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Cytodifferentiation of atypical adenomatous hyperplasia and bronchioloalveolar lung carcinoma: immunohistochemical and ultrastructural studies

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Abstract We used immunohistochemistry and electron microscopy to evaluate the differentiation of cells comprising atypical adenomatous hyperplasia (AAH; n = 26), early bronchioloalveolar lung carcinoma (BAC; n = 11), and overt BAC (n = 16), which are assumed to constitute a continuous spectrum of developmental steps of BAC. Surfactant apoprotein (SAP), a marker for type 2 alveolar cells, was expressed in cells from all the lesions of AAH, early BAC, and overt BAC. However, the proportion of SAP-positive cells decreased and their distribution became more heterogeneous with advancing lesion grade. Urine protein 1, which is identical to the Clara cell-specific 10 kDa protein, was expressed in 70% of overt BAC, whereas only 20% of early BAC showed weak reactivity and none of AAH lesions showed any reactivity at all. Ultrastructurally, type 2 alveolar cell differentiation was predominant among cells from AAH and early BAC. Our results suggest that precursor cells of BAC differentiate predominantly towards type 2 alveolar cells. Cells comprising overt BAC retain this differentiation phenotype, but to a reduced extent. In contrast, concomitantly with progression, cells with Clara cell differentiation emerge and their proportion increases. Such phenotypic changes may reflect metaplasia occurring in tumour cells during the development of BAC.

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Introduction

Atypical adenomatous hyperplasia (AAH) of the lung, a proliferative lesion of cuboid or low columnar cells with variable grades of atypia along the alveolar septa, is considered to be a precursor of bronchioloalveolar lung carcinoma (BAC) [3, 6, 8, 10, 14, 17, 19, 20, 26, 27, 29], as AAH and BAC represent a continuous spectrum with regard to atypia grade, lesion size, and proliferative activity [12, 13]. In addition, variable proportions of AAH cells exhibit expression of carcinoembryonic antigen [3, 13, 21, 26], overexpression of tumour suppressor gene product p53 [10, 12, 13] and of c-*erb*B-2 oncogene product [10], aneuploidy [22, 34], and point mutations of the K-*ras* oncogene [25, 33]. Early BAC is considered to be a potentially malignant lesion intermediate between AAH and overt BAC [12, 13]. The differentiation phenotype of AAH cells has been assumed to be the type 2 alveolar cell and/or the nonciliated bronchiolar (Clara) cell. To date, however, few studies have focused upon this issue [8, 14, 17, 21], and their results are inconsistent. Kodama et al. [14] evaluated the expression of surfactant apoprotein (SAP), which is specific for the type 2 alveolar cells [15, 30, 31], immunohistochemically and found that 15 of 16 AAH lesions showed positive reactivity. Miller [17] and Hammar [8] examined a few lesions of bronchioloalveolar cell adenomas (BAA) (identical to AAH in the present study) by electron microscopy and found type 2 alveolar cell differentiation in BAA cells. In contrast, Nakanishi [21] examined 4 AAH lesions in the same way and found features of Clara cells in the vast majority of AAH cells; only rarely were cells with features of type 2 alveolar cells seen. It is important to identify the progenitor cell of BAC, as this would help us to identify the mechanism of oncogenesis in BAC. In-

vestigation of precursor lesions of BAC would be a suitable strategy for this purpose, since the component cells may retain the differentiation phenotype of progenitor cells better than fully developed malignant cells.

We investigated the differentiation phenotype of cells comprising AAH lesions against BAC cells, to determine whether these cells belong to the same cell lineage. Using immunohistochemical methods, we evaluated the expression of SAP and of urine protein 1 (UP1), which has recently been demonstrated to be identical to the Clara cell-specific 10-kDa protein (CC10) [1, 2]. Furthermore, several lesions of AAH and early BAC were studied by transmission electron microscopy along with immunohistochemical evaluation of the expression of SAP and UP1.

Materials and methods

We selected16 overt BAC lesions, 11 early BAC, and 26 AAH lesions from 26 patients listed in the surgical pathology files at Yokohama City University Hospital, Kanagawa Cancer Centre, and Yokohama Municipal Citizen's Hospital between 1987 and 1996. Among the 26 AAH lesions, 11 were classified as low-grade AAH, and 15 were classified as high-grade AAH, as described previously [12, 13]. All the overt BAC lesions were of a sclerosing subtype [5] (see below) and appeared from light microscopic observation of tumour cells to be of a Clara cell type [27, 28].

During the same period we saw 262 primary lung cancer cases treated by lobectomy or pneumonectomy, which included 156 adenocarcinomas, 71 squamous cell carcinomas, and 35 cases of other types of lung cancer. The diagnosis and subtyping of BAC were based on the definition of Clayton [5]; there were 57 cases of BAC, including 10 mucinous, 8 nonmucinous, and 39 sclerosing BAC. Forty-three AAH lesions were found in 32 patients with primary lung cancer (30 adenocarcinomas, 1 squamous cell carcinoma, and 1 adenosquamous carcinoma) and in 2 patients with metastatic lung neoplasm. Fifteen lesions of early BAC were found in 8 patients, 4 of whom had a concurrent overt adenocarcinoma as the primary lesion and the remaining 4 patients underwent lobectomy for their early BAC. Distinction of early BAC from nonmucinous, nonsclerosing overt BAC was based on less anaplasia of the tumour cells and the smaller lesion size (usually <1.5 cm) [13].

Specimens were taken from the surgically resected lobes and were fixed by intrabronchial instillation of 20% neutral buffered formalin solution. Formalin-fixed tissues were routinely embedded in paraffin, and 4-μm-thick sections were stained with haematoxylin and eosin or used for immunostaining of SAP and UP1. Briefly, deparaffinized and rehydrated sections were treated with hydrogen peroxide in methanol to block endogenous peroxidase activity and then incubated with normal goat serum. The sections were reacted with anti-SAP mouse monoclonal antibody (PE10, Teijin, Tokyo) [15] at 1:800 dilution or anti-UP1 rabbit polyclonal antibody (Dako, Glostrup, Denmark) [24] at 1:400 dilution for 1 h at room temperature. The sections were then reacted with appropriate biotinylated secondary antibodies, and the antibody-binding sites were visualized by the labelled streptavidin-biotin peroxidase complex method using diaminobenzidine as the chromogen. Type 2 alveolar cells and Clara cells served as internal positive controls for SAP and UP1, respectively. As negative controls, sections were similarly treated with nonimmune mouse or rabbit serum in place of the primary antibodies.

Staining intensity for SAP and UP1 was graded as negative (–), weak (+), moderate (++), or strong (+++). The proportions of cells positive for SAP or UP1 were determined by observing the entire field of each lesion. Lesions were classified as focal or diffuse in terms of the distribution pattern of cells positive for SAP. When positive cells were distributed uniformly within the lesions they were classified as diffuse; otherwise, the lesions were classified as focal.

An additional 8 lung lesions were evaluated by both immunohistochemistry and electron microscopy. These were obtained from 3 patients and included 2 overt BAC lesions (one Clara cell type and another type 2 alveolar cell type), 3 lesions of early BAC, and 3 lesions of high-grade AAH. The lungs were fixed in buffered formalin in the same manner as above. Tissues obtained from the largest cut surface of the lesions were processed for light microscopy, and the remaining parts were used for electron microscopic analysis. For transmission electron microscopy small pieces of formalin-fixed tissue were washed in phosphate buffered saline (pH 7.2) and then postfixed in osmium tetroxide. The tissues were embedded in epoxy resin, and semi-thin sections were observed by light microscopy. Thin sections were made from selected blocks (3–5 blocks per lesion), stained with uranyl acetate and lead citrate, and observed with a transmission electron microscope.

For statistical analysis of differences in frequency, the Chi-square test and Fisher's exact test were used. When *P*-values were less than 0.05, the differences were considered significant.

Results

The histological and cytological features of AAH, early BAC, and overt BAC lesions examined in this study were essentially the same as described previously [12, 13]. The lung lesions from each category exhibited different grades of atypia in terms of cell density, cell size, nuclear size, chromatin pattern, and nucleolar morphology. Briefly, in both overt (Fig. 1A) and early BAC (Fig. 1B), the cell density was high, and the nuclei were so highly crowded as to make contact with, or even to overlap, the nuclei of neighbouring cells. The cell size was obviously larger than that of normal type 2 alveolar or Clara cells. The nuclear size (the largest dimension) was 3-fold that of small lymphocytes or even greater. Chromatin was coarsely aggregated. The nucleoli were irregular in shape, acidophilic, peripherally located, multiple, and often surrounded by a halo. These characteristics were more prominent in overt BAC than in early BAC (Fig. 1A, B). High-grade AAH was characterized by relatively high cell density (Fig. 1C). The cell borders were in contact with each other, but the nuclei were well separated. The cells were slightly larger than or approximately the same size as type 2 alveolar or Clara cells. The nuclear size ranged from 1.5-fold to 3-fold that of small lymphocytes. The nucleoli were conspicuous, slightly enlarged, round, usually single, and centrally located. Chromatin aggregates were fine. In low-grade AAH, the cellularity was low and the neighbouring cells were well separated from each other (Fig. 1D). The cells were similar in size to or even smaller than type 2 alveolar or Clara cells. The nuclear size was less than 1.5-fold that of small lymphocytes. The nucleoli were inconspicuous and small if present. Chromatin aggregation was not obvious. In all of the 4 categories of lung lesions, cells with intranuclear inclusion were observed at varying frequencies.

The results of immunostaining for SAP and UP1 in the lung lesions are summarized in Table 1. Virtually all the normal type 2 alveolar cells showed strong diffuse cytoplasmic staining for SAP. The bronchiolar and bronchial surface epithelial cells and bronchial gland cells

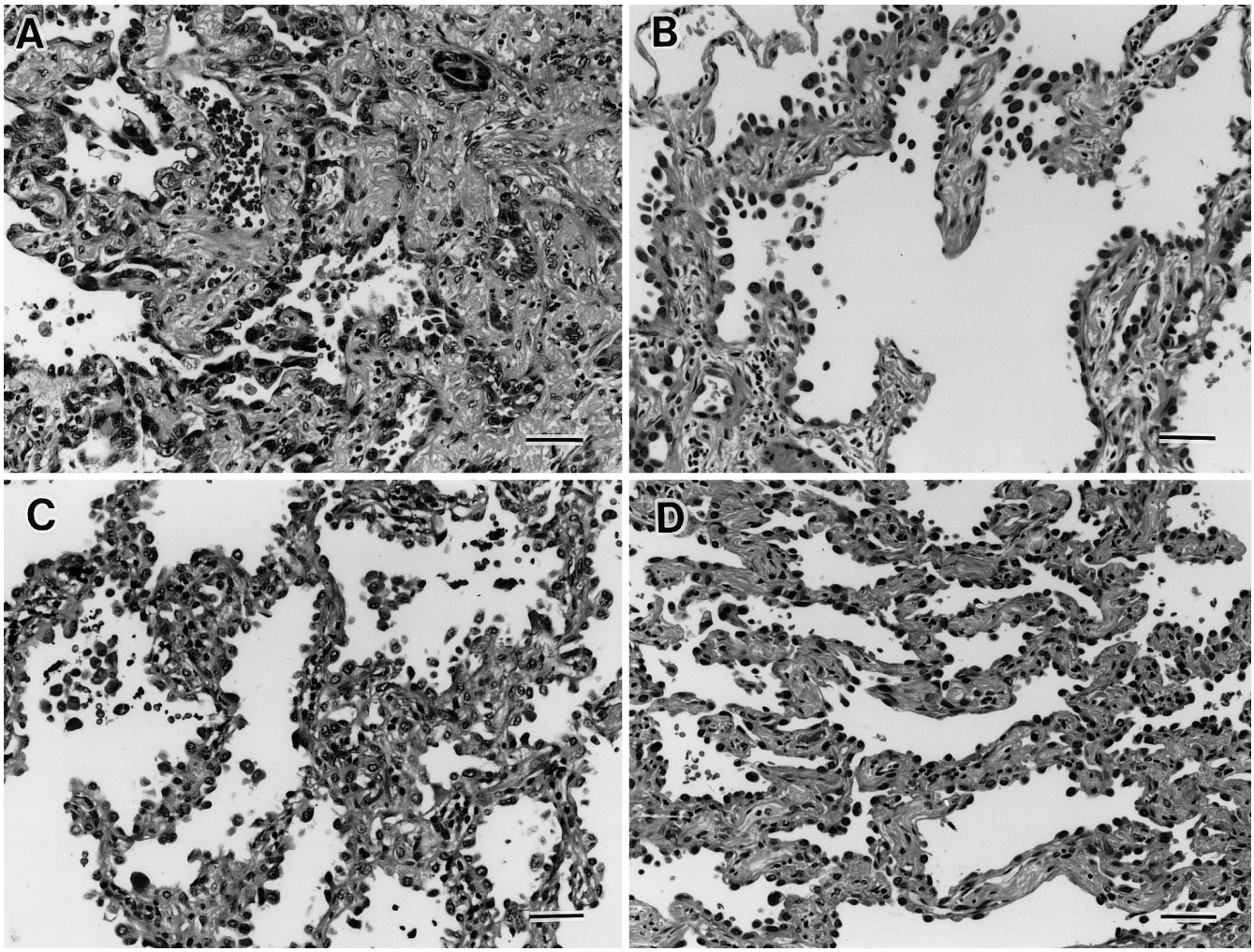

Fig. 1 **A–D** Representative light micrographs of **A** overt bronchioloalveolar carcinoma (BAC), **B** early BAC, **C** high-grade atypical adenomatous hyperplasia (AAH), and **D** low-grade AAH. Note invasion of tumour cells into the desmoplastic stroma in overt BAC (**A**). In overt BAC (**A**) and early BAC (**B**), the cell density was high and the nuclei of tumour cells were heaped. The tumour cells and their nuclei were much larger than normal type 2 alveolar or Clara cells and their nuclei. These characteristics were more prominent in overt BAC (**A**) than in early BAC (**B**). High-grade AAH showed relatively high cell density and slightly larger cellular and nuclear size compared to type 2 alveolar or Clara cells (**C**). Low-grade AAH showed relatively low cell density and small cellular and nuclear size (**D**). Haematoxylin-eosin staining, ×160, *bars* 50 μm

were negative for SAP. All of the 26 AAH lesions, all of the 11 early BAC lesions, and all of the 16 overt BAC lesions contained cells positive for SAP (Fig. 2A, B). The majority of overt BAC lesions and approximately half of early BAC lesions showed a focal pattern of distribution of positive cells, while the remaining half of early BAC lesions and the majority of AAH lesions showed a diffuse pattern. There was a statistically significant difference in the distribution pattern between overt BAC and both low- and high-grade AAH ($P<0.005$ for each). The staining intensity for SAP varied from lesion to lesion, but there were no significant differences between each of the categories. A tendency was noted for overt and early BAC lesions to contain cells positive for SAP at lower frequencies than those of AAH. There were statistically significant differences between high-grade AAH and early BAC ($P<0.05$) and between high-grade AAH and overt BAC ($P<0.005$). The nuclear inclusions invariably stained positively for SAP (Fig. 2A, B).

The cytoplasm of Clara cells showed intense positive staining for UP1, and nonciliated columnar cells in the bronchial surface epithelia, including goblet cells, stained positively at a moderate intensity for this antigen. Type 2 alveolar cells were consistently negative for this antigen. In all 26 AAH lesions, no cells were positive for UP1. There were 2 among the 11 lesions of early BAC that contained cells weakly positive for UP1 (Fig. 3A), while the remaining 9 lesions were negative for this antigen. Among the 16 lesions of overt BAC, 10 contained cells moderately to strongly positive for UP1 (Fig. 3B), and one lesion contained cells weakly positive for this antigen. The frequency of overt BAC lesions containing cells positive for UP1 was significantly greater than those of low-grade AAH, high-grade AAH, and early BAC ($P<0.005$ for each). The proportion of cells positive

Table 1 Immunoreactivity for surfactant apoprotein and urine protein 1 in lung lesions (*AAH* atypical adenomatous hyperplasia, *BAC* bronchioloalveolar carcinoma, – negative, + weakly positive, ++ moderately positive, +++ strongly positive)

Lung lesions (no. of lesions)	Surfactant apoprotein								Urine protein 1		
	Distribution of positive cells		Staining intensity			Frequency of positive cells			Staining intensity		
	Focal	Diffuse	+	++	+++	<33%	33–67%	67%<	–	+	++/+++
Low-grade AAH (n = 11)	2	9	2	2	7	1	2	8	11	0	0
High-grade AAH (n = 15)	4	11	1	4	10	0	2	13[b,c]	15	0	0
Early BAC (n = 11)	5	6	4	0	7	4	3	4	9	2	0
Overt BAC (n = 16)	13	3[a]	3	2	11	7	4	5	5	1	10[d]

[a]: significantly different from the corresponding values in low-grade AAH and high-grade AAH (P<0.005)
[b]: significantly different from the corresponding value in early BAC (P <0.05)
[c]: significantly different from the corresponding value in overt BAC (P <0.005)
[d]: significantly different from the corresponding values in low-grade AAH, high-grade AAH, and early BAC (P <0.005)

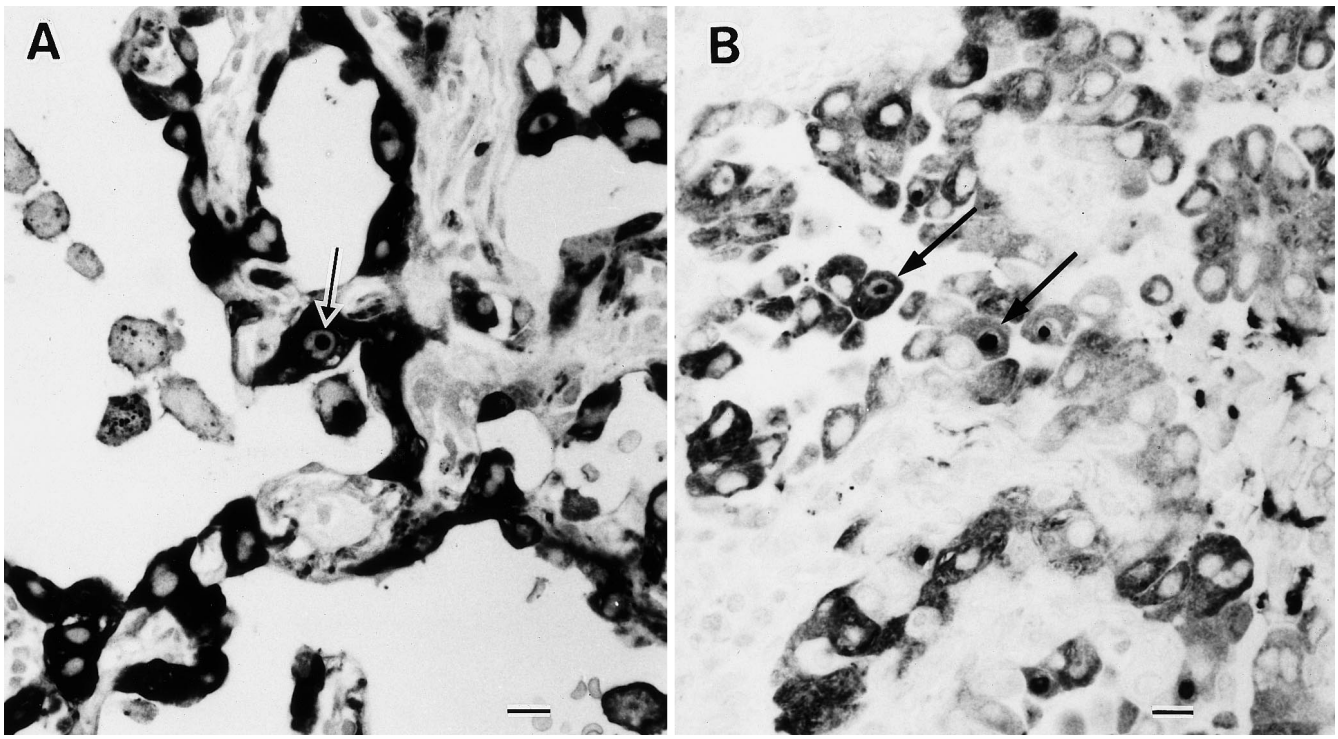

Fig. 2 A, B Immunostaining of surfactant apoprotein (SAP) in (**A**) high-grade AAH and (**B**) overt BAC. **A** Virtually all cells in high-grade AAH were positively stained for SAP at a strong and uniform intensity. **B** Almost all cells in overt BAC were also positively stained for SAP, but the staining intensity varied from cell to cell. *Arrows* indicate nuclear inclusions which were positively stained for SAP (**A** and **B**). Immunoperoxidase staining with haematoxylin counterstaining, ×460, *bars* 10 µm

for UP1 was 10% to 30% in 2 of the 10 strongly positive overt BAC lesions, and was less than 10% in the remaining 8 lesions.

The electron microscopic findings of cells in the lung lesions are summarized in Table 2. Tumour cells of Clara cell adenocarcinoma were columnar and had cytoplasmic protrusions. Their nuclei were oval, and the contours were irregular and frequently indented. Intranuclear inclusions were not seen in this tumour. Many membrane-bound dense granules (300–700 nm), occasionally associated with fingerprint-like structures, were observed in the supranuclear portion of the cytoplasm. Occasional cells also contained small numbers of lamellar bodies. Tumour cells of type 2 alveolar cell adenocarcinoma were cuboid or dome-shaped. Their nuclei were round and the outline was rather smooth. The cytoplasm contained many lamellar bodies (300–1,200 nm). Rare cells also contained membrane-bound dense granules. Intranu-

Table 2 Transmission electron and light microscopic findings in selected lung lesions

Lung lesion	Electron microscopy		Light microscopy			Lesion size (mm)
	Nuclear inclusion	Secretory granules[a]	Nuclear inclusion	SAP[b]	UP1[c]	
Adenocarcinoma Clara cell type	No	Dense granules +++ Lamellar bodies ±	No	10%	+	16
Adenocarcinoma Type 2 cell type	Yes	Lamellar bodies +++ Dense granules ±	Yes	100%	–	24
Early BAC	No	Dense granules ++	Yes	1%	–	6.3
Early BAC	No	Lamellar bodies +	Yes	5%	–	7.5
Early BAC	Yes	Lamellar bodies +++	Yes	30%	–	14.5
AAH, high grade	Yes	Lamellar bodies +++	Yes	20%	–	4.8
AAH, high grade	Yes	Lamellar bodies +++	Yes	80%	–	5.7
AAH, high grade	No	Lamellar bodies ++	Yes	5%	–	6.3

[a] ± Small numbers in occasional cells: +, ++, and +++ small, moderate, and large numbers in frequent cells, respectively

[b] Percentage of positive cells

[c] + Positive cells exist, – no positive cells

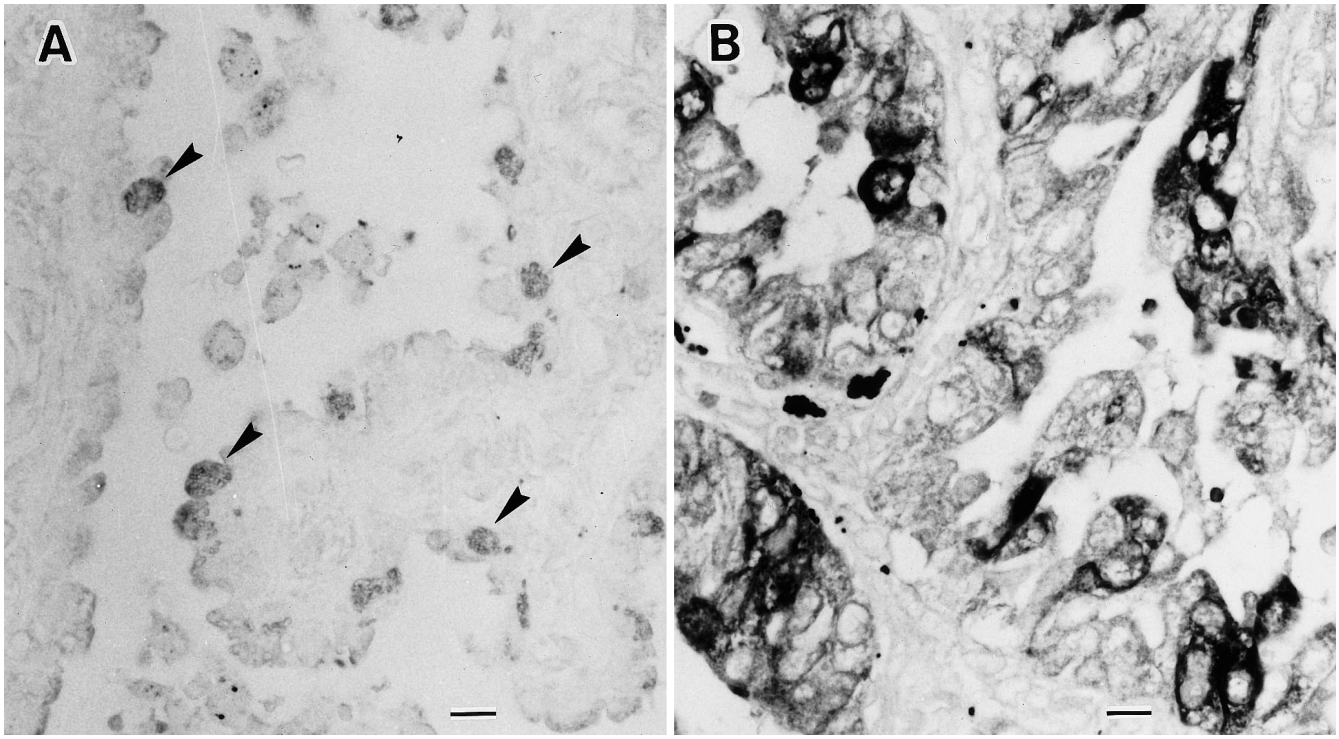

Fig. 3 A, B Immunostaining of urine protein 1 (UP1) in (**A**) early BAC and (**B**) overt BAC. **A** In early BAC, tumour cells weakly positive for UP1 (*arrowheads*) were scattered on the surface of thickened alveolar septa. **B** Tumour cells strongly positive for UP1 were observed in overt BAC. Immunoperoxidase staining with haematoxylin counterstaining, ×460, *bars* 10 μm

clear inclusions composed of branching microtubular structures were frequently seen. Cells of early BAC were cuboid to low columnar and frequently had cytoplasmic protrusions (Fig. 4). Their nuclei were round to oval, and the outline was irregular and sometimes indented. Among the 3 early BAC lesions, 3 contained cells with intranuclear microtubular inclusions. One lesion contained many cells with membrane-bound dense granules (200–500 nm) similar to those seen in cells from Clara cell adenocarcinoma (Fig. 4, inset). A few cells had both dense granules and lamellar bodies (Fig. 5). The remaining 2 lesions contained many cells with lamellar bodies which were similar to those seen in cells from type 2 alveolar cell adenocarcinoma. However, they were small in size (200–700 nm) and low in number compared to with those seen in the latter. Cells of high-grade AAH were cuboid to low-columnar and occasionally showed short microvilli (Figs. 6, 7). Their nuclei were round to oval and the outline was rather smooth. Binucleated cells (Fig. 7) and cells with an apical nucleus (Fig. 8) were occasionally seen. Of the 3 high-grade AAH lesions, 2 contained cells with intranuclear microtubular inclusions (Fig. 8). The cytoplasm was abundant and contained

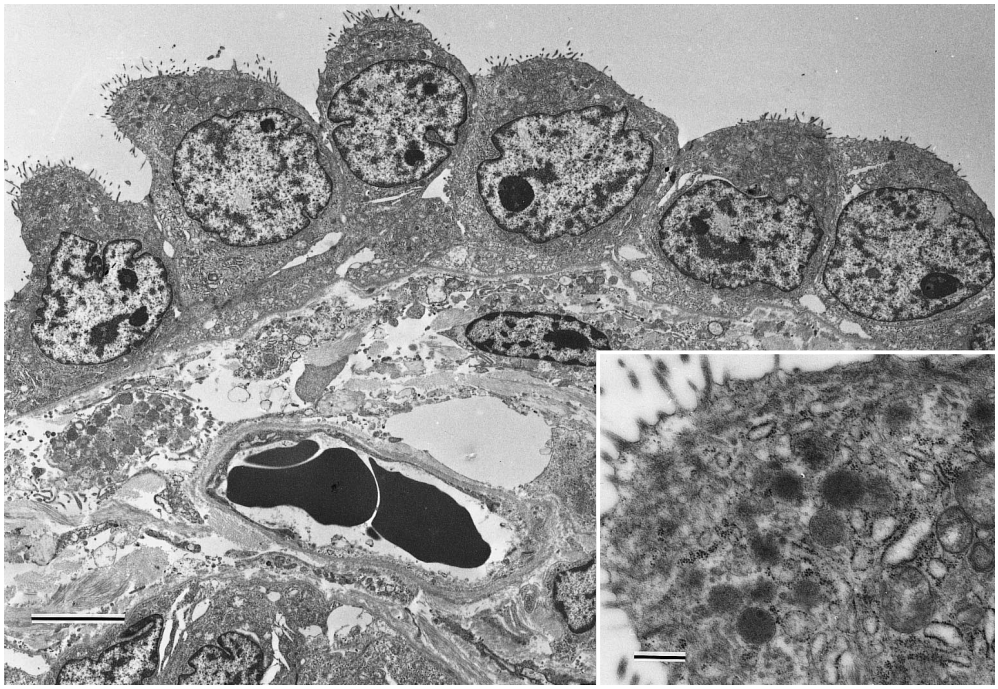

Fig. 4 Representative electron micrograph of early BAC. Low columnar tumour cells with cytoplasmic protrusions, microvilli, and dense granules were arranged in a single layer on the surface of thickened alveolar septa. *Bar* 5 μm. *Inset* A higher power view of a tumour cell, showing several membrane-bound dense granules and some profiles of rough endoplasmic reticulum in the supranuclear cytoplasm. *Bar* 0.5 μm

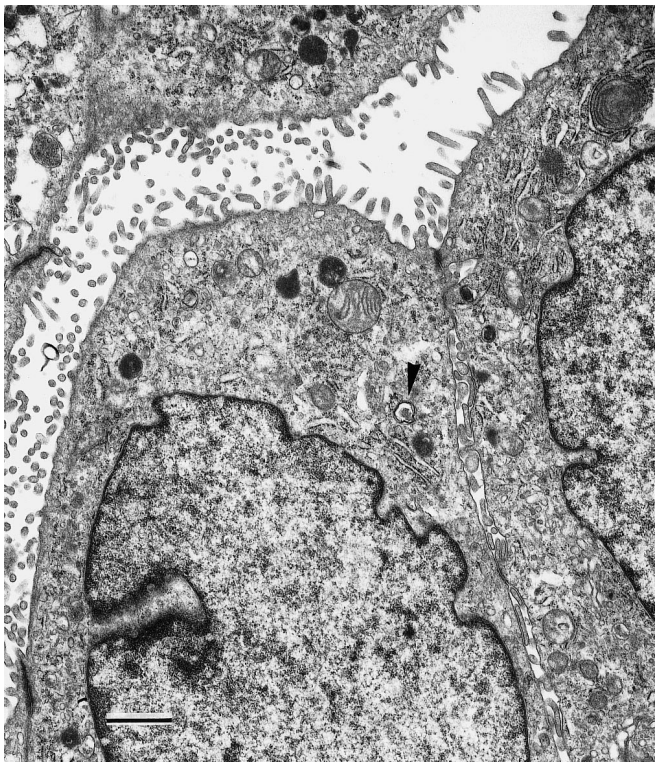

Fig. 5 A different portion of the same early BAC lesion as shown in Fig. 4 revealed tumour cells containing both membrane-bound dense granules and lamellar bodies (*arrowhead*). Note microvilli on the free surface of cells. *Bar* 1 μm

varying numbers of small lamellar bodies (200–500 nm; Fig. 6, inset, Fig. 7, inset), numerous microvesicles derived from rough endoplasmic reticulum, and many electron-dense small bodies (100–200 nm; Figs. 6–8). These features of AAH cells were considered to represent aberrant type 2 alveolar cells. The small dense bodies differed from lamellar bodies or Clara cell granules, and their nature remains unclear.

Lesions containing many cells with abundant lamellar inclusion bodies (+++) showed high proportions of cells positive for SAP (20–100%), while those lesions containing cells with less abundant lamellar inclusion bodies (–, ±, +, or ++) showed low proportions of cells positive for SAP (1–10%). Only the Clara cell type adenocarcinoma, in which many cells had numerous membrane-bound dense granules, exhibited positive immunoreactivity for UP1. Thus, in general, the immunoexpression of SAP or UP1 and the ultrastructural features of type 2 alveolar cell or Clara cell differentiation appeared to be correlated with each other.

Discussion

Although many studies have been conducted, the cell type of the progenitors of BAC has not been identified. Type 2 alveolar cells, Clara cells, or an as yet unidentified common progenitor cell for both these cell types have been suggested as likely candidates [24, 28]. While evidence has accumulated with regard to the differentiation phenotype of BAC cells, the differentiation pathway of BAC still seems to be controversial. For example, there have been some discrepancies and/or overlap be-

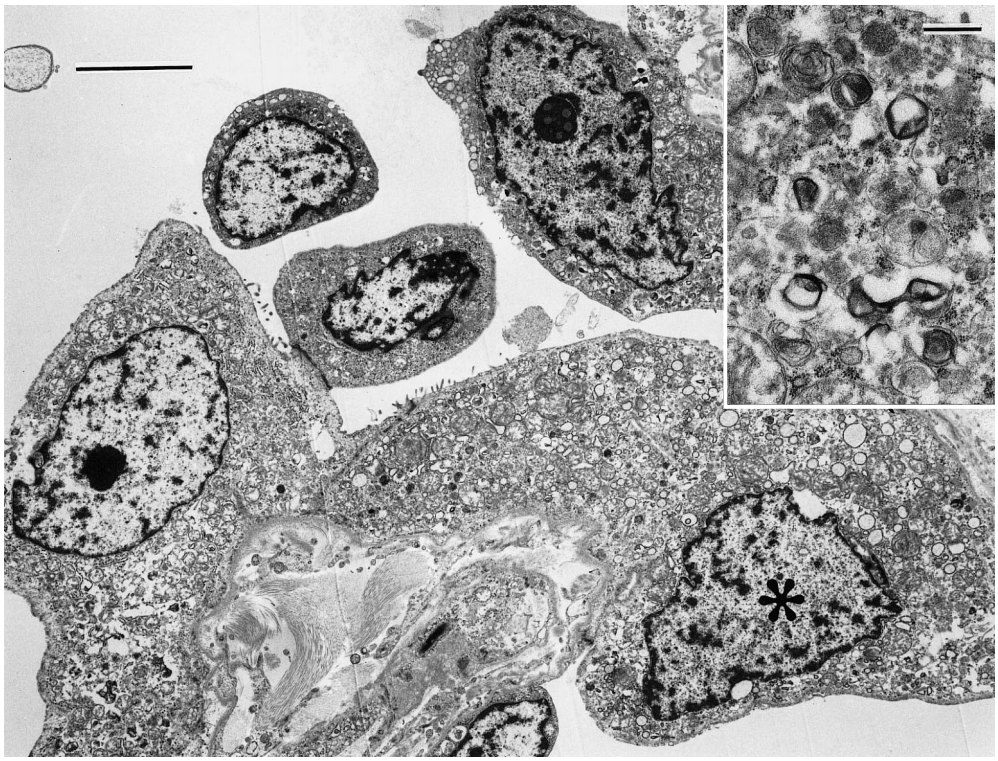

Fig. 6 Representative electron micrograph of high-grade AAH. Cuboid or polygonal cells had proliferated on the surface of alveolar septa. One cell was seen in a pore of Kohn (*asterisk*). *Bar* 5 μm. *Inset* A higher power view, showing small lamellar bodies in the cytoplasm of an AAH cell. *Bar* 0.5 μm

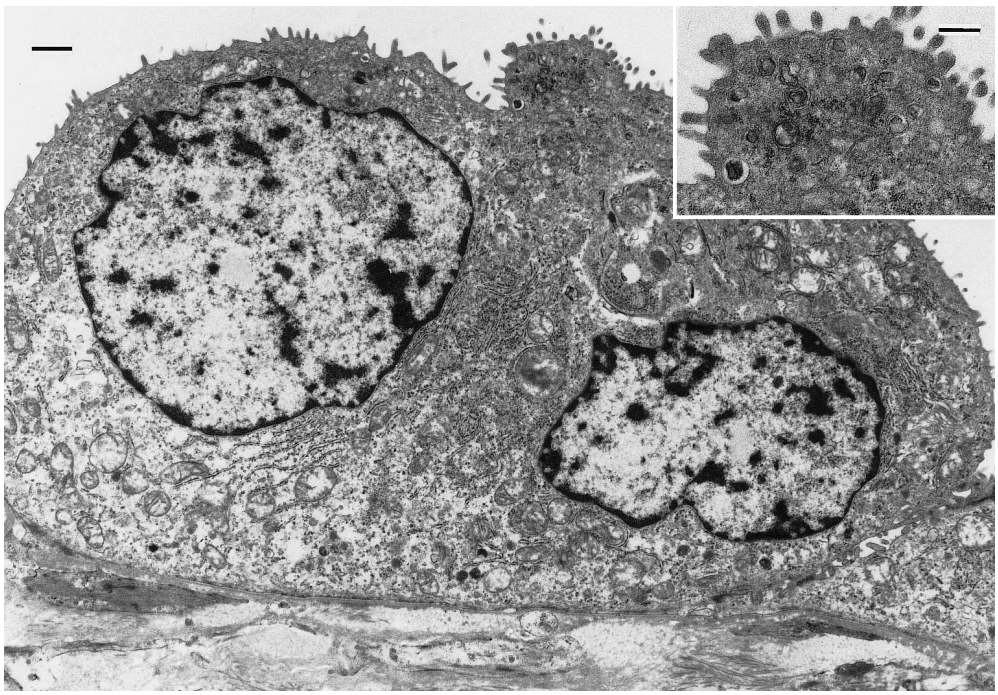

Fig. 7 A binucleated cell in high-grade AAH with surface microvilli, rather abundant rough endoplasmic reticulum, and round mitochondria. *Bar* 1 μm. *Inset* A higher power view, showing several small lamellar bodies in the apex of cytoplasm. *Bar* 0.5 μm

tween the phenotype of BAC cells determined by immunohistochemistry and that determined by electron microscopy. The results of electron microscopic studies of many lung adenocarcinomas [4, 11, 27] have suggested that a predominant pathway of differentiation of cells comprising BAC is towards Clara cells. However, SAP, a specific marker for type 2 alveolar cell differentiation, is detected immunohistochemically in the majority of lung adenocarcinomas, most of which are of a Clara cell type [18, 23, 24, 27]. These results, taken together, may reflect the heterogeneity of differentiation phenotype of cells within BAC lesions. In fact, both Clara cell gran-

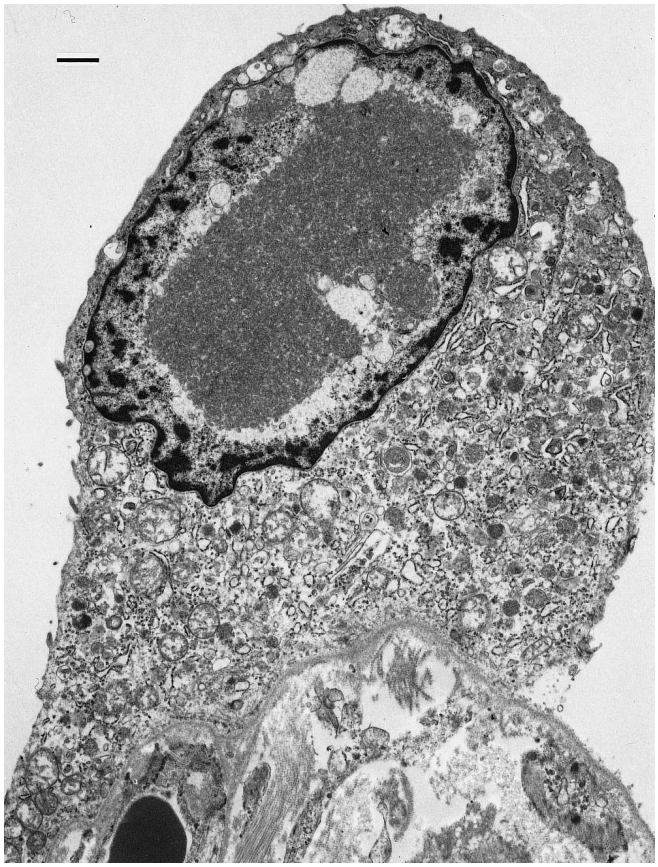

Fig. 8 A cell in high-grade AAH, with an apical nucleus and a nuclear inclusion consisting of branching microtubular structures. Note many small lamellar bodies, small dense granules, and abundant rough endoplasmic reticulum in the cytoplasm. *Bar* 1 μm

ules and lamellar inclusion bodies are often observed in a single tumour cell and/or different tumour cells within the same lesions of BAC [4, 6, 27, 28].

In the present study, we demonstrated that SAP was expressed in cells from all the lesions of AAH, early BAC, and overt BAC. Furthermore, SAP-positive nuclear inclusions were also seen in almost all the lesions studied from each category. These results indicate that cells comprising those lung lesions consistently exhibit a differentiation phenotype of type 2 alveolar cells in terms of immunoexpression of SAP. However, we also demonstrated that focal staining for SAP was predominant in overt BAC lesions, whereas diffuse staining was predominant in AAH lesions and the staining pattern among early BAC was intermediate between the two categories. The proportions of cells positive for SAP in overt and in early BAC were smaller than that in AAH. Similar results were previously reported by Kodama et al. [14]. It thus appears likely that as the grade of lung lesions advances cells expressing SAP becomes less frequent and their distribution becomes more heterogeneous.

A 10-kDa protein, designated as CC10, isolated from human lung lavage by Singh et al. [32] has been claimed to be a specific marker for Clara cells, since antibodies directed against this protein specifically and exclusively stained Clara cells in man, dogs, and cats. Singh et al. [32] reported that 10% of human lung adenocarcinomas were positive for CC10 immunohistochemically, and Linnoila et al. [16] found the expression of CC10 in approximately 30% or more of lung adenocarcinomas. In the present study, we used an antibody raised against UP1 as a potential marker for Clara cells. UP1 is an α-microprotein; it was originally discovered and isolated from the urine of patients with renal tubular disease (Product list, DAKO) and was subsequently revealed to be significantly homologous with rabbit uteroglobin [9] and CC10 [1, 2]. Immunohistochemical studies by Nomori et al. [24] using antibodies against CC10 and UP1 confirmed identical staining results in both normal lung cells and lung adenocarcinoma cells. Thus, UP1 expression seems to be a potentially useful marker for Clara cell differentiation. Nomori et al.[24] showed that only 7% of Clara cell type adenocarcinomas were positive for CC10 and for UP1. We found expression, albeit only focal, of UP1 in 70% of overt BAC lesions. In contrast, UP1 expression was absent in AAH lesions and rare, and weak if present, in early BAC lesions. Our results here thus suggest that cells in overt BAC lesions express the immunophenotype of Clara cells and/or nonciliated bronchial cells at a high frequency, but that such phenotypic expression is infrequent in early BAC cells and is lacking in AAH cells.

Mori et al. [19] classified premalignant and malignant lung lesions by morphometry and multivariate cluster analysis into AAH, Clara cell type adenocarcinomas, and type 2 cell type adenocarcinomas, concluding that AAH is an important lesion, corresponding to a step in carcinogenesis for both types of lung adenocarcinoma. More recently, they evaluated differentiation of the cells comprising these lesions by immunohistochemistry, using antibodies to SAP, UP1, and cytochrome P-450. They found no difference in immunoreactivity among these three categories [20]. The differences between their and our results may be attributable to the differences in staining technique, criteria for evaluating the results, and classification of lung lesions.

The results of the electron microscopic study were consistent with those of our immunohistochemical study, though we observed a coexistence of Clara cell granules and lamellar bodies not only in cells from BAC of both Clara cell type and alveolar type 2 cell type but also in cells from early BAC. We found type 2 alveolar cell differentiation in all the 3 high-grade AAH lesions and 2 of the 3 early BAC lesions, although they exhibited aberrant features of type 2 alveolar cells. In the remaining early BAC lesion, cells exhibited Clara cell differentiation. Immunohistochemically, these lesions were all positive for SAP at varied frequencies. Again, these results were mostly consistent with the ultrastructural findings. However, the early BAC lesion, which contained cells with dense granules showed no immunoreactivity for UP1. Thus, UP1 expression does not seem to be correlated exclusively with ultrastructural findings of Clara cell fea-

tures. It is likely that electron microscopic examination is more sensitive than the immunohistochemical analysis using the antibody to UP1 for identification of Clara cell differentiation.

We have shown that cells comprising AAH, early BAC, and overt BAC shared the common immunophenotype of type 2 alveolar cells, suggesting that these cells all belong to the type 2 alveolar cell lineage. Decreased frequencies of cells expressing SAP and increased heterogeneity of SAP expression were also found as the grade of lung lesions advanced. In parallel, cells showing Clara cell differentiation emerged and increased in frequency with the advance of lung lesions. This phenotypic change may reflect a kind of metaplasia that occurs during the development of BAC. Shimosato [27] commented that many adenocarcinomas less than 1.5 cm in size are composed of tumour cells of a single cell type, whereas larger tumours often consist of two or more cell types. He considered this phenomenon to be due to metaplastic changes in tumour cells, but although the mechanism for this phenotypic change is not clear, it may represent a genetic heterogeneity of the tumour cell population occurring during growth. It may also occur in association with fibrosis of tumour stroma, which progresses with the advance of lung lesions [28, 29], and may be mediated by interactions between extracellular matrix components, such as laminin and fibronectin and their cellular receptors the integrins [7]. Our results discussed here are not only consistent with, but also support, our proposal that AAH, early BAC, and overt BAC represent a continuous spectrum of development of BAC, though further studies are required to identify the precise progenitor cell of BAC.

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