Use of 11p15 Mucins as Prognostic Factors in Small Adenocarcinoma of the Lung

Noboru Nishiumi,1 Yoshiyuki Abe,3 Yoshimasa Inoue,2 Hiroyuki Hatanaka,2 Ken-ichi Inada,4 Hiroshi Kijima,2 Hitoshi Yamazaki,2 Masae Tatematsu,4 Yoshito Ueyama,2 Masayuki Iwashiki,1 Hiroshi Inoue,1 and Masato Nakamura2

Departments of 1General Thoracic Surgery and 2Pathology, Tokai University School of Medicine, Isehara, Kanagawa; 3Department of Respiratory Disease, National Kanagawa Hospital, Hadano, Kanagawa; and 4Laboratory of Pathology, Aichi Cancer Center Research Institute, Nagoya, Aichi, Japan

ABSTRACT
Purpose: Patients with small adenocarcinoma of the lung (SACL) generally have a good prognosis. However, some SACL cases show lymph node metastasis, with poor prognosis. The expression pattern of 11p15 mucins (clustered on the p15 arm of the chromosome 11) is known to change during carcinogenesis in lung cancer.

Experimental Design: We evaluated the expression of the 11p15 mucins (MUC2, MUC5AC, and MUC6) in 79 surgical specimens of SACL cases by immunohistochemical analysis. Lymph node metastasis was estimated by pathological staging.

Results: Six (7.6%) and 11 (13.9%) of the 79 SACL cases showed MUC2 and MUC6 expression, respectively. Three SACL cases showed both MUC2 and MUC6 expression, and a significant correlation was found between MUC2 and MUC6 expression (Fisher’s test, P = 0.003). Six (7.6%) SACL cases showed MUC5AC expression. Five of the 6 cases with MUC2 expression and 6 of the 11 cases with MUC6 expression were had lymph node metastasis. SACL cases with MUC2 or MUC6 expression showed a significantly higher incidence of nodal metastasis than those without expression (P < 0.001 and P = 0.006, χ2 test, respectively). There was no significant correlation between MUC5AC expression and nodal involvement in SACL, whereas three of the six cases with MUC5AC expression showed lymph node metastasis. The SACL cases with MUC2 expression had a significantly poorer prognosis than those without MUC2 expression (P = 0.011, log-rank test).

Conclusions: These results suggest that 11p15 mucins MUC2 and MUC6 are related to lymph node metastasis in SACL.

INTRODUCTION
Lung cancer is one of the most common malignant diseases in the world, and its prognosis is generally poor. Among several histological types of lung carcinoma, adenocarcinoma of the lung makes up approximately half of the non-small cell lung cancer and shows a variety of histopathological features. SACL, measuring 2 cm or less at its greatest dimension, shows generally good prognosis. However, a few SACL form metastases of the mediastinal lymph nodes or systemic organs and showed very poor prognosis (1). It has been reported that the histopathological features of SACL are various and that some types of SALC showed poor prognosis (2). However, it is very difficult to distinguish the above histological types by routine histological examination, despite some immunohistochemical analyses (3). If SACL cases with good prognosis could be distinguished from those with poor prognosis, some cases of SACL could be cured by limited surgical removal. It is therefore very important to reveal the factors that distinguish between SACL cases with good prognosis and those with poor prognosis.

Mucins have been postulated to be important molecules in maintaining epithelial homeostasis in various diseases (4, 5). In cancer, mucins are large O-glycoproteins expressed either at the cell surface or as secreted molecules that form a protective gel (6, 7). Core proteins for several human mucins (MUC1–MUC9 and MUC11–MUC13) have been identified (8–12). The mucin genes MUC2, MUC5AC, MUC5B, and MUC6 are clustered on the p15 arm of chromosome 11 and are therefore called 11p15 mucin genes (13). The 11p15 mucin genes possess a cell-specific pattern of expression in normal lungs, and the expression pattern is altered during carcinogenesis (14).

In this study, we immunohistochemically examined the 11p15 mucins MUC2, MUC5A, and MUC6 in SACL and explored the relationship between mucin expression and lymph node metastasis and prognosis.

MATERIALS AND METHODS
SACL Surgical Specimens. The 79 SACL specimens were obtained by surgical resection from previously untreated patients with their informed consent. Surgical specimens were processed for routine histopathological analysis. Morphological classification was based on histological typing of lung tumors. The nodal metastases were evaluated histologically.

Immunohistochemistry. Immunohistochemical analysis was performed with the Catalyzed Signal Amplification system.

Received 3/10/03; revised 7/22/03; accepted 8/4/03.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Requests for reprints: Dr. Masato Nakamura, M.D., Department of Pathology, Tokai University School of Medicine, Bohseidai, Isehara, Kanagawa 259-1193, Japan. Phone: 81-463-93-1121, ext. 2570; Fax: 81-463-91-1370; E-mail: mnakamura@is.icc.u-tokai.ac.jp.

The abbreviation used is: SACL, small adenocarcinoma of the lung.
monoclonal antibodies, 1:100 dilution): MUC2 glycoprotein/clone (Ccp58), MUC5AC glycoprotein/clone (CLH2), and MUC6 glycoprotein/clone (CLH5; Novocastra Laboratories Ltd.). The immune complex on the sections was then amplified with the biotinylated secondary antibodies (goat antimouse immunoglobulin; DAKO EnVision), streptavidin–biotin complex, and streptavidin peroxidase. The amplified products were visualized by the 3,3’-diaminobenzidine tetrahydrochloride reaction.

**Statistical Analysis.** We examined the statistical significance of the relationship between the expression of the MUC proteins by Fisher’s test. We examined the statistical significance of the relationship between MUC expression and lymph node metastasis by $\chi^2$. The survival rate of patients with SACL was estimated from Kaplan–Meier life tables plotted to compare survival and mucin production. The curves were analyzed for statistically significant differences by the log-rank test.

**RESULTS**

**Expression of MUC2, MUC5AC, and MUC6.** We evaluated the expression of MUC2, MUC5AC, and MUC6, in 79 surgical specimens by immunohistochemical analyses (Fig. 1). Six (7.6%) of 79 SACL cases showed MUC2 expression, and 11 (13.9%) of the 79 SACL cases showed MUC6 expression. Three SACL cases showed both MUC2 and MUC6 expression, and a significant correlation was noted between MUC2 and MUC6 expression (Fisher’s test, $P = 0.033$). Six (7.6%) SACL cases showed MUC5AC expression. No significant correlation was observed between MUC expression and histopathological features.

Five of the 6 SACL cases with MUC2 expression showed lymph node metastasis, and 6 of the 11 cases with MUC6 expression showed lymph node metastasis (Table 1). SACL cases with MUC2 or MUC6 expression showed a significantly higher incidence of nodal metastasis than those without expression ($P < 0.001$ or $P = 0.006$, respectively, $\chi^2$ test). Three of the six cases with MUC5AC expression showed lymph node metastasis. However, there was no significant correlation between MUC5AC expression and nodal involvement.

**MUC Expression and Prognosis.** Fig. 2 shows the survival curves of the 79 patients obtained with the Kaplan–Meier method. The mean observation period was 5.1 years. The SACL cases with MUC2 expression showed a significantly poorer

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Immunohistochemical analysis of MUC expression in SACL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lymph node metastasis, n (%)</td>
</tr>
<tr>
<td></td>
<td>+ (n = 19)</td>
</tr>
<tr>
<td>MUC2</td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>5 (26.3%)</td>
</tr>
<tr>
<td>-</td>
<td>14 (74.7%)</td>
</tr>
<tr>
<td>MUC5AC</td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>3 (15.8%)</td>
</tr>
<tr>
<td>-</td>
<td>16 (84.2%)</td>
</tr>
<tr>
<td>MUC6</td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>6 (31.6%)</td>
</tr>
<tr>
<td>-</td>
<td>13 (68.4%)</td>
</tr>
</tbody>
</table>

$^a$ Statistically significant by $\chi^2$ test.
prognosis than those without MUC2 expression (P = 0.011, log-rank test). Neither MUC6- nor MUC5AC-positive SACL show a significant correlation with prognosis.

**DISCUSSION**

We examined the expression of 11p15 mucins in 79 patients with SACL. SACL cases with MUC2 (7.6%) and MUC6 (13.9%) expression showed a significantly higher incidence of nodal metastasis than those without expression (P < 0.001 or P = 0.006, respectively, χ2 test). The SACL cases with MUC2 expression showed a significantly poorer prognosis than those without MUC2 expression (P = 0.011, log-rank test). These results suggest that production of the 11p15 mucins MUC2 and MUC6 is related to lymph node metastasis and affects prognosis in SACL.

Mucins are high-molecular-weight glycoproteins that have oligosaccharides attached to serine or threonine residues of the mucin core protein backbone by O-glycosidic linkages. The aberrant expression of mucins and mucin-related antigens has been noted to be a factor in poor survival in various human cancers, such as colon and breast cancer (15, 16). In lung cancer, some studies regarding MUC expression have been reported (17–19). Yu et al. reported that sialomucin expression is related to a relapse in non-small cell lung cancer (20) and that the expression of MUC5AC is positively correlated with the expression of sialomucin (17). The 11p15 mucin genes (MUC2, MUC5AC, MUC5B, and MUC6) possess a cell-specific pattern of expression in normal lungs that is altered during carcinogenesis (14). MUC2 is an intestinal type secretory mucin, is expressed mainly in the goblet cells of the intestine (21, 22), and functions as a cell differentiation molecule or regulator of cell proliferation (23). In gastrointestinal tumors, MUC2 mucin expression is related to noninvasive proliferation of the tumors and a favorable outcome for the patient (24–27). Perrais et al. (14) reported that MUC2 and MUC5AC are target genes of epidermal growth factor receptor ligands in lung cancer cells and that the up-regulation of these two genes is through the concomitant activation of the epidermal growth factor receptor/Ras/Raf extracellular signal-regulated kinase signaling pathway.

Since 1988, we have removed 79 SACL samples by the two-windows method (28). Few SACL have metastasis of the mediastinal lymph nodes or systemic organs and show poor prognosis (1). In this study, we showed that both MUC2 and MUC6 production was related to lymph node metastasis and poor prognosis. We conclude that these two mucins are markers for predicting the prognosis of SACL. However, the sample size of the mucin-positive specimens was rather small. The number of SACL cases are increasing in our institute, and we are continuing studies with these SACL samples. We therefore hope to confirm the results with a larger series in the future. At present we are also examining the expression levels of the 11p15 mucin genes by real-time PCR method.

**ACKNOWLEDGMENTS**

We thank Yuichi Tada, Johbu Itoh, and Koutatsu Nomura for technical assistance.

**REFERENCES**


Use of 11p15 Mucins as Prognostic Factors in Small Adenocarcinoma of the Lung

Noboru Nishiumi, Yoshiyuki Abe, Yoshimasa Inoue, et al.