Monoclonal Antibody 44-3A6 as an Adjunct in Cytodiagnosis of Adenocarcinomas in Body Fluids

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Monoclonal antibody (MCA) 44-3A6 detects a cell-surface transmembrane phosphoprotein frequently expressed by pulmonary adenocarcinoma (AC) and associated with glandular differentiation. This antibody has been found to have utility in assessing routine formalin fixed paraffin-embedded pulmonary neoplasms, as well as the cytopathological evaluation of sputum and bronchial brushings. Recently, it has been shown to be useful in cytological diagnosis of pleural effusions. This study is directed at evaluating its effectiveness in detecting immunoreactive neoplastic cells in body fluids (BF) arising in other tissues. A retrospective cohort of 57 cases was studied, consisting of 36 pleural, 19 ascitic, and 2 pericardial BF. After evaluation of Papanicolaou-stained slides, the BF specimens were immunostained with MCA 44-3A6 using the avidin–biotin–peroxidase complex (ABC) method. In 29 cases, tissue sections of the primary tumors, were also available for immunostaining with MCA 44-3A6. Results showed that 39/42 (93%) of AC BF cases were positive and 28/42 (66%) stained intensely (3–4+) with 75–100% of the AC cells staining in each case. All of the 18 benign and non-AC malignant BF were negative. The staining patterns in the tissue sections of the 29 cases that had corresponding BF samples were similar. We conclude from this study that the MCA 44-3A6 (1) is useful in detecting cells consistent with AC in BF; (2) does not stain inflammatory cells or reactive mesothelial cells, thus helping distinguish reactive from malignant BF; and (3) frequency and pattern of expression in BF parallels its expression in tissue specimens. This study confirms that this MCA is a useful adjunct tool in the cytopathological evaluation of BF. Diagn Cytopathol 1993;9:179–183.

Key Words: MCA 44-3A6; Adenocarcinomas; Body fluids, cytodiagnosis, immunohistochemistry, monoclonal antibodies

The cytologic assessment of body fluids is an important tool in the study of disease processes affecting various body cavities and neighboring organs, particularly neoplastic conditions. This has been done for over a hundred years and its importance is well recognized especially in the identification of malignant cells. Different criteria have been established to aid in the correct identification of these cells.[1–7] However, problems may arise in the morphologic distinction between normal or reactive mesothelial cells and tumor cells, especially cells expressing an adenocarcinoma phenotype.[8–10] Different laboratories have reported false-positive results ranging from 0%–3%.[4,11–14] The use of various cytochemical and immunocytochemical techniques, as adjunct tools, has helped by providing additional information about the specimen, and therefore helped in providing a diagnosis consistent with histological findings.[15–19] The use of a panel of tests which include digested periodic acid-Schiff (D-PAS), mucicarmine stain, Leu-M1, and carcinoembryonic antigen (CEA) stains in distinguishing AC from mesothelial cells has been employed.[15,20] Although helpful in some instances, equivocal results occur in some cases.

The monoclonal antibody (MCA) 44-3A6 was raised against the human pulmonary adenocarcinoma cell line A549, is well characterized, and has been shown to react with human pulmonary adenocarcinomas (ACs) in histologic sections.[21–35] The present study was undertaken to determine the effectiveness of MCA 44-3A6 in detecting AC in body fluids (BF) arising in various tissues.

Materials and Methods

Monoclonal Antibody

MCA 44-3A6 was produced using the well-defined hybridoma technology and the details of this procedure, as well as the characterization of this MCA have been described elsewhere.[21–35]

Cytological and Histological Specimens

Fifty seven Papanicolaou-stained BF (36 pleural, 19 ascitic and 2 pericardial) were studied retrospectively. Based

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on the Papanicolaou-stained slides, the 57 cases consisted of 51 malignant and 9 benign or reactive BF. The malignant samples are composed of 42 AC (10 breast, 8 lung, 6 ovary, 5 endometrium, 1 stomach, 1 pancreas, 1 gallbladder, and 10 unknown primary tumors), 5 non-Hodgkin's lymphomas, 3 undifferentiated small cell carcinomas, and 1 melanoma. The 6 reactive samples were from patients with medical conditions other than neoplasm.

The Papanicolaou-stained cytological slides were screened, and the location of tumor cells were marked using a white ink pen. Photomicrographs were made to document the cell-staining pattern and morphological features. The slides were then photocopied and the coverslips removed by soaking in xylene. After immunostaining, each slide could be placed over the photocopy of the slides, and the approximate location of the ink marks relocated on the slides. This approach permits the study of the same cell or cell cluster for both traditional and immunological screening.

Tissue sections of the primary tumors were available in 29 cases for comparison to the BF specimens. The assessment of the tissue sections were done in a blind fashion, that is, without the knowledge of the original diagnosis or the correlation to the cytological material for both the H+E and immunostained sections.

Immunocytochemistry and Immunohistochemistry

Immunostaining was performed on 6-μm tissue sections of the formalin-fixed paraffin-embedded material or on routine cytological BF smears. Following conventional deparaffinization or xylene incubation to remove coverslips of cytologically prepared slides, the slides were stained with the MCA 44-3A6, using the avidin–biotin–complex (ABC) technique (Vector Laboratories, Burlingame, CA) as previously described.[37] Expression of the antigen recognized by the MCA 44-3A6 appears to be restricted to epithelial cells.[26,28,32] MCA 44-3A6 was used in a concentration of 1 μg/ml and applied to each section for 15 min at room temperature. Tissue sections were subsequently counterstained with hematoxylin. Negative controls were performed by omitting the primary antibody and by substituting MCA 44-3A6 with a irrelevant MCA of the same isotype on the tissue sections only, since the BF slides were restricted to only one round of immunostaining. Positivity was assessed with respect to intensity [zero (absent) to 4+ (most intense)] and percentage of cells stained.

Results

Immunohistochemistry and Cytochemistry

The antigen recognized by MCA 44-3A6 is well preserved after alcohol fixation and Papanicolaou staining and destaining procedures (Fig. C-1–C-4; see also Fig. C-6). This is readily apparent by the sharp contrast between immunoreactive and negative cells (Fig. C-3). There was a slight variability, however, in the intensity of the staining from cell to cell (Fig. C-4). It is also well preserved in formalin-fixed paraffin-embedded tissues (Fig. C-5).

Adenocarcinoma

Body fluid specimens consisted of 42 samples from various primaries (Table I). Microscopically, the malignant cells comprise 10–80% of the cells present. More than 50% of the samples exhibited complete or aborted gland formation. The malignant cells varied from about the size to 4–5 times the size of a benign mesothelial cell. Most of the cells had vesicular nuclei with prominent nucleoli. Immunocytochemical staining showed intense and diffuse positivity in 39/42 (93%) of cases with 28/42 (66.7%) staining strongly positive (3+ or 4+). Seventy-five to 100% of the AC cells showed immunostaining in the positive cases. It is interesting to note that the breast and lung primaries showed 8/10 and 7/8 strong positivity. The three gastrointestinal primaries (stomach, pancreas, and gallbladder) all showed strong positivity. A greater immunostaining spectrum was noted in the ovarian, endometrial and the unknown primaries, when compared to the other tumor types studied (Table I). The accompanying benign or reactive mesothelial cells also were negative to minimally or focally positive (+/−). These were easily distinguished from true positive staining.

Nonadenocarcinoma Primaries

The 9 malignant BF from nonadenocarcinoma primaries (5 non-Hodgkin's lymphomas, 3 undifferentiated small cell carcinomas, and 1 melanoma) did not stain for MCA 44-3A6.

Benign and Reactive Mesothelial Cells

All BF from patients with no history of neoplasm showed benign and/or reactive mesothelial cells, which either stained negative or focally but minimally positive (+/−) with MCA 44-3A6. Like the benign or reactive mesothelial cells, these cells were easily distinguished from true positive staining.

Correlation of BF and Primary Tissue Immunoreactivity

Twenty nine malignant BF had corresponding tissue sections (26 from the primary neoplasms and 3 tissue blocks from unknown primary). The known primaries were breast (8), lung (7), ovary (4), endometrium (3), malignant lymphoma (3), and stomach (1). After immunostaining with MCA 44-3A6, the immunoreactivity pattern was assessed and correlated to the corresponding BF. Results showed similar staining patterns, that is, those BF that

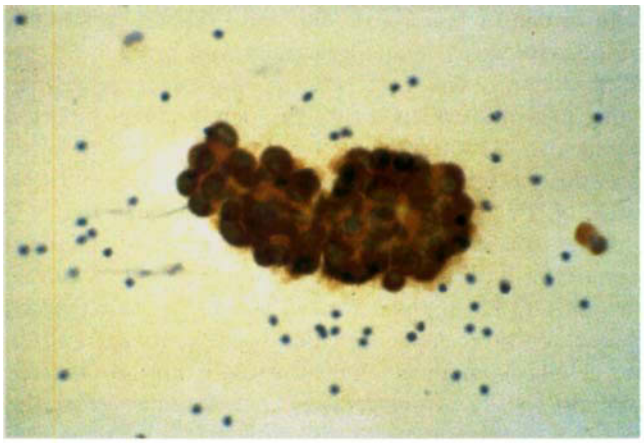

Fig. C-1

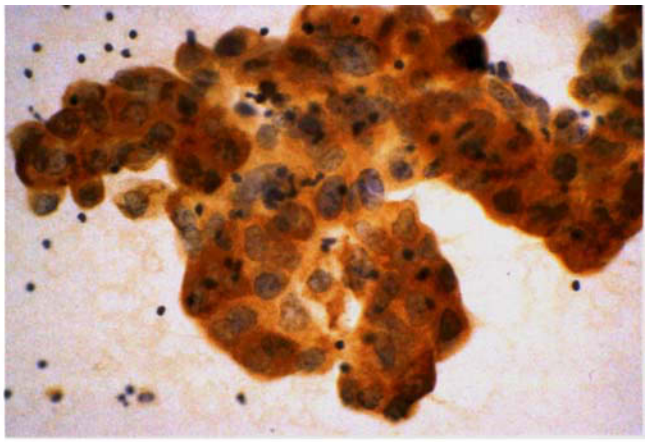

Fig. C-2

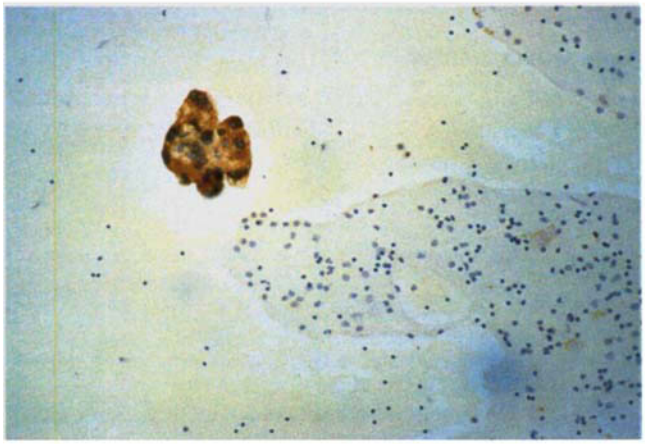

Fig. C-3

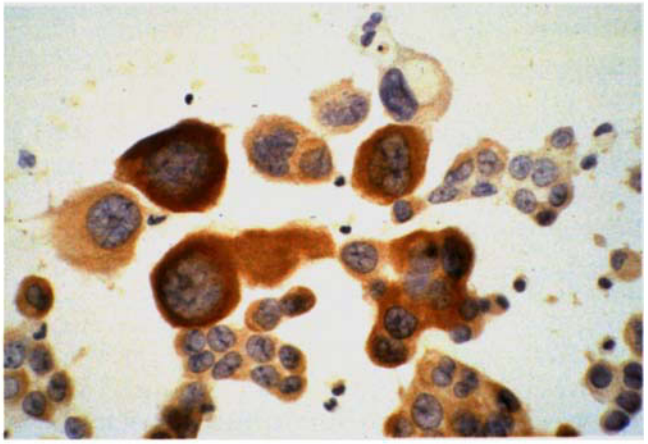

Fig. C-4

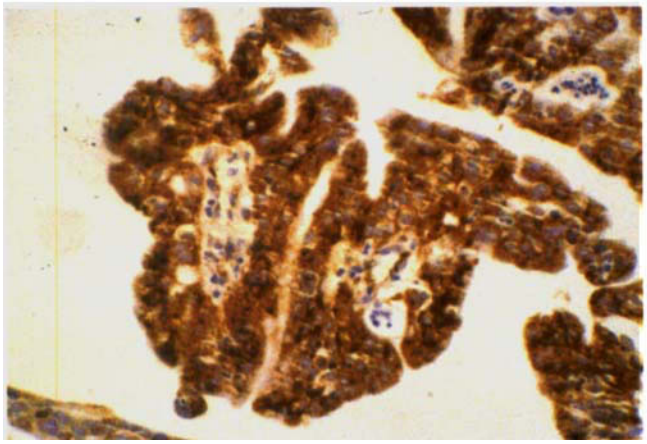

Fig. C-5

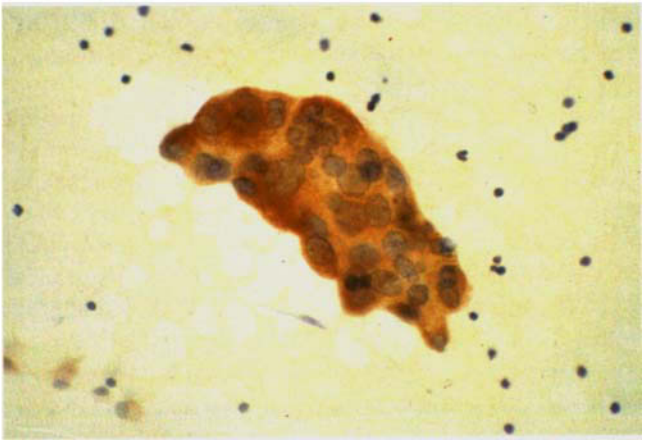

Fig. C-6

Figs. C-1–C-6. Fig. C-1. Adenocarcinoma of the lung (pleural fluid) showing positive immunoreactivity with MCA 44-3A6 (×200). **Fig. C-2.** Endometrial adenocarcinoma (ascitic fluid) showing intense (4+) immunoreactivity with MCA 44-3A6 (×200). **Fig. C-3.** Adenocarcinoma of the lung (pleural fluid) showing intense (4+) immunoreactivity with MCA 44-3A6 of malignant cells and negative staining of benign mesothelial cells (×125). **Fig. C-4.** Adenocarcinoma of breast (pleural fluid) showing a spectrum of immunoreactivity (1–4+) for MCA 44-3A6 staining from cell to cell (×200). **Fig. C-5.** Endometrial carcinoma tissue immunostained with MCA 44-3A6 which has been formalin-fixed–paraffin-embedded (×125). **Fig. C-6.** MCA 44-3A6 immunostained ascitic fluid from the same case as shown in Fig. 5. Note the similar immunoreactivity in both the tissue and cytological preparation (×200).

Table I. Metastatic Adenocarcinoma to Body Cavities

Primaries (no. of cases)	*Positivity*
Breast (10)	2(+); 8(++++)
Lung (8)	1(+); 2(+++); 5(++++)
Ovary (6)	1(0); 1(+); 3(++); 1(+++)
Endometrium (5)	1(0); 1(++); 2(+++); 1(++++)
Stomach (1)	++++
Pancreas (1)	++++
Gallbladder (1)	++++
Unknown (10)	1(0); 3(+); 1(+++); 5(++++)

showed intense and diffused staining also showed a similar staining pattern in the corresponding tissue sections and those BF which were negative or weakly positive showed identical results in the tissue sections.

Discussion

The examination of BF has become a standard of care procedure and is routinely carried out in sophisticated cytology laboratories. Therapeutic protocols demand a high diagnostic sensitivity and specificity in the detection of malignant cells in BF. Most malignant cells present in BF are metastatic AC, in which the primary tumor can arise in a variety of tissues. One study found that 66% of the positive pleural effusions and 82% of the positive peritoneal effusions were AC.[38] Another study has shown that the most frequent neoplasm seen in malignant pleural effusions include lung, breast, ovarian, gastric, and pancreatic AC, while those seen in malignant paritoneal effusion include ovarian, gastric, colonic, breast, and pancreatic AC.[12] In part, these finding were confirmed by a study that showed that the most common primaries of malignant pleural effusions include lung, breast and gastrointestinal AC.[11]

In most instances when all the pertinent information including the history of a histologically proven primary neoplasm are known, the pathologist can render a diagnosis based on morphology. However, there are times when other conditions may give rise to the presence of atypical cells that are not neoplastic, such as reactive mesothelial cells or neoplastic cells, other than AC, such as mesothelioma. Various studies have shown that the use of histochemical and immunocytochemical stains are beneficial in resolving this problem in most instances.[15,20] However, this is not the case when the staining is negative or weakly positive because of low sensitivity. In addition, the use of biological probes as adjunct tools in the cytological evaluation of specimens, aids in removing subjective bias and can provide supporting diagnostic evidence.

Previous studies have shown the utility of this MCA in the evaluation of both histological and cytological specimens.[22-29,31-33] The findings of these studies were again, in part confirmed, since there was (1) selective reactivity found within the neoplastic cases, (2) limited reactivity with benign or reactive tissues, and (3) good correlation of the cytological immunoreactivity with histological immunostaining. The conformation of these expected results, provides evidence that the new findings reported herein can be interpreted in a consistent manner with previously reported data. That is, this report extends the value of the MCA 44-3A6 to the evaluation of BF.

Like many of the tumor-associated antigens, the function of this antigen is unknown. However, it does appear that this 40-Kd transmembrane phosphoprotein may be linked to intracellular calcium concentrations, and/or the cellular components which regulate intracellular free calcium levels.[35] Considerable work remains before the function of this important AC antigen is known, as well as what role it plays in the growth of antigen positive cells.

Acknowledgments

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