G III = poorly differentiated; [2]Clinical stage was based on the 7th edition Tumor-Node-Metastasis (TNM) classification of the International Union Against Cancer (UICC) (IASLC 2009).

samples and 61 lung squamous cell carcinoma samples. The Tumor-Node-Metastasis (TNM) staging of NSCLC was determined based on the 7th edition criterion of TNM classification of the International Union Against Cancer (UICC) (IASLC 2009) [12, 13]. There were 32 cases in IA, 40 cases in IB, 38 cases in IIA, 10 cases in IIB, 29 cases in IIIA, and 1 case in IIIB. Based on the histological grade, tumors were divided into well differentiated (Edmondson grade I, 7 cases), moderately differentiated (Edmondson grade II, 96 cases), and poorly differentiated (Edmondson grade III, 47 cases) groups. There were 50 patients with lymph node metastasis, and 100 without lymph node metastasis.

Immunohistochemistry

PTP4A1 protein levels in the tissues were detected by immunohistochemical analysis. Tumor tissue, precancerous tissue, and normal lung tissue were fixed in 10% formalin, embedded in paraffin, and cut into 4 μm sections. Then the sections were stained with anti-PTP4A1 antibody (Abcam, Cambridge, MA, USA) at 1:200 dilution, followed by horseradish peroxidase-conjugated goat anti-Rabbit IgG (H+L) (Beyotime, Shanghai, China) at 1:400 dilution. Assessment of immunohistochemical staining was carried out on the basis of the previous method of Pang *et al*. [14]. Briefly, positive staining of PTP4A1 was evaluated by a light microscope (BX45-72H05). Staining results were divided into the following category based on percentage of positive cells (stained with brown) in total cells: < 50% as low expression and ≥ 50% as high expression.

Statistical analysis

All analyses were performed using SPSS19.0 software (SPSS Inc, Chicago, IL, USA). The results were presented as mean ± standard deviation (SD). PTP4A1 expression between lung cancer tissues and precancerous tissues as well as normal lung tissue was compared by χ^2. Survival analysis was determined by the Kaplan-Meier method and log-rank test. In order to confirm the prognostic role of PTP4A1, Cox proportional hazards regression analysis was applied to calculate the hazard ratio (HR). $P < 0.05$ was considered statistically significant.

Results

PTP4A1 expression in different tissues

The immunohistochemistry results showed that the expression of PTP4A1 could be detected

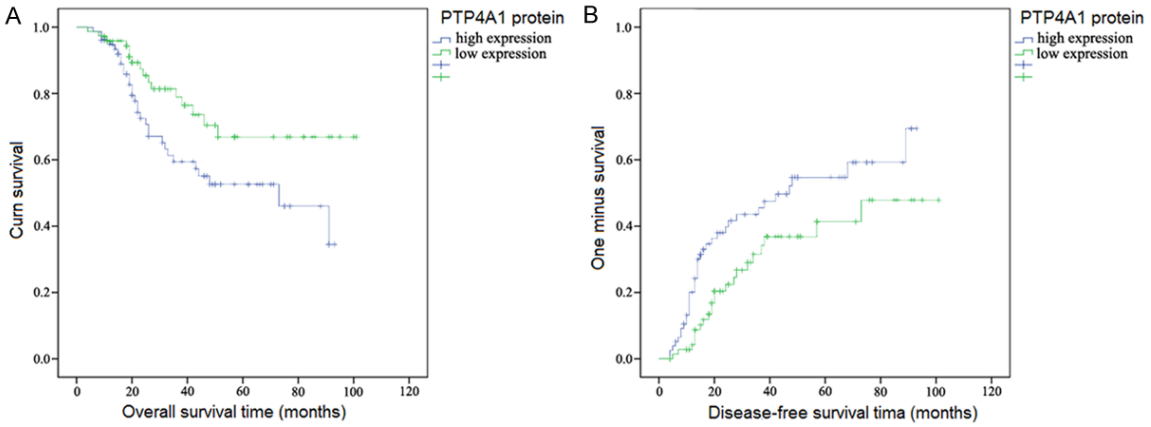

Figure 2. The overall survival (A, log-rank test, P = 0.006) and recurrence rate (B, log-rank test, P = 0.014) among patients with high PTP4A1 expression in tumor tissue.

Table 3. Survival rate and median survival time of patients with different PTP4A1 protein levels in lung cancer tissues

PTP4A1 protein	n	Survival rate (%)			Median survival time (months)
		1-year	3-year	5-year	
High expression	77	93.5	44.7	29.9	34.0
Low expression	73	97.3	66.5	43.4	51.0

in the nuclei of different tissues (**Figure 1**). In particular, PTP4A1 expression was significantly higher in the tumor tissues compared with the normal tissues (P = 0.008, **Table 1**), while no significant difference was observed between the tumor tissues and precancerous tissues (P = 0.341, data not shown).

PTP4A1 expression level and characteristics of lung cancer patients

The NSCLC patients in this study were further grouped according to the clinicopathologic characteristics, including age, gender, smoking history, pathology type, histological grade, clinical stage, and lymphatic metastasis. The results showed that PTP4A1 was overexpressed in NSCLC patients with lymphatic metastasis compared with those NSCLC patients without lymphatic metastasis (P = 0. 011, **Table 2**). Moreover, patients in the late stage present higher expression level of PTP4A1 compared with those in the early stage (P = 0. 022). In addition, no significant correlation was observed between PTP4A1 expression and other clinical characteristics, such as age, smoking history, and histological grade (all P > 0.05).

Prognostic role of PTP4A1 expression level on OS of NSCLC patients

The OS rate of patients with high PTP4A1 expression was lower than that of the patients with low PTP4A1 expression (**Figure 2A**, P = 0. 006). The median survival times of patients with high PTP4A1 expression and low PTP4A1 expression were 34 months and 51 months, respectively (**Table 3**). Patients with high PTP4A1 expression had a higher recurrence rate of NSCLC than those with low PTP4A1 expression (**Figure 2B**, P = 0. 014).

Moreover, the results showed that the OS rate of patients with squamous cell lung cancer was lower than that of patients with squamous cell carcinoma (**Figure 3A**, P = 0.035), and patients with adenocarcinoma lung cancer exhibited a higher recurrence rate compared with patients with squamous cell carcinoma (**Figure 3B**, P = 0.020). The lung cancer patients with poorly-differentiated tumors had a lower survival rate (**Figure 3C**, P = 0.031) and a higher recurrence rate (**Figure 3D**, P = 0.017) compared with patients with well-differentiated tumors. Additionally, patients with lymph node metastasis or late stage cancer also exhibited a low survival rate and high recurrence rate (all P < 0.001, **Tables 4** and **5**).

Multivariate survival analysis showed that PTP4A1 expression (HR = 1.660, P = 0.043, **Table 4**) and lymphatic metastasis (HR = 4.308, P < 0.001) were independent prognostic factors for OS time, suggesting that the NSCLC

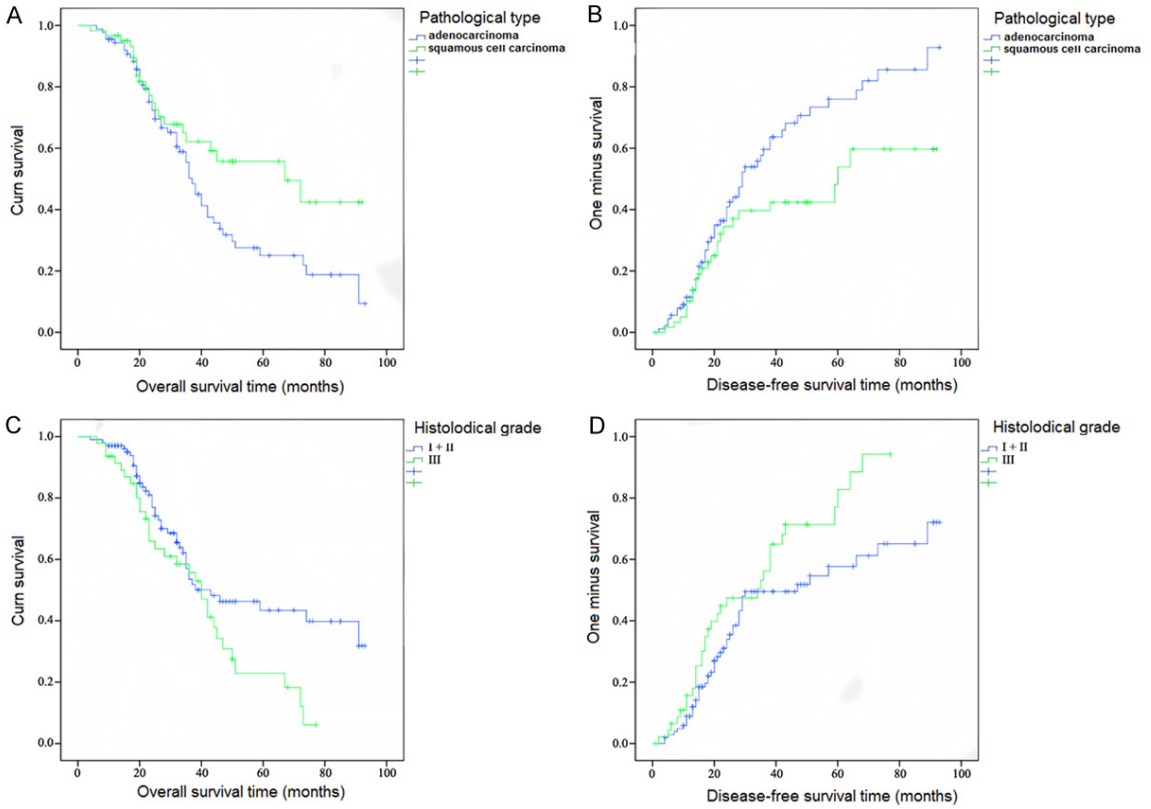

Figure 3. The overall survival (A, C) and recurrence rate (B, D) among patients with adenocarcinoma and squamous-cell carcinoma (A, log-rank test, P = 0.035; B, log-rank test, P = 0.020) and patients with well-differentiated and poorly-differentiated tumor (C, log-rank test, P = 0.031; D, log-rank test, P = 0.017).

Table 4. Analysis of the correlation between overall survival and clinicopathologic factors

Clinicopathologic characteristics	Univariate analysis	Multivariate analysis		
	p-value	Hazard ratio (HR)	95% CI	*p*-value
Age (< 58/≥ 58)	0.490			-
Gender (male/female)	0.180			-
Smoking history (Yes/No)	0.766			-
Pathological type (adenocarcinoma/squamous cell carcinoma)	0.035			
Histological grade (III/I+II)	0.031			
Lymphatic metastasis (Yes/No)	0.000	4.308	2.412-7.695	0.000
Clinical stage (III+IV/I+II)	0.000			
PTP4A1 expression in cancer tissue (high/low)	0.006	1.660	1.016-2.712	0.043

P < 0.05 was considered statistically significant.

patients with overexpressed PTP4A1 or who developed lymphatic metastasis were more likely to have poor OS following surgical therapy. Moreover, Cox proportional hazards regression analysis also showed that pathological type (P = 0.042, **Table 5**) and lymphatic metastasis (P < 0.001, **Table 5**) were independent prognostic factors for postoperative DFS.

Discussion

In spite of numerous studies demonstrating that PTP4A1 expression affects tumor progression, metastasis, and patient survival rate of lung cancer [10, 15, 16], the clinical or prognostic role of the PTP4A1 protein in NSCLC has rarely been reported. In the present study, we

Table 5. Analysis of the correlation between disease free survival (DFS) and clinicopathological factors

Clinicopathologic characteristic	Univariate analysis	Multivariate analysis		
	p-value	Hazard ratio (HR)	95% CI	*p*-value
Age (< 58/≥ 58)	0.419			-
Gender (male/female)	0.389			-
Smoking history (Yes/No)	0.927			-
Pathological type (adenocarcinoma/squamous cell carcinomas)	0.020	1.669	1.018-2.736	0.042
Histological grade (III/I+II)	0.017			
Lymphatic metastasis (Yes/No)	0.000	4.341	2.448-7.698	0.000
Clinical stage (III+IV/I+II)	0.000			
PTP4A1 expression in cancer tissue (high/low)	0.014			

P < 0.05 was considered statistically significant.

conducted a retrospective analysis of PTP4A1 expression in 150 lung cancer tissues, 20 precancerous tissues, and normal lung tissues. Consequently, it was confirmed that the overexpression of PTP4A1 was associated with high risks of poor OS.

Nakashima *et al*. found that *PTP4A1* mRNA levels were overexpressed in human lung cancer cells [17]. *In vitro*, the overexpression of PTP4A1 protein promotes migration and invasion of A549 lung cancer cell lines, while the migration and invasion of A549 lung cancer cell line decreased after knocking down PTPTA1 expression [17]. *In vivo*, it was also found that the PTP4A1 protein level was significantly higher in tumor tissues of NSCLC compared with that in precancerous and normal tissues, suggesting a possible carcinogenic effect of overexpressed PTP4A1 on NSCLC. However, these results are converse to a previous study which revealed that PRL-2 expression, rather than PRL-1 and PRL-3 expression, were increased in cancer tissues [18]. Racial differences or different research conditions were likely causes of the differences in these results.

Jin and his colleagues revealed that overexpressed PRL-1 level was significantly correlated with a more aggressive tumor phenotype measured by TNM stage [9]. Consistent with that study, our results showed that NSCLC patients in the late stage had a higher expression level of PTP4A1 compared with those in the early stage. Furthermore, we also found that NSCLC patients with lymphatic metastasis exhibited a higher PTP4A1 expression level compared with those without lymphatic metastasis. Therefore, the preoperative PTP4A1 expression level might be affected by clinical stage and lymphatic metastasis. In addition, our results showed that patients with overexpressed PTP4A1 presented a lower survival rate and a higher recurrence rate compared to those with low PTP4A1 expression. These results were further verified by the Cox proportional hazards regression analysis which indicated that PTP4A1 expression was an independent prognostic factor for OS. Notably, the phenomenon might be explained by a positive correlation between PTP4A1 expression and lymphatic metastasis. Based on the above results, we speculated that the determination of preoperative PTP4A1 expression level may be important for predicting the therapeutic effect and postoperative OS for patients with NSCLC.

In the current study, Cox analysis also suggested that patients with squamous cell carcinoma were likely to live longer with low recurrence or metastasis. Generally, lung squamous cell carcinoma mainly occurs in the central part of the lung, and the early appearance of the symptoms facilitates an early detection and treatment; whereas lung adenocarcinoma mostly occurs in the periphery and its symptoms often appear after the occurrence of distant metastasis. On the other hand, squamous cell carcinoma is more inclined to recur locally, while adenocarcinoma is prone to distant metastasis.

As recurrence and metastasis are two related hallmarks of cancer [19], it is easy to presume that patients with lymphatic metastasis would have low survival rates and high recurrence rates. Both recurrence and metastasis are major causes of hepatocellular carcinoma-related mortality [9]. Interestingly, no correlation between OS (or DFS) and smoking history

was found in this study. Here, patients with a history of smoking were often males, and the patients were not distributed symmetrically with respect togender (115 male vs. 35 female). These findings elucidate this phenomenon, while more experiments will be essential to validate our results.

In conclusion, PTP4A1 expression level might be a potential prognostic biomarker for NSCLC, and preoperative PTP4A1 overexpression in tumor tissue indicated a poor prognosis. Lymphatic metastasis had an adverseeffect on survival time.

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Disclosure of conflict of interest

None.

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