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CASE REPORT

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Papillary adenoma of type II pneumocytes might have malignant potential

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Abstract Papillary adenoma of type II pneumocytes is a rare tumour. It is considered to be a benign neoplasm and is derived from immature cells in the bronchioloalveolar epithelium, however, its biological nature has not been elucidated. We report a case of an adenomatous tumour; a papillary adenoma of type II pneumocytes, which we regard as possessing malignant potential. Light microscopically, as well circumscribed, papillary tumour of predominantly cuboidal cells resembling type II pneumocytes was found, but Clara type and ciliated cells were also present. Immunohistochemically, the tumour cells reacted positively with antibodies to surfactant apoproteins (A, B), carcinoembryonic antigen, cytochrome P-450 1A1-2 and 2B1-2. Ultrastructurally, many osmiophilic lamellar bodies and electron-dense granules were demonstrated. Semi-serial sections revealed signs of transbronchial dissemination and vascular invasion. Morphometry using 12-dimensional cluster analysis disclosed features of the tumour cells which resembled those of pneumocyte type II adenocarcinoma. These findings suggest that the present case has some malignant characteristics and originates from immature bronchiolar or alveolar cells, with a potential to develop into both type II pneumocyte and Clara cell type adenocarcinomas.

Key words Papillary adenoma of type II pneumocytes · Immunohistochemistry · Electron microscopy · Morphometry · Multivariate cluster analysis

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Introduction

Papillary adenoma of the peripheral lung is uncommon although several cases have been reported. Spencer et al. described two cases of papillary tumours arising from the bronchi or bronchioles and considered them to be of Clara cell origin [16]. However, immunohistochemical and ultrastructural investigations have disclosed that the tumour cells tend to differentiate not only toward Clara cells but also toward type II pneumocytes or ciliated cells [1–3, 5, 11]. Because of this multidirectional differentiation, the tumour is considered to derive from immature or "stem" cells in the bronchioloalveolar epithelium. Its expansive growth pattern, rare mitoses, minimal nuclear atypia and relatively good prognosis support the assumption that the tumour is benign, however, much remains to be defined about its biological nature.

Recently, we observed a lung tumour histopathologically consistent with what has been described as a papillary adenoma of type II pneumocytes. The tumour was subjected to immunohistochemical and electron microscopic studies to gain insight into its origin. Moreover, we re-examined the form of the tumour cells, applying the techniques of morphometry and multivariate analysis [6] which we used in our recent study of bronchioalveolar lung tumours [10]. The results allow us to consider the position of this papillary adenoma in a variety of lung tumours, and to define the grade of malignancy in accurate morphological terms.

Case report

A 35-year-old Japanese male was admitted for evaluation of a coin lesion in the left lung. The lesion was detected by chance in a chest radiograph taken when he had symptoms of a common cold. On admission, he was free from respiratory symptoms or weight loss. He had been smoking 20 cigarettes a day for the past 15 years. Chest radiograph and computed tomography showed a well-defined density approximately 2 cm in diameter in the low-

er field of the left lung. Physical examination disclosed nothing abnormal and the results of routine laboratory tests were normal. Bronchoscopy revealed no significant findings. Curettage smears were inappropriate because of the inaccessibility of the lesion. At thoracotomy performed in September 1992, a lower lobectomy was performed, because the possibility of malignancy could not be ruled out from an imprint cytology taken during the operation. Lymph nodes were not swollen. The postoperative course was uneventful, and there is no evidence of recurrence 3 years later.

Materials and methods

The resected lung was immediately fixed in 10% formalin solution, and embedded in paraffin. Sections were stained with haematoxylin and eosin, alcian blue-PAS, and elastic-Goldner stains. Selected sections were immunostained by the streptavidin-biotin complex peroxidase method (Histofine kit, Nichirei, Tokyo, Japan), used primary mouse antibodies for surfactant apoprotein A (SPA) [9] and B (SPB) [8], carcinoembryonic antigen (CEA, DAKO, Glostrup, Denmark) and thyroglobulin (DAKO), and primary rabbit antibodies for urine protein 1 (UP1, DAKO), cytochrome P-450 1A1-2, and 2B1-2 [4] respectively. The grade of staining pattern was determined by the rate of positive cells: grade 0, 0%; grade 1, 1, 0–25%; grade 2, 25–50%; grade 3, 50–75%; grade 4, 75–100%; grade 5, 100%.

For electron microscopy, a small sample was fixed in 1.5% cold glutaraldehyde in phosphate buffer at pH 7.4, post-fixed in 1.5% osmium tetroxide, dehydrated in graded alcohols, and embedded in Epon 812. Ultra-thin sections were stained with uranyl acetate and lead citrate and examined with a transmission electron microscope (H-600, Hitachi, Tokyo, Japan).

In order to examine the growth pattern of this tumour, about 100 serial sections were prepared from the paraffin block at 3 μm thickness, 10 sections of which, at an interval of 10 sections, were stained with elastic-Goldner stain.

The cellular features of papillary adenoma were quantified by morphometry and multivariate cluster analysis. In our previous report [10], 97 lesions which were diagnosed before morphometry as atypical adenomatous hyperplasia (AAH), type II pneumocyte and Clara cell type adenocarcinomas were subjected to cluster analysis which gave rise to a separation of the lesions into three clusters. The results of morphometry in the present case were add-

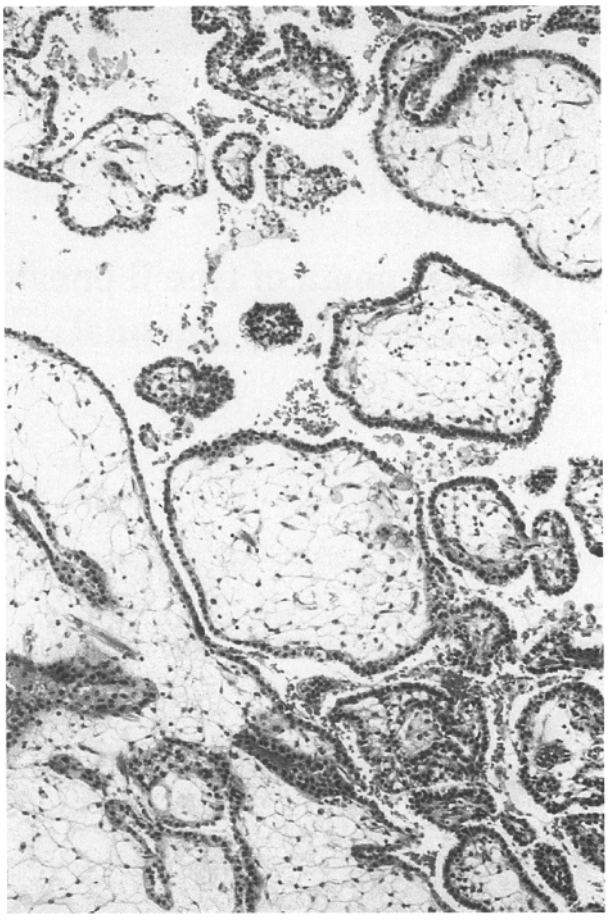
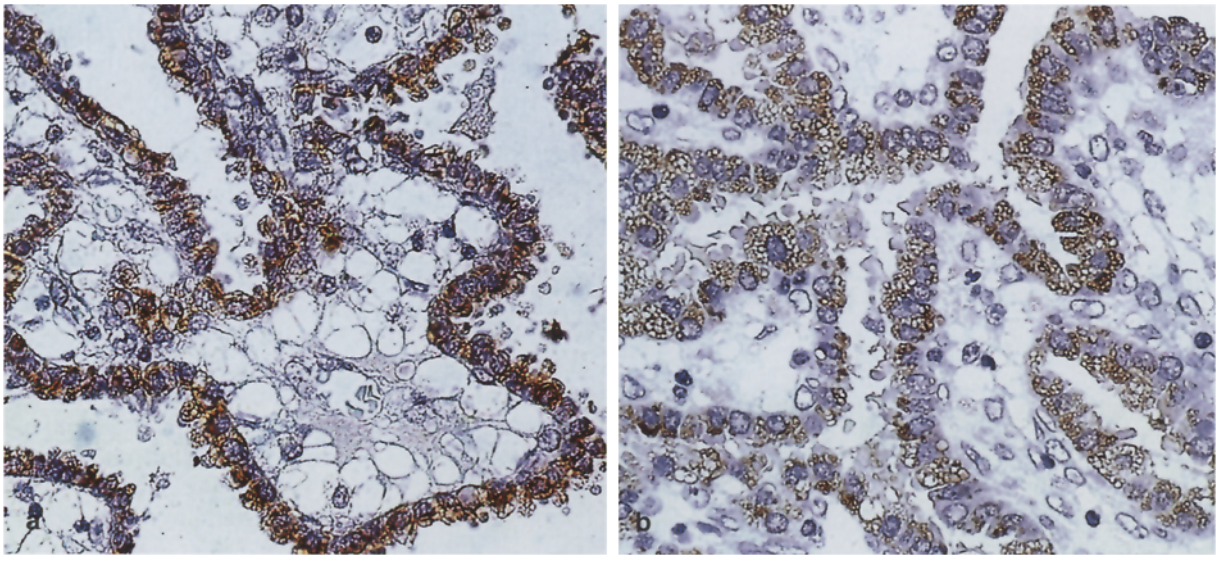


Fig. 1 Low-power view of adenoma. It shows a papillary growth pattern with oedematous stroma. H&E, original magnification, ×33

Fig. 2a, b Immunohistochemical analyses. **a** Surfactant apoprotein A, grade 4; **b** surfactant apoprotein B, grade 4. Immunoperoxidase-haematoxylin, ×132



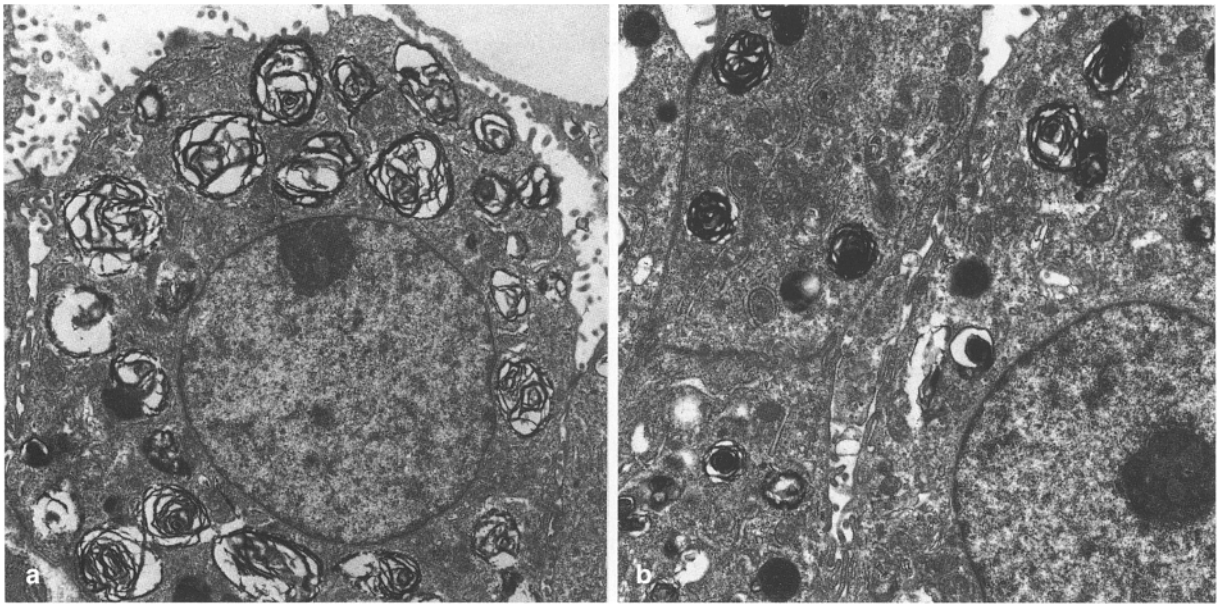


Fig. 3a, b Ultrastructural demonstrations of this tumour. **a** The cuboidal tumour cells have many osmiophilic lamellar bodies in cytoplasm similar to those seen in normal type II pneumocytes, ×6420. **b** A few tumour cells have membrane-bound, electron-dense granules in cytoplasm similar to those seen in Clara cells, as well as lamellar bodies, ×7490

ed to the above data for re-trial, in order to examine into which cluster the lesion was to be classified. In addition, the validity and reproducibility of the cluster analysis was tested by the discriminant analysis, including the canonical discriminant analysis which was performed to visualize the distribution of the sample. Details of the methods have been published previously [10].

Results

The tumour was located in the basal segment ($S_{10}{}^{b}$) of the left lung as a spherical mass 2 cm diameter. The cut surface was grayish white, soft and partially haemorrhagic. It was not encapsulated but was well-circumscribed.

The tumour was growing expansively, having no capsule but growing along alveolar walls and protruding into a few bronchioles. Mild haemorrhage was detected but no necrosis was seen. The cells displayed a papillary growth pattern, overlying an oedematous stroma containing stellate cells and thin-walled blood vessels (Fig. 1). The individual cells were uniformly cuboidal, having central nuclei with slight atypia. Intranuclear eosinophilic inclusions were sometimes present. The cytoplasm was foamy, resembling type II pneumocytes. Cells having the characteristics of Clara cells were also detected, which were cuboidal to low columnar, peg or slub shaped and projecting into the lumina beyond the level of the lateral cell junction. There were also a few ciliated cells and tall columnar cells without cilia or cytoplasmic protrusions into the lumina. Mitotic figures were rare. Histiocytes and lymphocytes infiltrated into the oedematous stroma. The tumour cells were negative for mucin. Elastic fibres were not present in the stroma. Sections of lymph nodes revealed no metastasis. Immunohistochemically, the tumour cells reacted positively with antibody to SPA (grade 4), SPB (grade 4) (Fig. 2), cytochrome P-450 1A1-2 (grade 1) and 2B1-2 (grade 1). CEA was also positive in the cytoplasm with polarity. Thyroglobulin and urine protein 1 were not detected. Control sections showed no specific reactivity.

Ultrastructurally the tumour cells were cuboidal with short microvilli. The cytoplasm had a moderate number of mitochondria and rough endoplasmic reticulum. The nuclei were round with evenly dispersed chromatin and contained nucleoli. The most characteristic feature was the presence of many osmiophilic lamellar bodies similar

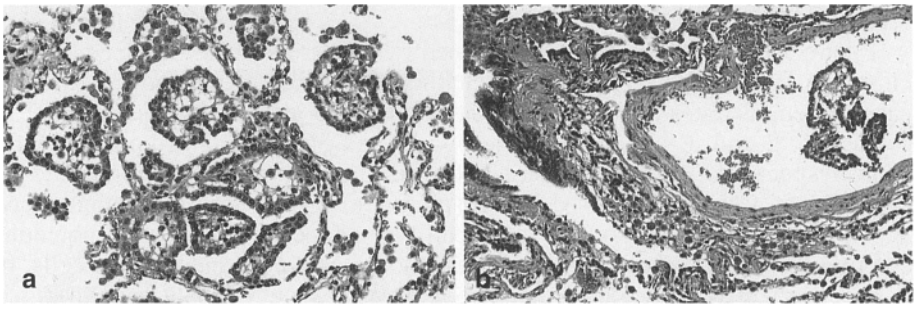


Fig. 4 Tumour cells found in normal lung surrounding the tumour (**a**) and in a small venule in the lung (**b**). H&E, original magnification, **a** ×66, **b** ×33

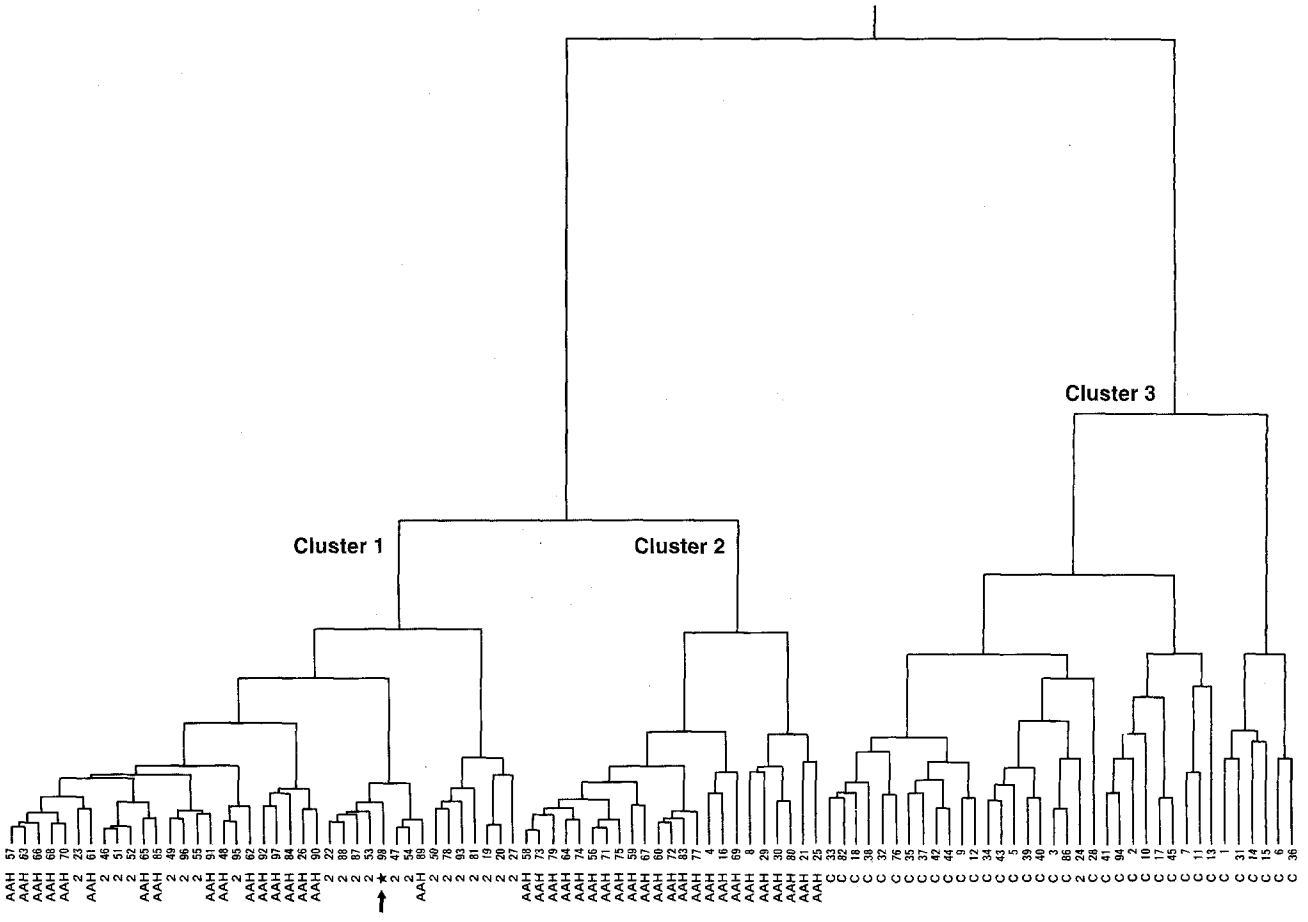


Fig. 5 The dendrogram obtained from 98 lesions including the present case (no. 98). These are shown to be classifiable into three clusters. Shown along the lower margin are the lesion numbers and pre-morphometry diagnoses. *AAH* Atypical adenomatous hyperplasia, *2* type II pneumocyte type adenocarcinoma, *C* Clara cell type adenocarcinoma. The present case (indicated with an *arrow*) was classified into cluster 1

to those seen in normal type II pneumocytes (Fig. 3a). Moreover, a few tumour cells contained membrane-bound, electron-dense granules similar to those seen in Clara cells (Fig. 3b). A few ciliated cells were also detected. Nuclear inclusions consisting of tubular structures, 50–60 nm in diameter, were also present in some of the tumour cells.

In several of the semi-serial sections, the tumour protruded into small bronchioles. There were several clusters of cells in alveolar air spaces in normal zones surrounding the tumour (Fig. 4a). Tumour cells were also seen in several small venules (Fig. 4b).

Figure 5 is a new dendrogram obtained by 12-variate cluster analysis of the 98 lesions including the present case. It demonstrates that the lesions are classfiable into clusters 1, 2 and 3, which comprise 39, 23 and 36 lesions, respectively. Of these, cluster 2 consists almost exclusively of lesions diagnosed before morphometry as AAH, and cluster 3, of those as Clara cell type adnocarcinoma. In cluster 1, however, lesions diagnosed as type II pneumocyte type adenocarcinoma co-exist with about the same number of lesions considered to be AAH. The present case, lesion no. 98, was classified into this cluster. The present case was also correctly rediscriminated by the discriminant analysis. The rates of correct rediscrimination by the discriminant analysis were 94.9% for cluster 1, 95.7% for cluster 2 and 94.4% for cluster 3, respectively. A two-dimensional scattergram containing the results of canonical discriminant analysis revealed that this case belonged to the marginal area of cluster 1.

Discussion

Papillary adenoma of the lung is a recently defined rare tumour. Spencer et al. [16] reported 19 cases of papillary tumours arising from bronchial epithelium, two of which arose from peripheral bronchi or bronchioles and were considered to be of Clara cell origin. Fantone et al. [1] and Noguchi et al. [11] reported papillary adenomas of the peripheral lung and demonstrated ultrastructurally a tendency to differentiate toward both Clara cells and type II pneumocytes. Immunohistochemically, the cells stained positive for surfactant apoprotein [2, 11] and Clara cell protein [5]. These results led to the assumption that the tumour arose from a common progenitor cell having the potential to differentiate toward bronchiolar and alveolar cells, (Clara cells and type II pneumocytes). Nuclear inclusions of tubular arrangement, ciliated cells,

and oncocytic cells were also found ultrastructurally [2, 3, 11]. The lesion was considered to be benign, because of its expansive growth pattern, slight nuclear atypia, rare mitotic figures and a favorable clinical course. The present case appears similar to the above reports of papillary adenoma of type II pneumocytes.

At light microscopic observation, the present case may resemble a metastatic papillary carcinoma of the thyroid. However, this possibility could be easily ruled out by clinical features, negative staining for thyroglobulin, and clear signs of differentiation mainly toward type II pneumocytes with positive immunostaining not only for SPA but also for SPB. Although SPA sometimes reacts positively in papillary carcinoma of the thyroid [14, 15], SPB is never expressed in thyroid carcinoma. Papillary adenoma can also resemble sclerosing haemangioma (pneumocytoma) of the lung, which sometimes shows papillary growth pattern with tumour cells having some characteristics similar to type II pneumocytes. Although the possibility remains that the papillary adenoma is one variant of sclerosing haemangioma, the latter is usually distinguishable from the former with its sclerotic interstitium and various features of solid proliferation of tumour cells.

We used UP1 as a marker of differentiation for Clara cells, whose immunoreactivity was confirmed to be similar to CC10, the 10 kDa Clara cell protein [12]. Besides normal Clara cells which specifically react with CC10 and UP1, about 10% of lung adenocarcinomas were shown to have some reactivity with UP1 [12]. The present tumour had no positive staining, however, reacting intensely in Clara cells but weakly in lung tumours, UP1 is likely to disappear in the course of carcinogenesis.

Cytochrome P-450-associated monooxygenases, localized in the endoplasmic reticulum of Clara cells, are implicated in the metabolism of a variety of xenobiotics including inhaled tobacco carcinogens [13]. In the rat, 1A1 and 2B1 are found in normal Clara cells and 2B1 has been demonstrated in some type II pneumocytes, expressed constitutively [7]. In a preliminary study, we examined the pattern of immunostaining for P-450s using an apparently normal human lung tissue surrounding tumour resected from a current smoker with lung cancer. An intense reactivity was demonstrated in Clara cells, whereas the ciliated columnar epithelia, macrophages and type I and II pneumocytes showed only faint staining (unpublished data). The expression of cytochrome P-450 1A1-2 and 2B1-2 in this tumour suggests that the cells tend to differentiate toward Clara cells. Ultrastructurally, the tumour cells were shown to possess not only lamellar inclusions which were characteristic of type II pneumocytes together with electron-dense granules of Clara cells. All these observations suggest that this tumour originates from multipotential stem cells which are distributed over a range from bronchioles to alveoli and can differentiate into Clara cells and type II pneumocytes, in agreement with previous reports [1–3, 5, 11]. It supports the diagnosis of this lesion as papillary adenoma of type II pneumocytes.

Although papillary adenoma of the lung has been regarded as a benign neoplasm, its malignant potential remains. Analysis of semi-serial sections disclosed dissemination of this tumour via airways and vascular invasion in zones of surrounding alveolar tissue, findings suggesting that the tumour had some characteristics of malignancy. The morphometry and multivariate analysis assigned the present case to cluster 1; the group mainly consisting of II type pneumocyte type adenocarcinomas. However, in this cluster, lesions diagnosed as type II pneumocyte type adenocarcinoma co-exist with 17 lesions considered to be AAH. In view of the grade of atypia which proved to be comparable to type II pneumocyte type adenocarcinoma, the latter lesions are likely to have been adenocarcinomas from the very beginning, and we think it justifiable to assume cluster 1 as a cluster of type II pneumocyte type adenocarcinoma [10]. Thus the present case may be a lesion closely related to adenocarcinoma. The rate of correct re-discrimination was sufficiently high to ensure that the above clustering is a reproducible, and therefore an adequate classification. The two-dimensional scattergram of canonical discriminant analysis showing that this case belongs to the territory of cluster 1 also indicated that it might have a malignant potential.

The present lesion differs from AAH in its papillomatous growth pattern instead of the bronchioalveolar or adenomatous pattern of the latter. However, these two lesions seem similar not only in relation to carcinoma but in their potential to differentiate towards both Clara cells and type II pneumocytes.

From a cellular morphological point of view, this case appears to have some malignant potential. These patients should be submitted to careful examination but, more cases and a long term follow-up are required to elucidate the characteristics of papillary adenoma of type II pneumocytes.

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