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Cell cycle and electron microscopic evaluation of the adenocarcinoma antigen recognised by the monoclonal antibody 44-3A6

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Summary Adenocarcinomas represent one group of tumours which are seen in the broad spectrum of pulmonary neoplasms. We have produced a monoclonal antibody (44-3A6) which reacts with a 40 kD protein expressed by human primary pulmonary adenocarcinomas and cell lines. Using FACS analysis, this antigen does not appear to be cell cycle specific, and is exposed to the external cell surface. This subcellular localisation has been confirmed using a whole cell, pre-embedding, EM-immunogold labelling technique.

Our laboratory has produced a monoclonal antibody (Mab) (44-3A6), and has focused on the study of this antigen as a marker for human non-small cell pulmonary neoplasms (Radosevich *et al.*, 1985; 1990). This antigen is a 40 kD protein, that by immunoperoxidase, immunofluorescence, and other methods, appears to be exposed to the cell surface (Radosevich *et al.*, 1985; 1988). It has unique reactive patterns separate from those antibodies in cluster 2. While 44-3A6 has been shown to be selective for their 'adenocarcinoma' phenotype, among non-small cell tumours, it does detect some expression of this antigen among small cell and other neoplasms (Combs *et al.*, 1988; Radosevich, *et al.*, 1988). This report is directed at demonstrating the subcellular localisation and cell cycle properties of the unique antigen recognised by the Mab 44-3A6.

Materials and methods

44-3A6

The mouse Mab 44-3A6 detects a 40 kD protein expressed by the human pulmonary adenocarcinoma cell line, A549. The expression of this antigen by pulmonary neoplasms, normal tissues, and by other tumours arising outside the pleural cavity have been well studied (Combs *et al.*, 1988). Production of culture supernatant from this hybridoma, and growth of the A549 cell line in tissue culture was as previously described (Radosevich *et al.*, 1985).

Antigen modulation

A549 cells were grown under standard conditions, and in the presence of media supplemented with SP2/0 or Mab 44-3A6 ascitic fluid (diluted 1:1000). After growth in culture for 3 days, the cells were prepared for FACS analysis as previously described (Radosevich *et al.*, 1985).

Cell cycle analysis

Asynchronously growing, mid-log phase, A549 cells were prepared for live cell immunofluorescence labelling. The cells were then fixed using 1% glutaraldehyde for 10 min at 4°C. After being permeabilised, using 0.1% Triton X-100, the cells were stained for DNA content using propidium iodide. Simultaneous, immunofluorescence/DNA content FACS analysis was performed on a Coulter EPICS V.

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EM-immunogold labelling

Asynchronously growing, mid-log phase A549 cells were prepared for pre-embedded immunogold labelling, using either 44-3A6 or SP2/O (negative) control supernatant, as essentially previously described (Bendayan, 1984).

Results

When A549 cells were grown in the presence of Mab 44-3A6, and subsequently analysed using FACS immunostaining, the cells remained positive for the cell surface expression of this antigen (data not shown). This finding suggests that

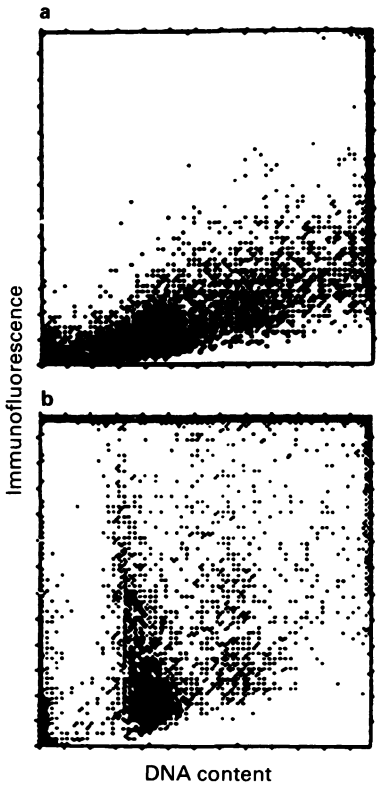

Figure 1 A549 cells were immunolabelled using the liver cell method as outlined in the Materials and methods. After immunolabelling, the cells were fixed and stained for DNA content. **a**, shows a histogram of SP2/O (negative antibody control) immunolabelled A549 cells as a function of DNA content. **b**, shows a sample of these cells immunolabelled with Mab 44-3A6, showing immunoreactive cells in all portions of the cell cycle.

the antigen recognised by 44-3A6 is continuously present on these cells, and is not modulated from the cell surface.

The DNA content of the asynchronously growing, mid-log phase A549 cells used to assess the cell cycle expression of this antigen, confirms the presence of cells within each of the portions of the cell cycle (Figure 1a). These cells were also treated with SP2/O conditioned media, which serves as the negative control for immunoexpression, resulting in minimal immunofluorescence reactivity. Figure 1b shows the 44-3A6 treated A549 cells, as resolved by FACS analysis. This histogram demonstrates immunolabelling of whole cells in all portions of the cell cycle. The degree of immunolabelling is influenced by the concentration of primary antibody used, but does not alter the distribution of staining with in various cell cycle subpopulations (data not shown). The ratio of stained to unstained cells in each cell cycle subpopulation is constant (data not shown).

Pre-embedded immunogold labelling of A549 cells, using SP2/O conditioned media is shown in Figure 2a. Few immunogold particles are seen on the cell surface of these A549 cells, as noted by the electron dense, uniformly shaped, 15 nm gold particles. Figure 2b demonstrates the cell surface localisation of the antigen recognised by 44-3A6. Numerous immunogold particles are seen on the cells, and are frequently seen in small clusters. Note that these particles localise specifically to the cell surface. This is consistent with specific staining and demonstrates that the staining is not due an inherently high background, but rather to the specific expression of the antigen recognised by 44-3A6.

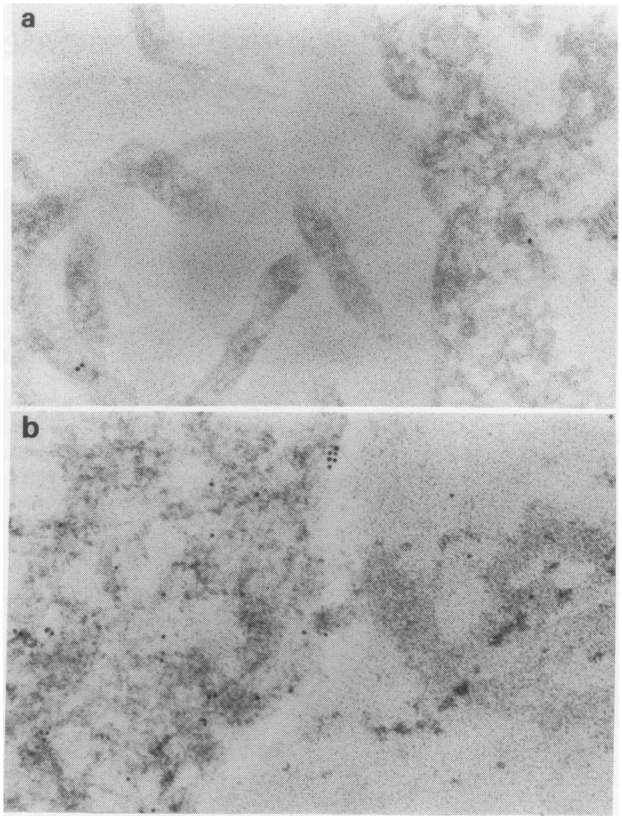

Figure 2 Electron micrograph of A549 cells using a pre-embedding immunogold method (15 nm particles). **a**, shows the SP2/O (negative antibody control) at 50,000 diameters. **b**, shows the MCA 44-3A6 sample, with numerous gold particle clusters on the cell surface (50,000 diameters).

Discussion

The nature and function of the antigen recognised by 44-3A6 is unknown. The molecular data to date, suggest that this antigen is a cell surface, non-modulating, 40 kD integral protein which has little or no carbohydrate associated with it. The present finding that this cell surface antigen is not readily removed from the cell surface, leads us to speculate that this antigen may be important in the growth of adenocarcinomas. The expression of this antigen throughout the cell cycle also supports this idea, and provides one explanation why this antigen is detected at a high frequency on all pulmonary adenocarcinoma cells. Additional studies are directed to further characterising this antigen at the molecular and cellular level, correlating changes in its expression to various biological response modifiers, and at cloning the gene which expresses this antigen.

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