Immunohistochemical distinction between pleural malignant mesothelioma and non-mucinous-type poorly differentiated adenocarcinoma of the lung

Koichi YASHIMA¹, Takashi HIRANO¹, Koichi YOSHIDA¹, Hiroaki KATABA¹, Yasuhumi KATO³, TatsuO OHIRA¹, Hiromi SERIZAWA², Harubumi KATO¹

¹Department of Surgery, Tokyo Medical University
²Department of Diagnostic Pathology, Tokyo Medical University

Abstract

Recently, the incidences of pleural malignant mesothelioma and primary lung adenocarcinoma resulting from exposure to asbestos have been increasing. As the two diseases require completely different surgical and chemotherapeutic strategies, it is very important to distinguish between them. However, differential diagnosis based upon morphology alone is sometimes very difficult. We therefore attempted to establish some immunohistochemical criteria that would be diagnostically useful for this purpose.

We evaluated 11 cases of mesothelioma and 32 cases of non-mucinous-type poorly differentiated (PD) adenocarcinoma immunohistochemically. Positive immunohistochemical reactivity for calretinin, mesothelin, cytokeratin 5/6, napsin A, TTF-1 and SpA was seen in 54.5%, 54.5%, 90.0%, 0%, 9.1% and 0% of mesothelioma cases, and 9.4%, 31.3%, 25.0%, 90.0%, 87.5% and 71.9% in PD adenocarcinoma cases, respectively.

As there are no biomarkers for mesothelioma with sufficiently high sensitivity and specificity, we considered that combined evaluation of positivity and negativity for these markers would be valuable for differential diagnosis between mesothelioma and PD adenocarcinoma.

On the basis of immunohistochemistry, we evaluated a case as mesothelioma when two or more markers for mesothelioma were positive and all the markers for primary lung adenocarcinoma were negative. Use of these immunohistochemical diagnostic criteria yielded a sensitivity of 63.6%, a specificity of 100% and an accuracy of 90.7%. We conclude that this combination of markers is valuable for distinction between mesothelioma and primary lung adenocarcinoma, even though more specific markers for mesothelioma are needed.

Introduction

Recently, the incidences of malignant pleural mesothelioma and lung diseases resulting from exposure to asbestos have been increasing. In the first half of the last century, it was established that exposure to asbestos carried an extremely high risk of mesothelioma and lung diseases. In spite of strong epidemiological evidence, asbestos production increased between 1960 and 1974. Therefore, it is predicted that asbestos-related lung diseases will increase rapidly for at least the next 30 years, because these diseases have latent periods of approximately 30 to 50 years.

As the surgical and chemotherapeutic strategies for pleural malignant mesothelioma and primary lung carcinoma differ completely, it is very important to distinguish between them. However, differential diagnosis is sometimes very difficult, because the clinical behavior as

Received November 15, 2005, Accepted December 19, 2005

Key words: pleural malignant mesothelioma, non-mucinous-type poorly differentiated adenocarcinoma of the lung, calretinin, mesothelin, cytokeratin 5/6, napsin A

Corresponding author: Koichi Yashima, Department of Surgery, Tokyo Medical University, 6-7-1 Nishi-shinjuku, Shinjuku-ku, Tokyo 160-0023, Japan
well as roentgenological and pathological features of malignant mesothelioma are similar to those of primary lung adenocarcinoma. Therefore, auxiliary techniques such as staining for immunohistochemical markers are needed for definitive diagnosis. Although many immunohistochemical markers are currently in clinical use, it is still difficult to distinguish mesothelioma from poorly differentiated non-mucinous-type primary lung adenocarcinoma. To diagnose malignant mesothelioma, not only positive markers that confirm malignant mesothelioma but also negative markers characteristic of primary lung adenocarcinoma that can rule out mesothelioma are necessary. Approximately 50% of all primary lung carcinomas in Japan are adenocarcinoma, and the incidence of adenocarcinoma continues to increase. Furthermore, most cases of peripheral-type primary lung carcinoma are adenocarcinoma. Therefore, differential diagnosis between malignant mesothelioma and poorly differentiated adenocarcinoma has become extremely important.

Primary lung adenocarcinoma arises mainly from type II pneumocytes in the alveolar sacs and from Clara cells in the bronchioles. Previously, we identified TA02 polyepitide as a histopathological biomarker for primary lung adenocarcinoma using two-dimensional polyacrylamide gel electrophoresis, and produced a mouse monoclonal antibody against it. When we evaluated the levels of TA02 expression in normal tissue, we found that its distribution was limited to lung (type II pneumocytes) and kidney (part of the renal tubules). Among malignant tumors, 90.7% of primary lung adenocarcinomas and 28.6% of large cell carcinomas showed positive immunohistochemical reactivity, but all other tumors, including other types of primary lung adenocarcinoma, metastatic lung adenocarcinoma and renal cell tumor, were completely negative. It has since been recognized that TA02 is homologous with napsin A, one of the human aspartic proteinases. Therefore, there is a high likelihood that napsin A would be a useful biomarker for discrimination between pleural malignant mesothelioma and poorly differentiated adenocarcinoma. In the present investigation, we evaluated differences in the protein expression profile between malignant mesothelioma and non-mucinous-type poorly differentiated adenocarcinoma of the lung.

Materials and Methods

Surgical specimens

The surgical materials used in this study were obtained from 11 cases of malignant mesothelioma (8 cases of epithelial type, 2 cases of mixed type and one case of sarcomatoid type) and 32 cases of non-mucinous-type poorly differentiated (PD) adenocarcinoma of the lung; resected at Tokyo Medical University Hospital between 1996 and 2004.

Immunohistochemical markers

We selected calretinin, mesothelin and cytokeratin 5/6 as positive markers for mesothelioma. Napsin A, thyroid transcription factor-1 (TTF-1) and surfactant apoprotein A (SpA), which were originally characterized as markers for primary lung adenocarcinoma, were used as negative markers for mesothelioma. The primary antibodies used for immunohistochemical staining were Pab, a rabbit polyclonal antibody against calretinin (Swant, Bellinzona, Switzerland), clone SP143 mouse monoclonal antibody against mesothelin (Spring Bioscience, Fremont, CA, USA), clone D5/16B4 mouse monoclonal antibody against cytokeratin 5/6 (Chemicon, Temecula, CA, USA), TMU-Ad02 mouse monoclonal antibody against TA02 (napsin A) (IBL, Fujioka, Japan), clone 8G7G3/1 mouse monoclonal antibody against thyroid transcription factor-1 (TTF-1) (DAKO), and clone PE-10 mouse monoclonal antibody against surfactant apoprotein A (SpA) (DAKO, Copenhagen, Denmark).

Immunohistochemical analysis

Surgically resected specimens were fixed in 10% formalin and subsequently embedded in paraffin, then 4-micrometer-thick tissue sections were cut and collected on glass slides. After deparaffinization, the specimens were stained immunohistochemically by the avidin-biotin peroxidase complex (ABC) method.

We evaluated these specimens microscopically, and classified them into four groups according to the percentage of immunohistochemically reactive cells (trace <10%; 1+ 10–40%; 2+ 40–75%; 3+ >76–100%).

Results

Figure 1 shows the representative features of immunohistochemical staining in specimens of mesothelioma and PD adenocarcinoma. The immunohistochemical reactivities for each antibody are summarized in Table 1.

Calretinin, mesothelin, cytokeratin 5/6, napsin A, TTF-1 and SpA were immunohistochemically positive in 54.5%, 54.5%, 90.9%, 0%, 9.1% and 0% of mesotheliomas, and in 9.4%, 31.3%, 25.0%, 90.6%, 87.5% and 71.9% of PD adenocarcinomas, respectively.

When more than two immunohistochemical markers for mesothelioma were positive and more than two markers for primary lung adenocarcinoma were negative, we diagnosed the case as mesothelioma. We evaluated the sensitivity, specificity and accuracy of these immunohistochemical criteria to be 72.7%, 96.9% and 90.7%, respectively (Table 2).

Discussion

Malignant mesothelioma is one of the most difficult
tumors to diagnose because it shows a wide variety of pathological and morphological patterns. In general, pleural malignant mesothelioma is morphologically subclassified into the epithelial type, sarcomatoid type or mixed type. Morphological distinction between mesothelioma and primary lung adenocarcinoma is particularly difficult, especially the differential diagnosis between epithelial-type mesothelioma and poorly differentiated adenocarcinoma without mucus production. In routine pathological examinations, immunohistochemical diagnosis is necessary as an ancillary technique for differential diagnosis. However, it is
Mesothelin is a cell surface antigen of unknown physiological function, and its distribution is restricted to cells of mesothelial origin. Recently it has been recognized as a new marker for mesothelioma, showing relatively high sensitivity. However, it is known that mesothelin is also expressed in serous carcinoma of the ovary, pancreatic adenocarcinoma, some squamous carcinomas and other kinds of carcinoma. Furthermore, it is reported to show immunohistochemical reactivity in 38–53% of primary adenocarcinomas of the lung\(^\text{11}\). In our investigation, mesothelin was immunohistochemically positive in 31% of PD adenocarcinomas. Therefore, it seems difficult to distinguish mesothelioma from PD adenocarcinoma using mesothelin alone.

Cytokeratin 5/6 has been used routinely in diagnostic pathology not only as a marker of mesothelial cells, but also for histological differentiation of basement membrane, squamous cells and myoepithelia in several human neoplasms\(^\text{12,13}\). In the present study, CK5/6 reactivity was demonstrated in 90.9% of malignant mesotheliomas, and its sensitivity for malignant mesothelioma was the highest among the three mesothelioma biomarkers investigated. On the other hand, we found that its false positivity rate was 25.0%. As we evaluated only non-mucinous-type poorly differentiated adenocarcinoma of the lung immunohistochemically, there is a possibility that part of these lesions showing morphologically undifferentiated nests may have either squamous cell differentiation or the potential to differentiate to squamous epithelium. Therefore, it is difficult to discriminate between mesothelioma and PD adenocarcinoma using immunohistochemical staining for CK5/6 alone.

For the differential diagnosis of mesothelioma from PD adenocarcinoma, it is very important to use not only positive markers for mesothelioma but also negative markers that can identify PD adenocarcinoma at the same time. Molecular markers for primary lung adenocarcinoma such as TAO2 (napsin A), thyroid transcription factor-1 (TTF-1), and surfactant protein-A (Sp-A) have been listed as candidate negative markers for mesothelioma.

The usefulness of TAO2 for diagnosing primary lung adenocarcinoma has been reported previously. We detected this molecule during investigation of primary lung cancer proteins using two-dimensional polyacrylamide gel electrophoresis, and at present it is acknowledged that TAO2 is synonymous with napsin A, a human aspartic proteinase\(^\text{14}\). Napsin A is distributed immunohistochemically in type II pneumocytes and part of the renal tubule. When we investigated the expression of napsin A in malignant tumors, we found it was present only in primary lung adenocarcinoma and a few large cell carcinomas, and not in other kinds of tumor including metastatic lung adenocarcinoma\(^\text{15}\). In the

---

**Table 1** Immunohistochemical expression of markers for malignant mesothelioma and poorly differentiated primary lung adenocarcinoma

<table>
<thead>
<tr>
<th>Expression levels in malignant mesothelioma</th>
<th>1+</th>
<th>2+</th>
<th>3+</th>
<th>total positivity rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calretinin</td>
<td>5</td>
<td>2</td>
<td>2</td>
<td>54.50%</td>
</tr>
<tr>
<td>Mesothelin</td>
<td>5</td>
<td>0</td>
<td>4</td>
<td>54.50%</td>
</tr>
<tr>
<td>CK5/6</td>
<td>1</td>
<td>0</td>
<td>4</td>
<td>90.90%</td>
</tr>
<tr>
<td>Napsin A</td>
<td>11</td>
<td>0</td>
<td>0</td>
<td>0.00%</td>
</tr>
<tr>
<td>TTF-1</td>
<td>10</td>
<td>1</td>
<td>0</td>
<td>9.10%</td>
</tr>
<tr>
<td>SP-A</td>
<td>11</td>
<td>0</td>
<td>0</td>
<td>0.00%</td>
</tr>
</tbody>
</table>

**Table 2** Immunohistochemical diagnosis of mesothelioma, evaluated in terms of positivity for two or more of the markers calretinin, mesothelin, and CK5/6, as well as negativity for more than two markers for primary lung adenocarcinoma

<table>
<thead>
<tr>
<th>Histological diagnosis</th>
<th>Immunohistochemical diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malignant mesothelioma</td>
<td>Mesothelioma</td>
</tr>
<tr>
<td>NM-PDAdc</td>
<td>Non-mucinous-type poorly</td>
</tr>
<tr>
<td></td>
<td>differentiated primary lung</td>
</tr>
<tr>
<td></td>
<td>adenocarcinoma</td>
</tr>
</tbody>
</table>

---

well known that some positive markers for mesothelioma, such as calretinin, mesothelin and cytokeratin 5/6, are expressed in some parts of primary lung adenocarcinoma of the lung\(^\text{6,7}\).

Calretinin is used most frequently for immunohistochemical diagnosis of malignant mesothelioma, and many investigators have reported its usefulness for this purpose\(^\text{8,9}\). However, calretinin can also be expressed to some degree in any histological type of pulmonary carcinoma, and a previous study has reported a positive expression rate of 69% in primary lung adenocarcinoma\(^\text{10}\). In our present study, calretinin was positive in 9.4% of PD adenocarcinomas. Thus, calretinin may not be clinically useful as a single marker for mesothelioma because of its low specificity.

Mesothelin is a cell surface antigen of unknown
In the present study, non of the 11 malignant mesotheliomas showed any reactivity for napsin A, whereas more than 90% of PD adenocarcinomas were immunohistochemically reactive. This level of reactivity was much higher than that in our previous study. In the present investigation we excluded mucinous-type adenocarcinoma because it is very easy to distinguish from mesothelioma morphologically. In this context we believe that more than 90% of PD adenocarcinomas showed positive immunohistochemical reactivity. The immunohistochemical staining pattern of napsin A is cytoplasmic and granular, corresponding to the lamellar bodies of pulmonary type II alveolar cells.

TTF-1 is a homeodomain-contacting nuclear transcription protein of the Nkx2 family that is expressed in bronchiolar and alveolar type II cells of the lung, follicular and C cells of the thyroid, and the developing brain. Immunochemical expression of TTF-1 has been demonstrated in primary lung adenocarcinoma and neuroendocrine carcinoma, but not in squamous cell carcinoma or metastatic adenocarcinoma, except that from the thyroid. Therefore, TTF-1 is often used for distinction between primary lung adenocarcinoma and metastatic lung adenocarcinoma. Although only a few reports have compared the immunohistochemical expressions of napsin A and TTF-1, the sensitivity of napsin A as a marker for primary lung adenocarcinoma seems to be the same as that of TTF-1. However, the specificity of napsin A for primary lung adenocarcinoma was higher than that of TTF-1.

Sp-A is a well-known immunohistochemical marker of alveolar type II cells and has been detected in atypical adenomatous hyperplasia and adenocarcinoma arising from type II pneumocytes. The histological distribution of Sp-A is similar to that of napsin A. Previous studies that compared napsin A with Sp-A found that 84.6-90.7% of primary lung adenocarcinomas expressed napsin A immunohistochemically, and that 61.5-69.8% of primary lung adenocarcinomas expressed Sp-A. In the present study, the positivity rate for napsin A was also 90.6%, despite the fact that the adenocarcinoma was poorly differentiated, and that for Sp-A was 71.9%. Napsin A may be more useful than Sp-A for distinguishing between malignant mesothelioma and primary lung adenocarcinoma, because the sensitivity of napsin A for PD adenocarcinoma is superior to that of Sp-A.

In this context, we immunohistochemically evaluated a case as mesothelioma when two or more markers for mesothelioma were positive and all the markers for primary lung adenocarcinoma were negative. These immunohistochemical diagnostic criteria gave a sensitivity of 63.6%, a specificity of 100% and an accuracy of 90.7%. In this study we evaluated one case of sarcomatoid-type mesothelioma, and found that it was completely immunohistochemically negative for all of the antigens investigated. Therefore, we were unable to diagnose this case as mesothelioma on the basis of our diagnostic criteria. However, it is easy to distinguish sarcomatoid-type mesothelioma from PD adenocarcinoma morphologically, as the former has a fibrosarcoma-like appearance and is composed of cells with elongated cytoplasm, arranged in parallel bundles. It accounts for only 10-30% of all mesotheliomas. When we excluded sarcomatoid-type mesothelioma from this immunohistochemical investigation, the sensitivity of our diagnostic criteria was 70.0%, the specificity was 100% and the accuracy was 92.9%.

Unfortunately, there are currently no biomarkers for mesothelioma that show sufficiently high sensitivity and specificity. Therefore, we conclude that combined use of positive and negative biomarkers for mesothelioma is valuable for differential diagnosis of mesothelioma from PD adenocarcinoma.

Acknowledgement

This study was supported by a grant from the Japan Society for the Promotion of Science (10671271) and a grant from Tokyo Medical University.

References


7) Miettinen: [DDD2] Expression of calretinin, thrombomodulin, keratin 5, and mesothelin in lung carcinomas of different types: An immunohistochemical analysis of 596 tumors in comparison with
胸膜悪性中皮腫と非粘液産生性低分化肺腺癌の免疫組織化学的鑑別

八島孝一1) 平野隆1) 吉田浩一1)
片場寛明3) 加藤靖文1) 大平達夫1)
芹沢博美2) 加藤治文1)

1) 東京医科大外科学第一講座
2) 東京医科大病理診断学講座

【要旨】近年アスベスト暴露による悪性中皮腫や原発性肺癌が増加している。この2つの疾患は手術方法や化学療法の選択が異なるため、鑑別診断が非常に重要である。しかししながら悪性中皮腫と原発性肺癌は病理像が類似していることが多く、形態学的な診断のみでは鑑別が困難であることが多い。そこで今回はこれらを鑑別のため、多数の抗体を用いた免疫組織化学的診断法の確立を試みた。悪性中皮腫11例および非粘液産生性の低分化肺腺癌32例を対象にcalretinin、mesothelin、cytokeratin5/6、napsin A、TTF-1、SpAに対する免疫組織化学染色の反応性を評価した。各分子に対する免疫組織化学染色の反応性は悪性中皮腫で54.5%、54.5%、90.9%、0%、9.1%、0%、非粘液産生性の低分化肺腺癌で9.4%、31.3%、25.0%、90.6%、87.5%、71.9%の症例であった。

悪性中皮腫に対して単独で診断しうる特異度と特異度をもったバイオマーカーはなく、悪性中皮腫と低分化肺腺癌の鑑別診断ではそれぞれに対するpositive makerおよびnegative makerを組み合わせて評価する必要があると考えられた。今回これらのマーカーの組み合わせによる悪性中皮腫の免疫組織化学的診断法の特異性63%、特異性100%、正確率90.7%であった。今後は悪性中皮腫に対して特異性の高いバイオマーカーが必要と考えるもの、本診断法は臨床的に有用性が高いと考えられた。

〈キーワード〉悪性中皮腫、非粘液産生性低分化肺腺癌、カルレチニン、メソセリン、サイトケラチン5/6、ナプシンA