Detection of methylation and deletion of p16 gene in non-small cell lung cancer

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Objective To investigate the methylation and deletion of p16 gene and its diagnostic value in non-small cell lung cancer.

Methods A total of 50 lung cancer tissues and 54 normal lung tissues were examined for p16 gene methylation in exon 1 and deletion in exon 2 by PCR based methylation analysis and duplex PCR respectively.

Results Out of 50 lung cancer tissues, 16 were positive for the p16 gene exon 1 methylation and 14 for the p16 gene exon 2 deletion. However, in 54 cases of normal lung tissues, only 2 showed the p16 gene exon 1 methylation and none showed the p16 gene exon 2 deletion. There were significant differences in methylation rates and deletion rates between the two groups.

Conclusion The methylation and deletion may be important mechanisms for p16 gene inactivation in non-small cell lung cancer. The detection of p16 gene status may contribute to the diagnosis of lung cancer.

Key words Lung neoplasms p16 gene Methylation Deletion

This work was supported by a grant from the Natural Sciences Foundation of Jiangsu Province to WANG Xu and by a grant from the Health Office of Jiangsu Province to WANG Xu.

DOI: 10.3779/j.issn.1009-3419.2002.04.03

p16 1994 multiple tumor suppressor 1 MTS1 5′ CpG 3′ PCR NSCLC p16 1 5′ 2 Sma I Sac II 2 9808H 4 221002 223800
1.2 DNA genotyping

Genomic DNA containing 200 mg of tissue and 752 mg of DNA, was prepared using an RNAIQ DNA extraction kit. DNA samples were prepared using the Quant-IT Pico Green DNA assay kit. DNA concentration was measured using an UV spectrophotometer.

1.3 Primers

The primers used in this study were designed with Primer3 software (http://frodo.wi.mit.edu/) and were synthesized by Sangon Biotech (China).

1.4.1 PCRs

DNA was extracted from the samples using a standard phenol-chloroform method. The PCR reaction was performed in a final volume of 30 μL containing 1 μL of DNA template, 1.5 units of Taq DNA polymerase, 1.5 μM of each primer, 1× Taq buffer, and deoxynucleotide triphosphates (dNTPs) (Promega). The reaction was performed in a thermal cycler with the following profile: initial denaturation at 94°C for 5 minutes, followed by 35 cycles of 94°C for 30 seconds, 55°C for 40 seconds, and 72°C for 40 seconds, and a final extension at 72°C for 10 minutes.

1.4.2 Gene cyclers

The PCR products were analyzed on 2% agarose gels and stained with ethidium bromide. The gels were photographed under UV light. The bands were excised from the gels and subjected to DNA sequencing.

1.5 DNA sequencing

DNA sequencing was performed using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). The PCR products were purified using the QIAquick PCR Purification Kit (QIAGEN) and then sequenced using the previously designed primers. The sequencing reactions were run on an ABI 3730 DNA Analyzer (Applied Biosystems).

1.6 Analysis

Statistical analysis was performed using the chi-square test (Fisher's exact test). The significance level was set at p < 0.05.

2 Results

2.1 DNA methylation patterns were analyzed using methylation-specific PCR (MSP) (1-3). The results showed that the methylation patterns were specific to cancer cells. The methylation patterns were also specific to the location of the DNA methylation sites.

Fig 1 The methylation of p16 gene. Sma I site.

Molecular markers: 1 Squamous cell carcinoma; 2 Adenosquamous carcinoma; 3 Adenocarcinoma. Lane 2 was 100 bp ladder. Lane 5 showed p16 gene methylation.

Fig 2 The methylation of p16 gene. Sac II site.

Molecular markers: 1-4 Squamous cell carcinoma; 5-6 Adenocarcinoma. Lane 2 was 100 bp ladder. Lane 3 showed p16 gene methylation.
### Tab 1 Methylation and deletion of p16 gene in NSCLC

<table>
<thead>
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<th>Clinic data</th>
<th>n</th>
<th>Exon 1 methylation</th>
<th>Exon 2 deletion</th>
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<tbody>
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<td></td>
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<td>Ra%</td>
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<tr>
<td>Age years ≤ 50</td>
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<tr>
<td>≥ 50</td>
<td>39</td>
<td>33.3</td>
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</table>

2.2 p16 and β-actin were co-amplified 250 bp products for NSCLC. p16 28.0% 14/50. S4 PCR amplified 456 bp. p16 1006 bp oligonucleotides. *Fisher’s exact test* = 0.000. p16 vs 1006 bp oligonucleotides. *Fisher’s exact test* 0.022 0.047 3 1

![Fig 3](image_url) The deletion of p16 gene exon 2. Molecular markers 1/2/4/5/8 Squamous cell carcinoma, 3/6 Adenocarcinoma. Lane 4/6 showed exon 2 deletion of p16 gene.

3 p16 40 kb 9p21 40 kb CDK4 5p16 19% 5/26 p16 100% 10% 90% Belinsky 70% p16 43% 3/7 p16 19% Palmisano 80% p16 100% MGMT 5~35 0

![Diagram](image_url)
分别为而与组织学类型和临床分期有密切关系（*+）。

基因的缺失是基因甲基化, 该基因的研究可外显子的缺失, 总缺失4-6例正常肺组织无缺失患者中, 鳞癌腺鳞癌无缺失;4-7等位点的基因纯合缺失的研究。...

第25.8% 23/89例 例缺失6例。...

癌组织中可能已有癌细胞的浸润, 而病理学检查未能发现。周清华, 易成, 侯梅, 等在肺癌组织...等甲基化位点, 而本研究仅检测4例患者。

基因的甲基化可以用于预测肿瘤行为。...

美国癌症协会国际肿瘤研究所和美国国家癌症研究所报道相近,

*+* Fisher’ s exact test 0.022 0.047 42.9% 5% 47.1% p16 ～ p16 ～ p16 ～ p16 ～ p16 ～ p16 ～ p16 ～ p16 ～ p16 ～ p16 ～ p16 ～ p16 ～ p16 ～ p16 ～ p16 ～ p16