Identification of Epigenetic Aberrant Promoter Methylation in Serum DNA Is Useful for Early Detection of Lung Cancer

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ABSTRACT

Purpose: The purpose of this study is to evaluate the usefulness of serum DNA methylation of five tumor suppressor genes for early detection of lung cancer.

Experimental Design: Methylation status in serum DNA from 200 patients undergoing bronchoscopy examination for abnormal findings on chest radiograph detected by lung cancer screening or surveillance was examined using methylation-specific PCR.

Results: Ninety-one patients were given a pathologic diagnosis of lung cancer, 9 other malignant diseases, and 100 nonmalignant pulmonary diseases. In patients with lung cancer, methylation was detected in 18.7% for MGMT, 15.4% for p16 INK4a, 12.1% for RASSF1A, 11.0% for DAPK, and 6.6% for RAR-β, which was higher compared with that in patients with nonmalignant diseases. Age and smoking status seemed to associate with methylation status. Sensitivity, specificity, and predictive value of methylation in at least one gene for diagnosis of lung cancer were 49.5%, 85.0%, and 75.0%, respectively. Adjusted odds ratio (95% confidence interval) for having lung cancer was 5.28 (2.39-11.7) for patients with methylation in one gene and 5.89 (1.53-22.7) for those with methylation in two or more genes. It is of note that methylation was identified in 50.9% of stage I lung cancer patients, whereas serum protein tumor markers were positive in 11.3% of them.

Conclusions: These results suggest that identification of promoter methylation of tumor suppressor genes in serum DNA could be useful for early detection of lung cancer.

INTRODUCTION

Despite intensive treatment, the prognosis of patients with lung cancer is poor. The 5-year survival rate of patients with clinical stage I disease is ~60%, but in those with clinical stage II to IV diseases, the 5-year survival rate ranges from 40% to <5% (1). Thus, the prognosis of lung cancer is strongly correlated to its clinical stage. Over two thirds of lung cancer patients have an advanced disease at the time of initial presentation (2), and lack of efficient diagnostic methods for early detection is considered to be the major reasons for the poor prognosis of lung cancer.

Although lung cancer screening with annual chest radiograph and sputum cytology is currently conducted in many municipalities in Japan (3), the usefulness of mass screening is yet to be fully confirmed. The previous screening trials sponsored by the National Cancer Institute failed to show that screening with sputum cytology and chest radiography reduced mortality from lung cancer (4–7). However, because one of these trials indicated more favorable survival rates associated with the diagnosis of resectable tumors, the American Cancer Society maintains that physicians and patients may decide to have these screening tests on an individual basis (8). Therefore, the development of more useful method in addition to the chest radiograph and sputum cytography for lung cancer screening is urgently required.

Aberrant methylation of CpG islands, which are in or near the promoter region of various genes, is a common feature in various neoplasms and is associated with the transcriptional silencing of tumor suppressor genes (9–11). In addition, this alteration has been described to occur in the very early stage of carcinogenesis (12). Recent advances in techniques simplified the methods for identification of promoter methylation, among which methylation-specific PCR (MSP) is a simple, sensitive, and specific method to determine the methylation status of any CpG-rich region (13).

Several studies have shown that several genes, including tumor suppressor genes, such as retinoic acid receptor β (RARβ; ref. 14) and p16 INK4a (15, 16), apoptosis-associated genes, such as death-associated protein kinase (DAPK; ref. 17) and ras association domain family 1A (RASSF1A; refs. 18, 19), and the DNA repair gene O6-methylguanine DNA methyltransferase (MGMT; refs. 20, 21), were frequently methylated in lung cancer cells. Zochbauer-Muller et al. showed that 82% of the non–small cell lung cancer tissues had methylation of at least one gene from eight genes and rarely identified methylation of these genes in nonmalignant lung tissue (20). These findings suggest the potential use of DNA methylation as a marker for lung cancer.
It has been shown that cancer patients have increased levels of free DNA in their sera, which is thought to be released from cancer cells (22–24). Many investigators have reported that microsatellite alterations and p53 and/or ras gene mutations could be identified in the serum and/or plasma DNA of patients with various cancers (25–28). Thus, circulating tumor-derived DNA might be used as a source for tumor detection by PCR analysis, including MSP.

In the present study, we attempted to identify methylated DNA in sera of patients with abnormal findings on their chest radiograph as detected by lung cancer screening or physician surveillance. Although there are some recent reports of DNA methylation analyses carried out with remote medium, including serum, plasma, sputum, and bronchoalveolar lavage fluid or brushing samples, this is the first report that has examined the methylation status of serum DNA on a population basis and showed the usefulness of the approach as a diagnostic tool for early detection of lung cancer.

MATERIALS AND METHODS

Sample Collection and DNA Extraction. In this study, 200 patients undergoing fiberoptic bronchoscopy for abnormal findings on chest radiograph were investigated. The examinations were carried out as part of the lung cancer mass screening program in Okayama Prefecture (3) or through physician surveillance. Diagnosis of these patients was completely blinded to the laboratory researchers. Peripheral blood samples (6 mL) were collected to investigate methylation status of serum DNA with written informed consent. Serum (2 mL) was isolated after centrifugation at 3,000 rpm for 10 minutes and stored at −20°C until use. Serum DNA was extracted using QIAamp DNA Blood Midi kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions. We also examined methylation status of the tumor tissues. Of 200 patients evaluated in this study, 30 with lung cancer underwent surgical resection at Department of Cancer and Thoracic Surgery of Okayama University Hospital. Tumor tissues from these patients were investigated with written informed consent. Tumor DNA was extracted from formalin-fixed, paraffin-embedded lung cancer tissues using QIAamp DNA Mini kit (Qiagen) according to manufacturer’s instructions.

Methylation-Specific PCR. Sample DNA was treated with sodium bisulfite using the CpGenome DNA Modification kit (Intergen, Purchase, NY) according to the manufacturer’s instructions. All bisulfite-modified DNA was resuspended in TE buffer [10 mmol/L Tris-0.1 mmol/L EDTA (pH 7.5)] and used immediately or stored at −20°C until subsequent MSP. Primer sequences for the RARB, p16INK4a, DAPK, RASSF1A, and MGMT were as described elsewhere (18, 20). DNA from a small cell lung cancer cell line SBC-3 (29), which has promoter methylation of all tested genes, was used as a positive control for the methylated form and that from serum of normal volunteer, positive control for methylated and unmethylated sequences, respectively; DNA from SBC-3 cell line and normal volunteer, positive control for methylated and unmethylated forms, respectively.

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Fig. 1 Representative results showing promoter methylation amplified by MSP. Lanes M and U, amplified product with primers recognizing methylated and unmethylated sequences, respectively; DNA from SBC-3 cell line and normal volunteer, positive control for methylated and unmethylated forms, respectively.
Statistical Analysis. The methylation status in five genes was scored in each patient as the total number of methylated genes. The methylation score of patients with lung cancer was compared with those with nonmalignant diseases using a t test with unequal variance. An unconditional logistic regression model was applied to estimate the odds ratios and its 95% confidence intervals for the occurrence of lung cancer. We used the patients without methylation in every five genes as a reference group and estimated the odds ratios for patients having one methylated gene and those having two or more methylated genes in five genes tested. Crude and multivariate models were examined. The factors adjusted in the multivariate model included age as a continuous variable divided by 10, sex, smoking status divided into quartiles (pack-years calculated by number of packs smoked per day multiplied by the number of years of smoking), and results of three tumor markers (carcinoembryonic antigen, cytokeratin 19 fragment, and progastrin releasing peptide) in a binary variable. \( \chi^2 \) or Fisher’s exact test were applied to examine the distribution in categorical variables. A t test was applied to test the continuous variables. The statistical significance was defined as \( P < 0.05 \). All the statistical analyses were implemented by Stata version 8 (College Station, TX).

RESULTS

Patients Characteristics. Between January 2001 and December 2002, a total of 200 peripheral blood samples were collected from consecutive patients undergoing fiberoptic bronchoscopy. Of these patients, 91 were given a pathologic diagnosis of lung cancer (median age, 71 years; range, 45-92 years; male/female 61:30), 100 nonmalignant diseases (median age, 65.5 years; range, 26-89 years; male/female 64:36), and 9 other malignancies. The histologic subtypes of the lung cancers, based on WHO classification (30), were adenocarcinoma in 64 patients, squamous cell carcinoma in 21 patients, small cell carcinoma in 4 patients, and carcinoid in 2 patients. Clinical stage classifications, based on the International Staging System (1), were as follows: 53 patients had stage I disease, 7 patients stage II, 22 patients stage III, and 9 patients stage IV. Nonmalignant diseases mostly consisted of benign pulmonary diseases, such as tuberculosis, atypical mycobacteriosis, pneumoconiosis, interstitial pneumonia, bronchitis, organizing pneumonia, and bronchiectasis. Malignant diseases other than lung cancer included pulmonary metastasis of laryngeal cancer in 3 patients, invasive thymoma in 2 patients, and non-Hodgkin’s lymphoma, thyroid cancer, breast cancer, and rectal cancer in 1 patient each. Elderly patients were more frequently represented among patients with lung cancer than those with nonmalignant diseases (median age, 71 versus 65.5 years; \( P < 0.001 \)). Current smokers were more commonly represented among patients with lung cancer than those with nonmalignant diseases (67% versus 54%; \( P = 0.046 \)).

Methylation Status of Five Genes. As shown in Table 1, serum methylated DNA was detected in 18.7% for MGMT, 15.4% for \( p16^{INK4a} \), 12.1% for RASSF1A, 11.0% for DAPK, and 6.6% for RAR\( \beta \) in lung cancer patients. When analyzed individually, the proportions of patients with methylated serum DNA were higher in patients with lung cancer than those with nonmalignant diseases in every five genes. Difference is especially evident for \( p16^{INK4a} (P = 0.003) \) and MGMT (\( P < 0.001 \)). Of 91 lung cancer patients, 45 (49.5%) had methylation of at least one gene. When methylation of at least one gene was assessed as positive, specificity and predictive values of methylation were 85.0% and 75.0%, respectively. The total number of methylations in five genes per patient was 0.64 in patients with lung cancer, which was higher than that in patients with nonmalignant diseases (0.19; \( P < 0.0001; \) Table 2). Of 9 patients with malignant diseases other than lung cancer, 6 (66.7%) had at least one methylation in five genes (data not shown). These 9 cases were excluded from subsequent analyses.

Twenty-three of 30 (77%) tumor tissues obtained from lung cancer patients showed methylation of at least one gene (Fig. 2). Sixteen of 18 (89%) tissues from patients with serum DNA methylation also had methylated genes. Methylation of RASSF1A gene was identified in one serum sample and MGMT gene in two samples, but they were not identified in the corresponding tumor.

Association with Clinicopathologic Features. We analyzed the correlations between methylation status in serum DNA and clinicopathologic variables of the patients. There was no correlation between methylation status and sex or histology in this study. In addition, frequency of serum DNA methylation between smokers and nonsmokers was not significantly different in both patients with lung cancer (48.3% in nonsmokers, 45.8% in <40 pack-years smokers, and 52.6% in \( \geq 40 \) pack-years smokers; \( P = 0.863 \)) and those with nonmalignant diseases (Table 3). These findings were also observed when analyzed in individual gene. Although serum DNA methylation in \( \geq 40 \) pack-years smokers with nonmalignant diseases tended to be more frequent than that in <40 pack-years smokers, this trend was particularly obvious in DAPK and RASSF1A genes. In control group, we found significant correlation between methylation and age (Table 3).

Methylation Status and Risk of Lung Cancer. Table 4 shows the results of a crude and adjusted logistic regression analyses evaluating correlation between number of methylated genes and risk of lung cancer. In the crude model, the patients with one methylated gene showed 5.08 (95% confidence interval, 2.28-11.3) times higher probability of having lung cancer compared with patients without any methylated genes. The odds ratio was higher in patients with two or more methylated genes. To consider the imbalance in baseline characteristics, we conducted similar analysis adjusting for age,

<table>
<thead>
<tr>
<th>Gene</th>
<th>Lung cancer (( n = 100 ))</th>
<th>Nonmalignancy (( n = 100 ))</th>
<th>Total (( n = 191 ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>MGMT</td>
<td>17 (18.7)</td>
<td>2 (2.0)</td>
<td>19 (9.9)</td>
</tr>
<tr>
<td>( p16^{INK4a} )</td>
<td>14 (15.4)</td>
<td>3 (3.0)</td>
<td>17 (8.9)</td>
</tr>
<tr>
<td>RASSF1A</td>
<td>11 (12.1)</td>
<td>8 (8.0)</td>
<td>19 (9.9)</td>
</tr>
<tr>
<td>DAPK</td>
<td>10 (11.0)</td>
<td>5 (5.0)</td>
<td>15 (7.9)</td>
</tr>
<tr>
<td>RAR( \beta )</td>
<td>6 (6.6)</td>
<td>1 (1.0)</td>
<td>7 (3.7)</td>
</tr>
</tbody>
</table>
sex, smoking status, and protein tumor marker results. The patients with methylation in at least one gene and two or more of five genes showed 5.28 (2.39-11.7; \( P < 0.001 \)) and 5.89 (1.53-22.7; \( P = 0.010 \)) times higher probability of having lung cancer, respectively. 

**Frequencies of Methylation According to Clinical Stage of Lung Cancer.** We investigated the correlation between clinical stage and methylation status in five genes or conventional serum protein tumor markers (Table 5). Of 53 patients with stage I disease, 27 (50.9\%) patients had methylated serum DNA in at least one gene, whereas only 6 (11.3\%) patients showed elevation of at least one serum protein tumor marker. In patients with stage II, III, or IV diseases, the difference was not evident.

**DISCUSSION**

This study shows that identification of serum DNA methylation is a potentially useful approach to detect lung cancer patients from subjects screened by chest radiograph. Serum DNA methylation was more frequently observed in patients with lung cancer than those with nonmalignant diseases. Although the sensitivity for the diagnosis of lung cancer was only 49.5\% when analyzed by a combination of five genes, the relatively high specificity (85.0\%) indicates the usefulness for subjects screened by with chest radiograph. The odds ratio for diagnosis of lung cancer was >5.0 in patients with at least one methylated gene even after statistical adjustment by other clinicopathologic risk factors, such as smoking, age, sex, and results of tumor marker tests. Of note, serum DNA methylation could be identified even in patients in the early stages of lung cancer, whereas conventional serum protein tumor markers were rarely elevated, indicating that this DNA-based method is more sensitive than protein-based method for diagnosis of lung cancer in early stage.

In former studies, methylation in tumor tissues was detected in 40\% to 43\% of non–small cell lung cancer patients for **RARβ**, 25\% to 41\% for **p16INK4a**, 16\% to 44\% for **DAPK**, 30\% to 40\% for **RASSF1A**, and 16\% to 27\% for **MGMT** (31). These results were consistent with our data in 30 corresponding tissue and serum samples. In our experiment, the frequency of detecting methylated genes in serum was about half to two thirds compared with that in tumor tissues. However, when we consider that tumor-derived DNA in blood is generally detectable in less than half of cancer patients (32), the frequency of methylation in serum DNA in our study may be reasonable. Laird reviewed the studies examining methylation status of serum/plasma DNA in patients with various neoplasms and indicated that clinical sensitivity of DNA methylation was ~50\% (33). Esteller et al. did methylation analysis in serum DNA from patients with non–small cell lung cancer for multiple genes and showed 33\% to 80\% of clinical sensitivity by combination analysis of these genes (34). Our results are consistent with the results of these studies, indicating that similar sensitivity is achievable even after mass screening.

Among various techniques used for methylation analysis, we adopted a simple method of qualitative MSP analysis. The specificity of the primers we used in this study had been verified using genomic sequencing and/or restriction analysis in previous reports (13, 35). Recently, several studies showed improved detection rates of methylation status using a nested PCR approach or a quantitative real-time PCR technique (36–38). Particularly, sensitivity of the Taqman method was reported to be 10-fold higher than conventional qualitative MSP (39). To apply DNA methylation as tumor marker for detection of lung cancer, the use of these improved methods is an attractive strategy.

Although promoter methylation was observed predominantly in lung cancer patients, 1\% to 8\% of patients with nonmalignant disease were methylation positive for each gene in this study. In addition, three lung cancer patients with serum DNA methylation did not show same alteration in the corresponding tumor tissues. We considered the following as possible explanation of these positive results. Firstly, the methylated serum DNA might be derived from undetected precancerous lesions in these cases. According to the previous reports, aberrant promoter methylation is detectable in precancerous lesions, such as dysplasia and nonmalignant lung tissues of patients with lung cancer (20, 37). Methylation-positive nonmalignant patients may develop malignant diseases in the near future. Secondly, aberrant methylation might be caused by environmental factors, such as smoking (12, 40). In

**Table 2** Comparison of methylation status between lung cancer and nonmalignant cases

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>SE</th>
<th>SD</th>
<th>95% Confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonmalignancy</td>
<td>0.19</td>
<td>0.0506</td>
<td>0.0506</td>
<td>0.09-0.29</td>
</tr>
<tr>
<td>Lung cancer</td>
<td>0.64</td>
<td>0.0774</td>
<td>0.738</td>
<td>0.48-0.79</td>
</tr>
</tbody>
</table>

*Total number of methylations in five genes per patient.

\( t \) test with unequal variance.

![Fig. 2](clincancerreses.aacrjournals.org) Summary of methylation of **RARβ**, **p16INK4a**, **DAPK**, **RASSF1A**, and **MGMT** in 30 corresponding tissue and serum samples. **Black boxes**, methylated samples; **white boxes**, unmethylated samples.
published series, controversial correlations between smoking and methylation have been reported (20, 41–44). We did not find any statistically significant correlation between smoking history and methylation; however, heavy smokers had at least one serum DNA methylation more frequently than mild to moderate smokers in patients with nonmalignant disease. The third explanation is possible occurrence of other occult malignancies. Indeed, we observed methylated serum DNA in 66.7% of the patients who were diagnosed as having another malignant disease. The final explanation is the possibility of detecting age-related methylation in control group.

To use this serum DNA methylation as a marker in lung cancer mass screening, several issues must be considered. The fairy good specificity even in patients screened by chest radiograph suggests the advantage of this approach. On the other hand, poor sensitivity may compromise the advantage of specificity. Improving sensitivity as a mass screening test might be achieved by two approaches. One is to increase sensitivity of DNA methylation itself by using large number of tested genes or applying a quantitative methylation assay. The other is to combine the methylation with highly sensitive screening method such as low-dose spiral computed tomography (45–47). Because one of the serious limitations of low-dose spiral computed tomography is its poor specificity (48), combination with the serum DNA methylation may overcome the limitation. Indeed, physicians often experience difficulty in sampling tumor specimen from small legions detected by computed tomography scans for pathologic diagnosis by invasive procedures, such as fiberoptic bronchoscopic examination or computed tomography–guided fine needle aspiration. Accordingly, in consideration of relatively higher frequency of serum DNA methylation in early-stage disease (50.9% in stage I) and serious complications after invasive procedures (49), serum DNA methylation may be a test to be conducted before invasive procedure. Although further evaluation is essential, the results in this study indicate the substantial usefulness for detection of lung cancer.

In conclusion, we examined the aberrant promoter methylation status in serum DNA and showed the usefulness of this approach as a tool for detection of lung cancer in patients screened by chest radiograph. Further studies are warranted to confirm the efficiency of the procedure and search for best combination of genes for methylation analysis. Moreover, it is important to investigate prospectively whether methylation-positive noncancer cases will have malignancies in the near future.

**ACKNOWLEDGMENTS**

We thank Dr. Tsuyoshi Kodani and colleagues (Okayama Institute of Health and Science) for collecting clinical and pathologic data and Drs. Haruyuki Kawai (Okayama Saiseikai General Hospital) and Youichiro Ogama (Okayama University Medical School) for technical advice.

<table>
<thead>
<tr>
<th>Smoking status</th>
<th>RARβ</th>
<th>p16INK4a</th>
<th>DAPK</th>
<th>RASSF1A</th>
<th>MGMT</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonsmoker</td>
<td>0</td>
<td>4.4</td>
<td>2.2</td>
<td>6.5</td>
<td>2.2</td>
<td>13.0</td>
</tr>
<tr>
<td>Pack-years &lt;40</td>
<td>0</td>
<td>0</td>
<td>4.8</td>
<td>4.8</td>
<td>0</td>
<td>9.5</td>
</tr>
<tr>
<td>Pack-years ≥40</td>
<td>3</td>
<td>3</td>
<td>9.1</td>
<td>12.1</td>
<td>3.0</td>
<td>21.2</td>
</tr>
<tr>
<td>Odds ratio</td>
<td>0.359</td>
<td>0.626</td>
<td>0.379</td>
<td>0.550</td>
<td>0.736</td>
<td>0.442</td>
</tr>
</tbody>
</table>

*Age quartile was defined as follows: 1, <59; 2, ≥59 and <69; 3, ≥69 and <73; and 4, ≥73.

**Table 3** Correlation between clinical features and methylation of the different genes in nonmalignant cases

<table>
<thead>
<tr>
<th>Smoking status</th>
<th>RARβ</th>
<th>p16INK4a</th>
<th>DAPK</th>
<th>RASSF1A</th>
<th>MGMT</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonsmoker</td>
<td>0</td>
<td>4.4</td>
<td>2.2</td>
<td>6.5</td>
<td>2.2</td>
<td>13.0</td>
</tr>
<tr>
<td>Pack-years &lt;40</td>
<td>0</td>
<td>0</td>
<td>4.8</td>
<td>4.8</td>
<td>0</td>
<td>9.5</td>
</tr>
<tr>
<td>Pack-years ≥40</td>
<td>3</td>
<td>3</td>
<td>9.1</td>
<td>12.1</td>
<td>3.0</td>
<td>21.2</td>
</tr>
<tr>
<td>Odds ratio</td>
<td>0.359</td>
<td>0.626</td>
<td>0.379</td>
<td>0.550</td>
<td>0.736</td>
<td>0.442</td>
</tr>
</tbody>
</table>

*Age quartile was defined as follows: 1, <59; 2, ≥59 and <69; 3, ≥69 and <73; and 4, ≥73.

**Table 4** Methylation status and risk of lung cancer

<table>
<thead>
<tr>
<th>No. methylations</th>
<th>Cases*</th>
<th>Controls*</th>
<th>Odds ratio</th>
<th>95% Confidence interval</th>
<th>P</th>
<th>Odds ratio</th>
<th>95% Confidence interval</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>46</td>
<td>85</td>
<td>1.00</td>
<td>2.28-11.3</td>
<td>&lt;0.0001</td>
<td>1.00</td>
<td>2.39-11.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>1</td>
<td>33</td>
<td>12</td>
<td>5.08</td>
<td>1.87-29.2</td>
<td>0.0008</td>
<td>5.89</td>
<td>1.53-22.7</td>
<td>0.010</td>
</tr>
<tr>
<td>≥2</td>
<td>12</td>
<td>3</td>
<td>7.39</td>
<td>5.89</td>
<td>0.010</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (10-y increase)</td>
<td>—</td>
<td>—</td>
<td>1.03</td>
<td>1.00-1.07</td>
<td>0.023</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex (male relative to female)</td>
<td>—</td>
<td>—</td>
<td>1.49</td>
<td>0.61-3.64</td>
<td>0.386</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pack-years (one level increase quartile)</td>
<td>—</td>
<td>—</td>
<td>1.00</td>
<td>0.99-1.00</td>
<td>0.207</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tumor marker positive</td>
<td>—</td>
<td>—</td>
<td>3.08</td>
<td>1.32-7.17</td>
<td>0.009</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Cases, lung cancers; controls, nonmalignant diseases.
†Model 1 included number of methylation only. Model 2 included age, sex, pack-years of smoking, and tumor marker result in addition to number of methylation.
Table 5 Frequency of DNA methylation and elevation of conventional serum protein tumor markers according to clinical stage

<table>
<thead>
<tr>
<th>Clinical stage</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methylation</td>
<td>27</td>
<td>3</td>
<td>45</td>
<td>55</td>
</tr>
<tr>
<td>MGMT</td>
<td>10</td>
<td>14</td>
<td>18</td>
<td>22</td>
</tr>
<tr>
<td>p16INK4a</td>
<td>7</td>
<td>0</td>
<td>25</td>
<td>22</td>
</tr>
<tr>
<td>RASSF1A</td>
<td>7</td>
<td>1</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>DAPK</td>
<td>5</td>
<td>1</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>RARβ</td>
<td>3</td>
<td>0</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Tumor marker</td>
<td>6</td>
<td>4</td>
<td>45</td>
<td>77</td>
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<tr>
<td>Carcinoembryonic antigen</td>
<td>5</td>
<td>9</td>
<td>40</td>
<td>55</td>
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<tr>
<td>Cytokeratin 19 fragment</td>
<td>0</td>
<td>0</td>
<td>9</td>
<td>22</td>
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<tr>
<td>Progastrin releasing peptide</td>
<td>1</td>
<td>2.8</td>
<td>9.1</td>
<td>2.2</td>
</tr>
<tr>
<td>Total</td>
<td>53</td>
<td>7</td>
<td>22</td>
<td>9</td>
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REFERENCES

Identification of Epigenetic Aberrant Promoter Methylation in Serum DNA Is Useful for Early Detection of Lung Cancer

Keiichi Fujiwara, Nobukazu Fujimoto, Masahiro Tabata, et al.


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