Immunohistochemical Evidence of Urokinase-type Plasminogen Activator in Primary and Metastatic Tumors of Pulmonary Adenocarcinoma

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ABSTRACT

The urokinase-type plasminogen activator (u-PA) was studied in 96 cases of lung adenocarcinoma and 49 cases of lymph node metastatic adenocarcinoma. The activity of u-PA was detected in the cytoplasm of tumor cells, and the number of positive cells was higher in patients with T1 or T2 than in those with T3 disease (P < 0.01). These u-PA-positive tumor cells were more frequent in patients with N0 than in those with N1 disease (P < 0.01). Such cells were also detected in patients with N2 rather than N0 disease (P < 0.01). When we compared the frequency of u-PA-positive tumor cells in metastatic lesions with that in primary ones, the former tended to be higher. On the other hand, the frequency of u-PA-positive cells in primary tumors of patients with a recurrence was higher than in those with no recurrence. Thus, u-PA is an important prognostic indicator associated with tumor growth and lymph node spread. If u-PA is detected in a tumor, a recurrence can be expected.

INTRODUCTION

PA³ activities are important factors linked to the spread of a tumor including local growth, lymphatic metastases, and hematogenous metastases (1–5). The PA content in extracts of excised malignant tissues exceeded that in normal tissues (5–8). The activity was also noted in cell lines originating from the colon, breast, and lung (9–11).

Immunohistochemical studies revealed that u-PA plays a role in invasion and metastasis of adenocarcinomas of the colon and breast (1, 2, 12). In patients with a lung carcinoma, Markus et al. found no relationship between PA levels and tumor spread (7). To further examine the u-PA content in adenocarcinoma of the lung, we made use of an immunohistochemical technique, and our findings are described herein.

MATERIALS AND METHODS

Ninety-six Japanese patients with adenocarcinoma of the lung underwent resection in our department from April 1974 through March 1987. There were 62 men and 34 women with an age range of 39 to 81 years (mean, 63 years). The stage of the lung cancer was pathologically classified according to the tumor-node-metastasis classification of the International Union Against Cancer (13). There were 38 patients with stage I, 15 with stage II, and 43 with stage III disease. Seventy-seven of the 96 patients underwent complete resection. Resected specimens of the primary tumor and of the regional lymph nodes were fixed in 10% formalin prior to embedding in paraffin. These sections were stained with hematoxylin and eosin, and all tumors were reviewed based on the current WHO criteria (14).

The primary monoclonal antibody to urokinase (M, 54,000) was obtained from Maruzen Oil Biochemical Co. (Tokyo, Japan; lot 02), and an indirect staining technique and the avidin-biotin-peroxidase complex method were used (15). The deparaffinized sections were immersed in 0.3% H2O2 in 100% methanol for 30 min at room temperature to block the endogenous peroxidase activity (16). Possible background staining was also removed by applying normal mouse serum, diluted 1:10, for 20 min at room temperature. Anti-urokinase serum was applied to the sections at a 1:50 dilution in phosphate-buffered saline, and the preparations were incubated at room temperature in a moist chamber for 14 h. Visualization of the peroxidase was achieved by the diaminobenzidine method. The sections were then stained with methyl green and examined under a transmitted light microscope.

Nonimmune mouse serum was used for the controls. Specificity of the primary monoclonal antibody against u-PA was tested by the absorption test, using anti-u-PA antisera and purified u-PA conjugates (Green Cross, Osaka, Japan), diluted antisera (1:50), and 2 units/ml of urokinase. When the sections were incubated with the purified u-PA conjugates and then with anti-u-PA antisera, staining results were negative.

The extent of the cytoplasmic reactivity was divided into four groups, as follows: −, no tumor staining; +, staining of 1 to 25% of the tumor cells; ++, staining of 26 to 75% of the tumor cells; ++++, staining of 76 to 100% of the tumor cells.

Statistical analyses were performed using the x² test and the Mann-Whitney rank-sum test (17). The difference was considered significant when P was less than 0.05.

RESULTS

Staining Pattern of u-PA. In primary tumor tissues, immunoperoxidase reactivity was present in the cytoplasm of some of the cancer cells (Fig. 1). However, there was no difference in reactivity between the histological degrees of differentiation of the pulmonary adenocarcinomas. The bronchial epithelium was weakly stained, and there was no stain on the alveolar epithelium.

In metastatic lymph node tissue, the reactivity was also assessed by grading of the primary tumor tissue. In some parts of the tumor, u-PA-positive tumor cells were heavily distributed at the growing edge of the tumor (Fig. 2). The intraluminal tumor cells were heavily stained in many areas of the tumor (Fig. 3).

Correlation of u-PA with Tumor Spread. Immunoreactivity was graded on a − to +++ scale for the tumor status of the primary lesions (Table 1). Of 36 with T1, 14 (40%) had a positive reactivity of + to ++++; of 36 with T2, 23 (64%) were positive; and of 24 with T3, 17 (70%) were positive. There were significant differences in immunoreactivity between T1 versus T2 and T1 versus T3, as determined by the Mann-Whitney rank-sum test (P < 0.01).

The immunoreactivity also correlated with lymph node status (Table 2). Of 47 with N0, 12 (25%) had a positive reactivity; of 20 with N1, 14 (70%) were positive; and of 29 with N2, 28 (96%) were positive. The number of u-PA-positive tumor cells increased with the increasing degree of lymph node status. There were statistically significant differences between N0 versus N1 and N1 versus N2, as determined by the Mann-Whitney rank-sum test (P < 0.01).

Correlation of u-PA in Primary Tumor with Metastases. The immunoreactivities of primary tumors and metastatic lesions
Fig. 1. Immunostaining for u-PA in well-differentiated adenocarcinoma of the lung; the pattern is papillary or tubular. A, +++, intense immunoreactivity in 100% of tumor cells, × 200. B, ++, moderate immunoreactivity staining in 42% of tumor cells, × 200. C, +, focal immunoreactivity staining to 9% in tumor cells. × 200.

were compared (Table 3). The frequency of positive tumor cells was higher in metastatic lesions than in primary tumors in 12 cases (29%), comparable in 27 (67%), and lower in 2 (4%). Data on each case of 16 primary tumors with both N₁ and N₂ node metastases are shown in Table 4. The frequency of positive tumor cells was higher in N₂ node metastases than in primary tumors in 7 cases (N₂ node/primary = number of cases: ++/+ = 3, +++/++ = 4), comparable in 8 (+/+ = 1, +++/+++ = 7), and lower in 1 (++/+++ = 1). However, it was higher in N₁ node metastases than in the primary tumor in 2 cases (N₁ node/primary = number of cases: ++/+ = 2), comparable in 8 (+/+ = 2, ++/+ = 2, +++/+++ = 4), and lower in 6 (−/++ = 2, −/+++ = 2, ++/+++ = 1, +++/+++ = 1).

Correlation of u-PA with Recurrence. In 77 patients who underwent “curative” resection and were followed for over 2 years, findings in the primary tumors which recurred were compared with cases of no recurrence (Table 5). The frequency of positive tumor cells in the group with a recurrence was significantly greater than that in the group with no recurrence (P < 0.01).

DISCUSSION

We used the monoclonal anti-u-PA antibody and immunohistochemically examined primary tumors and metastatic lymph nodes in cases of adenocarcinoma of the lung. Our
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and then noted the lack of any correlation between the activity and the degree of invasion or metastasis. On the other hand, in colon and breast cancer, u-PA was seen to play an important role in the invasiveness of tumor cells (1, 3, 5, 18), as was the case with the adenocarcinomas examined in the present study.

However, concerning PA contents in the primary tumor and the metastatic lesion, findings have remained controversial. We found that the frequency of u-PA-positive tumor cells for N2 nodes was greater than in the N1 nodes and primary tumors. Here it must be noted that the difference is apparent in the frequency of u-PA-positive tumor cells. PA secretion was reported by Marcus et al. (6) to be less in cases of a metastasis than in the primary tumor, as deduced from studies on human colon cancer cells in a short-term culture. They stated that the low secretion rates of metastatic tumors, presumably reflecting the property in the original cell that gave rise to the metastatic focus, could be of advantage to circulating cancer cells. More

Table 1 Immunoreactirity of u-PA in adenocarcinoma of the lung according to tumor factor

<table>
<thead>
<tr>
<th>Staining intensity of u-PA</th>
<th>No. of patients according to tumor factor (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>T1 60</td>
</tr>
<tr>
<td>Positive</td>
<td>T2 40</td>
</tr>
<tr>
<td>+++</td>
<td>T3 30</td>
</tr>
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* Mann-Whitney rank-sum test: T2 > T1 (P< 0.01); T3 > T1 (P< 0.01).

Table 2 Immunoreactirity of u-PA in adenocarcinoma of the lung according to lymph node factor

<table>
<thead>
<tr>
<th>Staining intensity of u-PA</th>
<th>No. of patients according to lymph node factor (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>N0 35</td>
</tr>
<tr>
<td>Positive</td>
<td>N1 22</td>
</tr>
<tr>
<td>+++</td>
<td>N2 44</td>
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* The staining intensity of u-PA was established as given in Table 1.

Present findings of u-PA-positive tumor cells in primary and metastatic tumors are important, because to our knowledge the correlation between these tumor cells, invasion, metastasis, and recurrence has not been reported.

Some workers who found PA in lung cancer using biochemical approaches noted an elevated activity in cases of adenocarcinoma (7, 10). They purified the M, 55,000 urinary urokinase

Fig. 3. Poorly differentiated adenocarcinoma of the lung, invading the lumen of a lymphatic vessel. A, tumor cells present in the lymphatic vessel. H & E. x 400. B, the same tumor cells strongly stained. u-PA immunostain, x 400.

Table 3 Comparison of immunoreactivity between primary tumors and the metastatic lesion

<table>
<thead>
<tr>
<th>Cases (Te)</th>
<th>M &gt; P*</th>
<th>M = P</th>
<th>M &lt; P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>12 (29)</td>
<td>27 (67)</td>
<td>2 (4)</td>
</tr>
</tbody>
</table>

* Comparison was made as follows. M > P, frequency of u-PA-positive tumor cells greater in M than in P; M = P, the same; M < P, less intensive in M than in P.

Table 4 Immunoreactirity of 16 primary tumors with both hilar and mediastinal lymph node metastases

See Table 1 for factors related to staining intensity.

Table 5 Comparison of immunoreactivity

<table>
<thead>
<tr>
<th>Recurrence</th>
<th>No. of patients</th>
<th>Cases (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>With</td>
<td>44</td>
<td>15 (34)</td>
</tr>
<tr>
<td>Without</td>
<td>33</td>
<td>23 (70)</td>
</tr>
<tr>
<td>Total</td>
<td>77</td>
<td>38 (39)</td>
</tr>
</tbody>
</table>

χ² test: P< 0.01.
recent immunofluorescence studies done by Burtin et al. (12) showed that PA-positive cells were more numerous in the case of a metastasis than in the primary tumor. They stated that PA produced by tumor cells was only partially secreted during short-term culture and that the production was not proportional to the secretion.

Other investigators described the PAI, purified from 20 human tumor cell lines (9), hepatoma (19, 20), breast carcinoma (21), and colon carcinoma (22). The most important mechanism involved in the regulation of PA activity seemed to be the inhibition by PAI (23). The presence of complexes of PA with PAI in human carcinomas was assessed by zymography (8, 22, 24). In the human tumor cell lines, the tumors with high metastatic activity showed not only secretion of high levels of u-PA but also expression of procoagulant and PAI activities (9). If all these findings are relevant to our results, then the production of PAI in primary and metastatic tumors may differ. Cancer cells with high secretion rates of u-PA may secrete a large amount of PAI; hence a distant metastasis could occur.

In 1988, Nishino et al. (25) demonstrated that urinary u-PA levels continued to increase in patients with a recurrence of gastrointestinal tract cancer, although the levels did decrease after excision of the tumor. Thus, cancer cells with high secretion rates of u-PA apparently give rise to a recurrence.

In conclusion, use of a specific antibody to u-PA revealed the presence of u-PA in more advanced and invasive adenocarcinomas of the lung. Retrospectively, the same detections were made in cases of a recurrence. u-PA appears to be a significant prognostic and recurrent indicator of adenocarcinoma of the lung.

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REFERENCES

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