Enzyme cytology of alkaline phosphatase in lung cancer

Department of Pathology, Gifu University School of Medicine

Kazuo KATO, Shigeyuki SUGIE, Naoki YOSHIMI, Masashiro YAMAZAKI and Masayoshi TAKAHASHI

Introduction

The human alkaline phosphatases (ALPs) are a group of similar enzymes with alkaline pH optima that hydrolyse a variety of phosphate esters. The best characterized are those from placenta, intestine, liver, bone and kidney. Recent evidence indicates that the protein moieties of these glycoproteins are coded by at least three distinct gene loci.

It is now well established that certain malignant tumors express a significant amount of a heat-stable placental-like ALP. However, there are only a few detailed descriptions of enzymatic activity patterns in lung specimens, particularly in cytological specimens. In the present study, we investigated possible correlation between the enzymatic behaviour and the histopathological pattern of tumors in over 70 specimens of human lung cancers.

Materials and Methods

Materials

Human materials were obtained from surgically resected specimens of 74 cases. Their histological types were determined with hematoxylin–eosin stained sections by WHO typing.

Methods

Direct imprint smears were prepared by gently pressing glass slides against the cut surface of the specimens within 1 hr after surgical removal. The cytochemical staining was carried out by a modified Burstone’s azo dye method. Five mg of naphthol AS-BI phosphoric acid (Sigma Chemical Co.,
Table 1 The distribution and the intensity of alkaline phosphatase activity

<table>
<thead>
<tr>
<th>Histological diagnosis</th>
<th>No. of cases</th>
<th>Activity of alkaline phosphatase*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0  1a 1b 1c 2a 2b 2c 3a 3b 3c</td>
</tr>
<tr>
<td>Adenocarcinomas</td>
<td>33</td>
<td>8 14 2 6 3</td>
</tr>
<tr>
<td>papillary adenocarcinoma</td>
<td>10</td>
<td>2 3 1 1 3</td>
</tr>
<tr>
<td>tubular adenocarcinoma</td>
<td>11</td>
<td>3 5 3 3</td>
</tr>
<tr>
<td>papillo-tubular adenocarcinoma</td>
<td>8</td>
<td>3 3 1 1</td>
</tr>
<tr>
<td>bronchiolo-alveolar carcinoma</td>
<td>4</td>
<td>3 3 3 3</td>
</tr>
<tr>
<td>Epidermoid carcinomas</td>
<td>29</td>
<td>10 11 2 2 2 1 1 1 1</td>
</tr>
<tr>
<td>well differentiated</td>
<td>4</td>
<td>1 1 1 1</td>
</tr>
<tr>
<td>moderately differentiated</td>
<td>19</td>
<td>6 7 1 2 2 1</td>
</tr>
<tr>
<td>poorly differentiated</td>
<td>6</td>
<td>3 1 1 1</td>
</tr>
<tr>
<td>Combined epidermoid and adenocarcinomas</td>
<td>5</td>
<td>1 1 2 1</td>
</tr>
<tr>
<td>Large cell carcinomas</td>
<td>6</td>
<td>2 3 1 1</td>
</tr>
<tr>
<td>Small cell carcinomas</td>
<td>1</td>
<td>1 1 1 1</td>
</tr>
<tr>
<td>Total</td>
<td>74</td>
<td>13 15 11 4 3 16 2 0 7 3</td>
</tr>
</tbody>
</table>

| Control                                |              |                                    |
|                                        |              | 0  1a 1b 1c 2a 2b 2c 3a 3b 3c     |
| bronchial epithelia                    | 17           | 11 3 2 1                           |
| alveolar epithelia                     | 22           | 4 1 9 2 6                         |

* ALP activity is expressed as a combination of the distribution (0, negative; 1, few; 2, moderate; 3, many) and activity of the highest population in the positive cells (a, weak; b, moderate; c, strong).

St. Louis, Mo.) was dissolved in 10 ml of 0.05 M, 2-amino-2-methyl-1, 3-propanediol buffer at pH 9.8 with 5 mg of Fast Blue RR salt (Sigma Chemical Co., St. Louis, Mo.) and incubated for 10 min at room temperature. Specimens were counterstained with a 1% solution of Safranin O. Some specimens were treated with heat at 65°C for 30 min in a 0.5 mM MgCl₂ solution prior to the cytochemical staining. Enzymatic activity was arbitrarily divided with distribution of reactive cells contained positive granules and ALP activity in a cell, and expressed as a combination of the distribution and activity of the highest population in the positive cells. The distribution of ALP active cells was divided into four grades:

0) negative
1) few : There were some reactive cells contained positive granules but they accounted for less than 10% of the total tumor cells.
2) moderate : 10% to 50% containing positive granules.
3) many : More than 50% containing positive granules.

The enzymatic activity was also divided into four grades:

a) weak : A few positive granules (less than 30 in a cell) were observed.
b) moderate : Positive granules were distributed more than 30 in a cell or heavily in an area of less than half of the cytoplasm, or granules were distributed throughout the cytoplasm with some adhesion.
c) strong : Positive granules were distributed heavily in more than half of the cytoplasm.

Results

The relation of ALP activities to histological types is shown in Table 1.

All 33 cases of adenocarcinomas showed more than moderate activities on the imprint specimens. There was no difference in stainability among the papillary type (Fig. 1, 2), tubular type, papillo-
Fig. 1 Papanicolaou's stain of imprint smear of moderately differentiated adenocarcinoma papillary type resected from right lobe of 74 year-old male. ×300

Fig. 2 ALP stain of the same specimen as in Fig. 1. About half the neoplastic cells have high activity. This stainability is expressed as 2 c. ×300

Fig. 3 Papanicolaou's stain of imprint smear of moderately differentiated adenocarcinoma papillo-tubular type resected from right upper lobe of 66 year-old female. ×300

Fig. 4 ALP stain of the same specimen as in Fig. 3. Almost all cells have activity at one side of the cytoplasm. This stainability is expressed as 3 b. ×300

Fig. 5 Papanicolaou's stain of imprint smear of bronchiolo-alveolar type carcinoma resected from left upper lobe of 56 year-old female. ×300

Fig. 6 ALP stain of the same specimen as in Fig. 5. Almost all cells have high activity. This stainability is expressed as 3 c. ×300
tubular type (Fig. 3, 4) and bronchiolo-alveolar type (Fig. 5, 6).

On the other hand, only six cases out of 29 epidermoid carcinomas (significantly different from the value of the adenocarcinomas, P<0.01) showed more than moderate activity of the enzyme. Weak reactivity was observed in 13 epidermoid carcinomas. The neoplastic cells of well-differentiated epidermoid carcinomas had a tendency to have very low ALP activity, and those of lower differentiation higher activity (Fig. 7, 8). The enzyme localized in whole cytoplasm, but no cells showed ALP activity localized at one side of the cytoplasm against the nucleus. This enzyme location were often observed in the adenocarcinomas. However, non-neoplastic cells, e.g., the histiocytes or the fibroblasts in the imprint smears of epidermoid carcinomas, displayed moderate ALP activity.

As for the combined epidermoid and adenocarcinoma, only the glandular component of the neoplasm displayed ALP activity.

Very low enzyme activity was observed in both large cell and small cell carcinomas.

The ALP activity of all cells in these 74 cases has proven to be easily inactivated by heating at 65°C for 30 min.

The normal epithelial cells of the alveoli displayed ALP activities which were observed in the whole cytoplasm, but bronchial cells usually did not display them.

**Discussion**

Several types of healthy glandular epithelia, e.g., mammary gland and intestinal epithelia, display ALP activities but lose the activities when they become malignant. On the contrary, our results showed that many cells of adenocarcinomas in the lung kept high activities of ALP.

Unlike adenocarcinoma cells, many epidermoid cells showed weak activity of the enzyme in the present examination. When moderate reaction was observed, the pattern of the enzyme activity was different from the pattern in adenocarcinomas. In normal squamous epithelia and squamous-cell carcinomas, ALP was generally absent from the epithelial elements. Many researchers have reported that there is abundant ALP activity in the stroma of squamous cell cancer.

It was also reported that in the uterine cervix, 32% of invasive squamous cell carcinomas had ALP activity in poorly differentiated tumor cells and none in well-differentiated ones. Our results suggest the mechanism of induction of ALP activity in epidermoid cancer of the lung is similar to that in squamous cell carcinomas of the uterine cervix.

Although only few bronchial epithelia showed ALP activity in this present study, transport of Na⁺ into an intravesicular space of apical membranes enriched in ALP from bovine tracheal epithelia was
demonstrated by a linear inverse correlation between Na$^+$ uptake and medium osmolarity. Results from a variety of studies indicate that the basic characteristics of tracheal secretion are common to those of other Cl$^-$-secreting epithelia. ALP is also present in lamellar bodies of the alveolar type II cells. We suspected epidermoid carcinomas, large cell carcinomas and small cell carcinomas lost the character of Cl$^-$-secreting epithelia more or less and also lost the strong ALP activity.

Our results suggest adenocarcinomas of the lung had ALP activity as usual bronchial or alveolar cells, or much more activity and that staining for ALP would be very useful in cytological examination to distinguish the character of glandular epithelial neoplasms in the lung.

**Summary**

Direct imprint smears were obtained from surgically resected specimens of 74 human lung carcinomas and cytochemical staining for alkaline phosphatase was carried out by a modified Burstone's azo dye method. All 33 adenocarcinomas of the lung had alkaline phosphatase activity more than usual bronchial and alveolar cells. However, only six cases out of 29 epidermoid carcinomas showed more than moderate activity of the enzyme.

The staining for alkaline phosphatase would be very useful in cytological examination to distinguish the character of glandular epithelial neoplasms in the lung.

**References**
