

Loss of PTEN Expression in Primary Lung Cancer

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Background : The *phosphatase and tensin homolog deleted on chromosome 10 (PTEN)* gene, a candidate tumor suppressor, is localized to chromosome 10q23 and shares extensive homology with cytoskeletal proteins auxilin and tensin. It appears to have multifunctional roles involved in cell proliferation, migration, and invasion. The role of *PTEN* alteration in the lung cancer and its relationship with other suppressor genes are not well established. **Methods :** Formalin-fixed, paraffin-embedded tissues from 105 patients with diagnosed with primary lung cancer were evaluated for PTEN and p53 protein expression using immunohistochemical methods. The results of the expression pattern of PTEN were compared with clinicopathological parameters and the expression pattern of p53. **Results :** Forty-seven (44.8%) of 105 cases had loss of PTEN expression. Loss of PTEN expression was significantly associated with histologic type ($p < 0.05$), but did not correlate with tumor size, lymph node metastasis, and stage. There was no significant relationship between loss of PTEN expression and p53 expression, and no significant difference in clinicopathologic characteristics between particular groups of patterns with the four possible tumor carrying PTEN/p53 phenotypes. **Conclusion :** It is suggested that loss of PTEN expression occurs commonly in primary lung cancers and correlates with histologic type. Our results also support the proposed role of *PTEN* as a candidate tumor suppressor in lung cancer, and we suggest that there is a need for further study of this gene.

Key Words : Lung Neoplasms-Immunohistochemistry-Tumor Suppressor Proteins

Lung cancer is the leading cause of cancer deaths worldwide, and the number of cases of lung cancer continues to increase.¹ Like any other human cancers, the development of lung cancer is associated with an accumulation of genetic alteration in the tumor suppressor genes.

Phosphatase and tensin homolog deleted on chromosome 10 (PTEN) gene (also called *MMAC1*-mutated in multiple advanced cancers) is a novel candidate tumor suppressor located on chromosomal band 10q23.3² and shares extensive homology with cytoskeletal protein tensin and the secretory vesicle protein auxilin.³ PTEN is a 55-kDa protein comprising an N-terminal catalytic domain and a dual specificity phosphatase that displays a pronounced preference for acidic substrates.³ Importantly, PTEN has a role in the modulation of the 1-phosphatidylinositol 3-kinase pathway (PI3K), which is involved in cell proliferation and survival, so it can inhibit cell cycle progression and induce G1 arrest.⁴ PTEN also dephosphorylates focal adhesion kinase (FAK), which results in inhibition of cell migration, spreading, and focal adhesion formation.⁵

Germ-line mutations of *PTEN* locus have been detected in the case of Cowden disease and Bannayan-Zonana syndrome, two related hamartoma syndromes.^{6,7} Somatic alterations at the *PTEN* locus have been described in a variety of neoplasms, including those of the primary central nervous system,^{8,9} breast,^{8,10} prostate,^{8,11} renal,¹² endometrial,¹³ thyroid,¹⁴ and urinary bladder tumors,¹⁵ melanoma,¹⁶ and non-Hodgkin's lymphoma.¹⁷ Yet, a few studies have investigated the role of *PTEN* in the tumorigenesis of primary lung cancer.^{18,19} Hosoya *et al.*¹⁸ reported that the overall deletion rate of the *PTEN* locus was 75% in small cell carcinomas and 33.3% in squamous cell carcinomas; whereas Petersen *et al.*¹⁹, using a microsatellite marker, reported that allelic loss was frequently seen in small cell carcinomas and that none of the non-small, non-squamous cell carcinomas had an allelic deletion at this locus.

The most common molecular alteration in lung cancer is mutation of the *p53* suppressor gene.²⁰ *p53* is involved in cell cycle control, DNA repair, apoptosis, cellular differentiation, senescence and angiogenesis.²¹ The level of *p53* protein in the nuclei

increases after cellular stresses such as DNA damage, hypoxia, nucleotide imbalance or oxidative damage, as well as in relation to various forms of oncogene imbalance.^{20,21}

The aim of this study is to assess the extent of loss of PTEN protein, to determine the involvement of alteration of the PTEN in carcinogenesis and the progression of primary lung cancers, and to define its relationship with the p53 protein using immunohistochemistry.

MATERIALS AND METHODS

Materials

The materials used in this study were obtained from the surgical pathology archival files of the Department of Pathology at Dong-A University Hospital. The records are from between 1994 and 1997, and they consisted of 105 primary lung cancer samples obtained from lobectomies or pneumonectomies. The clinical records, surgical pathological reports and follow-up informations were also obtained where available. HE-stained slides were reviewed in each case to confirm the original diagnosis with those based on the World Health Organization (WHO) criteria.²² Postoperative pathological staging was determined according to the guidelines of the American Joint Committee on Cancer.²³

Methods

Immunohistochemical staining

Immunohistochemical studies for PTEN and p53 were performed on formalin-fixed, paraffin-embedded, 4 μ m-thick tissue sections using the avidin-biotin-peroxidase complex method.

The primary antibodies used were monoclonal mouse anti-(human Ig) antibodies directed against PTEN (Neomarker, CA, U.S.A.) and p53 (Dako, Glostrup, Denmark). Dilutions were each 1:100 for both PTEN and p53, respectively. Deparaffinization of all sections was performed through a series of xylene baths, and rehydration was performed through graded alcohol. To enhance the immunoreactivity, microwave antigen retrieval was performed at 750W for 30 minutes in a citrate buffer (pH 6.0). After blocking the endogenous peroxidase activity with 5% hydrogen peroxidase for 10 minutes, the primary antibody incubation for PTEN was performed at 4°C overnight and that for p53 was performed for 1 hour at room temperature. Detection of the immunoreactive staining was carried out with the avidin-

biotin-peroxidase complex method using the Histostain-plus kit (Zymed, CA, U.S.A.). The antigen-antibody reaction was visualized using 3-amino-9-ethylcarbazole as a chromogen. A Mayer's hematoxylin counterstain was performed.

For each batch of immunohistochemistry, positive and negative control specimens were also incubated and reviewed along with the test slides. Peripheral nerve bundles were used in the internal control for PTEN, and sections from two colonic adenocarcinomas showing positive immunostaining were used as the positive control for p53. Negative controls were run without the primary antibody in order to monitor background staining.

Interpretation of immunohistochemical staining

For PTEN expression, positive cases were defined by the presence of granular, crisp cytoplasmic staining. Evaluation of PTEN expression was semiquantitative, based on staining intensity and distribution. Intensity was scored as strong, moderate, or weak. Distribution was scored as diffuse (>50% tumor staining), regional (15 to 50% tumor staining) and focal (<15% tumor staining). Tumors with intense to diffuse, intense to regional, intense to focal, and moderate to diffuse staining were considered positive for PTEN expression, whereas tumors with moderate to regional, moderate to focal, or weak staining with any distribution were considered negative.

For p53 expression, staining was positive if nuclei reactivity was >10% with any intensity.

Statistical analysis

Statistical analysis was performed with the Statistical Package Service Solution software (SPSS for Windows Standard version 10.1, Chicago, U.S.A.). The χ^2 -test was performed to assess the association between the loss of PTEN expression, clinicopathological characteristics and the overexpression of p53. p values less than 0.05 were considered to be significant.

RESULTS

Clinical and pathological characteristics

The ages of 105 patients ranged from between 31 to 74 years (median: 58 years). 74 were men and 31 were women. The tumor size ranged from 1 cm to 9 cm (median: 3.5 cm), and 24 cases involved tumors less than 3 cm, while 81 cases involved tumors that were more than 3 cm. Histologically, they consisted of 50 squamous cell carcinomas, 42 adenocarcinomas, 2 small cell car-

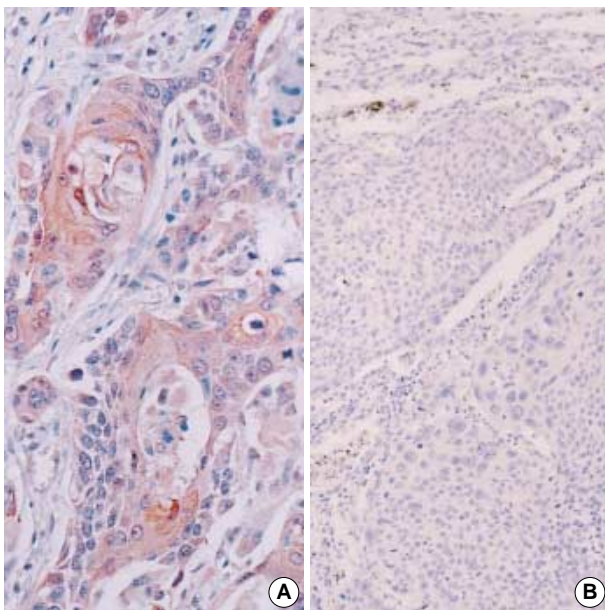


Fig. 1. Immunohistochemical findings of phosphatase and tensin homolog deleted on chromosome 10 (PTEN) for pulmonary squamous cell carcinomas: one tumor shows positive cytoplasmic immunostaining (A), and the other tumor shows loss of PTEN expression (B).

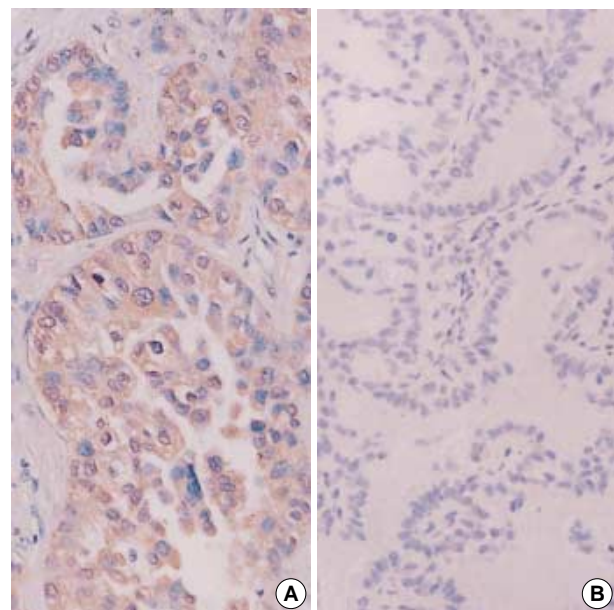


Fig. 2. Immunohistochemical findings of phosphatase and tensin homolog deleted on chromosome 10 (PTEN) for pulmonary adenocarcinomas: one tumor shows positive cytoplasmic immunostaining (A), and the other tumor shows loss of PTEN expression (B).

Table 1. Relation between loss of PTEN expression and clinicopathological characteristics in primary lung cancers

| Clinicopathological characteristics | No. of cases | PTEN expression | | p value |
|-------------------------------------|--------------|----------------------|----------------------|---------|
| | | Positive (%) n=58 | Negative (%) n=47 | |
| Histological subtype | | | | <0.05 |
| Squamous cell carcinoma | 50 | 18 (36.0) | 32 (64.0) | |
| Adenocarcinoma | 42 | 33 (78.6) | 9 (21.4) | |
| Small cell carcinoma | 2 | 0 (0) | 2 (100) | |
| Large cell carcinoma | 9 | 6 (67.0) | 3 (33) | |
| Others | 2 | 1 (50.0) | 1 (50) | |
| Tumor size | | | | NS |
| ≤3 cm | 24 | 15 (62.5) | 9 (37.5) | |
| >3 cm | 81 | 43 (53.1) | 38 (36.9) | |
| Lymph node metastasis | | | | NS |
| Negative | 59 | 36 (61.1) | 23 (38.9) | |
| Positive | 46 | 22 (47.8) | 24 (52.2) | |
| Stage | | | | NS |
| 1 | 30 | 19 (63.3) | 11 (36.7) | |
| 2 | 32 | 18 (56.3) | 14 (43.7) | |
| 3 | 43 | 21 (48.8) | 22 (51.2) | |

PTEN: phosphatase and tensin homolog deleted on chromosome 10.

cinomas, 9 large cell carcinomas, and 2 other tumors (1 atypical carcinoid and 1 carcinosarcoma). Forty six patients showed regional lymph node metastasis. Eleven patients were in stage 1A, 19 in stage 1B, 8 in stage 2A, 24 in stage 2B, 38 in stage 3A, and 5 in stage 3B.

Loss of PTEN expression and its relationship with clinicopathological characteristics

Loss of PTEN expression was seen in 47 (44.8%) of the 105 lung cancers evaluated. Loss of PTEN expression significantly correlated with histologic type ($p < 0.05$). 32 cases (64%) of the squamous cell carcinomas showed loss of PTEN expression (Fig. 1), whereas only 9 cases (21.4%) of adenocarcinomas showed loss of PTEN expression (Fig. 2). Although the number of studied cases is small, 100% of the small cell carcinomas showed loss of PTEN expression. There was no significant association between the loss of PTEN expression and lymph node metastasis, although a trend was observed. There was no correlation between tumor size and stage. The results of the pattern of immunohistochemical staining of PTEN protein and its relationship with clinicopathological characteristics are summarized in Table 1.

Relationship between loss of PTEN expression and expression of p53

Expression of p53 protein was detected in 55 (52.4%) of the 105 lung cancers evaluated (Fig. 3). There was no significant correlation between loss of PTEN expression and p53 expression (Table 2). However, p53 expression was more frequent in tumors

with loss of PTEN expression (61%) than in tumors with PTEN expression (44.8%). When the expressions of PTEN and p53 were analyzed together, no four possible combined PTEN/p53 phenotypes significantly correlated with tumor size, lymph node metastasis, and stage (Table 3).

DISCUSSION

PTEN is a candidate tumor suppressor that appears to have a multifunctional role involved in cell proliferation, migration, and invasion.⁵ *PTEN* shows homology at the amino terminus to

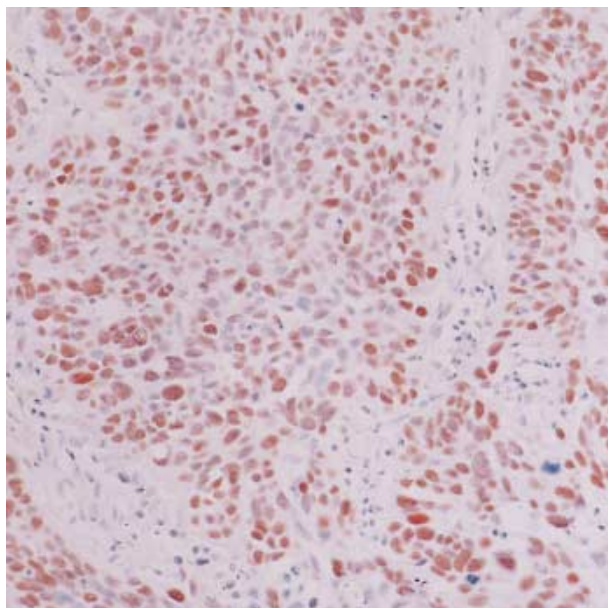


Fig. 3. The lung cancer shows overexpression of 53 by immunohistochemical study.

cytoskeletal proteins tensin and auxilin and contains a critical catalytic phosphatase core and two phosphotyrosine acceptor motifs. *PTEN* exhibits characteristics of a dual-specificity phosphatase and functional evidence that demonstrates its ability to suppress glioma cell growth through its phosphatase activity has been provided. Mutations present in any of the crucial domains of *PTEN* result in reduced phosphatase activity which affects its growth suppression activity.²⁴ Overexpression of *PTEN* suppresses tumor colony formation in certain cell lines and can suppress tumor formation in nude mice.²⁴ *PTEN* overexpression can also negatively regulate cellular adhesion and cell mobility on fibronectin-coated plates.⁵ This activity may result from *PTEN*-mediated dephosphorylation of focal adhesion kinase. *PTEN* may also alter mitogen-activated protein kinase signaling.²⁵

In non-small cell lung cancer (NSCLC), frequent chromosomal deletions were detected in 3p, 9p, and 17p, while relatively few 10q deletions were observed.²⁶ Yokomizo *et al.*²⁷ demonstrated that the *PTEN* gene had no alterations in 13 NSCLC cell lines and 10 tumors, which suggested that the *PTEN* gene did not contribute to NSCLC tumorigenesis.

However, in this study, a significant loss of *PTEN* expression (48.8%) in lung cancer study groups was found. A few studies reported allelic loss of *PTEN* in 41% of NSCLC and 45.8% of

Table 2. Relation between PTEN and p53 expression in primary lung cancers

| p53 | No. of cases | PTEN expression | | p value |
|----------|--------------|----------------------|----------------------|---------|
| | | Positive (%) n=58 | Negative (%) n=47 | |
| Positive | 55 | 26 (47.3) | 29 (52.7) | NS |
| Negative | 50 | 18 (36.0) | 32 (64.0) | |

PTEN: phosphatase and tensin homolog deleted on chromosome 10.

Table 3. Relation between combined PTEN and p53 expression and clinicopathological characteristics in primary lung cancers

| Clinicopathological characteristics | No. of cases | PTEN(+)/p53(-) n=32 | PTEN(+)/p53(+) n=26 | PTEN(-)/p53(+) n=29 | PTEN(-)/p53(-) n=18 | p value |
|-------------------------------------|--------------|------------------------|------------------------|------------------------|------------------------|---------|
| Tumor size | | | | | | NS |
| ≤3 cm | 24 | 9 (37.5) | 6 (25.0) | 5 (20.8) | 4 (16.7) | |
| >3 cm | 81 | 23 (28.4) | 20 (24.7) | 24 (29.6) | 14 (17.3) | |
| Lymph node metastasis | | | | | | NS |
| Negative | 59 | 22 (37.3) | 14 (23.7) | 12 (20.3) | 11 (18.7) | |
| Positive | 46 | 10 (21.7) | 12 (26.1) | 17 (37.0) | 7 (15.6) | |
| Stage | | | | | | NS |
| 1 | 30 | 13 (43.3) | 6 (20.0) | 6 (20.0) | 5 (16.7) | |
| 2 | 32 | 9 (28.1) | 9 (28.1) | 9 (28.1) | 5 (15.7) | |
| 3 | 43 | 10 (23.2) | 11 (25.6) | 14 (32.6) | 8 (18.6) | |

PTEN : phosphatase and tensin homolog deleted on chromosome 10.

and over 30% of the lung cancers.^{18,19,27} Hosoya *et al.*¹⁸ reported that the overall deletion rate of *PTEN* locus was 75% in small cell carcinomas, 33.3% in squamous cell carcinomas, and 27.3% in non-small non-squamous cell carcinomas, whereas Petersen *et al.*¹⁹ using a microsatellite marker, reported that allelic loss was frequently seen in small cell carcinomas and that none of the non-small non-squamous cell carcinomas had an allelic deletion at this locus. Our finding showed results that were relatively similar to those of previous studies.^{18,19,27} The cause for mild discrepancy may be explained by technical differences among the studies, including those arising from immunohistochemistry for protein product and microsatellite polymorphism analysis for the alteration of gene locus. Although a mild discrepancy between the previous reports and our study was noted, we define that *PTEN* may play a role in tumorigenesis of the lung.

Loss of *PTEN* expression significantly correlated with histologic type in this study group. 32 cases (64%) of squamous cell carcinomas showed loss of *PTEN* expression, whereas only 9 cases (21.4%) of adenocarcinomas showed loss of *PTEN* expression. Although the number of studied cases is small, 100% of small cell carcinomas showed loss of *PTEN* expression. This study and previous data^{18,19,27} indicate that *PTEN* mutations more commonly contribute to the pathogenesis and neoplastic evolution in small cell carcinomas than in NSCLC. As seen in this study showing different results of *PTEN* expression between squamous cell carcinoma and adenocarcinoma, the *PTEN* might have a cell-type specific, different role in the tumorigenesis of NSCLC.

The correlation of the loss of *PTEN* expression resulting in lung cancer has not been well described in the literature. *PTEN* alterations are more common in benign tumors than in malignant thyroid tumors¹⁴, and they also occur in a proportion of cases of endometrial hyperplasia, a precursor of endometrial carcinoma,¹³ which suggest that the genetic alterations may occur at an early stage in these tumors. In contrast, these mutations may evolve during the metastatic process of prostate cancer,¹¹ melanomas,¹⁶ and breast cancers.¹⁰ *PTEN* appears to play a role in the initiation of certain tumors, including a murine form of prostate cancer, and may play a role in the progression of other tumors such as gliomas and prostate cancer.¹¹ Although seemingly paradoxical, the role of *PTEN* loss as an initiating event versus its role as an agent of progression might arise from fundamental differences between tissues with respect to the order of additional various oncogenic events. Hosoya *et al.*¹⁸ reported that allelic loss at the primary site and that at the metastatic site of each patient was identical in the lung. In this study, loss of *PTEN* expression did not show any correlation with tumor size, lymph node metast-

sis, and stage. Therefore, it is suggested that *PTEN* may evolve during the initiating process of lung cancer.

Malignant transformation in the lung is associated with a number of molecular alterations including the activation of the *ras* and *myc* oncogene families and disruptions of tumor suppressor genes including *p53*, *pRb*, *CDKN2* and *FHIT*.²⁸ The most common molecular alteration in lung cancer is mutation of the *p53* suppressor gene.²⁰ *p53* is involved in cell cycle control, DNA repair, apoptosis, cellular differentiation, senescence and angiogenesis.²¹ A few studies about the relationship between *PTEN* and *p53* have been reported.^{29,30} Kato *et al.*²⁹ screened the mutations of two major tumor suppressor genes, *p53* and *PTEN*, in brain tumors using a yeast-based functional assay and cDNA-based direct sequencing, respectively. The frequency of *p53* mutations was higher in anaplastic astrocytoma than in glioblastoma multiforme; whereas *PTEN* mutation was observed mainly in glioblastoma multiforme rather than in anaplastic astrocytoma. They reported that the mutation of the *PTEN* gene is a later event than that of the *p53* gene in glioma progression. Stambolic *et al.*³⁰ reported that a *p53*-independent element controlling constitutive expression of *PTEN* was identified and that the induction of *p53* in primary and tumor cell lines with wild-type *p53* increased *PTEN* mRNA levels. This is in contrast to *p53* mutant cell lines. However, using immunohistochemistry, this study found no definitive relationships between the loss of *PTEN* expression and *p53* expression or between their combined phenotypes and clinicopathologic characteristics. Further studies of this gene in lung cancer patients and identification of the putative tumor suppressor gene close to *PTEN* on 10q23 in lung cancer appear to be warranted.

In conclusion, it is suggested that significant loss of *PTEN* expression occurs commonly in primary lung cancers and correlates with histologic type. Although the genetic status of *PTEN* in lung cancers was not assessed, loss of *PTEN* expression as assessed by immunohistochemistry might reflect a majority of the possible mechanisms resulting in *PTEN* inactivation. Our results support the proposed role of *PTEN* as a candidate tumor suppressor in lung cancer, and we suggest that further studies of this marker are needed.

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