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Immunohistochemical Analysis of Human Adenocarcinomas of the Lung Using the Monoclonal Antibody 44-3A6

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Abstract. In this study, 39 primary, surgically resected, pulmonary adenocarcinomas of hhe following cell types were investigated: 19 Clara cell, 4 bronchial-gland, 15 goblet cell, and 1 type II alveolar epithelial adenocarcinoma. They were analyzed for the expression of the glandular differentiation-associated antigen recognized by the monoclonal antibody 44-3A6. Formalin-fixed, paraffin-embedded tissue sections were immunostained using the avidin-biotin complex/peroxidase method. All of the Clara cell tumors (19/19) expressed this antigen as well as 3/4 bronchial-gland tumors, as compared to only 2/15 goblet cell tumors. The single type II pneumocyte neoplasm studied was found to express this antigen. These findings parallel our earlier observations based on cytological features, and provide supportive evidence that there may be biological differences between human pulmonary adenocarcinomas and bronchioloalveolar carcinomas.

Introduction

Traditionally, the diagnosis of a pulmonary neoplasm as a 'small-cell' or 'nonsmall-cell' carcinoma has had the greatest impact on the treatment course for a patient with lung cancer [1–8]. This is because it is generally held that the small-cell carcinomas of the lung are more responsive to chemotherapy than nonsmall-cell carcinomas. The nonsmall-cell carcinomas include the squamous-cell carcinoma, adenocarcinoma and large-cell carcinoma types as defined by the WHO, Veterans Administration Lung Study Group (VALG) and the Working Party for Therapy of Lung Cancer (WP-L) classifica-

tions [9–15]. Within all of these broad subclasses there are heterogeneous groups of neoplasms which express common morphological and cytological features to varying degrees. These sometimes subtle light- and electron-microscopic features have been the basis for several classification schemes [9–15].

In each classification, the same terms have been applied to describe slightly different tumor populations and subpopulations. This has led to confusion in accurately interpreting the reported diagnosis. In the broad meaning of the term, the 'adenocarcinomas' (ACs) have been further subclassified. Depending on the scheme, the bronchioloalveolar carcinoma (BAC) subgroup has been considered a distinct entity or part of the ACs [1–8]. The light-microscopic and ultrastructural morphological assessment of these neoplasms has resulted in the use of numerous terms to describe apparent variants of both the AC and BAC subgroups [16]. This is in part due to the expression of a wide spectrum of morphological features by these neoplasms [17–29].

There remains debate over the subclassification of the ACs, in particular the existence of the BAC subtype and the utility of such a classification if one does indeed exist [1, 4–6, 9, 11, 12, 25, 29]. The poor and varied response of BAC to therapy has added to the controversy. Some investigators have suggested that there is little or no utility in the subclassification of these neoplasms. In part, the utility of subclassifying these neoplasms has been obscured by the use of the above-mentioned subjective methods.

Monoclonal antibodies (MABs) can be used as molecular probes to assess the expression of specific gene products and provide a more objective approach to defining pulmonary-tumor subtypes [30–34]. MAbs which detect their respective antigens in routine formalin-fixed, paraffin-embedded tissues have the advantage of allowing the correlation of the histological and cytological features with their immunophenotypic characteristics. We have previously reported on an MAb (44-3A6) which recognizes an antigen in formalin-fixed, paraffin-embedded tissues and which has been shown to be associated with glandular differentiation in ACs [35]. The restricted expression of the epitope recognized by MAb 44-3A6 in primary carcinomas of the lung has been reported elsewhere, as well as its utility in distinguishing various histological subgroups of pulmonary neoplasms [36, 37]. It has also been shown to be of value in distinguishing mesotheliomas from ACs metastatic to the pleura, as well as in the cytological evaluation of pulmonary neoplasms [38, 39]. The MAb 44-3A6 recognizes a 40-kilodalton cell surface protein which has limited expression in neoplastic and nonneoplastic tissues [35, 40–42].

As previously reported, we have proposed a subclassification of ACs based on cytological features [43, 44]. In the current study, we tested its validity using MAb 44-3A6. Our hypothesis was that this antigen would be selectively expressed by cytologically defined subgroup(s) of ACs. If the antigenic pattern of expression, as defined by MAb 44-3A6, did correlate well with the previously reported subtypes, then it may be possible to use this MAb as a marker to confirm the subclassification and to clarify cases which express ambiguous cytological features. The differential expression of the 40-kilodalton protein by MAb 44-3A6 implies that those neoplasms expressing the antigen may be

biologically distinct from those lacking this expression. This expression may also allow a more precise identification of these neoplasms based on their expression of an epitope associated with glandular differentiation. Classifications based on the expression of specific gene products may lead to a more consistent selection of patients for different treatment protocols and, therefore, test their efficacy.

Materials and Methods

Thirty-nine primary, surgically resected, well-to-moderately differentiated pulmonary ACs were selected from the National Cancer Center Research Institute pathology files. Each case was reviewed by two pathologists independently and classified according to the scheme previously described [43, 44]. Each of the cases clearly demonstrated the criteria proposed for the subgroups assessed by light and electron microscopy. Mucus production was evaluated by the Alcian blue periodic-acid Schiff stain.

Immunohistochemical analysis was performed on routine formalin-fixed, paraffin-embedded, 5-μm thick tissue sections as previously described, using the avidin-biotin complex/peroxidase method [35, 45]. Immunoreactivity was scored on a scale of 0 to 3+, based on the percentage of positively stained tumor cells in the tissue section: +++ = > 50%; ++ = 50–6%; + = < 5%, and – = 0%. Culture supernatant produced by the hybridoma 44-3A6 contained approximately 1 μg/ml of the MAb. Negative controls included incubation without primary antibody, or with an irrelevant MAb on serial tissue sections stained under identical conditions.

Results

The antigen detected by MAb 44-3A6 was well preserved after routine formalin fixation and paraffin embedding. This was readily apparent by the sharp contrast between immunoreactive cells and negative cells. There was some variability, however, in the intensity of the staining from cell to cell. Nonneoplastic bronchial and bronchiolar epithelia, especially basal cells, and type II pneumocytes were positively immunostained by MAb 44-3A6, as had been previously reported [36, 37, 39]. These cell types served as internal controls, providing evidence that the tissue was adequately fixed and embedded. In total, 25 of the 39 tumors examined showed positive immunoreactivity; these results are outlined in table 1. Figures 1–5 show representative samples of hematoxylin/eosin (H/E)- or MAb-44-3A6-immunostained tissue sections which were counterstained with hematoxylin.

Table 1. MAb 44-3A6 positivity in various lung ACs

Cell type	Positivity				Number positive/ number examined
	+++	++	+	–	
Clara/BSE	12	4	3	0	19/19
Type II	1	0	0	0	1/1
Goblet	0 (0)	0 (0)	2 (0)	13 (5)	2/15 (0/5)
Bronchial gland	1	1	1	1	3/4

Positive reaction was seen in > 50% (+++), 50–6% (++), < 5% (+) and 0% (–) of tumor cells. Numbers in parentheses indicate the number of tumors showing lobar distribution, the remaining 10 cases being ACs of goblet cell type with solitary nodular growth, which can be classified as papillary AC. BSE = Bronchial surface epithelial cell type with scanty mucus; Type II = type II alveolar epithelial cell.

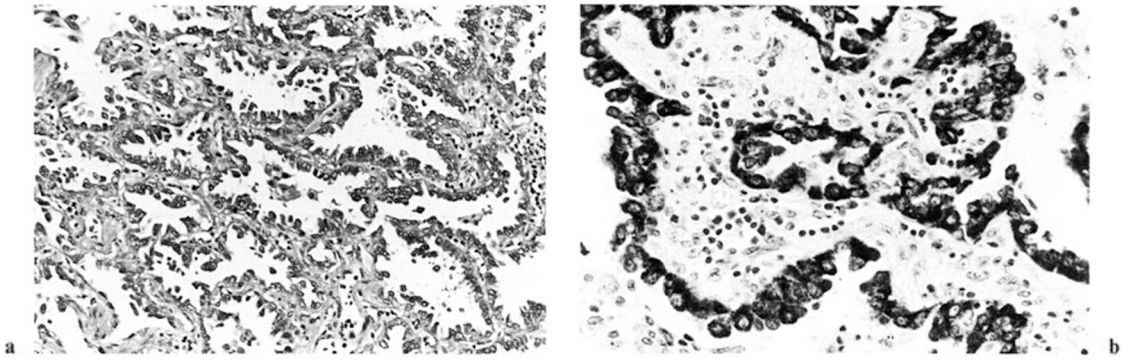

Fig. 1. Clara cell AC. **a** H/E. × 200. **b** Positive immunostaining with MAb 44-3A6. × 400.

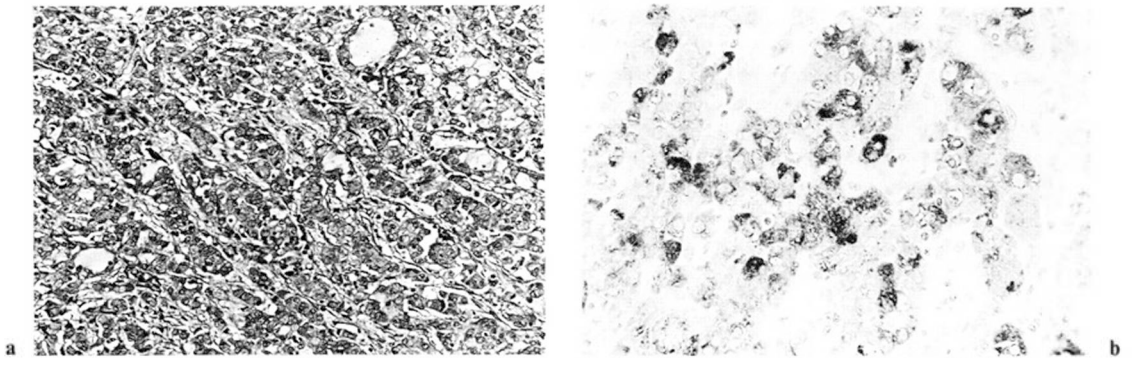

Fig. 2. Bronchial gland AC. **a** H/E. × 200. **b** Positive immunostaining with MAb 44-3A6. × 400.

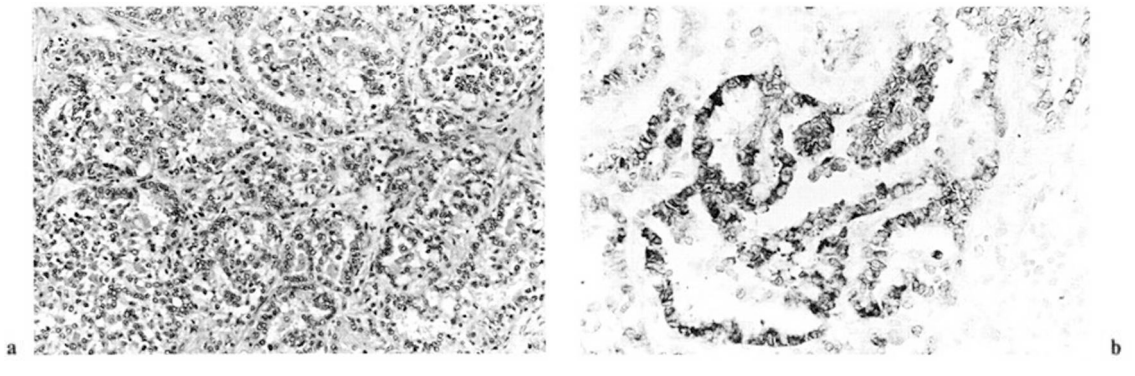

Fig. 3. Type II alveolar epithelial cell AC. **a** H/E. × 200. **b** Positive immunostaining with MAb 44-3A6. × 400.

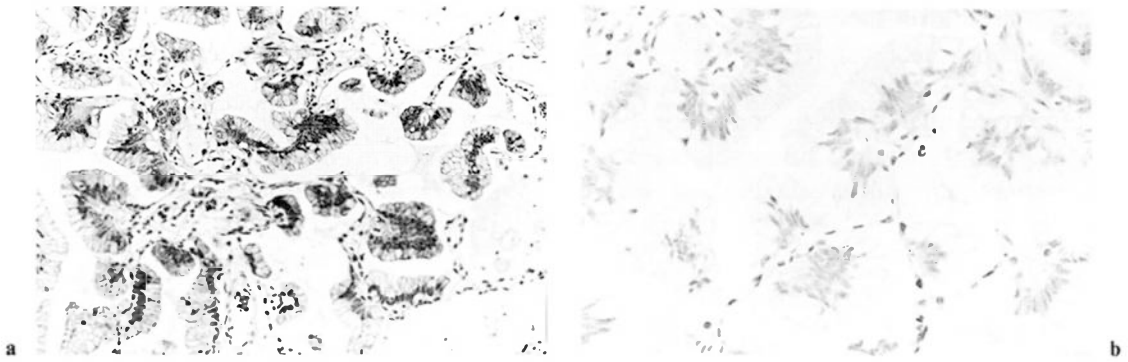

Fig. 4. Goblet cell AC with abundant mucin-producing cells. **a** H/E. × 200. **b** Negative immunoreactivity with MAb 44-3A6. × 400.

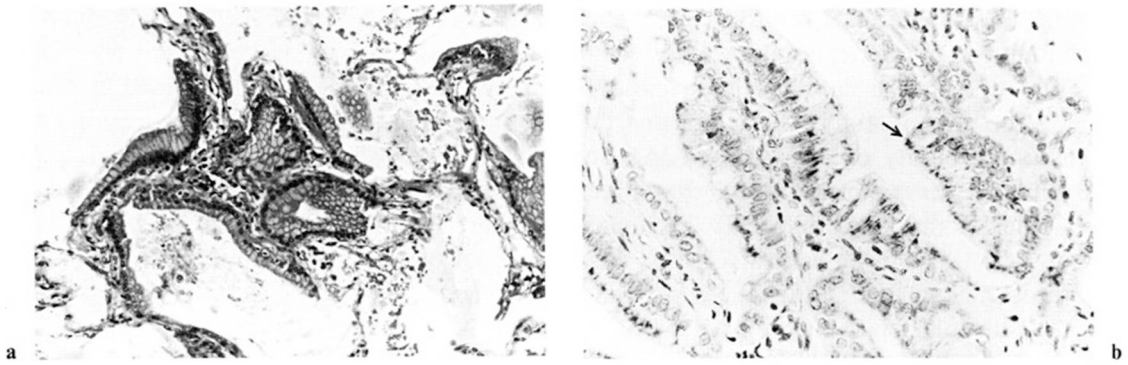

Fig. 5. Goblet cell AC with scanty mucin production. **a** H/E. × 200. **b** Arrow points to positive immunoreactivity with MAb 44-3A6. × 400.

Discussion

ACs of the lung can be divided histologically and cytologically into various subtypes. According to the WHO histological classification, these are acinar, papillary, bronchioloalveolar and solid tumors with mucus formation. The last type is considered to be a poorly differentiated form of mucus-producing ACs. BAC is thought to be a variant of papillary AC, exhibiting minimal destruction of the lung architecture as compared to the more aggressive papillary AC, frequently showing a lobar distribution.

Each histological type can be divided into cytological subtypes, based on the apparent direction of differentiation of the tumor cells which mimic the epithelial cell types present in the normal airway. Among the many epithelial cell types composing the normal air-

way are: ciliated columnar cells, goblet cells, nonciliated cuboidal cells (Clara cells), type II alveolar cells, and bronchial-gland cells. Accordingly, the cells in the majority of the well-to-moderately differentiated ACs of the lung can be subclassified into these five cell types, or mixed types of two or more cell types. BAC is a heterogeneous tumor, cytologically composed either of goblet cells, Clara cells or type II alveolar epithelial cells. However, there have been no reports of lung ACs having cells with visible cilia by light microscopy. Cilia, visible by light microscopy, have not yet been identified in tumor cells which appear to differentiate toward bronchial surface columnar cells, i.e. being reminiscent of bronchial surface epithelial cells with scanty or no mucus production.

In the present study, we investigated the immunoreactivity of the MAb 44-3A6 according to the histological and cytological subtypes as described above. It was found that ACs which were subclassified as Clara/bronchial surface epithelial cell type and type II alveolar epithelial cell type, that is, AC with no or scanty mucus production, were reactive with MAb 44-3A6 (20/20 cases studied), although the immunoreactivity varied within the tissue sections.

Mucus-producing ACs of the lung are composed of two subgroups: the bronchial gland cell type and the goblet cell type. The former contains some non-mucus-producing cells which may represent cells differentiating toward serous cells or bronchial gland duct cells. These were found to be positively stained with the MAb 44-3A6. Three of four cases of the bronchial gland cell type were positive for the expression of this antigen. In contrast, only 2 of 15 goblet cell types showed positive immunoreactivity, although very slight, and positive cells contained scanty mucin. It is interesting to note that normal goblet cells in the lungs do not express this antigen. Five cases classified as typical BAC of the lobar pneumonia type (with abundant mucus production) were completely negative for the expression of this antigen. The 2 slightly positive cases were found in the remaining 10 cases of the papillary AC of the solitary nodular type, with peripheral areas showing a bronchioloalveolar pattern. The negative or weak reactions in mucus-producing ACs may be due to the fact that much of the cytoplasm is occupied by mucous granules. This may reduce the available antibody-binding area within the tissue section, resulting in an antigen density which is below the level of detection under the conditions used herein. Alternatively, mucin production may mask or inhibit MAbs from detecting their respective epitopes. This is unlikely, however, because the MAb 44-3A6 has been used in a panel of MAbs to phenotype colon and lung neoplasms [37, 42]. In both of these studies, mucin-producing tumors which were negative for MAb 44-3A6 showed immunoreactivity for other MAbs. In addition, tumors which produced small amounts of mucin were immunoreactive in the present study.

In conclusion, ACs of the goblet cell type (with abundant mucin production) are negative for immunoreactivity with MAb 44-3A6, particularly those of the typical BAC subtype with lobar pneumonia-like distribution. In contrast, all of the ACs with no or scanty mucus production were found to express this epitope. These data support our previously reported subclassification scheme as well as the view that BACs of the goblet cell type may be biologically distinct from ACs arising in the lung.

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