Expression of Bmi-1 in NSCLC and Its Relationship to Clinicopathologic Characters

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ABSTRACT Objective: To investigate the relationship between the expression of Bmi-1 in nonsmall-cell lung cancer (NSCLC) and clinicopathologic characters. Methods: Reverse transcription-PCR RT-PCR was applied to detect Bmi-1 mRNA in 30 of NSCLC specimen and 20 of tumor-adjacent tissue. Immunohistochemistry was performed to detect the expression of Bmi-1 in 52 of NSCLC specimen and 30 of tumor-adjacent tissue. Results: The expression of Bmi-1mRNA in NSCLC specimen was remarkably higher than that in tumor-adjacent tissue $t=5.188$ $P<0.01$. The positive rate of Bmi-1 expression was 67.3% 35/52 in NSCLC specimen and 13.3% 4/30 in tumor-adjacent tissue, respectively. There was a statistically significant difference for the expression of Bmi-1mRNA between NSCLC specimen and tumor-adjacent tissue $Z=-4.837, P<0.01$. Bmi-1 expression in NSCLC was correlated with both TNM staging and lymph node metastasis $Z=-2.567, -2.366, P<0.05$, but which had no relationship with sex, age, histotype and differentiation $P>0.05$. Conclusion: High expression of Bmi-1 in NSCLC is related with the development and metastasis of tumorigenesis.

Key Words: lung cancer; Bmi-1; Immunohistochemistry; RT-PCR
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Introduction

Polycomb group genes (PcG) regulate cell differentiation and proliferation [1]. And they are involved in oncogenesis [2] and the maintenance of a variety of stem cells [3]. B-cell-specific Moloney murine leukemia virus integration site-1 (Bmi-1) gene was a core member of transcription inhibitors PcG, and it was found in 1991 when Haupt Y was looking for something that can offer synergistic action in inducing lymphoma in transgenic mice in the presence of oncogene C-myc [4]. At present, research of Bmi-1 in malignant tumors is a hot issue. Previous researches demonstrated that there was over-expression of Bmi-1 in leukemia [5] and some solid tumors such as gastric carcinoma [6,7] breast cancer [8], melanoma[9], Cervical Cancer[10] and Nasopharyngeal Carcinoma[11,12], and its expression levels were correlated with tumorigenesis and development as well as tumor invasiveness and prognosis. This study was to investigate the relation of Bmi-1 expression to clinicopathologic parameters of NSCLC at both mRNA and protein level with specimens of NSCLC and tumor-adjacent tissue.

1 Materials and Methods

1.1 Materials

52 NSCLC specimen and 30 tumor-adjacent tissue 5cm apart from cancer tissues resected by NSCLC operation treatment at Qingdao University Medical College affiliated hospital during 2009-2010 were selected. No cases underwent radiotherapy or chemotherapy. After tissues be removed from the body, all samples were labled and frozen in liquid nitrogen and stored at -80℃ for later use. All of 52 cases were confirmed NSCLC by histopathologic study, with 19 squamous cell carcinomas and 33 adenocarcinomas; 33 were well or moderately differentiated, 27 were node positive and 35 were in stage   ~   . Cancer masses resected from 35 cases were larger than 3cm in diameter. Clinic data showed among the 52 NSCLC patients, 37 were males, and 29 were below the age of 60.

1.2 Reagents and Methods

1.2.1 Reagents RNAiso Reagent was provided by Takara biotechnology DALIAN CO., LTD. Super RT Kit and PCR kit were bought from BioTeke Corporation   BEIJING . Bmi-1 and primer GAPDH were offered by Sangon Biotech Shanghai Co., Ltd. Mouse anti-Bmi-1 monoclonal antibody Abcam US , immunohistochemistry kit and DAB solution Zhongshan gold bridge Biotechnology Co. Ltd., P.R. China were also used.

1.2.2 RT－PCR Total RNA extraction from 30 cancer tissues and 20 tumor-adjacent tissues and RT-PCR were conducted after the instructions of the kits. Primer Bmi-1 with a length of 215bp, were as following: forward 5'-TACCCATAACCTATGTTGAGC-CT-3' reverse 5'-ATAGCCAATGGAAAATTAAGACG-3'. [13 The internal control with a length of 299bp were as follows forward 5'-CGGGAAACTGTGGCGTGAT-3' reverse 5'-AGTGGGTGTCGCTGTTGAAGT-3' [14]. PCR products were detected by electrophoresis in 1.5% agarose gels. PCR products of Bmi-1mRNA and GAPDH have a molecular weight of 215bp and 299bp respectively. Gray levels of band Bmi-1mRNA and band GAPDH were determined using Quantity One a software used in gray level analysis.

1.2.3 Immunohistochemical staining Serial sections of 4um in

thickness were cut from paraffin blocks, and immunohistochemical staining was conducted under the instruction of the kits. Antigen repair was peformed in an autoclave in the presence of EDTA pH9.0 for exposing antigen site adequately. Positive control were made by confirmed positive slide and negative control was made by PBS instead of the primary antibody. A positive reaction was detected as nucleic or plasmatic stain presenting in yellow or brown-yellow color. For each section, 10 high-power fields 400 x were randomly selected, and in each high-power field 100 cells were scored in terms of staining intensity and percentage of positive cells. Each section got its first score by staining intensity which was defined as 0 negative , 1 weak , 2 moderate , or 3 strong . The second score was determined on the basis of the percentage of positively stained cells. The criteria[15] are as following 0 ≤ 5% , 1 6%~30% , 2 31%~70% , 3 ≥ 71% . Each sec-

tion was then got a summed score which was derived from the two scores above ranging from 0 to 6. And the summed score was converted to a ranked value according to the following rules: 0 (-), 1~2 (+), 3~4 (++), 5~6 (+++).

1.2.4 Statistical analysis SPSS16.0 software was used. T-test was applied to RT-PCR data analysis. Mann-Whitney Test was used in analyzing ranked data. Statistical significance was considered as P-values below 0.05.

2 Results

2.1 RT－PCR

The expression of Bmi-1 mRNA in NSCLC was obviously higher than that in tumor-adjacent tissue and the difference was statistically significant (t=5.188, P<0.01) Table 1, Figure 1 .

Table 1 Expression of Bmi-1mRNA in NSCLC and tumor-adjacent tissue

Histotype	n	expression level (x± s)	t	P
NSCLC	30	0.606± 0.309	5.188	<0.01
tumor-adjacent	20	0.226± 0.130		

There was statistically significant differences between the two groups (t= 5.188, P<0.05)
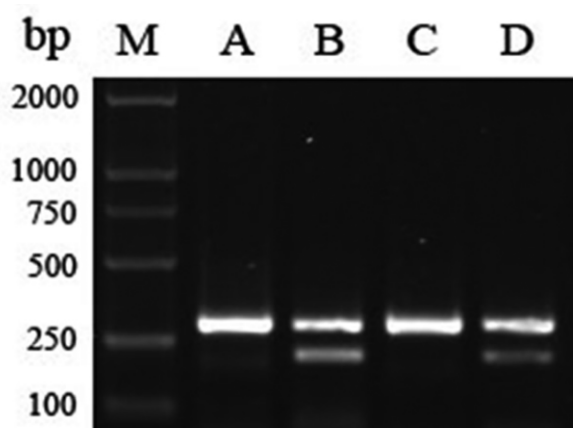

Fig 1 Expression of GAPDH band A and Bmi-1mRNA band C in tumor-adjacent tissue Expression of GAPDH band B and Bmi-1mRNA band D in NSCLC.

2.2 Immunohistochemistry

Positive expression rate of Bmi-1 in NSCLC 67.3% was remarkably higher than that in tumor-adjacent tissue 13.3% , and the difference was statistically significant (Z=-4.837 P<0.01) Table 2 .

2.3 Relation between Bmi－1 expression in NSCLC and clinicopathologic parameters

The expression of Bmi-1was associated with TNM staging and lymph node metastasis profile, NSCLC in stage 　~ 　 had higher Bmi-1 positive expression rates than that of stage 　~ 　 , lymph node positive NSCLC have higher Bmi-1 positive expression rates than that of lymph node negative NSCLC (Z=-2.567,-2. 366, p<0.05), (Table 3). There was no apparent correlation between the expression of Bmi-1 and parameters such as age sex differentiation tumor size and histotype p>0.05 .

Table 2 Expression of Bmi-1 in NSCLC and tumor-adjacent tissue

Histotype	n	Bmi-1				Z,P
		-	+	++	+++	
NSCLC	52	17	9	14	12	Z=-4.837,P<0.01
tumor-adjacent	30	26	3	1	0	

A significant difference was observed between the two groups (Z = -4.837, P < 0.05).

Table 3 Relation of Bmi-1 expression in NSCLC to clinicopathologic parameters

clinicopathologic parameters	n	Bmi-1				Z, P
		-	+	++	+++	
Sex						Z=-1.541,P>0.05
Male	37	14	7	9	7	
Female	15	3	2	5	5	

Age						Z=-0.382,P>0.05
≥ 60	23	7	3	8	5	
<60	29	10	6	6	7	
Differentiation						Z=-1.292,p>0.05
Well or moderate	33	13	6	7	7	
Low	19	4	3	7	5	
Tumor size cm						Z=-0.709,P>0.05
≤ 3	17	6	4	4	3	
>3	35	11	5	10	9	
Histotype						Z=-1.775,P>0.05
squamous cell carcinoma	19	5	1	6	7	
adenocarcinoma	33	12	8	8	5	
node involvement						Z=-2.366,P<0.05
Node positive	27	6	3	9	9	
Node negative	25	11	6	5	3	
TNM staging						Z=-2.567,P<0.05
~	35	15	9	5	6	
~	17	2	3	6	6	

3 Discussions

The highest incidence and mortality with lung cancer among malignant tumors has been proved by numerous experimental and clinic study[16]. Early diagnosis of lung cancer is the key step for its treatment [17]. It is imperative to find some molecular markers that may help in the early diagnosis and metastasis prediction of lung cancer.

PcG, an important gene family related to development, consists of several transcription inhibitors related to cell cycle and proliferation. PcG protein was closely correlated with cell reproduction and tumorogenesis. Bmi-1 is considered as a proto-onco gene and correlate with cell transformation and tumor formation [18]. As a core member of the PcG family, Bmi-1 plays major roles in the development of the skeletal, hematopoietic and nervous system of mammals in embryo period. And tumor cells with high expression of Bmi-1 are counted as tumor stem cells that renew tumor cells endlessly [19]. Multiple tumor suppressor (MTS) INK4a/ARF is a key negative regulator of Bmi-1 gene in the downstream and the two protein it encodes, namely cell cycle regulating factors p16INK4a and p14ARF, are critical in the balance of cell cycle when expressed at normal level and appropriate site [20,21]. Changes in the expression of Bmi-l gene lead to inactivation of INK4a /ARF gene, cell cycle disorder and possibly cancerization.

Researches have found abnormal expression of Bmi-l in many tumors viewed its high expression as a risk factor for bad prognosis. Studies have also discovered that the relation between high Bmi-1 expression and progression of breast cancer, suggesting Bmi-1 might become a new molecular marker in prognosis prediction of breast cancer[22,23]. The present study showed positive expression rate of Bmi-l in NSCLC was 67.3％, which was consistent with the findings of researches Vrzalikova K, [24]. This study

also found the difference in Bmi-1 expression between NSCLC and tumor-adjacent tissue had not correlated with age, sex, histotype, differentiation or tumor size, but had relationship with lymhp node involvement and staging. To be specific, node positive NSCLC had remarkably higher Bmi-1 expression than that of node negative NSCLC, and NSCLC in stage   ～   showed more Bmi-1 expression than that in stage   ～   . The fact that this study did not find the relation between high Bmi-1 expression and tumor size, which was different from some researches may be due to my small-numbers samples.

This study showed that high expression of Bmi-1 at protein and gene levels was closely related with tumorigenesis, progression, infiltration and metastasis of NSCLC. Bmi-1 might become a molecular marker in the early diagnosis of lung cancer and metastasis prediction of lung cancer. Further,there may give rise to a novel target in molecular therapy for lung cancer.

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Bmi-1

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Bmi-1　　　　　　　　(NSCLC)　　　　　　　　　　　　　　　。　　RT-PCR　　　30
　　20　　　　　　　Bmi-1mRNA　　　　　　　　　　　　SP　　52　　　　　　　　30
　Bmi-1　　　　　　　　　。　　　　　　　　Bmi-1mRNA　　　　　　　　t=5.188 P<0.01　。　　　　Bmi-1
　　　　67.3% 35/52　　　　　　13.3% 4/30　　　　　　　　　Z=-4.837,P<0.01
Bmi-1　　　　　　　　　TNM　　　　　Z=-2.567,-2.366, P<0.05　　　　　　　　`、`、`
　　　　P　>0.05　　　　Bmi-1

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　　Bmi-1　　　　　RT-PCR
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