Virchows Arch. A Path. Anat. and Histol. 377, 203–210 (1978)

Virchows Archiv A

Demonstration of Argyrophil Granules in Small Cell Carcinoma of the Lung

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Summary. The Grimelius silver nitrate stain has enabled us to demonstrate the presence of tumor cells with argyrophil granules (argyrophil cells) in small cell carcinoma of the lung. Of the 22 tumors, 11 showed varying numbers of argyrophil cells. The occurrence of the cells differed in frequency among the subtypes of small cell carcinoma. The fusiform cell type showed the cells more frequently than the other types. Both tumors with numerous argyrophil cells belonged to the fusiform cell type.

The number of positive cells seen under the light microscope did not correlate with the number of cells containing neurosecretory granules under the electron microscope, nor with the amount of either ACTH or serotonin in the tumor extracts.

The demonstration of these cells in a pulmonary carcinoma may be of help in making correct histological diagnosis.

Key words: Lung carcinoma – Small cell carcinoma – Argyrophil cells – Feyrter's paracrine cells.

Introduction

It has been recognised that bronchial carcinoids and small cell carcinomas of the lung arize from the Feyrter's paracrine (Kulchitzky-type) cells distributed throughout the bronchial tree. The paracrine cells are argyrophilic (Feyrter, 1954; Lauweryns et al., 1970; Tateishi, 1973), and contain neurosecretory granules (Bensch et al., 1965a; Tarzakis et al., 1972), and these granules and those found in bronchial carcinoids give a positive argyrophil reaction. Although small cell carcinomas of the lung possess neurosecretory granules

Supported in part by a Grant-in-Aid for Cancer Research from the Ministry of Education, Science and Culture, and the Ministry of Health and Welfare, Japan

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0340-1227/78/0377/0203/$01.60

identical to those found in the paracrine cells and bronchial carcinoids (Bensch et al., 1965b; Bensch et al., 1968; Hattori et al., 1968), it is uncertain whether or not they give a positive argyrophil reaction.

The purpose of the present study is to answer this question on small cell carcinomas of the lungs from 22 patients.

Materials and Methods

Twenty-two autopsied cases of small cell carcinomas of the lung and three cases of bronchial carcinoids were used for the present study. The 22 tumors were divided into the subtypes of the WHO's classification of lung tumors. Of the 22 neoplasmas, 4 belonged to the lymphocyte-like cell type, 5 to the fusiform cell type, 6 to the polygonal cell type, and 7 to the mixed cell type. In each case, three or more blocks from the primary and metastatic lesions were available for examination. The blocks were fixed in formalin and embedded in paraffin. Paraffin embedded sections were stained with hematoxylin and eosin, the Grimelius (1968) silver nitrate (argyrophil reaction) and the Fontana-Masson ammoniacal silver (argentaffin).

The following modification of the Grimelius method is recommended and was used in this study. 1. Place sections in a freshly prepared 0.03% silver nitrate solution (acetate buffer, pH 5.6), and leave for 40 hours at 42° C, 2. Place for 5 min in a freshly prepared reducing solution in room temperature, 3. Wash well in distilled water, dehydrate and mount in resin.

Since the above procedures stain the Feyrter's paracine cells and the axis cylinders of peripheral nerve fibers dark brown or black, these can be used as positive controls in a given slide. When they stain weakly or fail to stain, it is necessary to repeat the staining, as described in the original paper (Grimelius, 1968).

The number of argyrophil cells was recorded as 0 (absent), as 1+ (a small number of the cells scattered sparsely or occurring in small clusters), as 2+ (a moderate number of cells occurring in clusters), and as 3+ (over half of the tumor cells were argyrophilic).

The number of neurosecretory granules seen under electron microscope was recorded arbitrarily as 1+ (small numbers), as 2+ (moderate numbers), and as 3+ (large numbers). Methods for the assay of ACTH and serotonin, and those for the preparation of ultrathin sections for electron microscope were described in previous papers (Hattori et al., 1972; Horai et al., 1973).

Results

Of the 22 tumors, 11 showed varying numbers of tumor cells with argyrophil granules. The occurrence of argyrophil cells differed in frequency among the four subtypes. The fusiform cell type showed argyrophil cells more frequently and in larger number than the other three types and both tumors with numerous argyrophil cells belonged to this group (Figs. 1 and 2). Argyrophil cells occurred least frequently in the lymphocyte-like cell type, no argyrophil cells were detected in 3 of the 4 tumors composed of lymphocyte-like cells. In any given tumor, sections from the primary and metastatic lesions had a similar tendency to show argyrophil cells.

Argyrophil granules in the tumor cells of small cell carcinoma were frequently found to concentrate in the pseudopod-like, cytoplasmic processes, in the apical cytoplasm of the cells forming rosettes, or towards the peripheral cytoplasm which attached to the basement membrane (Figs. 1–3).

All tumor cells of the 3 carcinoids contained a large number of argyrophil granules distributed throughout the cytoplasm. One of these 3 tumors showed

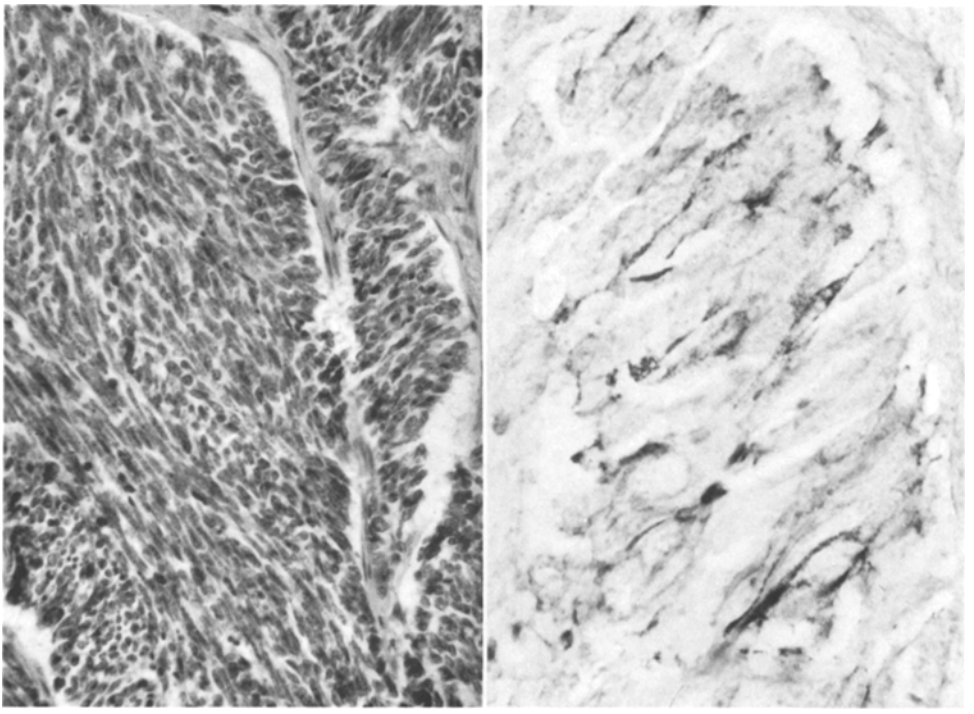

Fig. 1. Fusiform cell type (Case 22). *Left:* H.E stain, ×700. *Right:* Argyrophil granules concentrate in the long, cytoplasmic processes. Grimelius stain, ×1400

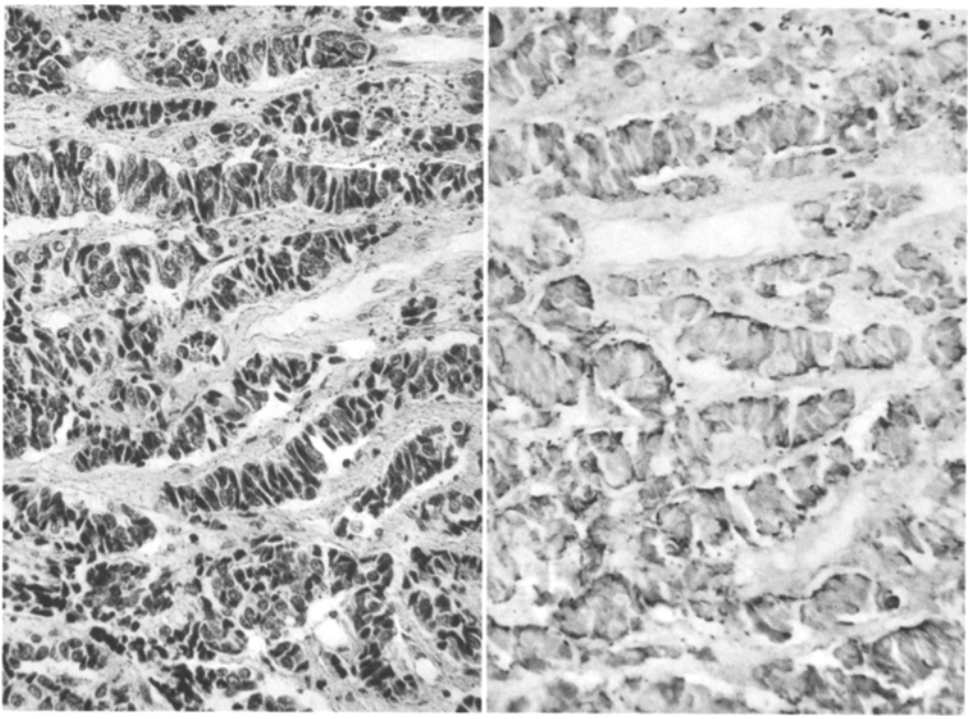

Fig. 2. Fusiform cells are arranged in ribbons (Case 12). *Left:* H.E stain, ×700. *Right:* Argyrophil granules concentrate towards the basement membrane. Grimelius stain, ×700

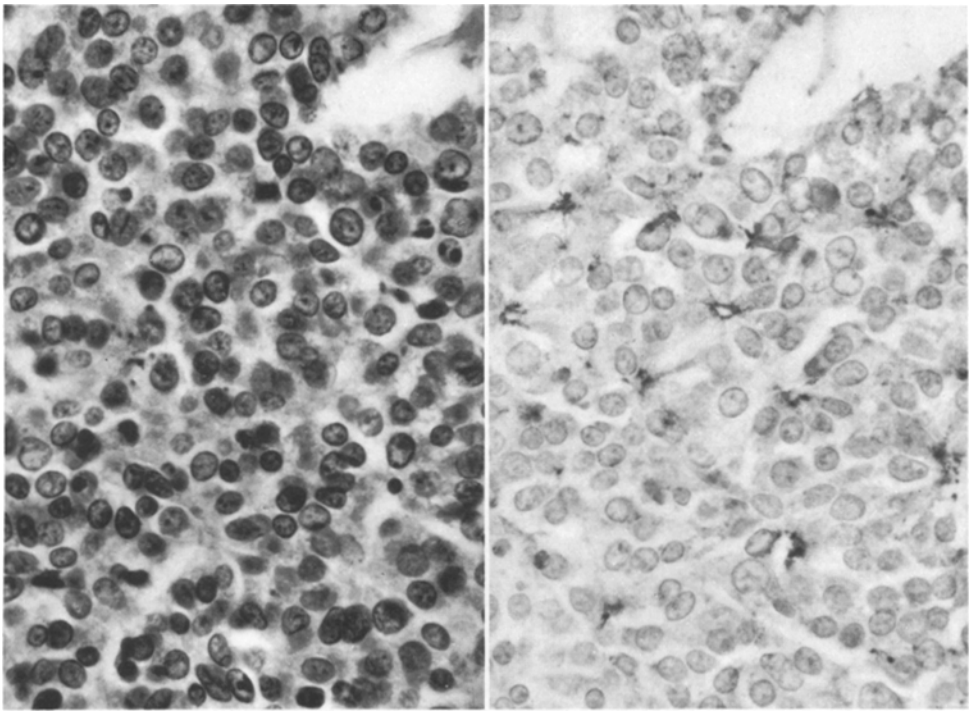

Fig. 3. Polygonal cell type (Case 7). *Left:* H.E stain, ×1400. *Right:* Grimelius stain unmasks rosettes which are indistinct in the photograph on the left. The granules concentrate in the apical cytoplasm of the cells forming rosettes, ×1400

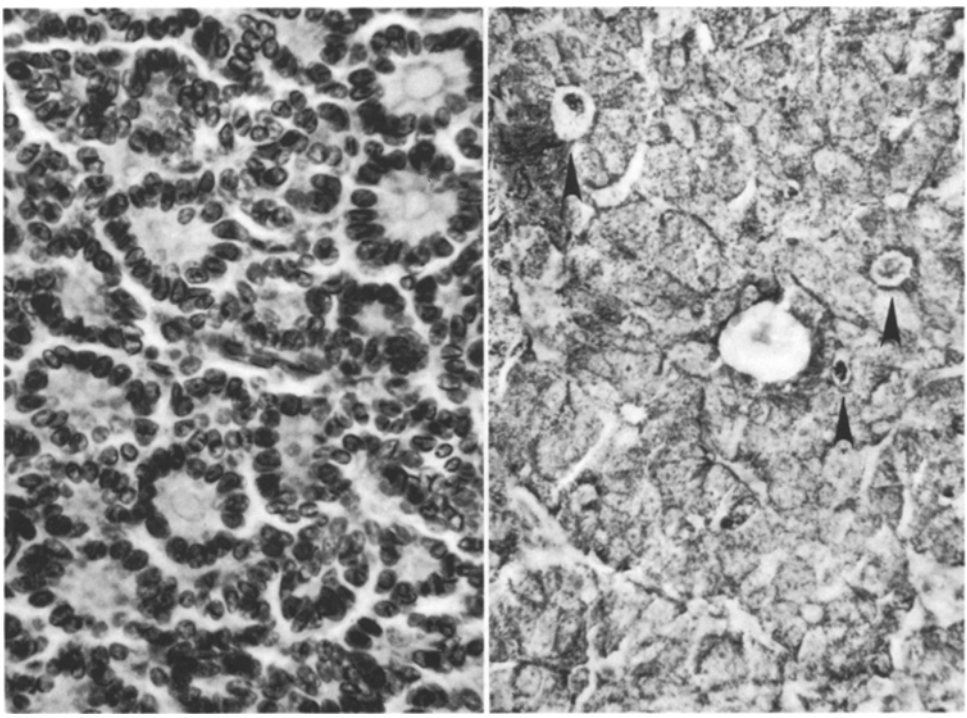

Fig. 4. Prominent rosette formation in a bronchial carcinoid. *Left:* H.E stain, ×700. *Right:* Grimelius stain reveals the granules to be more heavily concentrated in the apical cytoplasm of the cells forming rosettes, and even in the intraluminal spaces (*arrows*), ×700

Table 1. The number of argyrophil cells in relation to the cell types, number of neurosecretory granules, and amounts of ACTH or serotonin

Case no.	Age and Sex	Cell type	Number of argyrophil cell	Number of NSG	Tissue serotonin (μg/g.w.w.)	Tissue ACTH	
						Bioassay (mU/g.w.w.)	Radioimmunoassay (ng/g.w.w.)
1. KN	50 m	L	0	1+			
2. OC	65 m	F	2+	2+			
3. OD	57 m	P	0	2+	NS	0	0
4. IU	56 m	F	0	3+	29.6		
5. OY	46 m	P & F	2+	3+	3.1		
6. OM	62 m	P & F	2+	2+	NS	3.2	30.0
7. OG	53 m	P	2+	3+	7.4	0.02	28.0
8. IN	54 m	L & F	0	2+	8.81	0.14	5.0
9. SS	59 m	P	0	2+	1.42	1.60	6.5
10. KY	46 m	P & L	0	1+	1.33	1.35	5.0
11. MY	68 m	P	0	2+	5.2		
12. YZ	49 m	F	3+	3+	8.0	0	0
13. IR	63 m	P & F	2+			0.16	26.8
14. HR	51 f	P	1+				6.4
15. IG	59 m	F	1+			0.12	
16. OK	50 m	P & F	0				0.64
17. GT	69 m	L & P	0				2.1
18. NG	62 f	L	1+				0.30
19. OS	45 m	P	2+				8.0
20. BU	51 m	L	0				
21. NN	71 m	L	0				
22. AO	55 m	F	3+				

NSG = Neurosecretory granules, F = Fusiform, P = Polygonal, L = Lymphocyte-like, NS = Not significant amount

argyrophil granules which were concentrated in the apical cytoplasm of the cells forming rosettes. Granules were even observed in the intraluminal spaces of these rosettes (Fig. 4). There were no argentaffin-positive cells in the 3 bronchial carcinoids nor in the 22 small cell carcinomas. Table 1 summarizes the cell type, the number of argyrophil cells, the number of neurosecretory granules, and the assay data in all 22 cases of small cell carcinoma.

The number of neurosecretory granules seen by electron microscopy did not show a correlation with the number of argyrophil cells under the light microscope. In cases 3, 4, 8 and 9, no argyrophil cells were detected, though a sufficient number of neurosecretory granules were found to concentrate in the cytoplasm under the electron microscope (Fig. 5).

No correlation was observed between the number of argyrophil cells and the amount of either ACTH or serotonin in the tumor extracts.

Discussion

The Feyrter's paracine cells of the bronchus possess the cytochemical and ultrastructural characteristics of the APUD cell series of Pearse (Bensch et al., 1965a; Pearse, 1969; Hage, 1972; Tarzakis et al., 1972). They can be visualized

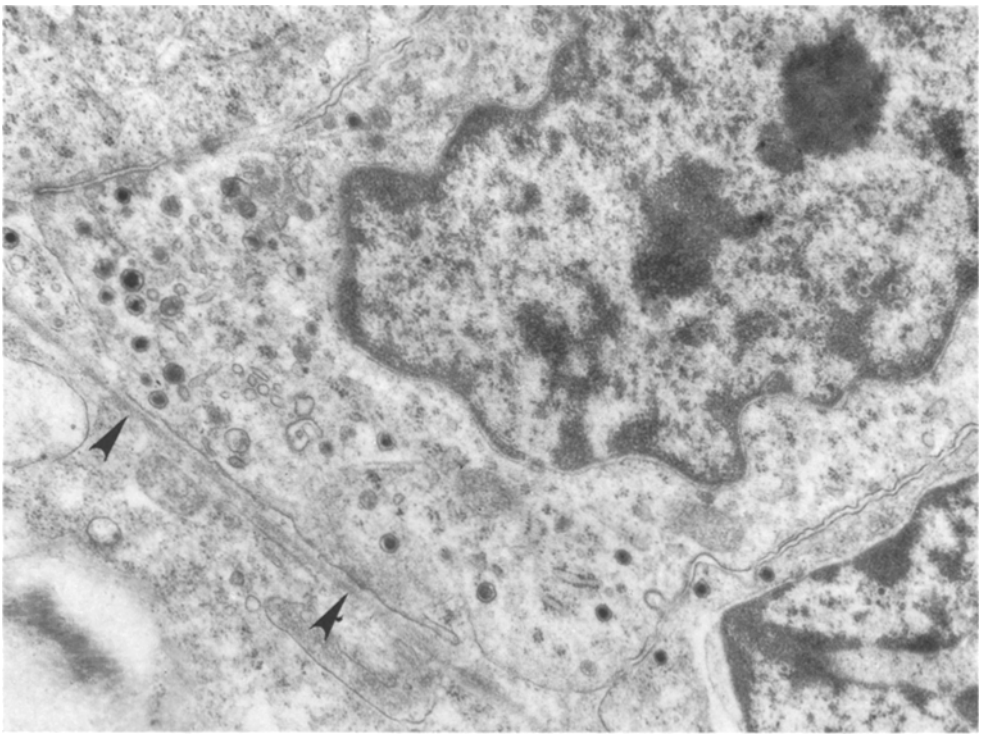

Fig. 5. Electron micrograph of Case 4. Neurosecretory granules concentrate towards the peripheral cytoplasm adjacent to the basement membrane (*arrows*), ×21,000

by argyrophil silver impregnation methods and are characterized light microscopically by the presence of pseudopod-like, cytoplasmic processes that extend over a considerable distance into the intercellular spaces among other epithelial cells. The processes usually reach the surface of the bronchial lumina (Tateishi, 1973). Feyrter has suggested that the paracine cells secrete a humoral substance that acts on neighboring cells (paracrine function).

The morphologic characteristics of the paracrine cells seem to be manifested in bronchial carcinoids and small cell carcinomas of the lung. The present study has demonstrated the presence of long, cytoplasmic processes that contain a large number of argyrophil granules, and extend for a considerable distance among other tumor cells. Similar observations have been described in electron microscopic studies on both bronchial carcinoids and small cell carcinomas of the lung (Bensch et al., 1965b; Bensch et al., 1968; Hattori et al., 1972).

The concentration of argyrophil granules towards the apical cytoplasm of the cells forming the rosettes, and the recognition of the granules in the intraluminal spaces of the rosettes may be regarded as a morphological indicator of the paracrine function inherent in Feyrter's paracrine cells.

Argyrophilia is dependent on some chemical substance stored in neurosecretory granules, and the recognition of argyrophil cells under light microscope

thus reflects the presence of neurosecretory granules visible by electron microscopy. Failure to demonstrate argyrophil cells, however, does not imply the absence of neurosecretory granules, since the demonstration of these cells depends mainly on a sufficient concentration of granules. In cases 1, 10 and 18, the number of granules appeared to be too small to be visualized by light microscopy. In cases 3, 4, 8, 9, and 11, though sufficient number of neurosecretory granules were concentrated in the cytoplasm, we failed to demonstrate argyrophil cells. It seems likely that this failure could be attributed to a relative difference in the chemical nature of neurosecretory granules in different tumors.

Argyrophilia is a common cytochemical characteristic shared by a number of the APUD cells producing low molecular weight polypeptide hormones. In the present series, the number of argyrophil cells bore no relation to the amount of either ACTH or serotonin in the tumor extracts. This discrepancy may depend on the production of multiple hormones, not all of the cells identified would necessarily produce ACTH or serotonin. Tumors arizing from the cells of APUD series (apudomas) are associated with multiple hormone production. Multiple peptide hormone production has been well documented in pancreatic islet-cell tumors (Larsson et al., 1975; Bordi and Bussolati, 1974; Polak et al., 1976), thyroid medullary carcinomas (Melvin et al., 1970; Bussolati et al., 1973), bronchial carcinoids and small cell carcinoma of the lung (Unger et al., 1964; O'Neal et al., 1968). It is conceivable that small cell carcinomas of the lung are capable of producing several peptide hormones with or without ACTH.

Although the normal function of Feyrter's paracine cells is still undetermined, it is suggested that they are likely to secrete a vasoactive peptide hormone which Pearse has tentatively called "pneumokinin" (Pearse, 1969). In this regard, biochemical, radioimmunological and immunofluorescence studies on small cell carcinomas, a not uncommon neoplasm of the lung, may help to throw light on the normal function of the paracrine cells.

The differential diagnosis between poorly differentiated epidermoid carcinoma and small cell carcinoma composed of fusiform cells and/or polygonal cells, may be difficult, especially if made from biopsy material. In differential diagnosis, the demonstration of argyrophil cells in lung carcinomas may help to establish correct histological diagnosis.

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Received October 3, 1977