Analysis of Expression Patterns of Breast Cancer–Specific Markers (Mammaglobin and Gross Cystic Disease Fluid Protein 15) in Lung and Pleural Tumors

Yuji Takeda, MD; Koji Tsuta, MD; Yasuo Shibuki, CT; Tatsuhiro Hoshino, MD; Naobumi Tochigi, MD; Akiko Miyagi Maeshima, MD; Hisao Asamura, MD; Yuko Sasajima, MD; Tsuyoshi Ito, MD; Yoshihiro Matsuno, MD

Context.—The lung is the most common site of metastasis during the natural history of malignant tumors.1 In particular, breast carcinoma has a propensity for distant metastasis, and the lung and pleura are the second most common metastatic sites following bone.2 It is also well known that breast carcinoma metastatic to the lung may be found even after a postoperative disease-free interval up to 20 years after resection of the primary lesion. Distinction of primary lung carcinoma from breast carcinoma metastatic to the lung is important because the treatment modalities are different.

Objective.—To elucidate the utility of mammaglobin and gross cystic disease fluid protein 15 (GCDFP-15), which are known to be breast-specific antigens, in distinguishing various primary lung and pleural tumors from breast carcinoma metastasizing to the lung.

Design.—A total of 20 cases of breast carcinoma metastatic to the lung and 263 tumors of nonbreast origin located in the lung and pleura were analyzed.

Results.—Of the 20 cases of breast carcinoma metastatic to the lung, 10 (50.0%) were immunoreactive for mammaglobin and 9 (45.0%) for GCDFP-15, the frequency of positivity being slightly higher for the former than for the latter. The area immunopositive for mammaglobin showed more diffuse staining than the area immunopositive for GCDFP-15. Furthermore, the specificity of mammaglobin for breast carcinoma metastatic to the lung was superior (98.9%) to that of GCDFP-15 (91.8%).

Conclusion.—The sensitivity of mammaglobin is equal or superior to that of GCDFP-15 for investigation of breast carcinoma. Immunopositivity for mammaglobin is more diffuse than that for GCDFP-15. In terms of practical diagnosis, mammaglobin immunohistochemistry can serve as a differential marker of breast carcinoma and should be added to the immunohistochemical panel.

Arch Pathol Lab Med. 2008;132:239–243

The lung is the most common site of metastasis during the natural history of malignant tumors.1 In particular, breast carcinoma has a propensity for distant metastasis, and the lung and pleura are among the most common metastatic sites. Although it is often difficult to make a clear-cut differential diagnosis between the two, distinguishing primary lung carcinoma from breast carcinoma metastatic to the lung is important because the treatment modalities are different.

Differentiating between primary lung carcinoma and breast carcinoma metastatic to the lung is often problematic when only a small amount of material is available, as the histologic features may not be sufficient to permit unequivocal distinction. Therefore, reliable immunohistochemical markers are required to facilitate the differentiation of these malignancies.

In breast cancer, the estrogen receptor (ER)/progesterone receptor (PgR) status of the tumor is useful for both prognosis and therapy, with more chemotherapeutic options being available for patients with hormone receptor–positive tumors.3,4 Breast adenocarcinoma has been shown to be positive for ER in 24% to 63% of cases and positive for PgR in 9% to 37% of cases.5,6 Breast adenocarcinoma may demonstrate immunophenotypic variability in its expression of ER and PgR, with differences that are dependent on the histologic grade, histologic subtype, antibody clone applied, and immunohistochemical techniques used. These factors limit the sensitivity of these markers for excluding metastatic breast adenocarcinoma in cases of unknown primary site.

When primary unknown metastatic tumor is suspected...
to have originated from the breast, ER, PgR, and gross cystic disease fluid protein 15 (GCDFP-15) have been shown to be useful immunohistochemical markers. However, ER and PgR have also been documented in many neoplasms from various organs. In lung tumors, ER and PgR expressions are reported to range from 0% to 96.7% and from 0% to 46.5%, respectively. A panel consisting of anticytokeratin 7 and anticytokeratin 20 (CK7/CK20) antibodies is useful for determining the origin of an unknown primary tumor. However, numerous tumors exhibit an identical CK7+/CK20+ immunophenotype, including nearly all breast carcinomas and adenocarcinomas of the ovary, lung, endometrial, thyroid, and salivary gland. Additionally, GCDFP-15 has also been documented in many neoplasms from various locations. In lung tumors, GCDFP-15 expression has been reported to range from 0% to 3.3%. GCDFP-15 on each tumor are listed in Tables 1 and 2, respectively. The mammaglobin gene sequence fragments were first isolated in 1994 by Watson and Fleming. The mammaglobin gene encodes a 10-kDa molecule, which is related to a family of secretory proteins, including rat prostatic steroid-binding protein subunit C3, human Clara cell 10-kDa protein, and rabbit uteroglobin. Mammaglobin is expressed specifically in breast tissue. Recently, an anti-mammaglobin antibody that can be applied to formalin-fixed, paraffin-embedded sections has become commercially available. In the present study, we elucidated the expression of mammaglobin and GCDFP-15 in order to distinguish various primary lung and pleural tumors from breast carcinoma metastatic to the lung.

**MATERIALS AND METHODS**

**Histologic Analysis**

Materials for the present study were extracted from the pathology files of the National Cancer Center Hospital (Tokyo, Japan). The specimens comprised 20 cases of breast carcinoma metastatic to the lung and 263 lung and pleural tumors other than metastatic breast carcinomas: 100 adenocarcinomas, 60 squamous cell carcinomas, 20 pleomorphic carcinomas, 20 large-cell neuroendocrine carcinomas, 15 small-cell carcinomas, 19 carcinoids (14 cases typical and 5 cases atypical), 16 salivary gland–type tumors of the bronchus and/or trachea (11 cases of adenoid cystic carcinoma and 5 cases of mucoepidermoid carcinoma), and 13 malignant pleural mesotheliomas.

The 100 cases of adenocarcinoma were divided into 2 subtypes according to the growth pattern: 60 cases showing lepidic growth and 40 cases without lepidic growth. The 60 cases showing lepidic growth were further divided into 20 cases of the nonmucinous type (tumor cells resembling Clara cells or type II pneumocytes) and 40 cases of the mucinous type (tumor cells resembling goblet cells and/or bronchial surface epithelial cells). Furthermore, the 40 cases without lepidic growth were divided into 20 cases of the acinar-cribriform type (tumor showing an acinar and/or cribriform growth pattern with some degree of cytoplasmic mucin) and 20 cases of the solid type (tumor showing solid growth with some degree of cytoplasmic mucin formation, such as intracytoplasmic lumina). The 60 cases of squamous cell carcinoma were divided into the well-differentiated type (tumor cells showing a stratified pattern and abundant keratinization), moderately differentiated type (cells showing a lower degree of stratification than that of the well-differentiated type), and poorly differentiated type (the tumor composed of more atypical cells that show only focal squamous cell differentiation).

**Immunohistochemistry**

For immunohistochemical staining of mammaglobin (clone 304-1A5, 1:200; DAKO, Carpinteria, Calif) and GCDFP-15 (clone D6, 1:200; Signet, Dedham, Mass), 5-μm-thick formalin-fixed sections from each paraffin block were routinely deparaffinized. The sections were exposed to 3% hydrogen peroxide for 15 minutes to block endogenous peroxidase activity, and then washed in deionized water for 2 to 3 minutes. Then, for heat-induced epitope retrieval, the sections stained for mammaglobin were subjected to a 0.02M concentration of citrate buffer (pH 6.0) in a steamer at 120°C for 20 minutes. The sections were washed in deionized water for 2 to 3 minutes. After rinsing with phosphate-buffered saline for 5 minutes, the slides were incubated with primary antibody for 1 hour at room temperature. Then the slides were washed in phosphate-buffered saline 3 times for 5 minutes each time. Subsequently, the slides were labeled with EnVision+/HRP system (DAKO). Diaminobenzidine was used as the chromogen, and Meyer hematoxylin as the counterstain.

Grading the intensity of immunostaining was performed using a sliding scale of 0 to 3+ according to the percentage of reactive cells (0 = <1%; 1+ = 1%–10%; 2+ = 20%–50%; 3+ = 51%–100%).

**RESULTS**

The results of the immunostains of mammaglobin and GCDFP-15 on each tumor are listed in Tables 1 and 2, respectively.

**Breast Carcinoma Metastatic to the Lung**

Of the 20 cases of breast carcinoma metastatic to the lung, mammaglobin (Figure 1, A and B) and GCDFP-15 (Figure 1, C and D) stained 10 cases (50.0%) and 9 cases (45.0%), respectively, with mammaglobin showing a...
Table 2. Immunoreactivity of Gross Cystic Disease Fluid Protein 15

<table>
<thead>
<tr>
<th></th>
<th>No. of Cases Examined</th>
<th>% Positive</th>
<th>Staining Area</th>
<th>0</th>
<th>1+</th>
<th>2+</th>
<th>3+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast carcinoma, metastasis to lung</td>
<td>20</td>
<td>45</td>
<td></td>
<td>11</td>
<td>5</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Primary lung carcinoma</td>
<td>250</td>
<td>8.2</td>
<td></td>
<td>231</td>
<td>19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>100</td>
<td>5</td>
<td></td>
<td>85</td>
<td>15</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>60</td>
<td>0</td>
<td></td>
<td>60</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pleomorphic carcinoma</td>
<td>20</td>
<td>5</td>
<td></td>
<td>19</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Carcinoid tumor</td>
<td>19</td>
<td>5.2</td>
<td></td>
<td>18</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Large-cell neuroendocrine carcinoma</td>
<td>20</td>
<td>0</td>
<td></td>
<td>20</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Small-cell carcinoma</td>
<td>15</td>
<td>0</td>
<td></td>
<td>15</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Adenoid cystic carcinoma</td>
<td>11</td>
<td>0</td>
<td></td>
<td>11</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mucoepidermoid carcinoma</td>
<td>5</td>
<td>40</td>
<td></td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Malignant mesothelioma</td>
<td>13</td>
<td>0</td>
<td></td>
<td>13</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Figure 1. Breast carcinoma metastatic to the lung, demonstrating solid growth. A to D represent the same case. A and B, Diffuse and strong staining for mammaglobin (original magnifications ×2 [A] and ×20 [B]). C and D, Focal but strong staining for gross cystic disease fluid protein 15 (original magnifications ×2 [C] and ×20 [D]).

The specificity of mammaglobin for detecting breast carcinoma metastatic to the lung (3/263 cases; 98.9%) was higher than that of GCDFP-15 (19/263 cases; 92.8%).

Primary Lung and Pleural Tumors

Primary Lung Adenocarcinomas.—All primary lung adenocarcinomas were immunonegative for mammaglobin. However, GCDFP-15 expression was observed in 15 (15%) of 100 cases of primary lung adenocarcinoma. The lepidic growth pattern was positive in 12 (20%) of 60 cases, and the nonlepidic growth type was positive in 3 (7.5%) of 40 cases. In the lepidic growth type, the non-
mammaglobin. All squamous cell carcinomas were immunonegative for GCDFP-15.

**Pleomorphic Carcinoma.**—In pleomorphic carcinomas, expression of GCDFP-15 was observed in 1 (5.0%) of 20 cases, whereas all cases were immunonegative for mammaglobin.

**Neuroendocrine Tumors.**—Expression of mammaglobin was observed in 1 (5.2%) of the 19 carcinoid tumors, but positivity was limited to a small area (Figure 2, B). Furthermore, expression of GCDFP-15 was observed in 1 different case (5.2%), but the positivity again was limited to a small area (Figure 2, C).

All high-grade neuroendocrine tumors (large-cell neuroendocrine carcinoma and small-cell carcinoma) were immunonegative for both mammaglobin and GCDFP-15.

**Salivary Gland-Type Tumors (Mucoepidermoid Carcinoma and Adenoid Cystic Carcinoma) of the Bronchus and/or Trachea**

In mucoepidermoid carcinomas, expression of GCDFP-15 was observed in 2 (40.0%) of 5 cases, whereas all cases were immunonegative for mammaglobin. In adenoid cystic carcinoma, 1 (9.1%) of 11 cases was immunopositive for mammaglobin, whereas all cases were immunonegative for GCDFP-15. Mammaglobin (Figure 2, C) and GCDFP-15 showed focal staining.

**Malignant Mesotheliomas**

All malignant mesotheliomas were immunonegative for both mammaglobin and GCDFP-15.

**COMMENT**

In the present study, we demonstrated 3 advantages of using mammaglobin over GCDFP-15 to identify breast carcinoma metastatic to the lung. The first advantage is that the sensitivity of mammaglobin is slightly higher than that of GCDFP-15. The mammaglobin positivity rate for primary breast cancer is reported to be between 47.9%\(^{19}\) and 71%\(^{20}\). Although we focused on analyzing breast carcinoma metastatic to the lung, the overall prevalence of mammaglobin expression in our series (50%) is almost accordant with the findings of previous reports\(^{19,20}\) on primary breast cancer. The expression status of some molecules may be altered between primary and metastatic lesions. For example, the expression of surfactant apoprotein in lung cancer is frequently reduced in metastatic sites.\(^{21}\) However, a previous report has demonstrated that the concordance rate of mammaglobin expression between the primary site and lymph node metastases was 93%\(^{20}\). These findings indicate that mammaglobin expression is not altered in the metastatic lesion.

Several studies have analyzed the mammaglobin expression pattern in breast carcinoma using immunohistochemical methods. Two of three studies used a noncommercial antibody\(^{15}\) or a cocktail of antibodies\(^{20}\) to identify mammaglobin, and the positivity rate in primary breast carcinoma was around 70%. However, Sasaki et al\(^{19}\) reported that the positivity rate for mammaglobin in primary breast carcinoma analyzed by commercially available monoclonal antibody (clone 304-IAS) was lower than that using a noncommercial antibody or a cocktail of antibodies. These findings suggest that commercially available monoclonal antibody has a lower sensitivity and that there might be differences in the patient population. According to analyses by histologic type, mammaglobin expression was positive in 2 (10.0%) of 20 cases, and the mucinous type was positive in 10 (25%) of 40 cases. In the nonlepidic growth type, the acinar-cribriform type was positive in 3 (15.0%) of 20 cases, and the solid type was negative in all cases.

**Squamous Cell Carcinomas.**—Mammaglobin expression was observed in only one case of moderately differentiated squamous cell carcinoma, but this positive area was restricted to a small part of the tumor (Figure 2, A). All other squamous cell carcinomas were negative for mammaglobin.
pression was reported to be higher in lobular carcinoma than in ductal carcinoma.19,20 Another study has suggested that mammaglobin expression is evident mainly in well-differentiated hormone receptor–positive breast carcinomas.

The second advantage of using mammaglobin is that it is a nonlepidic growth pattern.22 In particular, about 30% of cases of primary lung adenocarcinoma, and none of the primary lung adenocarcinomas were positive.

Immunopositive for mammaglobin and GCDFP-15 are partly exclusive, the combined use of both markers is important.

The third advantage of using mammaglobin is that its expression was found in only 1.1% of nonbreast tumors of the lung and pleura. One carcinoma tumor, squamous cell carcinoma, 1 adenoid cystic carcinoma of the trachea, and none of the primary lung adenocarcinomas were positive. In primary lung adenocarcinoma, about 30% of cases demonstrated a nonlepidic growth pattern.22 In particular, the cribriform and/or acinar and solid type might be confused with a lung metastasis from breast cancer. In the present study, we demonstrated that mammaglobin was negative in the cribriform and/or acinar and solid types of primary lung adenocarcinoma.

The mammaglobin expression rate in lung tumors is reported to range from 0% to 16.7%.15,19,20,23 Mammaglobin expression was reported to have been found in 20% of salivary gland tumors.15,20 Therefore, the finding that adenoid cystic carcinoma of the trachea showed immunoreactivity for mammaglobin is not surprising but does require attention.

The differential diagnosis of malignant effusion involving the serosal membrane may be difficult. In the present study, all malignant mesotheliomas were immunonegative for mammaglobin. These findings indicated that mammaglobin should be added as one of the mesothelioma-negative markers, especially in female patients and/or cases of peritoneal mesothelioma.

Thyroid transcription factor 1 (TTF-1) has been shown to play a crucial role in the morphogenesis and function of the lung by regulating gene expression of surfactant proteins.23 Most studies have reported finding TTF-1 expression in more than 70% of primary adenocarcinomas of the lung.24 Therefore, TTF-1 has been considered a reliable marker to distinguish between primary lung adenocarcinoma and metastatic adenocarcinoma. It is reasonable that TTF-1 should be added to the antibody panel as a negative marker for metastatic tumors.

In conclusion, we demonstrated that the sensitivity of mammaglobin is equal or superior to that of GCDFP-15 for investigation of breast carcinoma metastatic to the lung. Immunonegativity for mammaglobin is more diffuse than that for GCDFP-15. In terms of practical diagnosis, mammaglobin immunonegativity can serve as a differential marker of breast carcinoma and should be added to immunohistochemical panels.

We thank Sachiko Miura and Chizu Kina for their skillful technical assistance. This work was supported in part by a Grant-in-aid for Cancer Research (16-6) from the Ministry of Health, Welfare, and Labor of Japan.

References


