Monoclonal Antibody That Distinguishes Small-Cell Lung Cancer from Non-Small-Cell Lung Cancer

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ABSTRACT

To examine whether a monoclonal antibody, TFS-4, can distinguish small-cell lung cancer from non-small-cell lung cancers, an extensive survey of fresh lung tumors, cancers from other organs, and normal tissue specimens has been carried out. The antibody has been shown to react specifically with small-cell lung cancer (15 of 15) but not with squamous cell carcinoma (0 of 20), adenocarcinoma (0 of 20) of the lung, or large-cell lung cancer (0 of 2). It reacted neither with other malignancies, including colorectal cancer, gastric cancer, and malignant lymphoma, nor with such normal tissues as trachea, lung, liver, pancreas, colon, kidney, spleen, skin, striated muscle, bone marrow, or peripheral blood cells. Interestingly, the antibody cross-reacted with central nervous tissues. The antigenic determinant on small-cell lung cancer and that on human brain were both heat labile and trypsin sensitive, but resisted treatment with neuraminidase, suggesting that they represent similar peptides. TFS-4 may be of clinical use in the diagnosis of small-cell lung cancer, while the antigen may help investigate the nature and origin of small-cell lung cancer.

INTRODUCTION

With the advent of modern therapy of lung cancer, it became apparent that the histological type greatly influences the clinical presentation, response to therapy, and survival. SCLC has a much greater metastatic capacity and responsiveness to treatment than does NSCLC. In SCLC, even early stage cases are seldom cured by surgical resection. On the other hand, the majority of cases respond dramatically to chemotherapy and radiotherapy, and some suitably treated patients with extensive disease survive for long periods (1). Thus, it is essential to know whether the lung cancer is SCLC or NSCLC.

Currently, the distinction is made by light microscopy. However, even expert lung cancer pathologists can disagree on this subtyping of lung cancer (2). Since the development of hybridoma technology has been reported, monoclonal antibodies with high specificity have been used for the differential diagnosis of various diseases (3, 4). We have developed four monoclonal antibodies directed against SCLC (5). One of them has been found to be highly specific for SCLC. The most interesting possibility may be that this monoclonal antibody can distinguish SCLC from NSCLC tumors. To determine the usefulness of the antibody in diagnosis and treatment of SCLC, a large survey of lung tumors and normal tissue specimens has been carried out. In this paper, we describe the specificity of this antibody and the characterization of the antigenic determinant.

MATERIALS AND METHODS

Hybridoma. The production of TFS-4 has been previously reported (5).

RESULTS

Immunohistochemistry. The preliminary examinations revealed that routine formalin fixation and paraffin embedding of the tissue specimens destroyed the binding capacity of the antigen detected by TFS-4.4 Immunohistochemistry was performed on frozen sections with the ABC method (6). Samples taken at surgery or autopsy were fixed in 4% paraformaldehyde at 4°C for 4 h, incubated in increasing concentrations of sucrose (10 to 20%), and snap frozen in OCT compound (Miles Lab., Naperville, IL) at −70°C. Six-μm cryostat sections were treated with 10% NSS (Dakopatts, Copenhagen, Denmark) in phosphate-buffered saline prior to staining. The sections were incubated with TFS-4 at a concentration of 5 μg/ml for 60 min at room temperature. Control sections were treated with phosphate-buffered saline or irrelevant monoclonal mouse IgG1 at the same concentration. As a secondary antibody, biotinylated anti-mouse IgG (1:200 in 2% NSS in phosphate-buffered saline) (Vector, Burlingame, CA) was applied for 30 min. Then, the sections were treated with ABC complex (1:100 in 2% NSS in phosphate-buffered saline) for 30 min. Finally, they were reacted with diaminobenzidine for 5 to 10 min. Sodium azide (0.06%) was included to inhibit the intrinsic peroxidase. Sections were then washed, counterstained with Mayer's hematoxylin for 1 min, and mounted.

Histological diagnosis of tumors was made on conventional formalin-fixed and paraffin-embedded sections stained with hematoxylin-eosin.

Characterization of Antigen. Smears of cultured cells (NCI-H69 SCLC cell line), frozen sections (SCLC tumor), and tissue homogenates (human cerebrum) were used for the characterization studies. Samples were incubated with trypsin (Miles Lab.) at concentrations of 1 to 1000 μg per ml in phosphate-buffered saline (pH 7.4) for 60 min at 37°C. Then, to terminate the reaction of the enzyme, 1 mg of soybean trypsin inhibitor (Miles Lab.) was added to the samples. To examine the heat stability, samples were incubated in phosphate-buffered saline at 80°C for 60 min. Neuraminidase from Vibrio cholerae (Calbiochem, San Diego, CA) was added at concentrations of 0.05 to 50 units/ml in acetate buffer (pH 5.0) and incubated for 60 min at 37°C. Samples were incubated in methanol for 5 min and washed with phosphate-buffered saline. Reactivity of the antigen after treatments was assessed by the ABC method (smears and frozen sections) or by immunoblotting (tissue homogenates) described below.

Immunoblotting. Two μl of samples were blotted onto nitrocellulose membrane (Bio-Rad) and air dried. To prevent the nonspecific binding of antibodies, the membranes were incubated in 1% gelatin in phosphate-buffered saline for 20 min. Then, they were reacted with TFS-4 antibody (5 μg/ml in phosphate-buffered saline with 1% gelatin) for 1 h at room temperature. After 3 washes with phosphate-buffered saline, membranes were incubated with biotinylated anti-mouse IgG (Vector) (1:2000 in 1% gelatin in phosphate-buffered saline) for 30 min, washed 3 times, and then incubated with avidin D-peroxidase (Vector) (1:2000 in 1% gelatin in phosphate-buffered saline). Finally, the membranes were reacted with HRP reagent (Bio-Rad) according to the instructions of the manufacturer.

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2 To whom requests for reprints should be addressed.
3 The abbreviations used are: SCLC, small-cell lung cancer; NSCLC, non-small-cell lung cancer; NSS, normal swine serum; BASCA, brain-associated small-cell lung cancer antigen; ABC, avidin-biotin-peroxidase complex.

Unpublished data.
malignant cells, while the interstitial tissues were spared. TFS-4 was reactive with none of the conventional squamous cell carcinomas, nor large-cell carcinomas of the lung. In the positive cases the reaction appeared to be confined to cell membranes (Fig. 1).

Reactivity of TFS-4 with cancers from other organs is also shown in Table 1. None of the colorectal cancers, gastric cancers, or lymphomas was reactive with TFS-4. Interestingly, carcinoid tumors and a neuroblastoma showed positive reactions.

In normal tissues, most remarkable staining was demonstrated on central nervous tissues and neuroendocrine cells, including the adrenal gland (medulla and zona glomerulosa), glandular cells of the thyroid gland, Leydig cells of the testis, and stromal cells of the ovary (Table 2). In peripheral nerves, Schwann cells and nerve endings were weakly stained. No reactivity was detected with other tissues including trachea, lung, liver, kidney, colon, pancreas, skin, striated muscle, spleen, bone marrow, or peripheral blood cells. The islet cells of the pancreas, enteroendocrine cells, and Kulchitski cells of the lung were found to be unreactive. An unexpected finding was that the antibody was reactive with cell membranes of cardiac muscles and some smooth muscles (alimentary tract and myometrium of the uterus).

Characteristics of TFS-4 Antigen. To see whether the antigen recognized by TFS-4 on SCLC shares the common features with the antigen on central nervous tissues, an SCLC cell line (NCI-H69), SCLC tumors, and human cerebrum were subjected to treatment with various concentrations of trypsin and neuraminidase, methanol, and heat. As shown in Table 3, TFS-4 antigen(s) on an SCLC cell line, an SCLC tumor sample, and the cerebrum showed similar characteristics, i.e., trypsin sensitive, heat labile, but resistant to neuraminidase and methanol.

Species Specificity of TFS-4. Since TFS-4 seemed to recognize brain-associated antigen, its reactivity with cerebrum from other species was examined. TFS-4 was reactive with cerebrum from chimpanzee and night monkey, but not with that of cow, pig, dog, rabbit, or rat (Table 4).

DISCUSSION

In this paper, we demonstrated that TFS-4 was highly specific for SCLC. The antibody reacted with SCLC but not with NSCLC. With the exception of a neuroblastoma and carcinoid tumors, such cancer cells as carcinomas of the colorectum, stomach, breast, lymphomas, thymomas, leiomyosarcoma, or meningiomas were unreactive with TFS-4. The antibody failed to react with normal lungs, trachea, bronchus, liver, kidneys, colon, breast, skin, spleen, striated muscle, and blood cells. These observations suggest that TFS-4 recognizes an antigen specifically expressed on SCLC but not on NSCLC. Thus, TFS-4 appears to be able to distinguish SCLC from NSCLC tumors.

We have compared the antigenic determinant(s) on SCLC and that on central nervous tissues. Their determinant(s) shared some common properties; that is, it is heat labile, trypsin sensitive, but resists treatment with neuraminidase, suggesting that it is a peptide. The antigen on SCLC has been shown to have a molecular weight of 124,000 on sodium dodecyl sulfate-
polyacrylamide gel electrophoresis under reducing conditions (5). Purification studies of the antigen from human brain revealed the molecular weight of 124,000 under the same conditions (15). These observations suggest that SCLC and human brain share a common antigen on the cell surface. We designated TFS-4 antigen as BASCA. Immunohistochemical studies with normal tissues suggested that BASCA is expressed on cell membranes of central nervous tissues, neuroendocrine cells, and interestingly of cardiac muscles.

Species specificity studies revealed that the reactivity of TFS-4 appears to be restricted to primates.

Although there have been several reports concerning the monoclonal antibodies for SCLC (5, 7–13), few have been well investigated in terms of reactivity with fresh tumor tissues obtained directly from the patients. The reported results are summarized in Table 5.

HNK-1 was reported to show preferential specificity for SCLC (7, 13). However, a large survey of lung tumors has demonstrated that HNK-1 reacted with more than half of the well-differentiated adenocarcinomas of the lung (8). SM-1 and B10/12 reacted with breast cancers (10, 11). MOC-1 recognized more than half of adenocarcinomas (12). It appears that TFS-4 shows the higher specificity for SCLC among the hitherto reported monoclonal antibodies.

Another monoclonal antibody UJ13A may deserve comment. UJ13A, which was raised against human fetal brain, also reacted with SCLC and showed a similar staining pattern with normal tissues, recognizing central nervous tissues and thyroid gland epithelium. But the antigen is believed to be a glycolipid which is restricted to neuroendocrine cells, cardiac muscle, and some smooth muscle cells leads to speculation that BASCA might be associated with some specific function(s) in these cells that possess an excitable cell membrane.

In conclusion, TFS-4 may be of clinical importance in the differential diagnosis of SCLC from other cancers. BASCA (TFS-4 antigen) may also lead to better understanding of the origin and nature of SCLC cells.

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BRAIN-ASSOCIATED SMALL CELL LUNG CANCER ANTIGEN

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