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BPTF biomarker correlates with poor survival in human NSCLC

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Abstract. – OBJECTIVE: The aim of the present study was to identify the clinical significance of BPTF expression in the development and progression of NSCLC.

PATIENTS AND METHODS: The expression of BPTF in 189 pairs of NSCLC and adjacent normal lung tissues were detected by Real-time PCR. The expression of BPTF was investigated in NSCLC and normal control tissues by immunohistochemical staining and immunofluorescence staining. Then, we analyzed the potential relationship between BPTF levels in tumor tissues and existing clinicopathological features of NSCLC, and clinical outcome.

RESULTS: It was found that BPTF mRNA was significantly overexpressed in NSCLC tissues in comparison with paired normal control tissues ($p < 0.01$). Consistent with BPTF mRNA expression, up-expression of BPTF protein was also found in NSCLC tissues. Furthermore, BPTF expression was positively correlated with advanced lymph node metastasis ($p = 0.002$), clinical stage ($p = 0.004$), and differentiation ($p = 0.014$). Moreover, patients with high BPTF expression had significantly poorer survival by Kaplan-Meier method ($p < 0.001$). Finally, Cox regression analyses showed that high BPTF expression might be an independent prognostic parameter to predict poor prognosis ($p < 0.05$).

CONCLUSIONS: BPTF is important in predicting patient outcomes and is a potential target for the development of therapeutic approaches to NSCLC.

Key Words:
BPTF, Prognosis, NSCLC.

Introduction

Lung cancer is a leading cause of cancer-related deaths claiming lives of over 25% of cancer patients (26% in women and 28% in men)[1]. Non-small cell lung cancer (NSCLC) accounts for 80-85% of total lung cancers[2]. Despite the recent advances in diagnostic and therapeutic procedures, the 5-year survival of patients with stage I NSCLC is 54.8% while the 5-year survival of patients with stage IV drops to 4.2%[3,4]. Thus, there exists a high urgent medical need to develop novel molecular markers for accurately predicting prognosis for NSCLC patients and guiding treatment Bromodomain PHD finger transcription factor (BPTF), a ubiquitously expressed ATP-dependent chromatin-remodeling factor, is critical for epigenetically regulating DNA accessibility and gene expression[5]. BPTF was found to be overexpressed in several human cancers, including lung adenocarcinomas[6], hepatocellular carcinoma[7], and melanoma[8]. However, its effects in human NSCLC have not been fully elucidated. In the present study, we examined the expression level of BPTF mRNA in NSCLC tissue samples using qRT-PCR. Next, we used immunohistochemical staining and immunofluorescence staining to determine the expression of BPTF. Moreover, the relationship between BPTF protein and the clinicopathological features of gastric cancer and its prognostic value were also evaluated.

Patients and Methods

Patients and Tissue Samples

One hundred and eighty-nine patients who were diagnosed with NSCLC and underwent surgery at Linyi Central Hospital were recruited between July 2008 and January 2011. Patients were diagnosed with NSCLC based on the histopathological evaluation. None of the patients had received chemotherapy, radiotherapy or immunotherapy before the surgery. Demographical and clinical features of the patients were obtained from hospital charts. Detailed data including gender, age, smoking status, tumor size, lymph node metastasis, clinical stage, histological type and differentiation. Written informed consent

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was obtained from all of the patients. The present study was approved by the Ethical Committee of the Second Affiliated Hospital of Linyi Central Hospital, China.

Reverse Transcription and Real-time PCR

Total RNA was isolated using TrIzol (Invitrogen, Carlsbad, CA, USA). For analysis of BPTF mRNA expression, qRT-PCR was performed using Hairpin-it miRNAs qPCR Quantitation Kit (catalog number QPM-010, GenePharma, Shanghai, China) according to the manufacturer's instructions. The Real-time-PCR cycling conditions were as follows: 95˚C for 10 min, followed by 40 cycles at 95˚C for 15 sec and 60˚C for 45 sec and a final extension step of 95˚C for 15 sec, 60˚C for 1 min, 95˚C for 15 sec and 60˚C for 15 sec. The primers used for the expression analysis were as follows: BPTF mRNA, forward 5′-AATCGGAGAAGTCCAACGGG-3′; reverse 5′-TTGCCCTATGTGATGCCCAG-3′; GAPDH, forward, 5′-CTCATGACCACAGTCCATGC-3′; reverse, 5′-TTACTCCTTGGAGGCCATGT-3′. The mRNA expression of BPTF was normalized to GAPDH. Relative gene expression was calculated by the $2^{-\Delta\Delta Ct}$ method.

Immunohistochemistry

Immunohistochemical staining was performed as previously described[9]. Briefly, NSCLC tissue sections were deparaffinized in xylene and rehydrated by sequential incubation in ethanol/water solutions. Antigen retrieval was performed by heating the tissue in boiling EDTA buffer, pH 8.0, for 18 min. Endogenous peroxidase activity was blocked by 15 min of treatment with 3% hydrogen peroxide. Then the sections were blocked with 10% goat serum at room temperature for 15 min and then incubated with primary antibodies at 4°C overnight. The primary antibodies were BPTF (1:500) obtained from Abcam (Hyclone, Logan, UT, USA). After tri-wash with PBS, the sections were incubated with the peroxidase-conjugated Goat Anti-rabbit IgG (1:125, DGCS-BIO, Beijing, China) for 30 min at room temperature. Staining was accomplished using DAB reagent sets (ZSGB-BIO, Haiding, Beijing, China) for 2-5 min, and then counterstained with hematoxylin before coverslipping.

Immunofluorescence

Immunofluorescence staining was performed as previously described[10]. Attachment adhered to NSCLC was collected under dissecting microscope and fixed in 10% formaldehyde for 24 h, routine paraffin-embedded, and sectioned (5 μm). After dewaxing, the sections were incubated in FITC-ConA (Sigma, St. Louis, MO, USA) in the dark at room temperature for 30 min, followed by DAPI (Sigma, St. Louis, MO, USA) incubation in the dark at room temperature for 30 min. The images were taken using a confocal laser scanning microscope (BD Biosciences, San Jose, CA, USA).

Statistics Analysis

SPSS 20.0 software (IBM Corporation, Armonk, NY, USA) were used for data analysis. The χ^2-test was also used to analyze the association between BPTF protein expression and clinicopathological parameters. Kaplan-Meier analysis was used to evaluate survival curves, and the log-rank test was conducted to verify them. A Cox proportional hazards model was used for univariate and multivariate analysis. $p < 0.05$ was considered to be statistically significant in the patients with NSCLC.

Results

BPTF Expression in Human NSCLC Tissues

We first analyzed BPTF mRNA expression in NSCLC specimens and corresponding normal lung tissues by RT-PCR. As shown in Figure 1, the relative level of BPTF mRNA expression in NSCLC tissues was significantly higher than that in corresponding noncancerous tissues ($p <$

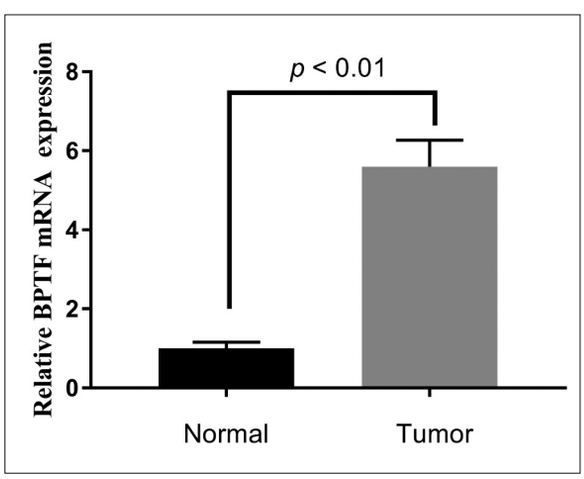

Figure 1. BPTF mRNA expression levels were analyzed by quantitative RT-PCR in 189 paired NSCLC with their corresponding non-cancerous tissues.

0.01). Next, we performed immunohistochemical staining and immunofluorescence to determine the expression of BPTF protein. In agreement with RNA expression results, BPTF protein expression is higher in NSCLC tissues than in normal tissues (Figure 2A-D).

Correlation Between BPTF Expression and Clinical Characteristics in NSCLC

We further determine if there was any correlation between BPTF expression and clinicopathological variables in NSCLC. The 189 patients were divided into two groups by the median value of relative BPTF expression levels. The associations of BPTF expression with clinicopathological features are summarized in Table I. The results showed that expression of BPTF was significantly associated with differentiation, clinical stage, and lymph node metastasis ($p < 0.05$). However, there was no significant correlation of BPTF expression with other clinical features ($p > 0.05$). Collectively, these results supported the notion that BPTF up-regulation might be associated with tumor progression and development.

The Association Between BPTF Expression and Overall Survival of Patients with NSCLC

Kaplan-Meier analysis was applied to examine the prognostic value of BPTF expression to the

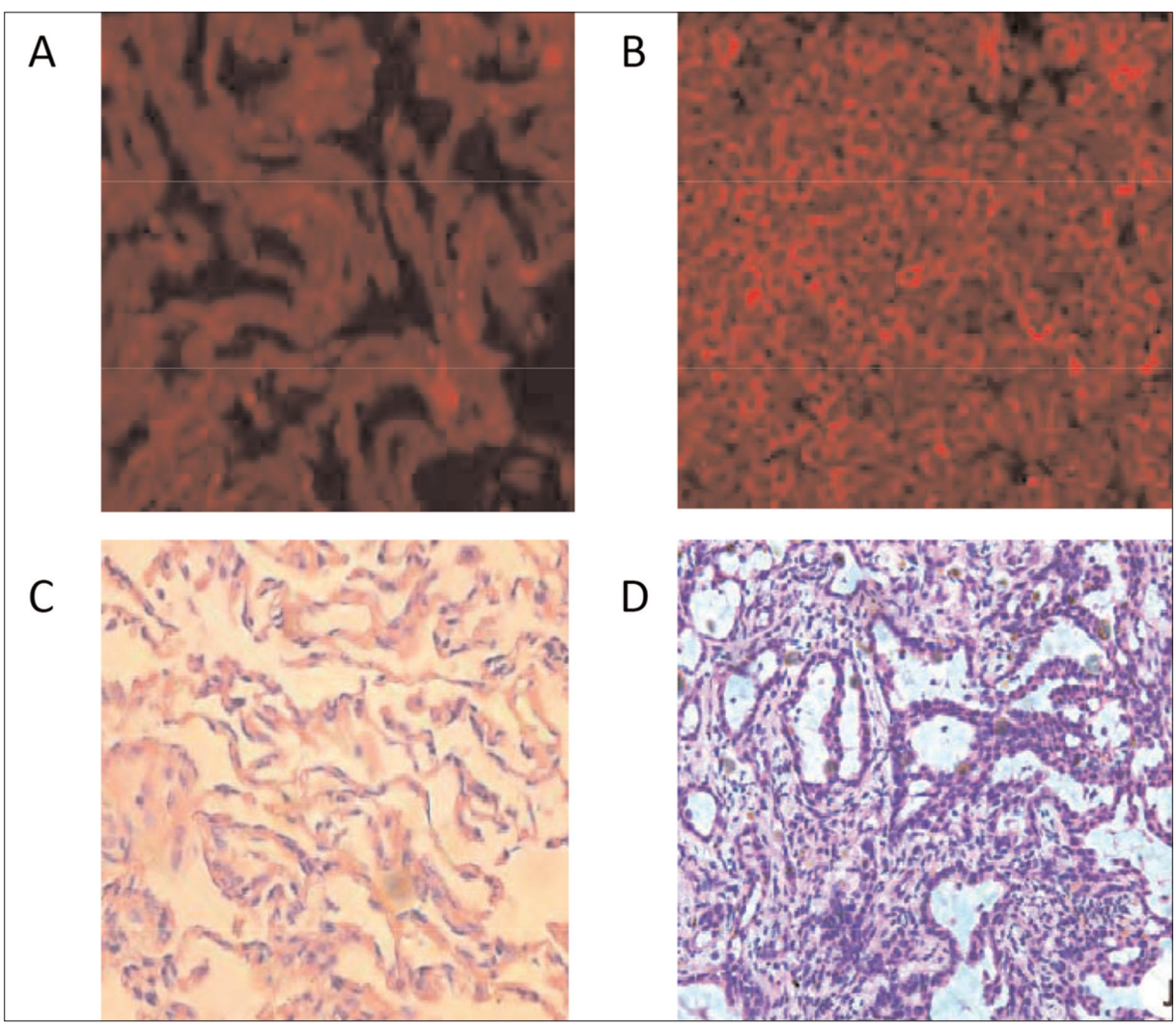

Figure 2. Expression of BPTF in normal lung and NSCLC tissues based on immunohistochemistry and immunofluorescence staining. ***A,*** Low expression of BPTF in normal lung tissues. ***B,*** High expression of BPTF in NSCLC. ***C,*** Moderate expression of BPTF in normal lung tissues. ***D,*** Strong expression of BPTF in NSCLC.

Table I. Correlation of BPTF expression with clinicopathological characteristics in NSCLC.

Variables	No. of cases (n = 189)	BPTF expression		p-value
		High	Low	
Age (years)				0.814
≤ 65	75	34	41	
> 65	104	49	55	
Gender				0.178
Male	126	51	75	
Female	63	32	31	
Smoking				0.734
Yes	95	41	55	
No	93	42	51	
Tumor size (cm)				0.370
≤ 3	64	31	33	
> 3	125	52	73	
Lymph node metastasis				0.002
Yes	90	50	40	
No	99	33	66	
Clinical stage				0.004
I/II	93	34	59	
III/IV	96	49	47	
Histological type				0.098
Squamous cell carcinoma	69	38	31	
Adenocarcinoma	120	45	75	
Differentiation				0.014
Well/Moderate	101	36	65	
Poor	88	47	41	

overall survival of patients with NSCLC. The results showed that NSCLC patients in the BPTF-high group have significantly shorter overall survival than those in the BPTF-low group (Figure 3). Furthermore, Multivariate analysis using the Cox proportional hazard model for all variables that were significant in the univariate analysis confirmed that lymph node metastasis, clinical stage, differentiation and BPTF expression ($p < 0.05$) were independent prognostic factors for patients with NSCLC (Table II). These findings supported the hypothesis that increased BPTF expression play a key role in NSCLC development and progression.

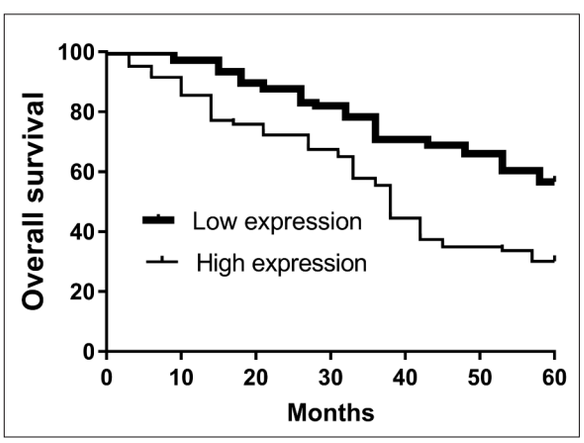

Figure 3. NSCLC patients with high BPTF expression had significantly shorter overall survival than those with low BPTF expression did ($p < 0.001$).

Discussion

The development of NSCLC is a multistep process involving multiple factors[11,12]. Despite many molecular markers of early diagnosis such as special genes, lncRNAs and miRNAs had been confirmed for NSCLC detection in previous studies, the low sensitivity and specificity still made the diagnosis of NSCLC inaccurate and not in time[13,14]. Thus, identifying new diagnostic and prognostic markers may enable earlier detection and better treatment of NSCLC.

The human BPTF gene is located on chromosome 17q24, presumed to contain oncogenic elements given the demonstration of chromosomal gains in this locus in various tumors[15,16]. In previous studies, researchers found that BPTF may

Table II. Univariate and multivariate analyses of different prognostic parameters on NSCLC survival.

Variables	Univariate analyses			Multivariate analyses		
	HR	95% CI	p	HR	95% CI	p
Age	1.82	0.73-3.56	0.29	1.34	0.93-3.11	0.43
Gender	2.15	1.17-3.92	0.37	1.75	0.87-3.22	0.35
Smoking	0.83	0.43-1.06	0.14	0.95	0.53-1.22	0.18
Tumor size	1.56	0.83-2.85	0.22	1.77	0.91-2.74	0.29
Lymph node metastasis	3.65	1.71-6.68	0.008	3.99	1.56-5.53	0.004
Clinical stage	1.83	0.92-4.45	0.013	2.23	1.33-4.03	0.008
Histological type	2.66	1.23-4.33	0.21	2.95	1.48-3.36	0.27
Differentiation	3.67	1.56-7.74	0.003	3.14	1.23-6.68	0.006
BPTF	6.63	2.15-9.55	0.001	5.57	2.43-8.81	0.001

regulate transcription via histone acetylation and could be involved in cancer progression[17,18]. Xiao et al[7] found that BPTF is remarkably increased in hepatocellular carcinoma and up-regulate expression of BPTF is correlated with poor prognosis in hepatocellular carcinoma patients. Dar et al[8] showed that knockdown of BPTF significantly inhibited the proliferation, metastasis of melanoma cell line. More importantly, Dai et al[6] indicated that BPTF was specifically overexpressed in NSCLC cell lines and lung adenocarcinoma tissues. In addition, they also showed that knockdown BPTF significantly inhibited cell proliferation, induced cell apoptosis and arrested cell cycle progress from G1 to S phase by regulating cyclin D, phospho-Rb, and phospho-cdc2. Those results revealed that BPTF might function as an oncogene in several tumors. On the basis of the previous reports, we hypothesized that BPTF could serve as a novel and reliable prognostic biomarker for NSCLC patients.

In the present study, we confirmed that the expression BPTF is upregulated in NSCLC tissues in comparison with that in normal lung tissues. Our results agree with the previous study[6]. By statistical analysis, the expression of BPTF was observed to be significantly correlated with poor differentiation, advanced clinical stage and higher incidence of lymph node metastasis. Furthermore, survival analyses demonstrated that patients with high BPTF expression had a low overall survival rate. Finally, multivariate analyses were utilized to evaluate whether the BPTF expression level and various clinicopathological features were independent prognostic parameters of patient outcomes. The results suggested that the expression level of BPTF might become an independent predictive factor for the prognosis of NSCLC.

Conclusions

These findings provide novel insights into developing potential alluring targets for prognostic and therapeutic interventions in NSCLC. However, the molecular mechanisms of BPTF involved in NSCLC need to be further studied.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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