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Prognostic significance of *BP1* mRNA expression level in patients with non-small cell lung cancer

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Abstract

Objectives: To examine the association of *BP1* mRNA level with tumor characteristics and clinical prognosis in non-small cell lung cancer (NSCLC) patients.

Design and methods: Tumor specimens from 98 NSCLC patients who underwent surgical resection were quantitatively determined for *BP1* mRNA expression by real-time RT-PCR.

Results: *BP1* mRNA was expressed at significantly higher levels in tumors than in adjacent nontumorous tissues and normal lung samples. The level of *BP1* transcript was significantly associated with tumor histological type and cell differentiation grade, but not related with other clinicopathological factors and *p53* mutations. Patients with high *BP1* mRNA expression had a poorer prognosis in terms of both disease-free survival (DFS) and overall survival (OS) rates. Additionally, *BP1* mRNA expression level was an independent prognostic factor for DFS.

Conclusions: BP1 may be part of a pathway contributing to NSCLC development and/or progression. *BP1* mRNA level could be a novel prognostic marker for NSCLC.

Keywords: BP1; Homeobox; NSCLC; Prognosis; Real-time RT-PCR

Introduction

Lung cancer is the most frequent cause of tumor-related deaths throughout the world [1]. In developed countries and some developing countries, the 5-year survival rates for this fatal disease are less than 15% and 5%, respectively [2]. Despite the great improvements made in early detection, surgical resection, and systemic therapeutic strategies over the past decades, the prognosis of patients diagnosed with lung cancer, especially for those with advanced disease, remains unfavorable. For instance, approximately 50% of early-stage lung tumor patients undergo local recurrences or develop distant metastases within 5 years after surgical treatment [3]. Therefore, identification of novel molecular events underlying the development of this malignancy and its poor prognosis is still crucial for providing potential targets of clinical intervention.

Homeobox genes encode a group of transcription factors governing pattern formation and cell fate commitment during embryonic development. Accumulating evidence shows that many homeobox genes are aberrantly expressed in a variety of tumors including lung cancer [4,5]. *Lung cancer-associated gene Y* (*LAGY*), a novel homeobox gene mapped on chromosome 4q11-13.1, is normally present in human lung tissues. Recently, Chen et al. [6] found that *LAGY* mRNA expression was widely lost in 18 lung tumor cell lines comprising all major histological types. Furthermore, down-regulation of *LAGY* expression was also identified in primary lung tumor tissues and associated with tumor grade and clinical stages [6,7], suggesting this gene might be involved in lung cancer development. The level of homeobox gene expression also alters the biological

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features of lung tumor cells. Overexpression of *HOXD3* in human lung cancer A549 cells could enhance cell motility, invasion, and metastasis *in vitro* by increasing the transcription of integrin αv*β3* and simultaneously depriving E-cadherin [8]. It is gradually accepted that *HOXD3* may function as a key motility- or metastasis-promoting molecule in lung cancer [9].

Beta Protein 1 (*BP1*) belongs to the superfamily of *distal-less* (*Dlx*) homeobox genes [10]. In normal erythroid lineage, BP1 acts as a repressor of the *β*-globin gene via two upstream silencers [11,12]. Previous studies revealed ectopic BP1 overexpression in the bone marrow of 63% of acute myeloid leukemia patients [13], 81% of invasive ductal breast tumors [14,15], as well as 78% of primary lung tumors [16]. Although no precise mechanism(s) has been defined, it was recently documented that BP1 impacts anti-apoptotic pathways in breast cancer MCF-7 cells: (1) up-regulated expression of BP1 could inhibit the TNF-α-induced cell death by reducing the processing and activation of caspase-7, caspase-8, and caspase-9; (2) BP1 protein binds to and directly activates the expression of *bcl-2*, a negative regulator of apoptosis, resulting in an increased tumor cell survival [17]. In addition, elevated levels of *BP1* in tumors also have potential implications for strategies of clinical interventions. In acute promyelocytic leukemia (APL) NB4 cells, *BP1* overexpression can reduce their sensitivity to the all-*trans* retinoic acid (ATRA) treatment [18], indicating that BP1 is a very promising anti-cancer therapeutic target in APL. Our recent data also suggested that high expression level of *BP1* mRNA is predicative for the poor prognosis of invasive breast cancer patients [19].

Until now, the level of *BP1* expression and its potential value in lung cancer has been poorly described. To ascertain whether BP1 plays an important role in the initiation and/or progression of lung tumor, in the present study, we detected the quantitative expression of *BP1* mRNA in 98 cases of non-small cell lung cancer (NSCLC) specimens by using real-time RT-PCR. The copy number of *BP1* mRNA was subsequently analyzed for the associations with various clinicopathological factors, *p53* mutations, and patient prognosis. To our knowledge, this is the first report addressing the levels of *BP1* transcript in NSCLC and its links with the patient outcomes.

Materials and methods

Patients and tissue samples

A total of 98 NSCLC patients (77 males and 21 females) who had undergone thoracic surgery at the Department of Surgical Oncology, Central Hospital of Chinese Petroleum Corporation between April 1996 and December 2004 were enrolled in this study. The median age of the patients were 62, with the range from 37 to 79. Totally, 128 tissue samples from 98 patients were used: 98 tumor specimens and a corresponding subset of 30 adjacent nontumorous tissues (5 cm way from the tumor during lobectomy and 5–7 cm in pneumonectomy). Two normal lung samples from noncancerous healthy subjects were obtained from the Tissue Bank at the Department of Forensic Medicine, Tianjin Gong'an Hospital. Tissues were immediately snap-frozen in liquid nitrogen after resection and stored at −80 °C until further use. All tumors were histopathologically confirmed to contain at least 70% malignant cells and none of the participants received preoperative treatment. The tumor type and the grade of cell differentiation were designated based on the criteria of World Health Organization (WHO), whereas the pathological stage of each tumor was determined by the International Union Against Cancer (UICC) TNM classification. Details of clinical and pathological characteristics of the patients are summarized in Table 1.

All patients were under a close follow-up observation for disease recurrence at 3-month intervals during the first 2 postoperative years, and every 6 months thereafter. Clinical evaluation consisted of physical examination, biochemical tests, standard chest radiography, CT of the chest, abdomen, and brain, bronchoscopy, abdominal ultrasonography, and bone scanning. The median follow-up duration was 58 months (range 47–68). Informed consent was obtained from each patient before surgery and this study was approved by the Review Board of Hospital Ethics Committee.

RNA isolation and cDNA synthesis

Briefly, total RNA samples were isolated from surgically removed NSCLC tumors, adjacent nonneoplastic tissues, and normal lung samples using an E.Z.N.A total RNA kit (Omega Bio-tek, Doraville, GA, USA) according to the manufacturer's instructions and quantified at A260/280 nm on a spectrophotometer. Subsequently, 3 μg of total RNA were reverse transcribed

Table 1
Associations between *BP1* mRNA levels and various clinicopathological factors in 98 NSCLC patients

Parameters	Group	Case No.	*BP1* mRNA level	*P*-value
Age (years)	<60	40	448±210	0.472
	≥60	58	526±178	
Gender	Male	77	457±204	0.556
	Female	21	515±180	
Smoking history	Smoker	74	499±191	0.801
	Nonsmoker	24	473±185	
Histological type	Ad	47	401±202	0.035
	Sq	43	558±146	
	La and others	8	482±193	
Differentiation grade	Well	23	385±197	0.029
	Moderate	33	476±208	
	Poor	42	569±151	
Pathological stage	I	61	518±192	0.427
	II	26	450±204	
	III	11	472±180	
pT classification	T1	29	504±175	0.603
	T2 and T3	69	456±226	
pN classification	Positive	35	512±184	0.504
	Negative	63	443±201	
p53 mutation	Positive	37	468±197	0.620
	Negative	61	515±173	

The *BP1* mRNA levels are shown as the mean±standard deviation. The Mann–Whitney *U*-test and the Kruskal–Wallis test (for histological type, differentiation grade, and pathological stage) were applied to compare the *BP1* mRNA levels between/among various clinicopathological groups. Ad: adenocarcinoma; BP1: Beta Protein 1; La: large-cell carcinoma; NSCLC: non-small cell lung cancer; pN classification: lymph node metastasis; pT classification: tumor size; Sq: squamous cell carcinoma.