An Immunohistochemical Study of Keratin, Carcinoembryonic Antigen, Human Chorionic Gonadotropin and Alpha-Fetoprotein in Lung Cancer

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Department of Pathology, Tohoku University School of Medicine, Sendai 980 and *Department of Pathology, University of Miami, Miami, Florida 33101, USA

KIMURA, N. and GHANDUR-MNAYMEN, L. An Immunohistochemical Study of Keratin, Carcinoembryonic Antigen, Human Chorionic Gonadotropin and Alpha-Fetoprotein in Lung Cancer. Tohoku J. exp. Med., 1985, 145(1), 23-38 — Immunohistochemical staining utilizing a peroxidase-antiperoxidase (PAP) technique for keratin, carcinoembryonic antigen (CEA), human chorionic gonadotropin (HCG) and alpha-fetoprotein (AFP) was performed on paraffin sections from 72 cases of lung cancer obtained at autopsy. Positive reaction was shown in 44% of the cases for keratin, 77% for CEA, and 58% for HCG. AFP was positive in only one case of large cell carcinoma. Keratin was positive in 100% of squamous cell carcinoma, 53% of adenocarcinoma, 15% of small cell carcinoma and 45% of large cell carcinoma. CEA showed positive staining in 90% of squamous cell carcinoma, 88% of adenocarcinoma, 58% of small cell carcinoma and 69% of large cell carcinoma. CEA was the most useful tumor marker for detection of all types of lung cancer. HCG was positive in 30% of squamous cell carcinoma, 100% of adenocarcinoma, 23% of small cell carcinoma and 56% of large cell carcinoma.

Pulmonary carcinoma is divided into four large groups; squamous cell carcinoma, adenocarcinoma, small cell carcinoma and large cell carcinoma. If tumors have morphologically characteristic features such as keratin pearl or glandular structure, it may not be difficult to make the diagnosis of typing. Diagnostic agreement in lung cancer was less than 50% for poorly differentiated tumors, especially in the polygonal small cell carcinoma, large cell carcinoma growing in sheets, poorly differentiated adenocarcinoma with little gland formation, and poorly differentiated squamous cell carcinoma without keratin (Yesner 1978). Unfortunately, these tumors comprise over 50% of all lung cancer (Yesner 1978).

The presence of keratin has been directly related to increased squamous
differentiation. Therefore keratin is reasonably known as a useful tumor marker of squamous cell carcinoma. On the other hand, keratin is detected in various normal epithelial cells, e.g. in the myoepithelial cells of the salivary gland and breast, basal cells of the bronchus, prostate, endocervix and transitional cells (Schlegel, Schlegel and Pinkus 1980). How about the immunoreactivity for keratin in other three types of lung carcinoma besides squamous cell carcinoma? Is keratin a useful marker to differentiate the squamous cell carcinoma from other types?

Oncodevelopmental proteins, such as carcinoembryonic antigen (CEA), human chorionic gonadotropin (HCG) and alpha-fetoprotein (AFP) have been detected in patients with malignant tumors including lung cancer. CEA is defined as an endodermally derived tumor associated antigen that is also present in fetal gastrointestinal tissues. CEA is a component of the apical membrane of the cancerous cell and of the normal columnar cell, as well as of the glycoproteins of the mucosecreting cell, whether cancerous or not (Goldenberg, Sharkey and Primus 1978). In lung cancer, 68% of the patients have an elevated concentration of CEA in their sera (Vincent, Chu, Fergen and Ostrander 1975).

In view of rather scanty and vague information on the tissue localization of keratin, CEA, HCG and AFP in pulmonary carcinomas and in the hope of adding objectivity to the assessment and diagnosis of this condition, we have undertaken to relate keratin and the inappropriate proteins to histological types. Serum levels of these proteins do not always reflect histological findings, because lung cancer exhibits varying degrees of differentiation from field to field, and transition among types (Yesner 1978). In order to detect the precise location of the keratin and these inappropriate proteins, we investigated autopsy materials which represented different degrees of differentiation and coexistence of different histological types, using an immunoperoxidase method. Results were discussed with comparison to ultrastructural findings and biochemical data of previous studies.

**Materials and Methods**

Formalin-fixed paraffin-embedded autopsy materials dated from 1974 to 1981 were obtained from files of Jackson Memorial Hospital, University of Miami, USA. Seventy-two cases of primary lung cancer were randomly selected for this study. Two cases of surgical materials were also examined. Each case had 3 to 5 blocks including areas of varying degrees of differentiation and different histological types. Histological type and degree of differentiation of tumors were assessed by reviewing sections stained with hematoxylin and eosin according to WHO classification of lung tumors (WHO 1982). These materials comprised 10 cases of squamous cell carcinoma (14%), 17 cases of adenocarcinoma (23%), 24 cases of small cell carcinoma (38%), 16 cases of large cell carcinoma (22%), 3 cases of atypical carcinoid (4%) and 2 cases of adenosquamous carcinoma (3%). Small cell carcinoma of combined type consisted of one case of squamous cell carcinoma, one case of adenocarcinoma and one of large cell carcinoma. Five cases of large cell carcinoma with poorly differentiated adenocarcinoma were included in large cell carcinoma depending on predominance of histological type.

The unlabeled antibody, peroxidase antiperoxidase (PAP) technique was performed by
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Sternberger’s method (Sternberger et al. 1970). Antiserum for keratin was provided by Dr. Penneys, N.S. (Ganjei, Nadji, Penneys, Averette and Morales 1983), University of Miami. Antiserum for CEA and AFP, normal swine serum, antirabbit IgG and rabbit peroxidase antiperoxidase were commercially obtained from DAKO Cooperation, Santa Babara, California, and HCG from Cappel Laboratories Inc., Cochranville, Pennsylvania. Monoclonal CEA antibody (Mochida Pharm., Tokyo) was additionally used. The commercial polyclonal CEA-antiserum was absorbed with a normal lung extract to prevent cross-reaction with nonspecific cross-reacting antigen. Positive controls were as follows: human skin for keratin, CEA producing tumor for CEA, placental tissue for HCG and endodermal sinus tumor for AFP. In each run, sections of these tissues were stained simultaneously as positive control, while substitution of rabbit antikeratin with non-immune rabbit serum constituted our antibodies control.

RESULTS

Results obtained are summarized in Table 1.

Keratin

Of the 71 cases, 44% were positive for keratin. All of the squamous cell carcinoma samples showed positive reaction, especially strong in keratinizing area of the tumor (Fig. 1). Intensity of the reaction was proportional to the degree of squamous differentiation. Poorly differentiated areas of th squamous cell carcinoma also showed positive reaction (Fig. 2).

Of the 17 cases of adenocarcinoma, 53% were positive for keratin. In the subclassifications of adenocarcinoma, positive reaction of keratin was demonstrat-

Table 1. Histological types and immunoreactive cells

<table>
<thead>
<tr>
<th>Classification WHO (1981)</th>
<th>Number of cases</th>
<th>Keratin positive cases</th>
<th>CEA positive cases</th>
<th>HCG positive cases</th>
<th>AFP positive cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Squamous cell carcinoma</td>
<td>10</td>
<td>10(100%)</td>
<td>9(90%)</td>
<td>3(30%)</td>
<td>0</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>17</td>
<td>9(53%)</td>
<td>15(88%)</td>
<td>17(100%)</td>
<td>0</td>
</tr>
<tr>
<td>Acinar</td>
<td>6</td>
<td>2</td>
<td>6(100%)</td>
<td>6(100%)</td>
<td></td>
</tr>
<tr>
<td>Papillary</td>
<td>6</td>
<td>4(67%)</td>
<td>4(67%)</td>
<td>6(100%)</td>
<td></td>
</tr>
<tr>
<td>Solid carcinoma*</td>
<td>3</td>
<td>1</td>
<td>3(100%)</td>
<td>3(100%)</td>
<td></td>
</tr>
<tr>
<td>Bronchiolo-alveolar</td>
<td>2</td>
<td>2(100%)</td>
<td>2(100%)</td>
<td>2(100%)</td>
<td></td>
</tr>
<tr>
<td>Small cell carcinoma</td>
<td>24</td>
<td>3(15%)</td>
<td>14(58%)</td>
<td>7(23%)</td>
<td>0</td>
</tr>
<tr>
<td>Intermediate</td>
<td>6</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Oat cell carcinoma</td>
<td>15</td>
<td>1</td>
<td>8</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Combined</td>
<td>3</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Large cell carcinoma</td>
<td>16</td>
<td>7(45%)</td>
<td>11(69%)</td>
<td>10(56%)</td>
<td>1(7%)</td>
</tr>
<tr>
<td>Atypical carcinoid</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Adenosquamous carcinoma</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>72</td>
<td>32(44%)</td>
<td>53(73%)</td>
<td>41(57%)</td>
<td>1(1.1%)</td>
</tr>
</tbody>
</table>

* Solid carcinoma with mucus formation of adenocarcinoma.
Fig. 1. Immunoreactive cells for keratin of well differentiated squamous cell carcinoma. Positive cells show strongly brown granularity in the cytoplasm. PAP method, counterstained with methylgreen, ×60.

Fig. 2. Poorly differentiated squamous cell carcinoma showing positive reaction for keratin. PAP method, counterstained with methylgreen, ×60.
Fig. 3. Immunoreactive cells for keratin in papillary adenocarcinoma. PAP method, counterstained with methylgreen, ×160.

Fig. 4. Immunoreactive cells for keratin in large cell carcinoma. PAP method, counterstained with methylgreen, ×400.
ed in 2 of 6 cases of acinar type, 4 of 6 cases of papillary type (Fig. 3) and in 2 of 2 cases of bronchiolo-alveolar type. One of 3 cases of solid carcinoma with mucus formation type was also positive. Papillary type with partly squamous metaplasia demonstrated a strongly positive reaction. Intensity was generally less in adenocarcinoma than in squamous cell carcinoma. However, some strongly positive cells in adenocarcinoma were also observed.

Only 3 of the 23 cases of small cell carcinoma showed focally positive staining (15%). They were oat cell carcinoma, intermediate type and combined with squamous cell carcinoma. In the oat cell carcinoma, the cytoplasmic border of tumor cells was distinctly positive and the cytoplasm was weakly positive. In the intermediate type, cytoplasm of the tumor cells was strongly positive (Fig. 4). The latter case had transitional areas from small cell carcinoma to squamous cell carcinoma. In this area, the tumor cells were slightly larger in size and showed positive reaction for keratin.

Of 16 cases of large cell carcinoma, 45% were positive. Most of the tumor cells demonstrated strongly positive reaction (Fig. 5). Some of them showed lamellar concentric structures in the cytoplasm.

Atypical carcinoid tumor was positive in one of 3 cases.

Both cases of adenosquamous carcinoma were positive (Fig. 6). The basal cells and intermediate cells of the bronchus were strongly positive. The ciliated tall columnar bronchial epithelium sometimes showed positive reaction.

**CEA**

Of 72 cases of lung cancer, 73% showed positive reaction for CEA. Of 10 cases of squamous cell carcinoma, 90% were positive. Only poorly differentiated type of squamous cell carcinoma was negative. Large superficial type cells and keratin pearls were always positive, whereas intermediate type cells and basal type cells of squamous cell carcinoma were negative (Fig. 7).

In adenocarcinoma, 88% of 18 cases showed strongly positive reaction. Acinar and bronchiolo-alveolar types showed mostly strong positive reaction. All of them showed diffusely positive cytoplasm (Fig. 8). In some of the papillary type and solid carcinoma of mucus formation type, the cell border showed more intense reaction than the perinuclear area, though the intense reaction was not attributable to the glycocalyx of the intestinal mucosa. Two cases of adenocarcinoma were negative for CEA. These cases were papillary adenocarcinoma and were positive for keratin.

Of 24 cases of small cell carcinoma 58% were positive for CEA. Both intermediate and oat cell subtypes had similar staining behavior showing intracytoplasmic granularity (Fig. 9).

Of 16 cases of large cell carcinoma 69% showed focally but strongly positive reaction.

Two of 3 cases of atypical carcinoid tumor were positive. One of them was
Fig. 5. Immunoreactive cells for keratin in large cell carcinoma. There are so many positive cells. PAP method, counterstained with methylgreen, ×400.

Fig. 6. The basal cells of the bronchus reacting strongly for keratin. PAP method, counterstained with methylgreen, ×250.
Fig. 7. Immunoreactive cells for CEA in squamous cell carcinoma. Large superficial type cells are strongly positive. PAP method, counterstained with methylgreen, ×250.

Fig. 8. Immunoreactive cells for CEA in adenocarcinoma. Tumor cells show diffusely intracytoplasmic reaction. PAP method, counterstained with methylgreen, ×60.
Fig. 9. Immunoreactive cells for CEA in small cell carcinoma. Most of the tumor cells strongly react for CEA. PAP method, counterstained with methylgreen, ×400.

Fig. 10. Immunoreactive cells for HCG in adenocarcinoma. Intensity of reaction is mostly strong in the acinar type. PAP method, counterstained with methylgreen, ×60.
mucus producing type. Both of the two adenosquamous carcinoma samples were diffusely and strongly positive. In the adjacent area of the tumor, atypical portions of the bronchial mucus gland showed positive staining. Bronchial epithelial cells were negative. Numbers of macrophages accompanying the tumor were also positive for CEA.

**HCG**

Of 72 cases of lung cancer, 56% were positive for HCG. Three of 10 cases of squamous cell carcinoma were positive. One of them had a tendency of cellular dissociation and transition to large cell carcinoma. The other positive cases were moderately differentiated type.

All of the cases of adenocarcinoma were positive. Intensity of reaction was mostly strong in the acinar type (Fig. 10). Only one patient with solid carcinoma with mucus formation presented gynecomastia clinically.

Of 24 cases of small cell carcinoma, 23% showed positive reaction. Positive cells were focally observed.

Of 16 cases of large cell carcinoma, 56% were positive.

Two of 3 cases of atypical carcinoid and both two cases of adenosquamous carcinoma demonstrated positive reaction.

Normal lung tissues adjacent to the tumors were consistently negative.

CEA and HCG were both positive in 33 cases; 2 squamous cell carcinomas, 13 adenocarcinomas, 6 small cell carcinomas, 7 large cell carcinomas, 2 adenosquamous carcinomas, and 3 atypical carcinoid tumors. Eleven cases were

<table>
<thead>
<tr>
<th>Number and Intensity of Immunoreactive Cells</th>
<th>Karatin</th>
<th>CEA</th>
<th>HCG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marked</td>
<td>Squamous cell carcinoma</td>
<td>Adenocarcinoma</td>
<td>Adenocarcinoma</td>
</tr>
<tr>
<td></td>
<td>Adenocarcinoma (acinar type)</td>
<td></td>
<td>(acinar type)</td>
</tr>
<tr>
<td>Moderate</td>
<td>Adenocarcinoma (papillary, mucus producing and bronchioloalveolar types)</td>
<td>Adenosquamous carcinoma</td>
<td>Adenocarcinoma</td>
</tr>
<tr>
<td></td>
<td>Adenosquamous carcinoma</td>
<td>Small cell carcinoma</td>
<td>Squamous cell carcinoma</td>
</tr>
<tr>
<td></td>
<td>Large cell carcinoma</td>
<td>Large cell carcinoma</td>
<td>Large cell carcinoma</td>
</tr>
<tr>
<td></td>
<td>Adenosquamous carcinoma</td>
<td>Squamous cell carcinoma</td>
<td></td>
</tr>
<tr>
<td>Slight</td>
<td>Adenocarcinoma (acinar type)</td>
<td>Atypical carcinoid</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Small cell carcinoma</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Correlation between intensity of staining, number of immunoreactive cells and histological types in the positive cases.
positive for CEA alone, and 6 cases were positive for HCG alone. Thus, the CEA positive cases and the HCG positive cases did not necessarily overlap each other. The relative intensity of staining of HCG was somewhat weaker than CEA staining.

AFP was positive in only one case of large cell carcinoma, giant cell type. All of the other cases were negative.

**Poorly Differentiated cases with sheet like arrangement**

These types were reluctantly classified into adenocarcinoma, squamous cell carcinoma and large cell carcinoma in this study. These were negative or very focally positive for CEA and diffusely but faintly positive for keratin. Therefore, this type was still difficult to classify by using immunohistochemical methods for CEA and keratin. Correlation between intensity of staining, number of immunoreactive cells and histological types in the positive cases is summarized in Table 2.

**Discussion**

**Keratin**

There have been several immunohistochemical investigations of keratin on the lung cancer. Strong staining for keratin is evident in all squamous cell carcinoma (Gusterson, Michell, Warburton and Sloane 1982; Said, Nash, Tepper and Banks-Schlegel 1983; Schlegel, Schlegel, McLeod and Pinkus 1980). This reaction is particularly helpful in identifying squamous differentiation in poorly differentiated areas and in cases in which tonofilaments were seen sparsely or absent on electron micrographs (Said, Nash, Tepper and Banks-Schlegel 1983). On the other hand, adenocarcinomas were reported to be either negative or weakly positive by Schlegel, Schlegel, McLeod and Pinkus (1980). Two of 6 cases of adenocarcinoma revealed focal or uneven staining for keratin, which was weak in intensity (Gusterson, Mitchell, Warburton and Sloane 1982; Said, Nash, Tepper and Banks-Schlegel 1983). In our series 53% of adenocarcinoma samples were positive for keratin. Some of them showed strongly positive staining for keratin, especially papillary adenocarcinoma exhibiting strong reaction. On electron-microscopic examination, half of the lung cancers were combined type of epidermoid and adenocarcinoma. Some of the adenocarcinoma had both tonofilaments and microvilli (McDowell, McLaughlin, Merenyi, Kieffer, Haris and Trump 1978). Our data were consistent with their ultrastructural findings.

Small cell carcinoma was negative for keratin by Schlegel, Schlegel, McLeod and Pinkus (1980), but focally positive by Gusterson, Mitchell, Warburton and Sloane (1982). Some of the small cell carcinoma had well developed desmosomes and tonofilament bundles (Churg, Johnson and Stulbarg 1980; McDowell and Trump 1981). In our study, 3 of 24 cases of small cell carcinoma were positive. In these positive cases, cytoplasmic borders were especially strongly positive, and
we believe this finding to be consistent with the presence of well developed desmosomes.

Forty-five percent of large cell carcinoma were found to be positive for keratin by Gusterson Michell, Warburton and Sloane (1982). This percentage was exactly the same as our data of 45%. On the ultrastructural basis, large cell carcinoma was subclassified into four subtypes; squamous type, adenosquamous type, adenocarcinoma type and giant cell type (Horie and Ohta 1981).

The criteria for this identification of squamous cell carcinoma should include demonstration of intracellular bridges with a desmosome-tonofilament complex, keratohyaline granules and poorly developed endoplasmic reticulum in a comparatively dense cytoplasm (Buckley and Fox 1979; Krauss, Macy and Ichiki 1981). In contrast, the criteria for identification of glandular differentiation of tumor cells were found to be demonstration of acini with microvilli, junctional complexes, and a well organized cytoplasmic membrane system for secretory activity (Horie and Ohta 1981; McDowell, McLaughlin, Merenyi, Kieffer, Haris and Trump 1978). In adenosquamous carcinoma, there was evidence of differentiation to both squamous and glandular directions. Giant cell carcinoma was identified by demonstration of marked nuclear pleomorphism and occasional signs of squamous differentiation (Horie and Ohta 1981). Based on these criteria, 26 cases of large cell carcinoma were subtyped into 5 squamous cell carcinomas, 10 adenosquamous carcinomas, 9 adenocarcinomas and 2 giant cell carcinomas (Hansen, Hansen, Hirsh, Arends and Christensen 1980). Since squamous cell carcinoma and adenosquamous carcinoma are thought to be positive for keratin, the possibility of positive reaction for keratin is at least 58% (15 out of 26 cases). This result resembles our data closely.

In conclusion, if keratin is negative, it is difficult to diagnose that tumor is squamous cell carcinoma. Positive keratin staining is not limited to squamous cell carcinomas, but it could be seen in all epithelial cells as tonofilaments and desmosomes are seen universally in epithelial cells. Frequency of the immunoreactivity is different between small cell carcinoma and large cell carcinoma.

CEA

In lung cancer 68% of the patients have an elevated concentration of CEA in their sera (Vincent, Chu and Lane 1979). Sixty-eight percent of lung cancer patients with adenocarcinoma will have CEA greater than 2.5 ng/ml while only 45% of the patients with either small cell, or large cell squamous cell carcinoma will have initial values greater than 2.5 ng/ml (Vincent, Chu, Fergen and Ostrander 1975).

Pascal, Mesa-Tejada, Bennet, Garces and Fenoglio (1977) reported that well-differentiated adenocarcinoma of the lung showed glycopalyx staining similar to that seen in colon cancer. However, in our study, though the intensity of the reaction was stronger at the peripheral area of the cytoplasm, diffuse intracyto-
plasmic staining was also observed in the well-differentiated adenocarcinoma. All of the positive cells showed a diffuse cytoplasmic reaction. Squamous cell carcinoma showed positive reaction only in the superficial type cells and keratin pearls but not in the intermediate or basal type cells. This result is consistent with the data of Pascal, Mesa-Tejada, Bennet, Garces and Fenoglio (1977) and of Obata, Kodama, Hando and Takeuchi (1980) on cervical squamous cell carcinoma. Positive reaction for CEA in squamous cell carcinoma could affirm it glandular epithelial origin.

In small cell carcinoma, 58% are positive for CEA and 15% for keratin in our series. Krauss, Macy and Ichiki (1981) reported 80% of small cell carcinoma showed a high level of CEA in the serum. Therefore, CEA is the most detectable and useful marker in any type of the lung cancer. However, it is not particularly helpful in making distinction in classification.

**HCG**

Elevation of HCG in the presence of non-trophoblastic malignant disease has been reported. A surprisingly high incidence of measurable levels was found in patients with gastrointestinal tract tumors, especially in those with stomach, liver, pancreas and large intestine tumors (Braunstein, Vaitukaitis, Carbone and Ross 1973; Buckley and Fox 1979). In the bronchogenic carcinoma, the frequency of HCG producing tumor is not so high as seen in the gastrointestinal tract tumors (Gailani, Ming Chu, Nussbaum, Ostrander and Christoff 1976). However, there are several reports of HCG-producing bronchogenic carcinoma without evidence of choriocarcinoma (Cottrell, Becker and Moore 1968). The reported cases of HCG-producing tumor include squamous cell carcinoma, adenocarcinoma and oat cell carcinoma. The ability to secrete gonadotropin is not confined to one cell type (Cottrell, Becker, Mathews and Moore 1969). In the malignant tumor tissue, the HCG-α content is almost the same as in normal tissue (Braunstein, Forsythe, Rasor, Van Scoy-Mosher, Thompson and Wade 1979; Demura, Jibiki, Odagiri, Demura and Shizume 1979), but HCG-β was significantly elevated in about 50% of gastrointestinal tract cancer and 15% of lung cancer (Demura, Jibiki, Odagiri, Demura and Shizume 1979). Urine HCG/LH was detectable in 43% of 75 patients with small cell carcinoma, but serum HCG/LH could be demonstrated in only 15% (Hansen, Hansen, Hirsh, Arends and Christensen 1980). In all cases the concentration of serum HCG was low (Hansen, Hansen, Hirsh, Arends and Christensen 1980). Our study showed a positive reaction in 57% of the cases of lung cancer. We believe that this result is slightly high for two specific reasons. First, this discrepancy between low level of serum HCG and high rate of positive reaction on immunohistochemistry may reflect a differential rate of tumor HCG biosynthesis and release, differences in host metabolism and degradation of circulating HCG, or both of them. Second, HCG-α subunit is known to cross react with α-subunit of LH,
FSH or TSH. Our data may include LH, FSH or TSH producing tumors in HCG positive cases.

Although, there are so many positive cases for HCG, only one case was suggestive of ectopic HCG-production in our series. The HCG level in serum or urine was not examined in any case of ours. It would be interpreted that the chemical structure of ectopically produced HCG possesses few carbohydrate substrates which normally play an important role in bioactivity (Yoshimoto, Wolfsen and Odell 1977). Compared to CEA, the intensity of staining and the number of the positive cells are less in HCG.

**AFP**

Ectopic production of AFP in the non-trophoblastic neoplasm is well known in gastric carcinoma (Kodama, Kameya, Hirota, Shimosato, Ohkura, Mukojima and Kitaoka 1981). However, no remarkable investigation about AFP in lung cancer has been reported. Only one case of large cell carcinoma in our series demonstrated positive reaction for AFP. This peculiar behavior suggests that some of the large cell carcinomas have a characteristic possibility of pluripotential differentiation.

Application of immunohistochemical techniques for keratin, CEA, HCG and AFP in the lung cancer revealed that the immunoactivity for these antigens itself could not contribute to the distinction of the histologic typing of the tumor. However, there is trend in the intensity of the immunoreactivity that keratin is strongly positive in squamous cell carcinoma and CEA and HCG strongly react in adenocarcinoma.

CEA is mostly detectable in lung cancer and it is a useful tool to differentiate the small cell carcinoma from the small cell sarcoma such as lymphoma, neuroblastoma and rhabdomyosarcoma.

**Acknowledgments**

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**References**


