Immuno-detection of Exfoliated Cells in Sputum with a Monoclonal Antibody

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Objective To develop a prompt and specific method for diagnosis of lung cancer.

Methods A murine monoclonal antibody against lung cancer cells was developed and characterized with the techniques of ELISA, immunohistochemistry and immunocytochemical detection of sputum.

Results The antibody selectively bound to lung cancer tissues and exfoliated cells in the sputum from the patients with lung cancer, but did not bind to normal lung tissues and the cells in sputum either from the patients with pneumonia or from normal individuals.

Conclusion The antibody 2C25 has potential application for immunocytological detection of lung cancer cells in sputum.

Key words monoclonal antibody; lung cancer; diagnosis

The morbidity and mortality of lung cancer has been risen steadily with environmental pollution and smoking habit. The morbidity has reached the first one in male at coast area of China, and at the rank of second or third in female[1], so it has been a menace to the health of human being. Hence, the early diagnosis of lung cancer is an important approach of accelerating cure rate and decreasing mortality. At present, many methods such as CT, bronchoscope[2], pathological check, cyto-check on sputum and serological test have been employed to detect the lung cancer. In order to detect the lung cancer specifically and sensitively, scientists are attempting to search specific marker on lung cancer cells and relevant antibody identical to the marker[3,4], such as monoclonal antibody against hnRNP-A2/B1[5], protein 14-3-3[6], protein Lewis X[7], and other biomacromolecule[8] and sugar-molecule[9]. Different methods based on detection of tumor maker[10,11], acetylneuraminate in serum[12], and tumor gene mutation in sputum had been employed to detect lung cancer in the country, but so far it still lacked a highly sensitive and specific method which can be applied in clinical practice. The purpose of this study was to generate a kind of specific antibody on lung cancer and set up a quick, sensitive detection system.

Materials and methods

Cell and antibody

A2 lung caner cell lines were devoted by Doctor Takako, Maopury institute of biochemistry, Germany; Alkaline-phosphatase-conjugated goat anti-mouse IgG antibody was purchased from Southern Biotechnology Associates Company, USA; Biotinlyated goat antimouse IgG and HRP conjugated avidin was purchased from Bio-Rad Corporation, USA. The first spitted sputum samples were obtained in the morning from patients with lung squamous carcinoma ($n = 5$) and adenocarcinoma ($n = 5$), patients with pneumonia ($n = 10$) and normal persons ($n = 10$).

Antibody preparation

Balb/c mice of 8 weeks old were immunized with A2 lung cancer cells mixed with Freund adjuvant, and then were reinforced immunity 3 times. Mouse spleen cells were merged with mouse SP2/0-Ag8.635 cells with 50% PEG-4000. Hybridoma cells were cultured in selected culture medium supplemented with 10% bovine serum and HAT-RPMI. Sreening monoclonal antibody which can combine with lung cancer cells specifically instead of normal lung cells by immunohistochemistry.

Immunohistochemistry

Tumor and normal tissue cryo-sections were fixed in acetone for 4 to 10 min, and then were treated with 0.3% H_2O_2 methyl alcohol at room temperature for 30 min in order to block endogenous peroxidase. The slide was washed with 0.3% Triton X-100/PBS at room temperature for 30 min. The sample was blocked with sheep serum for 1 h, incubated with culture supernatant of the monoclonal antibody for 24 h, washed 3 times with PBS, and then reacted with biotinlyated goat antimouse IgG(H+L) and ABC complex. The sections were dyed with 3, 3'-diaminobenzidine (DAB; Sigma Chemical Co., USA) and counterstained with Mayer hemotoxylin, and were observed and photographed finally.

ELISA

Sputum samples were diluted with 4 ml of 0.01 M phosphate-buffered saline (PBS) at pH 9.6 into 1:1, then diluted into 1:10, 1:100, 1:1000, 1:10 000. Micrometer plates were coated with sputum sample, overni-

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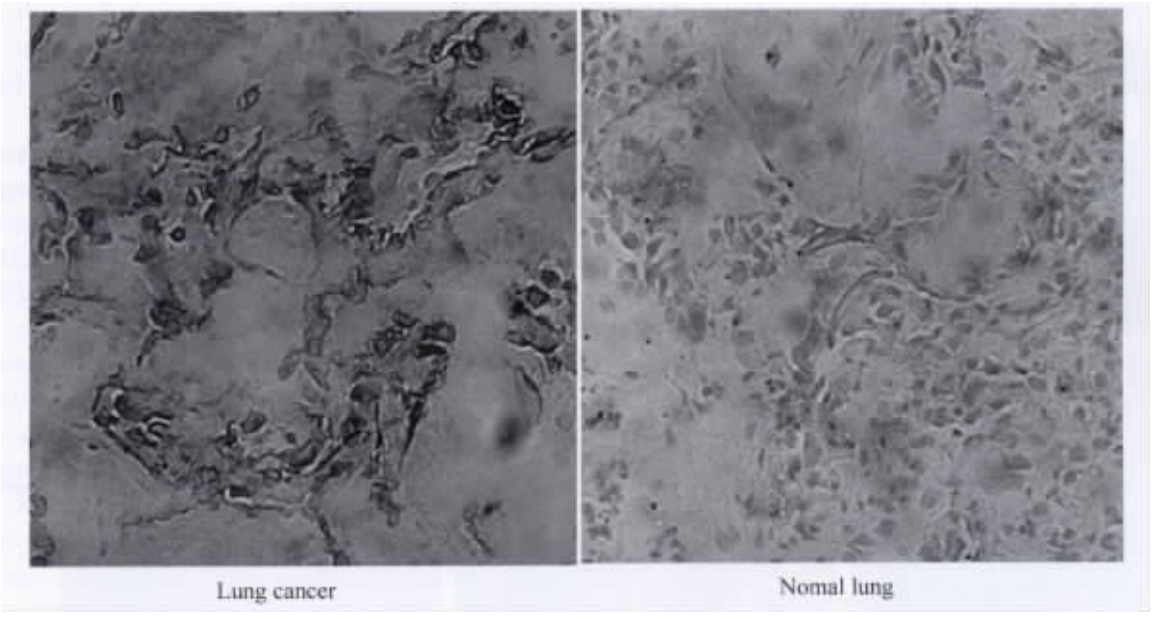

Fig. 1 Antibody binded with lung cancer tissues, but not with normal lung tissues (×100)

ght at 4 ℃. After being washed once with 0.01 M PBS (pH 7.4), the plates were blocked with 5% milk for 1 h. Purified mouse monoclonal antibody 2C25 hybridoma substrate (1∶100) was added (mouse IgG and PBS as negative control) and incubated for 2 h at 37 ℃. The plates were washed with PBS for 3 times and incubated with alkaline-phosphatase-conjugated goat anti-mouse IgG antibody(1∶2000) for 1 h at 37 ℃, then washed with PBS for 3 times. PNPP substrate was added to the wells. The absorbance A at 410nm (Bio-Rad Model 550) was measured. Each concentration was tripled tested.

Immunological staining of exfoliated cells in sputum

The sputum samples were mixed with 1 ml PBS (pH 9.6) and treated with adhesive reagent APES (purchased from Beijing Zhongshan Bio-technique Ltd. Corp., China). The treated samples smeared on three glass slides. The air-dried samples were fixed with acetone for 10 min at −20 ℃ and washed with PBS for 5 min. After addition of 0.3% H_2O_2 methyl alcohol, the slides were incubated at room temperature for 30 min in order to inactivate endogenous peroxidase, blocked with sheep serum for 60 min, 2C25 hybridoma cell culture supernatant was added and incubated at 4 ℃ overnight. Wash with PBS three times, add biotinlyated goat anti-mouse Fab antibody(1∶200) and incubate at 37 ℃ for 1 h. Wash with PBS three times, add HRP conjugated avidin and incu bate at 3 7 ℃ for 1 h . Wash with PBS three times, add

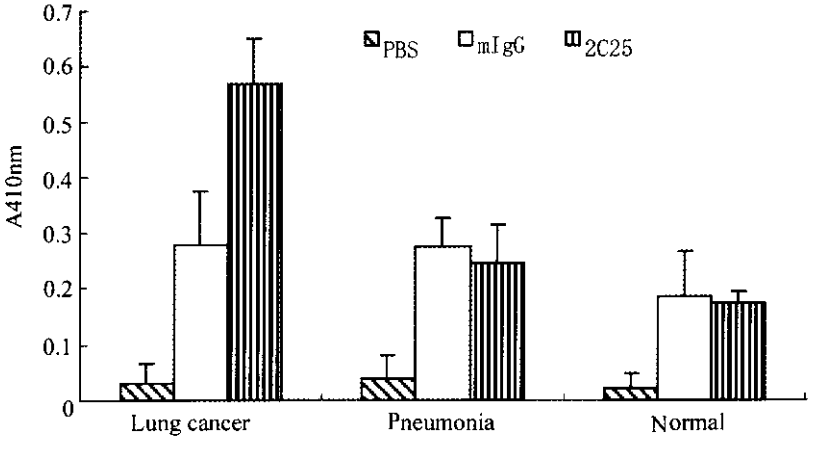

Fig. 2 ELISA analysed antibody 2C25 ,mIgG and PBS reacting with lung cancer,pneumonia and normal sputum samples

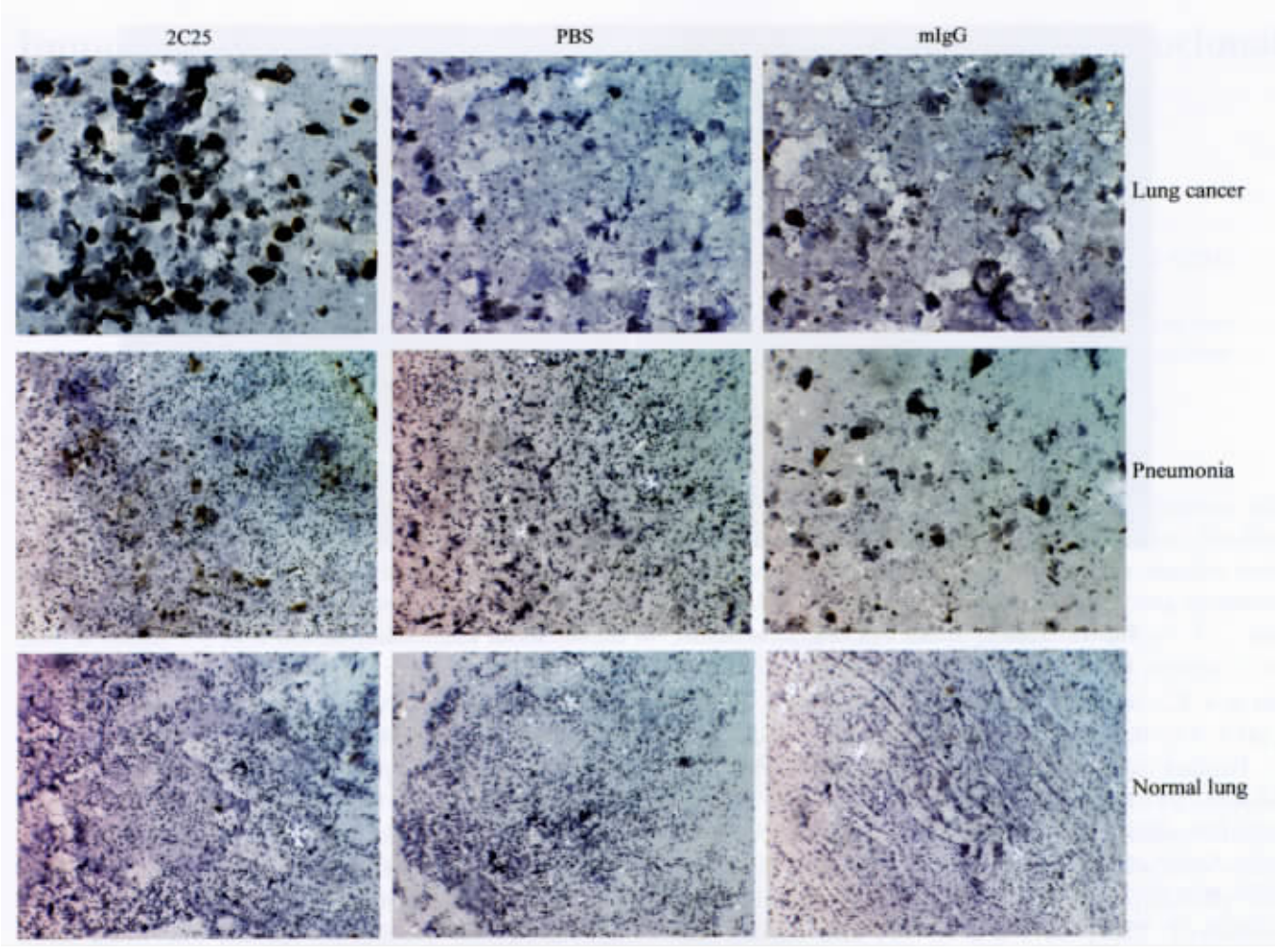

Fig. 3 Antibody 2C25, mIgG and PBS reacting with lung cancer, pneumonia and normal sputum samples (×200)

DAB to develop color 5 min. Wash the samples with water and stain again with haematine, wash with water for 10 min, dehydrate samples with 80%, 90%, 100% ethanol gradually, add dimethylbenzene twice on the slides for 5 min, then seal the slide.

Results

Preparation of specific monoclonal antibody against lung cancer cells

Lung cancer cells were used as antigen to prepare monoclonal antibody by hybridoma technique. To select antibody which can specifically bind lung cancer tissue instead of normal tissues, we have screened and obtained three strains of monoclonal antibody which can specifically combine with lung cancer tissue but not with normal lung tissue by way of immunohistochemistry. One of them was named as 2C25. It was found that antibody 2C25 could combine selectively with lung cancer tissue (Fig. 1).

Detection of sputum exfoliated cells by ELISA

The results showed that antibody 2C25 could combine positively with lung squamous carcinoma and adenocarcinoma but not with sputum exfoliated cells from pneumonia and normal persons (Fig. 2). Negative controls of isoforms mIgG and PBS showed no difference among lung cancer, pneumonia and normal group. These data suggested that antibody 2C25 could recognize specifically component in sputum from lung cancer patients.

Immunohistochemical assay of sputum exfoliated cells

To further analyze the component recognized by antibody 2C25, we smeared above slides by using cell immunological staining method, which reacted respectively with antibody 2C25 and mIgG, and observed the morphological results. As shown in Fig. 4, compared with mIgG and PBS group, only antibody 2C25 recognized sputum exfoliated cells from lung squamous carcinoma and adenocarcinoma patients, and the cells were stained dark brown. Neither antibody 2C25 nor mIgG combined with sputum exfoliated cells from pneumonia and normal persons. These data were consistent with the previous results

of ELISA, and showed that antibody 2C25 was not only able to distinguish normal lung epithelial cells from tumor cells, but also to distinguish normal and tumor cells from sputum as well, suggesting this antiboby might be a better reagent and target to diagnose lung cancer quickly.

Discussion

We found in this study a kind of monoclonal antibody 2C25 which was able to combine with lung carcinoma cells specifically. The results of immunohistochemistry revealed that the antibody combined specifically with various types of lung cancer cells, but not with normal lung tissue, suggesting that the antigen being combined with antibody 2C25 was over-expressed or specifically expressed in various types of lung cancers and may be a kind of target molecule of lung cancer. At present, the antigen evaluation work is proceeding.

Although antibody 2C25 had higher specificity in the immunohistochemistry detection of lung cancer, it still has obvious limitation such as it cannot diagnose lung cancer as early as possible. Collecting the sample from patients was a kind of trauma, might bring patients some distress and result in tumor cells metastasis. In contrast, sputum sample has the advantage of collecting easily. We have observed in this study the reaction between antibody 2C25 and sputum exfoliated cells through the quick and simple way of ELISA and cellular immunochemistry. We have confirmed that the antibody can selectively recognize sputum exfoliated cells from lung cells patients, but not the sputum samples from pneumonia patients and normal persons. These findings had established a basis of generating a kind of fast and simple diagnosis method to detect lung cancer, and we should analyze the accuracy and clinical coincidence of antibody 2C25 of lung cancer in the future.

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