ABSTRACT

The expression of fucosylceramide (PC47H antigen) in 97 lung cancers and 4 extrapulmonary squamous cell carcinomas was examined with the use of a novel monoclonal antibody, PC47H, recognizing fucosylceramide specifically. The observed variation in fucosylceramide content was dependent on the degree of glandular differentiation in adenocarcinoma of the lung. Fucosylceramide was abundantly expressed in well differentiated adenocarcinoma of the lung and poorly expressed in poorly differentiated adenocarcinoma. Some squamous cell carcinomas of the lung reacted with this monoclonal antibody weakly, but the reaction was noted only at the periphery of the epithelial sheets. Extrapulmonary squamous cell carcinoma and small cell carcinomas did not react with monoclonal antibody PC47H. Interestingly, large cell carcinomas with uncertain cell origin were all positive for fucosylceramide, which accumulated in the cytoplasm. At the ultrastructural level, fucosylceramide was located in the plasma membrane and unit membrane of the rough endoplasmic reticulum. On the other hand, carcinoembryonic antigen as an adenocarcinoma-associated tumor marker was expressed significantly in squamous cell carcinomas as well as adenocarcinomas. Taken together, fucosylceramide seems to be expressed preferentially in adenocarcinomas, and is closely linked to glandular differentiation. Thus it may be a better tumor marker than carcinoembryonic antigen.

INTRODUCTION

Although cases of lung cancer are increasing throughout the world, the disease is still classified based mainly on morphological features (1, 2). The main types of lung cancer are squamous cell carcinoma, small cell carcinoma, adenocarcinoma, large cell carcinoma, and carcinoid tumor. It is especially difficult for a pathologist to determine the type to which large cell carcinoma belongs, because this carcinoma lacks both glandular structures and epithelial sheets (3, 4).

On the other hand, many fucose-containing glycolipids have been found in human adenocarcinomas (5), and it has been shown that changes in the constitution of glycoconjugates are difficult for a pathologist to determine the type to which large cell carcinoma belongs, because this carcinoma lacks both glandular structures and epithelial sheets (3, 4).

Recently, one of the present authors established a mouse anti-fucosylceramide MAb,2 PC47H, using neutral glycolipids from human pancreatic cancer tissue as an immunogen (8). The antigen specificity of MAb PC47H was exclusively directed against fucosylceramide. Another anti-CEA monoclonal antibody, CA 208, was used as an adenocarcinoma-associated tumor marker (9).

Monoclonal Antibody. A murine antifucosylceramide monoclonal antibody, PC47H, was generated by immunization with glycolipids from human pancreatic cancer tissue as described elsewhere (8). The antigen specificity of MAb PC47H was exclusively directed against fucosylceramide. Another anti-CEA monoclonal antibody, CA 208, was used as an adenocarcinoma-associated tumor marker (9).

Materials and methods

Tissues. Ninety-seven formalin-fixed and paraffin-embedded lung cancers were obtained from Fukujuji Hospital, Japan Anti-Tuberculosis Association, Tokyo, Japan. They comprised 28 squamous cell carcinomas, 10 small cell carcinomas, 46 adenocarcinomas, 7 large cell carcinomas, 2 carcinoid tumors, 3 sarcomas, and 1 carcinosarcoma. They were classified according to the General Rules for Clinical and Pathological Recording of Lung Cancer of the Japan Lung Cancer Society (Table 1) (2). Four extrapulmonary squamous cell carcinomas (uterine cervix cancer, esophageal cancer, tongue cancer, and perineal cancer) and five fresh-frozen lung cancers were provided by the Department of Pathology, Saitama Medical School, Saitama, Japan.

Monoclonal Antibody. A murine antifucosylceramide monoclonal antibody, PC47H, was generated by immunization with glycolipids from human pancreatic cancer tissue as described elsewhere (8). The antigen specificity of MAb PC47H was exclusively directed against fucosylceramide. Another anti-CEA monoclonal antibody, CA 208, was used as an adenocarcinoma-associated tumor marker (9).

Immunohistochemistry. The cancer tissues embedded in O.C.T. compound were cut thinly with a cryostat. The paraffin sections were deparaffinized with xylene and ethanol. Mabs PC47H and CA 208 were diluted 1:4 and 1:100, respectively, in phosphate-buffered saline plus 1% bovine serum albumin for fresh-frozen and paraffin sections. As described in detail elsewhere, immunohistochemistry was performed by using the Histofine avidin-biotin complex procedure and reagents (Nichirei Co., Tokyo, Japan) (10). After completion of the entire immune reaction, the slides were counterstained with hematoxylin, dehydrated, cleared, and mounted. For negative control slides, all these steps were repeated, excluding the primary antibody, and substituting an irrelevant, isotype-matched, monoclonal antibody (antikeratin).

The immunostaining results were evaluated independently by three well-trained immunopathologists (H. Y., S. I., and I. S.), and were similar in all cases. Scoring of MAb PC47H and MAb MA 208 reactivities was based upon evaluation of tissue sections immunostained with the two Mabs. The intensity of immunostaining was scored as follows: -, no cancer cells immunostained; ±, less than 5% of cancer cells immunostained; +, 5-50% of cancer cells immunostained; ++, more than 50% of cancer cells immunostained.

Immunoelectron Microscopy. For immunoelectron microscopic examination, fresh lung cancer tissues were fixed with periodate-lysine-paraformaldehyde at 4°C overnight. Immunoelectron microscopy was then performed with the use of MAb PC47H and pulmonary adenocarcinoma tissue as described previously (11, 12).
Table 1 Immunoreactivity of monoclonal antibody PC47H with lung cancers and extrapulmonary squamous cell carcinomas

Lung cancers were classified according to the General Rules for Clinical and Pathological Recording of Lung Cancer, 1987, of the Japan Lung Cancer Society.Intensity of immunostaining was classified as follows: -, no staining; ±, less than 5% of cancer cells stained; +, 5 to 50% of cancer cells stained; ++, more than 50% of cancer cells stained. Percentage positivity was expressed as:

<table>
<thead>
<tr>
<th>Types of lung cancer</th>
<th>Cases</th>
<th>-</th>
<th>±</th>
<th>+</th>
<th>++</th>
<th>% of positivity (+ and ++)</th>
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<td>3</td>
<td>1</td>
<td>5</td>
<td>1</td>
<td>60</td>
</tr>
<tr>
<td>Poorly differentiated</td>
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<td>5</td>
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<td>0</td>
<td>40</td>
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<td>1</td>
<td>19</td>
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<tr>
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<td>4</td>
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<td>0</td>
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<tr>
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<td>5</td>
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</table>

RESULTS

Immunoelectron Microscopic Study. As shown in Fig. 1, immunoreactive fucosylceramide (PC47H antigen) was recognized in the cell membrane and unit membrane of the rough ER of adenocarcinoma of the lung. Immunoreactive fucosylceramide was not noted in two of the squamous cell carcinomas investigated.

Immunohistochemistry. First we compared PC47H antigen expression in fresh lung adenocarcinoma tissues with that in paraffin-embedded lung adenocarcinoma tissues. Both displayed similar immunostaining patterns. Therefore, we used mainly paraffin blocks of lung cancer tissue in our present large-scale study. PC47H antigen was present most extensively in well-differentiated adenocarcinoma of the lung (24 of 24) (Fig. 2, top). MAb PC47H was very strongly positive (+++) for 20 well-differentiated adenocarcinoma of the lung (83%) and 6 moderately differentiated adenocarcinoma of the lung (50%), and was positive (+) for 4 well-differentiated adenocarcinoma (17%) and 6 moderately differentiated adenocarcinoma (50%), respectively (Table 1).

PC47H antigen tended to decrease as glandular patterns in the adenocarcinoma were lost (Fig. 2, center and bottom). The staining for PC47H antigen showed a cytoplasmic granular pattern (Fig. 1). Well-differentiated squamous cell carcinomas reacted with MAb PC47H very weakly, and PC47H antigen-positive cancer cells of moderately and poorly differentiated squamous cell carcinoma were located at the periphery of epithelial sheets, accounting for less than 50% of the cancer cells present. No immunoreactivity with MAb PC47H was seen in small cell carcinomas, carcinoid tumor, or sarcoma. In the case of carcinosarcoma, the chondrosarcomatous parts were immunostained moderately.

Large cell carcinomas were all stained strongly with MAb PC47H, the immunoreaction appearing as a granular pattern (Fig. 3). Since fucosylceramide was absent or present to only a moderate degree in squamous cell carcinoma of the lung, we examined the immunoreactivity of MAb PC47H with extrapulmonary squamous cell carcinomas of the esophagus, tongue, uterine cervix, and perineum. All were negative for PC47H antigen (Fig. 3, B, C, D, and E).

CEA, commonly used as an adenocarcinoma-associated tumor marker, was expressed in 25 of 36 adenocarcinomas, 13 of 28 squamous cell carcinomas, and 1 of 7 large cell carcinomas (Table 2). Extrapulmonary squamous cell carcinomas of the esophagus, tongue, uterine cervix, and perineum reacted with MAb CA 208.

DISCUSSION

We have shown that immunoreactive fucosylceramide was highly expressed in various lung cancers, especially in adenocarcinoma. As adenocarcinoma occurs in other organs such as...
Fig. 2. Expression of PC47H Ag and CEA in adenocarcinoma of the lung based on the degree of glandular differentiation. × 190. Avidin-biotin complex-peroxidase method. Counterstained with hematoxylin. Top, well-differentiated adenocarcinoma of the lung; middle, moderately differentiated adenocarcinoma of the lung; bottom, poorly differentiated adenocarcinoma of the lung. Both PC47H antigen and CEA are abundantly expressed in well-differentiated adenocarcinoma, but not so highly expressed in poorly differentiated adenocarcinoma. Note that PC47H antigen is present in a granular pattern (→).
the stomach, colon, breast, pancreas, uterus, and ovary, it may also be of interest to examine fucosylceramide expression among them. It has already been shown that fucosylceramide is absent in fibroblasts, lymphocytes, granulocytes, and neuroblastoma (8), whereas it is abundantly expressed in colonic cancer but very poorly expressed in normal colonic mucosa; thus the authors speculate that the concentration of fucosylceramide is correlated with the severity of colonic cancer (13).

At the ultrastructural level, expression of immunoreactive fucosylceramide (PC47H antigen) was confined to the plasma membrane and unit membrane of the rough ER. The immuno-staining pattern produced by MAb PC47H in the cytoplasm was granular. As PC47H antigen is present in the rough ER, the granular pattern may correspond to the distribution of fucosylceramide in this organelle. Since the rough ER synthesizes protein(s), fucosylceramide, which is a type of glycolipid, cannot be produced there. Instead, it may be a constituent of the plasma membrane and rough ER unit membrane of cancer cells, where it is uniquely expressed. Therefore, the fucosylceramide may not be secreted by cancer cells.

Interestingly, the expression of immunoreactive fucosylceramide was closely associated with glandular differentiation of lung adenocarcinoma. So far, many researchers have shown that CEA, CA 19–9, CA125, sialyl SSEA-1 antigen, and other

Fig. 3. Expression of PC47H antigen in other types of lung cancer and extrapulmonary squamous cell carcinoma. × 190. Avidin-biotin complex-peroxidase method. Counterstained with hematoxylin. PC47H antigen is moderately expressed in large cell carcinoma of the lung (A), but hardly expressed in small cell carcinoma (B), squamous cell carcinoma of the lung (C), extrapulmonary squamous cell carcinoma of the esophagus (D), and perineum (E).
FUCOSYLCERAMIDE IN LUNG CANCER

proteins are expressed in various adenocarcinomas (5, 6, 14–16). However, their expression is also significantly higher in other types of carcinomas. Thus fucosylceramide is unique in that it is expressed preferentially in well-differentiated lung adenocarcinoma but poorly or barely expressed in other types of lung cancer. We thought it important to compare the level of fucosylceramide expression with that of fucosylceramide positivity of lung cancer and prognosis linked to prognosis.

The prognosis of lung cancer has been evaluated on the basis of clinical staging and morphological type. The prognosis in terms of 5-year survival is poor, especially for small cell carcinoma, poorly differentiated adenocarcinoma, and large cell carcinoma (17). It may be valuable to investigate the relationship between fucosylceramide positivity of lung cancer and prognosis to determine if the level of fucosylceramide expression is linked to prognosis.

REFERENCES

Preferential Expression of Immunoreactive Fucosylceramide in Adenocarcinoma of the Lung

Hiroyuki Yamada, Hideki Ishihara, Hiroshi Kitagawa, et al.


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