# Expressions of P33ING1 and P53 Protein in Human Lung Cancer Tissues

Haiyan Dong, Yongjun Wu, Yiming Wu

*College of Public Health, Zhengzhou University, Zhengzhou, Henan 450052, China*

**Abstract: Aim.** The inhibitor of growth 1 (*ING1*) gene is a novel candidate tumor suppressor gene and involve in the regulation of the cell cycle, senescence, and apoptosis. This study aims to evaluate the expression of P33ING1 protein, the product of *ING1*, with P53 protein in the same human lung cancer tissues. **Methods.** Human lung tissues were collected and classified into three groups, 31 cases of tumor tissues (A), 21 and 12 cases of tumor-adjacent lung tissues which were 3 cubic cm (B) and 5 cubic cm (C) by volume, respectively. 21 cases from group A were paired with those from group B (A-B); 12 cases from A-B were paired with those from group C (A-B-C). Immunoblotting was employed to detect P33ING1 expression in both tumor and tumor-adjacent lung tissues to ascertain the co-occurrence of P33ING1 with P53 protein. **Results.** The expression level of P53 was elevated of tissues in group A (12 of 21, 57.1%). In contrast, the expression level of P33ING1 decreased (14 of 21, 66.7%) of the tissues from the same group. There was not significant linear relationship between P53 and P33ING1 protein expression ($P>0.05$) by correlation analysis and McNemar's Test. **Conclusions.** This study first demonstrated that P33ING1 protein was down-regulated in lung cancer tissues, implying that it might play a role in carcinogenesis of lung cancer. In contrast to previous reports, this study didn't find the correlation between P33ING1 and P53 protein expression. Further studies will be conducted on gene polymorphism by larger volume of tissue samples. [Life Science Journal. 2006;3(4):23-26] (ISSN: 1097-8135).

**Keywords:** Lung cancer; P33ING1; P53

**Abbreviations:** ING1: inhibitor of growth 1

## 1 Introduction

Recently, studies on genes and their functional products have become hot in life science research. Exploration of interactions among diverse gene products is a critical issue in the field of proteomics. *ING1* (inhibitor of growth 1), a new candidate tumor suppressor gene, was identified in the subtelomeric region of human chromosome $13q^{33-34}$. Suppression of *ING1* expression is associated with increased proliferation and immortalization. This growth inhibitor participates in cell cycle regulation. Overexpression of *ING1* from transfected DNA constructs efficiently decreases S-phase fraction through blocking the entry of cell into S-phase, further leading to inhibition of normal cell growth. The repression of *ING1* expression frequently occurs with tumor development of breast cancer, stomach cancer, lymphoma, and so on. It has been reported that the growth inhibitory effect of P33ING1 directly cooperates with P53 protein *in vivo*. However, the expression of *ING1* gene has not been determined yet in lung carcinoma tissue. P53 protein plays a critical role in the regulation of cell proliferation, apoptosis and cellular aging, and is associated with tumor development. The mutation of *p53* gene is an early event in the pathogenesis of lung cancer[1], so *p53* could be applied for early lung cancer forecast. However, there are about 50% cases of lung cancer without *p53* gene mutation. It is shown that *p53* suppressing effect on tumor growth is regulated and modulated by other genes or proteins. So far, it is very obscure about the network for P53 protein regulation. Any efforts on uncovering one of those links will shed light on understanding of the development of lung cancer. So Western blot was used to detect protein expression of P33ING1 in tumor and tumor-adjacent tissues to unveil the correlation between P33ING1 and P53 protein.

## 2 Materials and Methods

### 2.1 Subjects

The fresh tissues including tumour tissues (A), 3 cubic cm (B) and 5 cubic cm (C) by volume, were harvested from the same patient and frozen in liquid nitrogen immediately after surgery and then stored at −80 ℃ until the extraction of protein. Three groups, 31 cases in group A, 21 cases in group B and 12 cases in group C, were harvested from 22 cases with lung squamous cell carcinoma, 7 cases with lung adenocarcinoma, 1 case with small cell lung cancer, and 1 case of large cell lung cancer. 21 cases from group A were pair-

matched with those from group B (A-B); 12 cases from A-B were pair-matched with those from group C (A-B-C).

### 2.2 Western blot

Protein isolation was conducted according to the published procedure in *Molecular Cloning: A Laboratory Manual*. Specifically, 0.1 g – 0.5 g lung tissue was washed with ice-cold PBS buffer, then homogenized immediately in lysis buffer at 0 ℃ (100 μg/mL PMSF, 1 μg/mL aprotinin, 0.001 mol/L EDTA(pH 8.0), 0.01 mol/L Tris·HCl(pH 7.6), 0.1 mol/L NaCl). The lysates were further sonicated to shear DNA prior to centrifugation with 10,000 rpm for 10 minutes at room temperature. The supernatants were collected and stored at −20 ℃. Protein contents were measured before use.

The supernatant was mixed with an equal volume of 2× SDS sample buffer (100 mmol/L Tris·HCl, (pH 6.8), 200 mmol/L dithiothreitol, 4% SDS, 20% glycerin) and boiled for 10 minutes. After normalization for protein content, cell extracts were subjected to 12% SDS-PAGE. Proteins were then electroblotted onto nitrocellulose.

The membrane was blocked with 20% fetal bovine serum in PBS, then washed and incubated with goat anti-ING1 antibody (sc-7566) at room temperature for 1 hour. After wash, the blots were incubated with a horseradish peroxidase-conjugated secondary antibody and counterstained with 3′3 diaminobenzidine (DAB). The images were taken with Bio-Imaging System. The horseradish peroxidase-conjugated secondary antibody and Kalerdoscope Prestained standards were purchased from Santa Cruz Biotechnology. DAB was obtained from Sigma.

### 2.3 Statistic analysis

Experiment data was processed using SAS software(6.12). Normal distribution was first tested. The data of Normal distribution was denominated by $\bar{x} \pm s$, and the data of abnormal distribution was denominated by 50% Med. Difference among three independent groups were determined by analysis of variance(ANOVA) and least significant difference $t$ test. Differences between independent ratios were determined by Chi-square test. Significance was set at $P<0.05$.

## 3 Results

### 3.1 Expression of P53 protein

The expression level of P53 was increased in 57.1%(12 of 21) of tissues from group A, which was consistent with the previous reports (50% – 60%); in A-B-C, there was no significant difference in P53 expression between group A and the combined group B ($P>0.05$) (Tables 1 – 3). However, the differences of P53 expression of group A *vs*. group C, group B *vs*. C were significant ($P<0.05$), respectively(Table 3).

**Table 1.** The different expression of P53 in different groups

| Group | $n$ | P53($\bar{x} \pm s$) |
|---|---|---|
| A | 31 | 33380.1±11841.3* |
| B | 21 | 33704.6±12984.9 |
| C | 12 | 25000.1±11961.7 |

* *vs*. group C, $P<0.05$; group A *vs*. group B, group A *vs*. group C, $P>0.05$

**Table 2.** The different expression of P53 in A-B

| Group | $n$ | P53($\bar{x} \pm s$) |
|---|---|---|
| A | 21 | 31139.8±13233.0 |
| B | 21 | 33704.6±12984.9 |

$t=-0.8325$ $P=0.4149$

**Table 3.** The different expression of P53 in A-B-C

| Group | $n$ | P53($\bar{x} \pm s$) |
|---|---|---|
| A | 12 | 33130.5±11572.8 |
| B | 12 | 32254.8±9703.9 |
| C | 12 | 25000.1±11961.7* |

* *vs*. group A, group B, $P<0.05$; group B *vs*. group A, $P>0.05$.

From Table 3, it was shown that compared with adjacent tissues which were 5 cubic cm from the cancer tissue, the expression level of P53 in tumor tissues and tissues which were 3 cubic cm from the cancer tissue were increased significantly.

### 3.2 Expression of P33ING1 protein

The expression level of P33ING1 was decreased in 66.7% (14 of 21) of the tissues from group A. In A, B, C and A-B-C, compared with group C, the expression level of P33ING1 in group A was decreased significantly ($P<0.05$) (Tables 4 – 6). However, the differences in P33ING1 expression between the group A and group B, group B and group C were not significant ($P>0.05$), respectively (Table 4).

**Table 4.** The different expression level of P33ING1 in different groups

| Group | $n$ | P33ING1($\bar{x} \pm s$) |
|---|---|---|
| A | 31 | 22156.8±4654.2* |
| B | 21 | 23983.5±5586.2 |
| C | 12 | 26021.9±5079.8 |

* *vs*. group C, $P<0.05$; group A *vs*. group B, group B *vs*. group C, $P>0.05$.

**Table 5.** The different expression of P33ING1 in A-B

| Group | *n* | P33ING1 (50% Med) | Significance M(sign) | *P* |
|---|---|---|---|---|
| A | 21 | 20654 | — | — |
| B | 21 | 23412 | 10.5 | 0.001 |

**Table 6.** The different expression of P33ING1 in A-B-C

| Group | *n* | P33ING1($\bar{x} \pm s$) |
|---|---|---|
| A | 12 | 22528.4±3716.4 |
| B | 12 | 25812.8±5278.3 |
| C | 12 | 26021.9±5079.8 |

Group A *vs*. group B, group A *vs*. group C, $P<0.05$; group B *vs*. group C, $P>0.05$

From Table 6, these results showed that compared with tissues which were 3 cubic cm and 5 cubic cm from the cancer tissues, the expression level of P33ING1 in tumor tissues were decreased significantly.

### 3.3 Correlation between P53 and P33ING1 expression

66.7% cancer tissues that expressed less P33ING1, P53 protein was expressed at a higher level. Meanwhile, 55.6% tissues with decreased P33ING1 levels did not exhibit an elevated expression of P53. However, there was no significant linear relationship between P53 and P33ING1 protein by correlation analysis and McNemar's Test ($P>0.05$) (Tables 7 and 8).

**Table 7.** The different expression level of P53 and P33ING1 in A-B

| P53 | P33ING1 | | Total |
|---|---|---|---|
| | Decreased | Increased | |
| Decreased | 6 | 3 | 9 |
| Increased | 8 | 4 | 12 |
| Total | 14 | 7 | 21 |

$\chi^2=2.273, P=0.132$

**Table 8.** Linear correlation analysis

| Group | *n* | *r* | *P* |
|---|---|---|---|
| A | 31 | 0.0358 | 0.8482 |
| B | 21 | 0.2646 | 0.2464 |
| C | 12 | 0.2723 | 0.3920 |

## 4 Discussion

The normal expression of P53 protein can induce growth suppression and apoptosis. Vicious cycle of abnormal cell proliferation, immortalization, and malignant tumor may take place if P53 loses its function or is mutated. Up to now, it has been accepted that there exists a close relationship between p53 mutation and human tumor development. Previous studies have shown that 50% – 60% of 5,000 cases of tumor derived from 43 kinds of tissues produced with mutated *p53* gene[2]. This study demonstrated an abnormal expression of P53 (57.1%), which was consistent with previous reports. There was no significant difference in P53 expression between lung cancer tissues and the cancer-adjacent tissues. The explanation for these phenomena may lie on two aspects. First, due to the irregular infiltration of malignant tumor, abnormal differentiation cell or cancer cell may exist in cancer-adjacent tissues, which may express mutated P53; second, wild type P53 protein might be combined with other molecular chaperones (such as MDM-2)[3], thus caused elongation of its half life, which may interfere the detection of mutated P53 protein. Further studies on gene expression need to be conducted to confirm these explanations.

The data demonstrated that the expression of P53 in lung cancer tissues (A) was significantly higher than that in the tissues which were 5 cm (C) from cancer tissues and the same was true of A-B-C of 12 patients. These are consistent with previous reports. In addition, an expression of P53 increased in lung cancer. And also the expression of P53 in the 3 cubic cm tissues from the cancer tissue was statistically higher than that in 5 cubic cm tissues away in A-B-C. However, analysis on groups A, B and C that were not paired, there were no significant differences in P53 expression between tissues that were 3 cubic cm and 5 cubic cm from the cancer tissue. These might be caused by unpaired groups A, B and C, since the result of analysis of variance in A-B-C showed that match factors could interfere the expression level of P53 protein ($P<0.05$), and would confound the results while taking the comparison in unpaired groups A, B and C.

*ING*1 gene is located in the subtelomeric region of human chromosome $13q^{33-34}$. Previous studies showed[4-8] that P33ING1 protein was the product of *ING*1 gene, located in nuclear, and participated in cell cycle regulation and its suppression was associated with increased cell proliferation and formation of neoplasm. Recently, it has been discovered that *ING*1 gene products had interaction with P53, and P53 protein took part in the regulation of cell proliferation, apoptosis and cellular aging[4].

Some investigations reported that the expression of *ING*1 gene was decreased in stomach cancer[9], breast cancer[10], and others, which implied that *ING*1 gene was relevant to the occurrence and

development of carcinoma. P33ING1 expression was determined in this study, which showed an increase in its expression in tumor tissues(A), tissues which were 3 cubic cm(B) and 5 cubic cm(C) from cancer tissue, respectively. It was assumed that the decreased expression of P33ING1 could be one of the mechanisms of abnormal cell growth, and played an important role in the procession of the occurrence and development of pulmonary carcinoma.

It has been reported that the expression of P33ING1 was correlated with P53 protein in stomach cancer, breast cancer, and so on; however, analysis on the correlation between P33ING1 and P53 in the study revealed that in 66.7% of the tissues that down-regulated P33ING1 the level of P53 protein was up-regulated. In addition, 55.6% of the tissues with decreased P33ING1 expression did not show altered P53 protein expression. There was no significant linear relationship between P53 and P33ING1 protein by correlation analysis and McNemar's Test ($P>0.05$). Further study needs to be carried out to confirm this observation based on the following reasons: *ING*1 gene encodes alternative transcripts of P47ING1, P33ING1 and P24ING1 in human cell, three of them exert different function through different channel.

In summary, the decreased expression of P33ING1 was determined in lung cancer tissues in the study; this suggested that it might play a role in the occurrence and development of lung cancer. This study provided a new clue for further study of pathogenesis and diagnosis of lung cancer, also gave a new idea and direction to gene therapy. But we did not find the correlation between P33ING1 and P53. Future studies on the gene polymorphism will be conducted. However, the exertion of tumor suppressor P53 function is regulated by multiple factors; most of them occur in different temporal and spatial segments of cell proliferation and interact with each other through expression modulation. Therefore, it is difficult to figure out their interactions by analyzing a single or few factors. Nevertheless, elucidation of certain events may be helpful in understanding carcinogenesis.

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**Correspondence to:**
Yiming Wu
College of Public Health
Zhengzhou University,
Zhengzhou, Henan 450001, China
Telephone: 86-371-6665-8011, 6665-8960
Email: wuym@zzu.edu.cn

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