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Application of Monoclonal Antibody 44-3A6 in the cytological diagnosis of pleural effusion and histological correlation in lung carcinoma

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Summary

113 patients with exudative pleural effusion had fluid examined cytologically by the conventional method and by the use of Monoclonal antibody (MCA) 44-3A6 using the alkaline phosphatase anti-alkaline phosphatase technique. Forty-two samples came from patients with tuberculosis to serve as control. Seventy-one samples came from patients with lung carcinoma. Of 49 adenocarcinoma cases (69%), 47 showed malignant cells in which positive MCA staining was demonstrated in 45. Of 10 squamous cell carcinoma cases (14%), one showed malignant and two showed suspicious cells: all negative for MCA. Of 6 large cell carcinoma cases (8.5%), one showed malignant cells and two showed suspicious cells: one in each group gave a positive MCA stain. Of 6 small cell carcinoma cases (8.5%), one showed positive malignant cells with negative MCA stain. Correlation of MCA immunostaining in pleural fluid and tissues of pleural and bronchial/transbronchial biopsies were obtained in 38 samples (that belonged to 33 patients), the majority of these were adenocarcinoma. The overall impression was of a significant correlation. This study reiterated adenocarcinoma as the predominant lung cancer cell type giving rise to pleural spread in Hong Kong and demonstrated the confirmatory value of MCA 44-3A6 in typing of 'glandular' carcinoma both in fluid and in tissue specimens. This may have a favourable impact in the study of the epidemiology of lung cancer.

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Introduction

A mouse IgG, monoclonal antibody 44-3A6 directed against a human adenocarcinoma cell line A549 and synthesized from a hybridoma of mouse myeloma SP 2/0 Ag 14 and spleen cells of a hyperimmunised BALB/C mouse [18] was found to have the potential to differentiate adenocarcinomas and some large cell carcinomas from other types of pulmonary carcinomas in histological sections [1,16]. We embarked on the present study in pleural effusions secondary to lung carcinomas with the following purposes. These included (1) refining the diagnosis of glandular carcinoma in fluid samples; (2) correlation of utility of MCA 44-3A6 in immunocytochemical staining of fluid and tissue specimens, and (3) to reconfirm adenocarcinoma as the commonest type of lung carcinoma spreading to the pleura in Hong Kong [10,23].

Materials and methods

Specimens

71 patients admitted consecutively between May 1988 and November 1989 and diagnosed to have pleural effusions secondary to primary lung carcinomas were assessed. The diagnostic criteria of primary lung carcinomas included: (1) positive cytology of pleural fluid; (2) positive cytology of sputum; (3) positive cytology of bronchial brush; (4) positive histology from lung (bronchial or transbronchial biopsies) or other tissue specimens, the pleura in particular, and (5) compatible radiographic picture. In fact, 62 patients met 3 or more of these criteria. Nine patients met criteria (1) and (5) only. In addition, 42 patients with tuberculous effusions were included as control. Each patient had at least 30 ml of pleural fluid aspirated for examination.

MCA 44-3A6

This monoclonal antibody was produced as previously described and stored with a final concentration of 0.02% sodium azide at 4°C [18]. The concentration of MCA 44-3A6 used for immunocytochemical staining, as will be subsequently described, was about 1 μg/ml [16].

Routine Papanicolaou stain

A portion of fresh fluid was fixed with an equal volume of 50% ethanol and centrifuged at 1500 rpm for 20 min. The supernatant was then discarded and sediment pipetted onto albuminized slides and spread evenly and immediately fixed in 95% ethanol for at least one hour. The Papanicolaou stain was then applied.

Preparation of specimens and immunocytochemical staining with MCA 44-3A6

(1) *Preparation of straw-coloured or lightly blood-stained fluid as cytospin.* The fresh fluid was centrifuged at 1500 rpm for 20 min. Supernatant was then discarded and the remaining sediment was resuspended in Hank's solution and roughly adjusted to a concentration of $0.6–1 \times 10^6$ cells/ml. 200 μl of the resuspension was spinned onto a 0.05% poly-l-lysine coated slide by Shandon Cytospin 2 at 600 rpm for 5 min. The cytospin was then air-dried and stored at –30°C.

(2) Preparation of heavily blood-stained fluid as cytospin. The fresh specimen was centrifuged at 1500 rpm for 20 mins and supernatant was pipetted out and the remaining sediment was resuspended in 5 ml of supernatant. 10 ml of Lympho-prep was placed in a centrifuge tube and the resuspended fluid was carefully layered onto it. The two-layered solution was centrifuged at 1000 rpm for 10 min. The fluid just above the surface of Lymphoprep was aspirated and transferred to another centrifuge tube and the cytospin was then prepared by repeating the procedure described under (1).

(3) Alkaline phosphatase anti-alkaline phosphatase (APAAP) technique. The cytospin was fixed in equal parts of acetone and methanol for 90 s. After washing with Tris-buffered saline (TBS), normal rabbit serum (NRS), 1:5 was applied for 10 min to block background non-specific staining activity. After draining, the preparation was incubated with MCA 44-3A6 for 30 min and rinsed with TBS thrice, for one min each. Rabbit anti-mouse Ig (1:50 in 10% NRS) was applied for 30 min. It was then rinsed with TBS thrice, for one min each and mouse alkaline phosphatase anti-alkaline phosphatase complex (1:100 in 10% NRS) was applied for 30 min and then rinsed with TBS again. The intensity of the labelling reaction was enhanced by repeating the anti-mouse bridging antibody and the APAAP complexes, but the incubation time was reduced to 10 min. The preparation was developed in appropriate fresh substrate. The preparation was then washed in running water, and counter-stained with Mayer's haematoxylin.

Thirty-eight paraffin blocks prepared from tissue specimens of 33 patients (31 adenocarcinomas and 2 large cell carcinomas) including those of pleural and bronchial/transbronchial origins were sectioned and stained with haematoxylin and eosin as well as MCA 44-3A6 stain, as described above, with the exception of elimination of the fixation step. As for the rest, 30 patients were diagnosed solely by cytological examination of sputum, bronchial brush and fluid, singly or in combination, and 8 patients did not have sufficient additional histological tissue specimens for immunocytochemical staining.

The slides were all independently reviewed by a cytologist and a pathologist in the Department of Pathology, University of Hong Kong. The former was responsible for the assessment of fluid samples and the latter for tissue specimens. The classification of histological/cytological types of lung carcinoma was largely based on morphology as laid down by the criteria of the World Health Organisation for histological typing of lung tumours [21]. A small number of equivocal cases of adenocarcinoma were confirmed by the mucin stains viz Southgate mucicarmine, Alcian blue and Periodic-Acid-Schiff. Cases classified as large cell carcinomas were all negative for mucin stain. Both investigators also agreed by visual assessment the following gradings of staining intensity by MCA 44-3A6 alkaline phosphatase anti-alkaline phosphatase technique: namely, weakly positive, moderately positive and strongly positive. The percentage of positive malignant cells was graded as negative (0–15%), + (16–50%), ++ (51–75%), +++ (>75%).

Results

The results of the pleural fluid examinations are as depicted in Table 1 and Table 2. To summarise, for adenocarcinoma cases, malignant cells were present in 47 out of 49 and in the former group, 45 showed positive and 2 negative staining. It is also of interest to note in the large cell carcinoma cases, that 2 showed positive staining as well. In the control group only 5 out of 42 cases showed weak positive staining in macrophages and/or mesothelial cells. As for histo-

Table 1
MCA 44-3A6 staining in malignant cells in pleural fluid of 45 adenocarcinoma cases

Percentage of positively stained cells	Grading of staining intensity		
	Weak (W)	Moderate (M)	Strong (S)
+	8	5	1
++	3	9	4
+++	0	5	10

Table 2
MCA 44-3A6 staining in cells of carcinomatous pleural effusions other than those of adenocarcinoma

	Total No. cases	Cytological status			
		malignant cells present	suspicious cells present	no abnormal cells	MCA 44-3A6 staining
Squamous cell carcinoma	10	1	2	7	All negative
Large cell carcinoma	6	1*	2*	3	*2 positive = =+W, +M
Small cell carcinoma	6	1	0	5	All negative

logical immunostaining correlation, the results are as depicted in Table 3. The majority were adenocarcinoma cases, only 2 were large cell carcinoma. For 19 cases of pleural biopsy, all correlated in immunostaining between tissue and fluid specimens. This included 1 case of adenocarcinoma with negative MCA stain in the malignant cells of pleural tissue and fluid. For bronchial/transbronchial biopsies, 18 cases correlated, 1 case did not: the lung biopsy showed negative staining whereas the fluid cytology showed positive staining. As for intensity, all cases with 'strong' immunostaining in fluid cytology exhibited a 'moderate-to-strong' degree of staining in the histological sections. However, for cases with 'weak' or 'moderate' staining in fluid cytology, the matching between staining intensity of the tissue and fluid was found to be quite variable. Figure 1 shows the staining with MCA 44-3A6 of adenocarcinoma cells in pleural

Table 3
Cytological/histological correlation of MCA 44-3A6 staining of malignant cells in pleural fluid and biopsy specimens

	Cytological/Histological Correlation		
Nature of specimen	+	–	No. of cases
Pleural biopsy	19**	0	19
Bronchial/Transbronchial biopsy	18	1	19
Both of the above	5*	0	5

**included 2 cases of large cell carcinoma; *included 1 case of large cell carcinoma; other cases all adenocarcinoma

fluid and Figs. 2 and 3 showed the staining of these cells in the pleural biopsy and transbronchial biopsy respectively.

Discussion

In Hong Kong, in the male population, for primary lung carcinoma, adenocarcinoma (33.6%) and squamous cell carcinoma (33.3%) co-dominate followed by small cell carcinoma (21%) and large cell carcinoma (7%). In women, the predominant cell type is adenocarcinoma (55%) followed by squamous cell carcinoma (23%), small cell (13%) and large cell (4%) [13]. The high incidence of adenocarcinoma in the non-smoking female is particularly striking [3,15] and is also causing a very high mortality rate [2]. This corresponds with the absolute and relative decline in incidence of squamous cell carcinoma and accompanying rise in incidence of adenocarcinoma in other parts of the world, e.g. the United States [8,19,22]. This escalation in incidence of adenocarcinoma might suggest major changes in pathogenesis of lung cancer [8]. Therefore, accurate diagnosis and classification would have significant impact in the study of this epidemiological shift.

A monoclonal antibody such as MCA 44-3A6 which was raised against an adenocarcinoma cell line [18] would obviously be of importance and interest in the study of tissue or cytological specimens and in its value in diagnosis and classification of lung carcinoma as has been previously reported for bronchial brush cytological specimens and bronchial biopsy histological specimens [1,16]. In this particular study, attention was focussed on pleural effusion because lung adenocarcinoma metastasize to pleura rather commonly [10,23] and in fact constitutes a

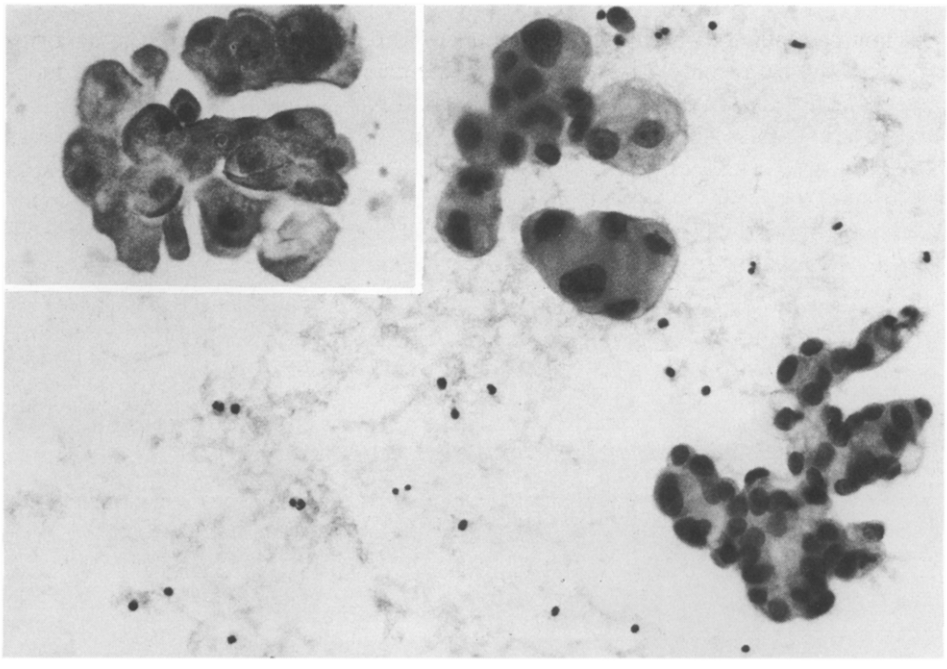

Fig. 1. Clusters of adenocarcinomatous cells in the pleural fluid. (Papanicolaou stain; magnification × 375). *Inset:* moderate-to-strong immunostaining of tumour cells. (APAAP stain for MCA 44-3A6; magnification × 337.5).

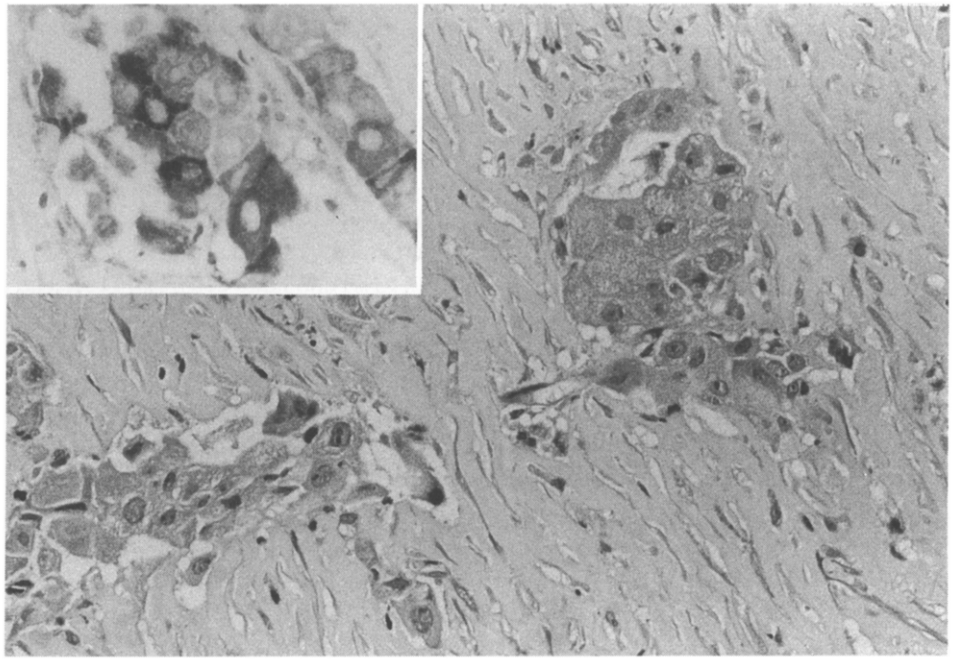

Fig. 2. Malignant glands infiltrated the parietal pleura (hematoxylin-eosin stain; magnification × 187.5). *Inset:* moderate-to-strong immunoreactivity to MCA 44-3A6. (APAAP stain; magnification × 337.5).

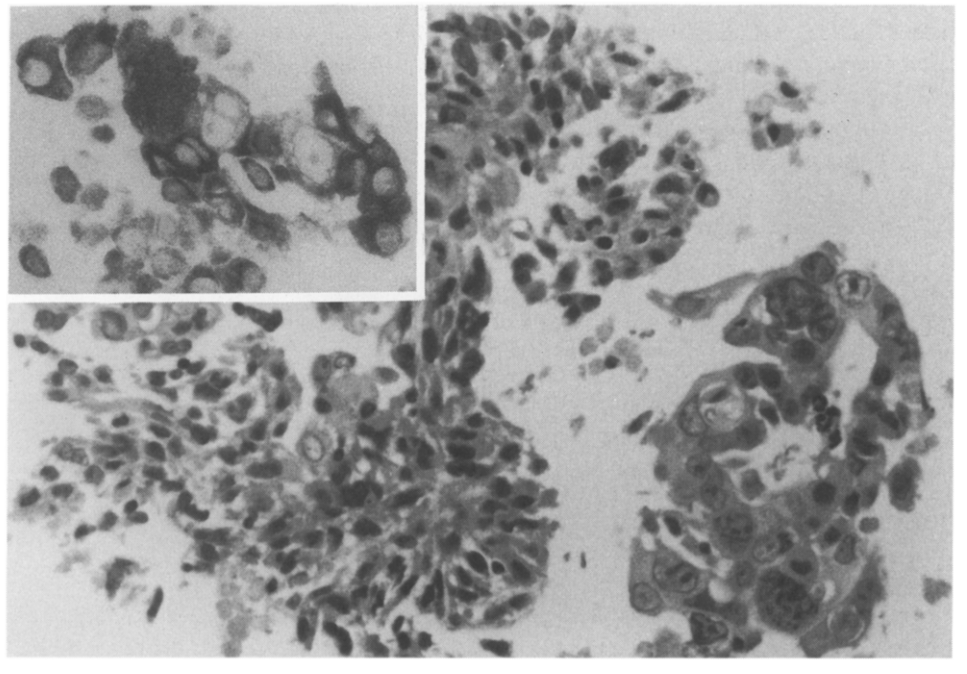

Fig. 3. The tumour cells in this transbronchial lung biopsy showed acinar structure. (Hematoxylin-eosin stain; magnification × 300). *Inset:* diffuse cytoplasmic staining in tumour cells (APAAP stain; magnification × 352.5).

significant presenting manifestation of that carcinoma in the Hong Kong population [14]. As the results demonstrated, the findings concurred with those reported earlier on for specimens utilising tissue from lung primary. Adenocarcinoma and some 'large' cell carcinomas which presumably were glandular in origin but lacked the typical features of adenocarcinoma on light microscopic examination (based on the criteria of the World Health Organisation for the histological typing of lung tumours [21]) were found to exhibit positive immunostaining with MCA 44-3A6. The reason for the negative immunostaining of the two cases of acinar adenocarcinoma was unclear. This illustrates the confirmatory value of MCA 44-3A6 in classification of malignant pleural effusion. Because the high percentage of positive cytology for malignant cells in pleural fluid in adenocarcinoma of lung in this study (47/49, i.e. 96%) was similar to another study [11], it was not possible to evaluate whether application of this monoclonal antibody could increase the diagnostic yield in equivocal cases as was demonstrated by reports using other kinds of monoclonal antibodies [5,9]. The cytological and histological correlation was found to be very good for pleural biopsies. This, apart from being just logical, also indicates that there had been no technical disparity resulting in interpretation difficulty when utilising MCA 44-3A6 immunocytochemical technique in the study of cytological and histological specimens that were processed somewhat differently. Finally, the correlation of positive immunocytochemical staining of the primary sites (bronchial/transbronchial biopsies) and the metastatic sites (pleural fluid) in virtually all cases signifies no change in expression of epitopes that react with MCA 44-3A6 when the tumour disseminates. The better cytological and histological correlation of staining intensity in cases that exhibited strong immunostaining in fluid cytology rather than the others could mean more florid antigenic expression in these cases, thus minimizing sampling bias when fluid specimens and tissue specimens were compared.

Further studies, particularly in the application of MCA 44-3A6 in (1) differentiation of non-epithelial tumours of lungs such as mesothelioma, lymphoma from adenocarcinoma of lung, and (2) classification of lung carcinomas, evaluation of heterogeneity [4,6–8,12,16,17,20] which is common in both small cell lung cancer and non-small cell lung cancer from percutaneous transthoracic needle lung biopsies and bronchial/transbronchial biopsies are, in our opinion, worth undertaking and in fact currently in progress.

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