# Clinincopathological Significance of Homeobox BP1 mRNA Expression in lung Cancer Tissue

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**Abstract** **Background & Objective** Beta-protein1 (BP1)gene, a novel member of DLX homeobox gene family, is located in 17q21-22 region and overexpressed in both acute myeloid leukemia and acute T cell lymphocytic leukemia. Up till now, there are more reports on the function of BP1 gene in breast cancer. The study was designed to explore the expression of BP1 gene in lung cancer tissue and to analyze its relationship with clinical parameters. **Methods** RT-PCR methods for detecting the expression of BP1 gene was established successfully, forty-six tissues from patients with lung cancer, tissues adjacent to cancer, non-cancer lung tissues was detected using reverse transcription-polymerase chain reaction(RT-PCR). **Results** Thirty-five specimens of lung cancer tissues were determined to express BP1 gene, 13 of which were moderately differentiated, 22 cases of which were poor differentiated. 12 of which were Stage Ⅰ-Ⅱ, 15 of which were Stage Ⅲ-Ⅳ. No specimen of tissues adjacent to cancer, non-cancer lung tissues and blood were screened out to be over expression of BP1 gene. There were significantly different rates between lung cancer and tissues adjacent to cancer, non-cancer lung tissues and blood. **Conclusion** Mark up-regulation of BP1 gene expression is found in human lung cancer. and the expression of BP1 gene may be related to differentiation level of lung cancer but not related to clinical stage.

**Key Words** Lung neoplasm; BP1 gene; Gene expression; RT–PCR

Lung cancer is one of the most common malignant cancer in world, its high morbidity and mortality, especially in male patients, are still the unsolved problems. Although many methods such as operation, chemotherapy, radiotherapy have been made big progress in treatment of lung cancer, the morbidity and mortality are tending to go up. Among lung cancer, about 85% belong to middle and later period, 5 years survival rate only about 13%[1]. Early diagnosis of lung cancer is very important to clinical treatment and prognosis. In early period of the cancer, many genes developed mutation, these mutation frequently appear early than clinical symptom. These gene marker can provide very important testify to early diagnosis[2].

BP1 (beta-protein)gene is a transcription factor which was found by Patricia E. Berg and his coworkers. They have cloned a human homeobox cDNA called BP1, which belong to the DLX family that encode a group of transcription factors with highly conserved 60 amino acid DNA[3]. It is located on chromosomes 17q21-22. BP1 is apparently involved in the regulation of diverse pathways. It was reported that BP1 was expressed in 47% of the adult and 81% of the pediatric acute myeloid leukemia patients and 80% of invasive ductal breast tumors expressed BP1. The activation of BP1 is recognized as a early event of breast cancer [3]. In this experiment, we use RT-PCR method to examine the BP1 mRNA expression level in 46 cases of lung cancer, tissues adjacent to cancer, and normal lung tissues. The aim is to explore the clinical meaning of BP1 gene expression in hung cancer.

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## MATERIALS AND METHODS

**Sample**

Tumor sample were randomly obtained from 46 primary lung cancer patients who were treated surgically from march, 2004 to may, 2005 at Oncology Department of first Hospital attached to Xi´an Jiao Tong university. Before operation, they have no received radiotherapy and (or) chemotherapy.

Among the patients, 32 patients were males and 14 females. Their ages ranged from 31 to 78 years old. According to the pathological types, 24 patients were squamous cell carcinoma (Among them, 11 were moderate and well differentiation and 13 were poor differentiation; 10 were Ⅰ-Ⅱ stage and 14 were Ⅲ-Ⅳ stage), 22 were adenocarcimona(Among them,10 were moderate and well differentiation and 12 were poor differentiation and 9 were Ⅰ-Ⅱ stage and 13 were Ⅲ-Ⅳ stage). In addition, 46 cases of the tissues adjacent to cancer, which were apart from the cancer lesion about 3cm, and 46 cases of the normal lung tissues part from cancerous lesion about 10cm were taken. It´s according to the asepsis, the samples were obtained from fresh surgical resection specimens and were snap-frozen in liquid nitrogen and were kept in −70℃ refrigerator until use. The TNM and clinical stage classification were determined retrospectively based on the guidelines of IUCC, 1997 revision.

**The measurement of BP1 mRNA expression**

Total RNA was extracted from 46 cases of frozen human lung cancer, 46 cases of tissues adjacent to cancer and 46 cases of normal lung tissue (every tissue was 0.2g) with Trizol regent (Invitrogen, Gaithersburg, MD, USA. Offered by Sidney W Fu) in accordance with the guide of Sidney W Fu [4]. The primers were synthesized by Shanghai Ouyi Biological Technology Limited Company. The PCR primers for BP1, which were span an intron, were 5´-GTATGGCCACCTCCTGTCTT-3´ (forward) and 5´-GAGTAGATGGT-CCTCGGCTT-3´(reverse), giving a product of 225 base pairs. The internal control for all sample was β-actin, which were designed by Shanghai Ouyi Biological Technology Limited Company, were 5´-ACCCCCACTGAAAAAGATGA-3´ (forward) and 5´-ATCTTCAAACCTCCATGATG-3´ (reverse), giving a product of 114 base pairs

In the 10ul reveres transcription polymerase chain reaction (RT-PCR) tube, we use the tissues from lung cancer, tissues adjacent to cancer and normal lung tissue 0.2ug respectively and 50mmol/LTris-HCl(pH8.3), 40mmol/LKCl, 7mmol/L $MgCl_2$, 1mmol/L, DTT, 0.01% BSA, 1mmol/L dNTPS, 20u MMLV reveres transcription enzyme (Sangon product), 10u RNA inhibition enzyme (Shanghai Ouyi Biological Technology Limited Company product) and reverse product of BP1gene 20pmol/L respectively. Total RNA(1ug) was used to synthesize cDNA with a Super Script Ⅱ reverse transcriptase system (Invitrogen). After synthesis of the first-strand cDNA, PCR was performed by initial denaturing at 94℃ for 2 min followed by 28 cycles of denaturing at 94℃ for 1 min, annealing at 60℃ for 1 min, and extension at 72℃ for 1.5 min.

The products of BP1 gene amplification were analysed by 1.5% and 6% garose gel electrophoresis, and took pictures using garose gel electrophoresis system. Randomly select the positive reaction product of BP1 gene to check up gene sequence in Sidney W Fu´s laboratory[3] and ShangHai Ouyi Biological Technology Limited Company. The results and BP1 gene cDNA´s whole sequence were contrasted by MegAlign 5.0 software of DNAstar 2.05, the products of BP1 gene amplification´s speciticity were determined.

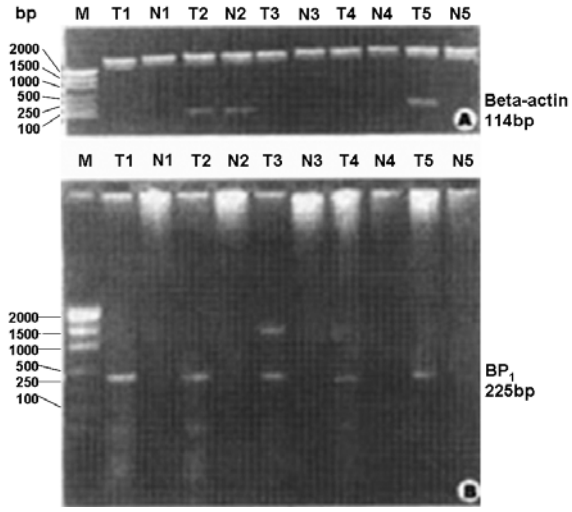
**Statistical analyses**

Statistical analyses were used to determine the relationship between BP1 positive expression, and clinico-pathological parameters. All P values were calculated with Fisher´s Exact Test. Tests were considered significant if P value was 0.05 or less. All statistical analyses were performed with SAS 8.2 (SAS Institute, Cary, NC, USA).

## RESULTS

**Expression of BP1 in lung cancer tissues**

BP1mRNA expression was examined and determined in 46 cases of lung cancer, tissues adjacent to cancer and normal lung tissue 46 cases respectively. The positive expression of BP1mRNA in lung cancer tissues were 35 cases (76.1%), in tissues adjacent to cancer were 6 cases (13.04%) and no BP1expression in normal lung tissues. There was significance difference between lung cancer tissues and tissues adjacent to cancer, normal lung tissues ($P$<0.05). There was no significance difference between tissues adjacent to cancer and normal lung




Comparison and difference of BP1 mRNA amplication determined by RT-PCR lung cancer, tissues adjacent to cancer, non-cancer lung tissues. A;1.5%agarose gel electrophoresis analysis of beta-actin expression in different lung tissues. B:6% agarose gel electrophoresis analysis of BP1 mRNA expression in different lung tissues; M: Marker; T: lung cancer; N1-3: adjacent to lung cancer (<3cm from carcinoma; N4-5: non-cancer lung tissues (>10cm from carcinoma).

tissues ($P$>0.05). (Tab1)

There were 13 cases expressed BP1 gene in 21 cases of moderate and well differentiation lung cancer tissues, and 22 cases expressed BP1 gene in 25 cases of poor differentiation lung cancer tissues. There was significance difference between moderate and well differentiation and poor differentiation lung cancer tissues ($P$<0.05). In 20 cases of Ⅰ-Ⅱ TNM Stage lung cancer tissues, 12 cases expressed BP1 gene and 15 expressed BP1 gene in 26 cases of Ⅲ-Ⅳ TNM Stage lung cancer tissues. There was no significance difference between Ⅰ-Ⅱ TNM Stage and Ⅲ-Ⅳ TNM Stage lung cancer tissues ($P$>0.05); In 24 cases of squamous cell carcinoma lung cancer tissues, 21 expressed BP1gene and 19 expressed BP1 gene in 22 cases of adenocarcinoma. There was no significance difference between squamous cell carcinoma and adenocarcinoma of lung cancer tissues ($P$>0.05).

## *DISCUSSION*

In the present study, we using RT-PCR method found that 76.1% of lung cancer expressed BP1. In contrast, only 13.04% of tissue adjacent to cancer expressed BP1 and no expressed in normal lung tissues. No significant relationship was detected between expression of BP1 and TNM stage, tumor size and histology. We also found that the poorer of lung cancer differentiation was the higher of the expression of BP1 expressed. There was significant difference between the poor and moderate and well differentiation

BP1 (beta-protein)gene is a transcription factor which was cloned by Patricia E. Berg in Department of Biochemistry and Molecular Biology, the Georg Washington University Medical Center[5]. It´s belonged to the DLX family which encode a group of transcription factors with highly conserved 60 amino acid DNA and located on chromosomes 17q21-22. The molecular weight of BP1 gene is

**Tab.1** Comparison of BP1expression in different kinds of pulmonary tissues

| Group | n | positive(%) | Negative(%) | *p* |
|---|---|---|---|---|
| Lung cancer tissue | 46 | 36 (78.3%) | 10(21.7) | <0.05 |
| Tissue adjacent to cancer | 46 | 6 (13.04%) | 40(87.96%) | <0.01 |
| Normal lung tissue | 46 | 0 (0.00) | 46(100%) | |

**Tab.2** Relation between expression of BP1 and clinical characteristics of lung cancer tissue

| Group | n | Positive | Negative | *p* |
|---|---|---|---|---|
| TNM Stage | | | | |
| Ⅰ–Ⅱ | 20 | 12(60%) | 8(40.0%) | >0.05 |
| Ⅲ–Ⅳ | 26 | 15(57.7%) | 11(42.3%) | |
| Differentiation | | | | |
| Poor | 25 | 22(88%) | 3(12.0%) | <0.05 |
| Moderate and Well | 21 | 13(61.9%) | 8(38.1%) | |
| Histology | | | | |
| Squamous cell carcinoma | 24 | 21(87.5%) | 3(12.5%) | >0.05 |
| Adenocarcinoma | 22 | 19(86.4%) | 3(13./6%) | |
| Tumor size | | | | |
| <3.0cm | 28 | 23(82.1%) | 5(17.8%) | |
| >3.0cm | 18 | 15(83.3%) | 3(16.6) | >0.05 |

26kDa and belonging to a protein which represses the human β-globin gene. BP1gene is isoforms with DLX7 which belong to DLX family and play an important role in modulate the expression of a variety of target gene (van Oostveen et al, 1999; Akam,1989). There is extensive sequence identity when BP1 is compared with DLX7, the DNA sequence of part of their open reading frames (ORF) are identical and their 3´flanking sequences differ by only a few bases [6]. Both are co-expressed in variety of malignant cell lines and tissues. Accumulating evidence suggests that homeobox gene are important in malignant. Not only have altered expression levels of HB genes been detected in a variety of human malignancies[7], but the ectopic expression of certain HOX genes can induce tumors[8]. In normal erythroid cells BP1 gene acts a repressor of the β-globin gene [9]. BP1 gene was also expressed in 47% of the adult and 81% of the pediatric acute myeloid leukemia patients and its overexpression increased the the leukemogenic potential of K562 cells in vitro [10]. There were evidences demonstrated that the repressor function of BP1 in K562 cells by co-transfection of a plasmid expressing BP1 protein (driven by the Rous sarcoma virus long terminal repeat)with target DNAs. Two of the target plasimds were used and contain the CAT reporter gene driven by the ε-glonbin promoter and include either Silencer Ⅱ or Silencer Ⅰ (located 300 or 500bp upstream of theβ-globin gene ,respectively)[11]. Molecular analysis revealed that BP1expression is required for the survival of K562 leukemia cells, which suggesting that BP1might be part of an anti-apoptotic pathway and aberrant BP1 expression might also be involved in other malignancies [12]. BP1 gene and DLX7are co-expressed in a high percentage of bone marrow sample from AML patients [13]. Yu Man et al recognized that BP1 gene affected from begin to clinical expression in breast cancer[14].

Lung cancer is a polygenes event, the change of P53 and P21WAF-1 gene, DNA base damage, telomerase activation and expression, microsatellite instability (MSI), loss of heterozygosity (LOH), the abnormal methylation of initiating factors and so on are play an important role in lung cancer [15]. The detail mechanism of BP1gene in the development of lung cancer is not clear.

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## *REFERENCES*

1. Lai BT. Several hotspots in basic investigation of lung cancer. Chin Med News, 2004, 19(3):12.
2. Chottenfeld D. Epidemiology of lung cancer. In: pass HI, Mitchell JB, Johnson DH, et al, eds. Lung cancer. Philadephia: Lippincott–Raven Publishers, 1996. 305–322.
3. Michael B, Chase, Sidong Fu, Susanne B, et al. BP1, a Homeodomain-Containing Isoform of DLX4, Represses the b-Globin Gene. Molecular and Cellular Biology, 2002, 22: 2505–2514.
4. Sidong Fu, Holly Stevenson, Jeff W, et al. Distinct function of two isoforms of a homeobox gene, BP1and DLX7. in the regulation of the beta-globin gene. Gene, 2001, 278(1–2):131–139.
5. Sidney W Fu, Arnold Schwratz, Holly Stevenson, et al. Correlation of expression of BP1, a homeobox gene, with receptor status in breast cancer. Breast Cancer Research, 2003, Vol 5 No 4.
6. Jemal A, Murray T, Samuels A, et al. Cancer statistics. CA Cancer J Clin, 2003, 53(1):5–26.
7. Haga SB, Fu s, Karp JE, BP1, a new homeobox gene, is frequently expressed in acute leukemias. leukemia, 2000, 14:1867–1875.
8. Thorsteinsdottir U, Sauvgaeau G, Hough MR, et al. Over expression of HOXA10 in murine hematopoietic cells perturbs both myeloid and lymphoid differentiation and leads to acute myeloid leukemia. Mol Cell Biol, 1997, 17:495–505
9. Chase MB, Fu S, Davenport G. BP1, a homeodommain–containing isoform of DLX4, represses the β-globin gene. Mol Cell Biol, 2002, 22(8): 2505–2564.
10. Haga SB, Fu S, Karp JE, et al. BP1, a new homeobox gene, is frequently expressed in acute leukemias. Leukemia, 2000, 14:1867–1875.
11. Drew L, D.C. Tang, PE Berg, et al. The role of trans–acting factors and DNA-bending in the silencing of human beta-glonbin gene expression. Nucleic Acids Res. 2000, 28: 2823–2830.
12. Ferrari N, Paleari L, Palmisano GL, et al. Induction of apoptosis by fenretinide in tumor cell lines correlated with DLX-2, DLX-3, and DLX-4 gene expression[J]. Oncol Rep, 2003, 10(4): 973–977.
13. Li H, HuangCJ, Chhoo KB; Expression of homeobox genes in cervical cancer. Gynecol Oncol 2002, 84: 216–221.
14. Yu Man, Yang Yi, Niu Rui-Fang, et al.Clinicopathological Significance of Homeobox Gene BP1mRNA Expression in Human Breast Cancer. Chinese Journal of Cancer, 2004, 23(7): 855–859.
15. NCI guide. Chemoprevention of tobaccorelated cancers in former smokers: clinical studies, 2001.