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Bronchial carcinoid with paranuclear fibrillary inclusions related to cytokeratins and vimentin

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Summary. A bronchial carcinoid with globular intracytoplasmic inclusions is reported. The inclusions stain brown with Grimelius silver impregnation and some show distinct immunoreactivity for chromogranin A. Tumour cells stain positively with antisera to neuron specific enolase, chromogranin A and not with antisera against ACTH, somatostatin or S-100 protein. The cells show distinct immunoreactivity for cytokeratins and vimentin, which is particularly intense in the intracytoplasmic inclusions. Desmin and glial fibrillary acidic protein are absent. Ultrastructural analysis reveals that the inclusions are composed of aggregates of filaments of 8–10 nm of diameter, intrapping a few neurosecretory granules. Immunohistochemical and ultrastructural data support the hypothesis that the inclusions are composed of intermediate filaments, whose metabolism and synthesis have somehow been deranged.

Key words: Bronchial neoplasms – Carcinoid – Immunohistochemistry – Inclusions

Introduction

Intracytoplasmic fibrillary inclusions have been demonstrated in a variety of endocrine cells and tumours (Schochet et al. 1972; An 1978; Carstens and Broghamer 1978; Horwarth and Kovacs 1978; Roy 1978; Alvarez 1980; Warner and Sed 1980; Wilander et al. 1980; Fetissof et al. 1982; Berger et al. 1984; Wick and Scheithauer 1984; Blobel et al. 1985; Ironside et al. 1985; Leff et al. 1985; Hoefler et al. 1986; Christen et al. 1987).

These inclusions consist of tangles of filaments with a diameter of 8–10 nm, which are generally related to cytokeratins and neurofilaments (Berger et al. 1984; Neumann et al. 1985; Hoefler et al. 1986; Christen et al. 1987). Ultrastructural analysis generally reveals the presence of neurosecretory granules engulfed in the meshes of the fibrillary inclusions.

We report a case of bronchial carcinoid tumour, with prominent intracytoplasmic fibrillary inclusions immunoreactive for neuroendocrine markers and cytocheratin and vimentin.

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Material and methods

The surgically resected lung tumour was routinely formalin fixed and paraffin embedded. Sections were stained with haemtoxylineosin, Alcian blue-PAS, Mallory trichrome, Congo red and Grimelius silver impregnation and immunostained with antisera detailed in Table 1.

Table 1. Primary antisera and antibodies used in the present study

Antiserum/Antibody	Source and code
Rabbit anti-NSE	Immunonuclear Corp. 3AO22
Rabbit anti-porcine CG	Immunonuclear Corp. 63H2TP
Mouse PHE-5 antibody	Ortho Diagnostic Syst. 01102
Rabbit anti-somatostatin	DAKO A566
Rabbit anti-ACTH	DAKO A571
Rabbit anti-S100	DAKO Z311
Mouse anti-vimentin	DAKO M725
Mouse anti-cytocheratin:	
52.5 kd	Ortho Diagnostic Syst. 00730
56-56.5–58 kd	Ortho Diagnostic Syst. 00731
68 kd	Ortho Diagnostic Syst. 00732
Rabbit anti-desmin	BIO-YEDA 015084
Rabbit anti-human keratin	DAKO A575

Sections were immunostained using either the indirect peroxidase conjugated method or the unlabelled peroxidase-antiperoxidase technique (Sternberger 1979).

Sections of formalin fixed human adrenal gland were positive controls for neuron specific enolase (NSE) and Chromogranin A (CGA). Pancreatic sections with numerous Langerhans islets were used as positive controls for somatostatin immunoreactivity. Positive controls for ACTH were sections of an human pituitary basophil adenoma. For S-100 protein (S-100), vimentin, desmin, keratin and cytokeratins the tumour sections provided various built-in positive controls; nerve fibers entrapped in the neoplasm, endothelial cells, smooth muscle cells and epithelial cells of nearby bronchioles. Negative controls were obtained by omitting primary antisera/antibody.

A fragment of the paraffin embedded specimen was carefully dewaxed, rehydrated, postfixed in 1% osmium tetroxide, dehydrated and embedded in Epon. Ultrathin sections were prepared with an LKB ultrotome, contrasted with uranyl acetate-lead citrate and viewed with a Zeiss EM9 electron microscope.

Results

The tumor is a typical insular bronchial carcinoid, classifiable as neuroendocrine carcinoma of the carcinoid type according to Gould et al. (1983) and Mosca et al. (1986). It consists of solid sheets, nests and festoons of uniform cells, separated by variably thin fibrovascular septa. Each cell shows an eccentric nucleus of fairly regular shape, without atypia or mitosis.

Cytoplasm is relatively abundant, eosinophilic and sometimes fairly hyalin. Fine PAS-positive intracytoplasmic granules are often found.

Grimelius silver impregnation shows strong black staining of argyrophilic granules in numerous tumour cells and reveals a dark brown intracytoplasmic inclusion in most cells (Fig. 1). These inclusions are 1 to 4 μm in diameter, are frequently paranuclear and are generally surrounded by a clear halo. Cells containing these inclusions sometimes show argyrophilic granules in the surrounding cytoplasmic ring. The inclusions do not stain with PAS, Alcian blue, Mallory trichrome and Congo red techniques. In semithin toluidine-blue stained sections, most cells show a paranuclear hyaline inclusion.

Immunohistochemical analysis of the tumour reveals diffuse but variable staining for NSE. ACTH and somatostatin immunoreactivity is absent. Immunostaining with polyclonal antiserum against native porcine chromogranin (CG) and with monoclonal PHE-5 antibody against chromogranin A (CGA) (Riddel et al. 1987; Gown 1988; Whittaker and Sandusky 1988) reveal moderate to strong reactivity in numerous cells, some of them showing a distinct immunoreactive paranuclear inclusion (Fig. 2). S-100 immunostaining does not evidence any sustentacular S-100 positive cells (Hoefler and Auboeck 1984; El-Salhy et al. 1986; Barbareschi et al. 1987, 1988). The tumour cells do not react with polyclonal antiserum to whole keratin or with monoclonal antibody for cytokeratins of 56, 56,5 and 58 kd molecular weight. Strong immunoreactivity is observed with monoclonal antibody against cytokeratin of 52.5 kd, which clearly decorates the paranuclear inclusions (Fig. 3). In some areas the inclusions strongly react with monoclonal antibody against vimentin (Fig. 4). The distribution of the areas of cells with vimentin-positive inclusions is quite irregular and does not overlap with the more ubiquitous distribution of cells with cytokeratin-positive inclusions. No immunostaining is observed with antisera against other intermediate filaments (GFAP and desmin).

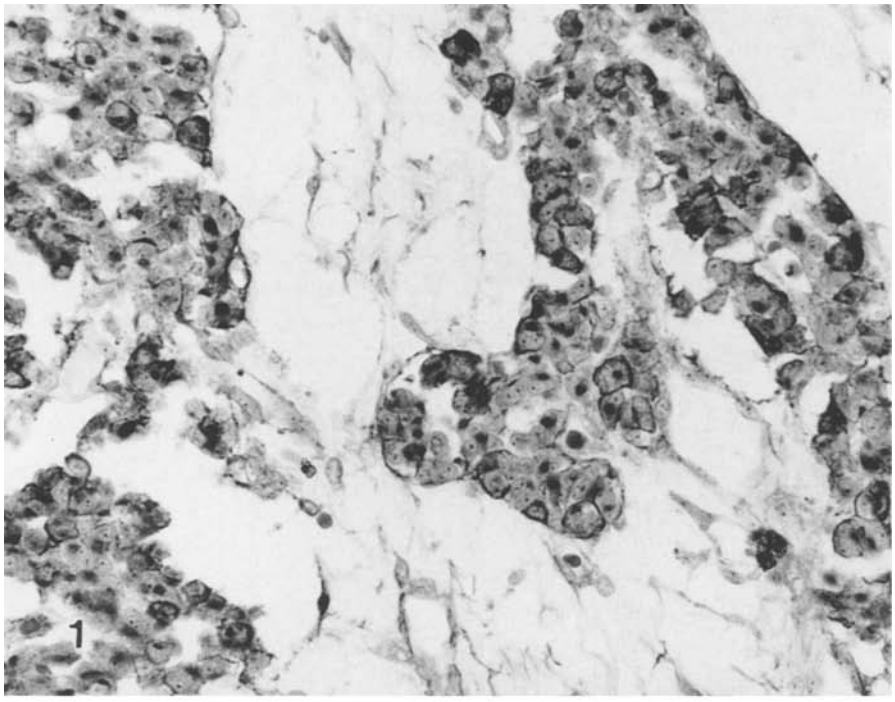
Ultrastructural morphology is quite poor due to retrieval of tissue samples from paraffin embedded specimens. However most cells reveal electron-dense round granules with a diameter of 100 to 150 nm, interpretable as neurosecretory granules. The cytoplasm of most cells contains large, well demarcated, spherical or elliptical aggregates of filaments, which are in the proximity of the nucleus and lack a limiting membrane (Fig. 5). The filaments are haphazardly arranged and form meshes, sometimes entrapping some endocrine granules. Filaments are not branching nor showing any periodicity.

Discussion

The present case of bronchial carcinoid is remarkable, since most tumour cells contain spherical intracytoplasmic granulofilamentous inclusions in the paranuclear area. Reported carcinoids containing such inclusions are few: the most typical are bronchial (An 1978; Alvarez 1980; Berger et al. 1984; Hoefler et al. 1986; Christen et al. 1987), thymic (Fetissof et al. 1982; Wick and Scheithauer 1984) and intestinal (Carstens and Broghamer 1978; Hoefler et al. 1986). Similar inclusions are described in other endocrine tumours and in some non-endocrine tumours.

The filaments are generally described as non-periodic, non branching, with a diameter of 8–10 nm, as found in the present case. Depending on these morphological characteristics and on the reported cytokeratin and neurofilaments immunoreactivity (Berger et al. 1984; Leff et al. 1985; Neumann et al. 1985; Hoefler et al. 1986; Christen et al. 1987), the filaments are generally interpreted as intermediate filaments.

In our case, the granulofilamentous inclusions show a peculiar immunoreactivity, not previously



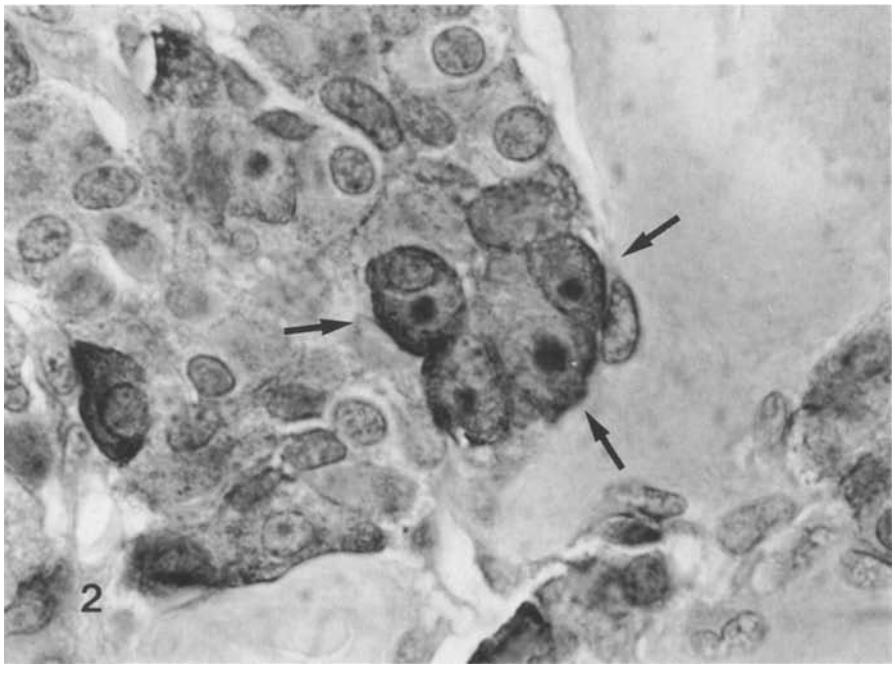


Fig. 1. Grimelius silver impregnation reveals distinct argyrophilia in most cells and dark brown paranuclear inclusions in many cells. Argyrophilic granules are generally confined to a peripheral cytoplasmic ring. Grimelius silver impregnation, 250 ×

Fig. 2. Immunostaining with monoclonal antibodies for CGA, shows a very similar pattern to that of Grimelius impregnation. Intensity of staining is variable and positivity is frequently confined to a peripheral cytoplasmic ring. Some cells show a clearly immunoreactive paranuclear inclusion (*arrows*). Immunoperoxidase stain for CGA; (**a**) 250 ×, (**b**) 1000 ×

described. Some of the inclusions are immunoreactive for CGA, marker of neurosecretory granules, almost all stain for cytokeratins of 52.5 kd molecular weight and a number of them are also reactive for vimentin. The pattern of distribution of vimentin-positive cells is different from that of cytokeratin-positive cells. This suggests that vimentin immunoreactivity is not due to cross-reactivity of antivimentin antibody with cytokeratins. Moreover no vimentin staining is appreciable in bron-

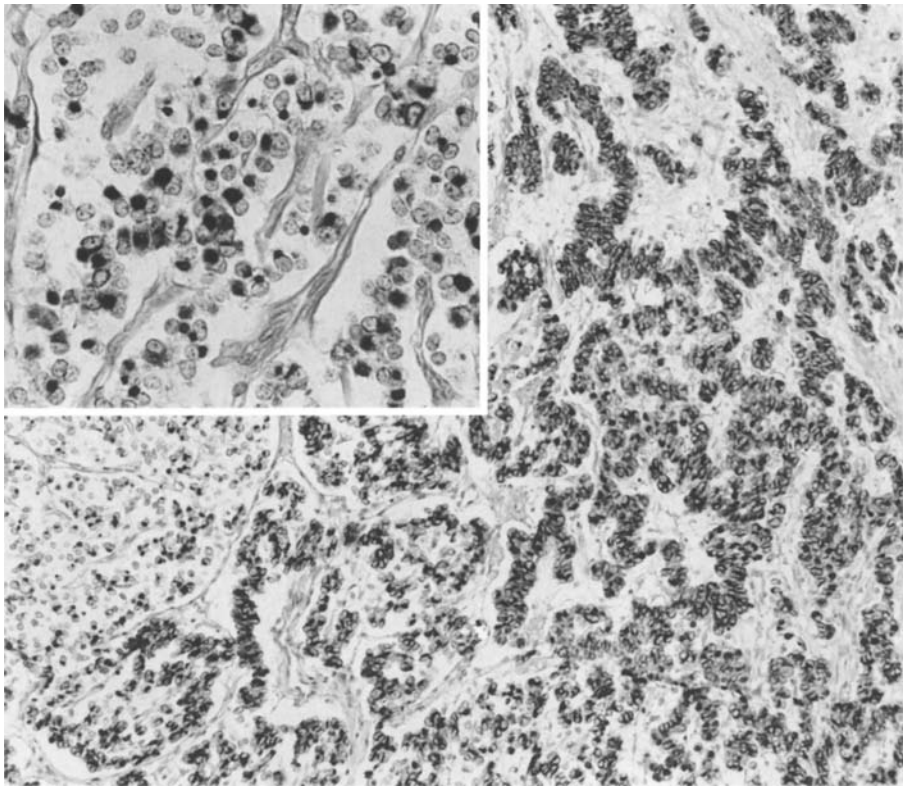


Fig. 3. Cytokeratin immunoreactivity is variable: some cells are diffusely stained, whereas other show only a paranuclear dot like reactivity (*insert*). Immunoperoxidase stain for cytokeratin (**a**) 100 × ; (**b**) 400 ×

chial epithelial cells, which are instead immunoreactive for cytokeratin polypeptides.

Our data are in keeping whith the hypothesis that the inclusions are composed of aggregates of intermediate filaments and neurosecretory granules. The contemporaneous presence of cytokeratin and vimentin immunoreactivity suggests a derangement of cytoskeletal function and synthesis.

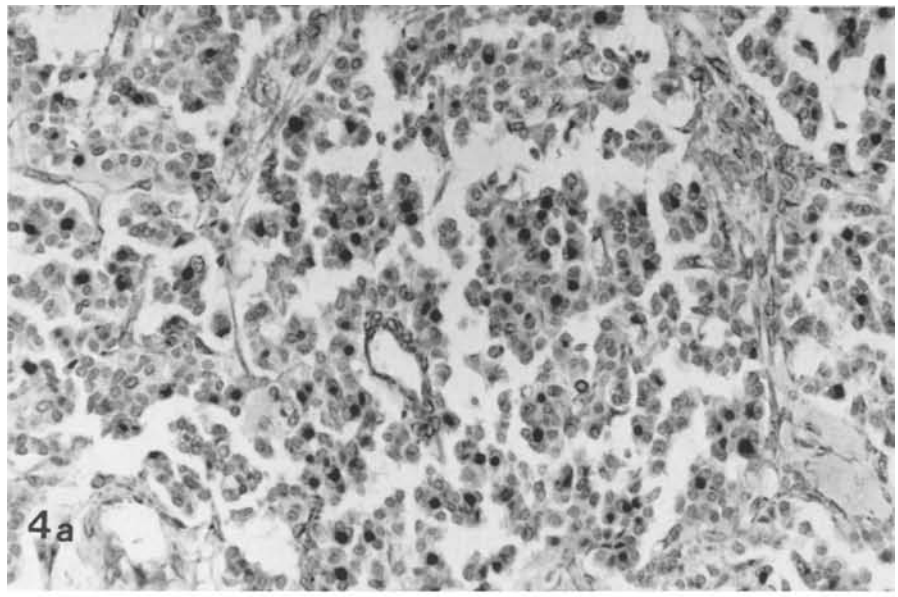
Data from literature support the hypothesis that the inclusions are due to deranged function and synthesis of intermediate filaments, microtubular disfunction and/or an altered excretory process. It has been shown that the inclusions may disappear in cultured cells in vitro (Alvarez 1980) or that they may otherwise be formed in cultured cells after colchicine-induced rupture of microtubles (Goldman et al. 1980). Fibrillary inclusions may be induced in APUD cells of hamsters' lungs after diethylnitrosamine treatment (Reznik-Schuller 1977) and may be induced by chemotherapy (Sajjad and Mackay 1982).

Recently Christen et al. (1987) demonstrated that intermediate filament aggregates of lung carcinoids are related to neurofilaments with abnormal preponderance of phosphorylated epitopes as compared with non-phosphorylated forms. In this view the abnormal phosphorylation status should be implicated in the formation of the filament aggregates.

The present case is the first reported lung carcinoid with double expression of cytokeratin and vimentin related to paranuclear fibrillary inclusions. Co-expression of cytokeratins and vimentin has been reported for lung adenocarcinomas and large cell carcinomas (Jasani et al. 1985; Upton et al. 1986; Azumi and Battifora 1987) and is very rare in small cell carcinomas (Lehto et al. 1983; Azumi and Battifora 1987; Leader et al. 1987). In a series of 35 lung neuroendocrine carcinomas (carcinoids, atypical carcinoids and small cell carcinomas) we found only one case of small cell carcinoma of the intermediate type with vimentin expression

Fig. 4. Vimentin immunostaining shows strong reacting paranuclear round areas whereas the remaining cytoplasmic is unreactive. Endothelial cells are also stained (*large arrows*). Immunoperoxidase stain for vimentin; (**a**) 250 × , (**b**) 400 ×

Fig. 5. Ultrastructural analysis reveals the structure of the paranuclear inclusions, which are composed of aggregates of haphazardly arranged filaments. Original magnification: 7500 ×



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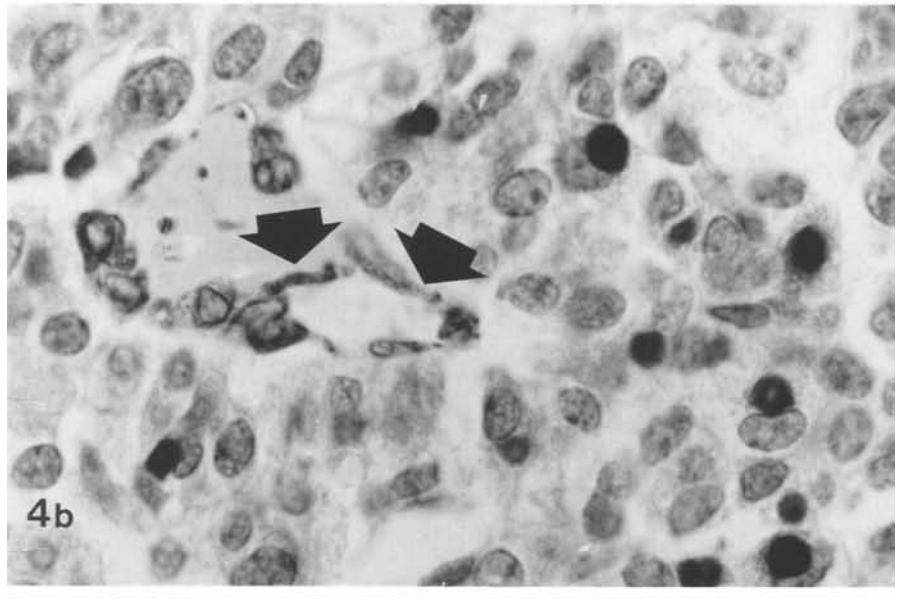

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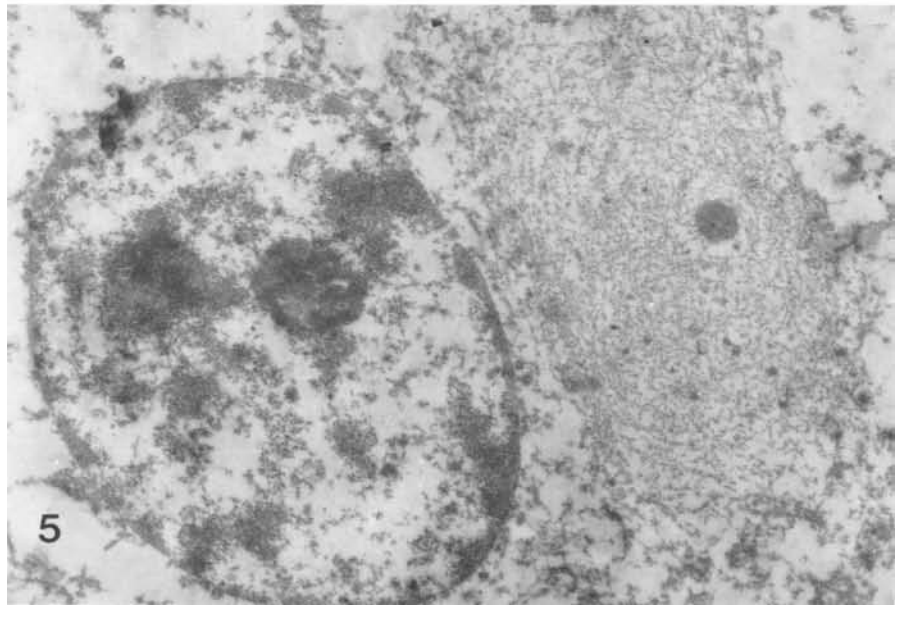

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(unpublished results). In that case the immunoreactivity was focal and stronger in the basal portions of the tumour cells, arranged in pseudorosettes around small blood vessels. The significance of these findings are obscure, but they may be related to the derangements of intermediate filament synthesis described above. Further studies are required to evaluate vimentin expression by neuroendocrine tumours, in view of their possible double expression of cytokeratins and neurofilaments (Lehto et al. 1985).

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