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Heterogeneity in a colonic carcinoid tumor *

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Summary. This report describes a colonic carcinoid tumor in which three, and possibly four, distinct cell types are distinguishable on the basis of their ultrastructure and granule morphology. These cell types closely resemble the normal endocrine cells of the large bowel, both in appearances and in relative frequency. The mixed composition of this tumor may have arisen either by parallel differentiation of distinct cell types, or by sequential maturation of one cell type.

Key Words: Heterogeneity – Ultrastructure – Colonic carcinoid

Introduction

The carcinoid tumors of the gut form a mixed group of neoplasms derived from neoplastic transformation of the endocrine cell of the mucosal crypt epithelium. Consequently these tumors are composed of cells characterised by cytoplasmic, electron dense secretory granules, containing a peptide hormone product. This feature may be demonstrated by electron microscopy and immunohistochemistry.

Until recently, the type of granule and product has been described to be homogeneous throughout individual carcinoid tumors of the hindgut (Black 1968), with a granule morphology that is related to the embryological origin of the region of the bowel from which the tumor arises (Williams and Sandler 1963). There is now increasing evidence that this is not always so (DeLellis et al. 1984).

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This paper describes a case of a colonic carcinoid tumor which is composed of a mixed population of cells and secretory granules. Such ultrastructural heterogeneity within one hindgut tumor has only been described twice previously (Williams 1979; Yoshida et al. 1981).

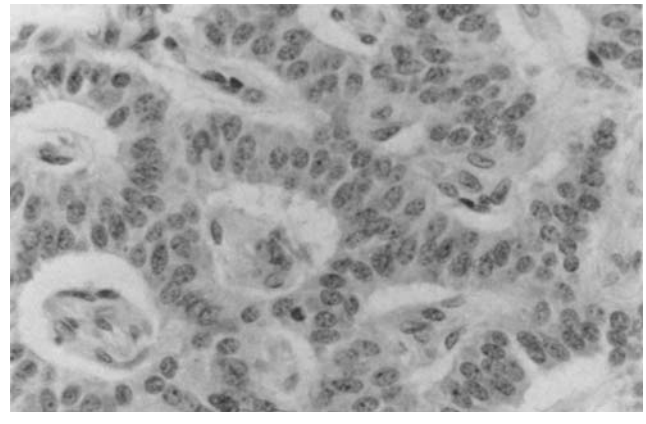


Fig. 1. Carcinoid tumor with a trabecular growth pattern. (×480)

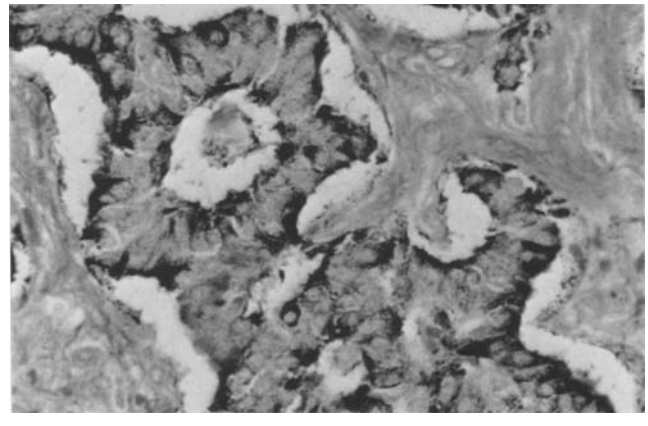
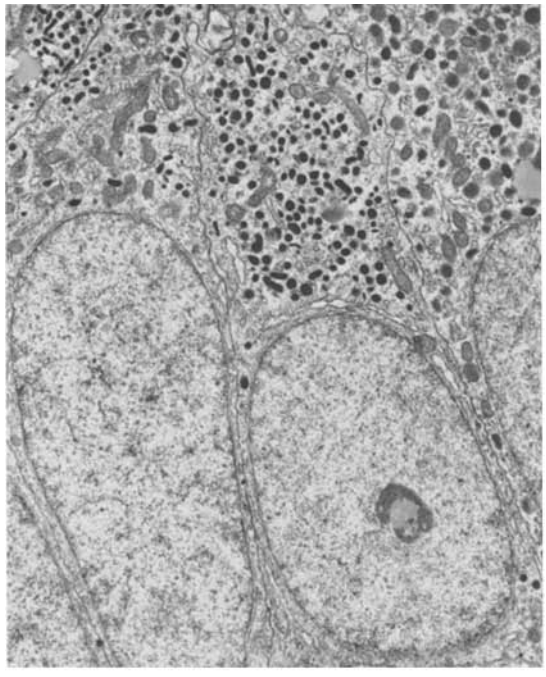


Fig. 2. Argyrophil positive granules in the peripheral cytoplasm of the tumor cells. (×480)



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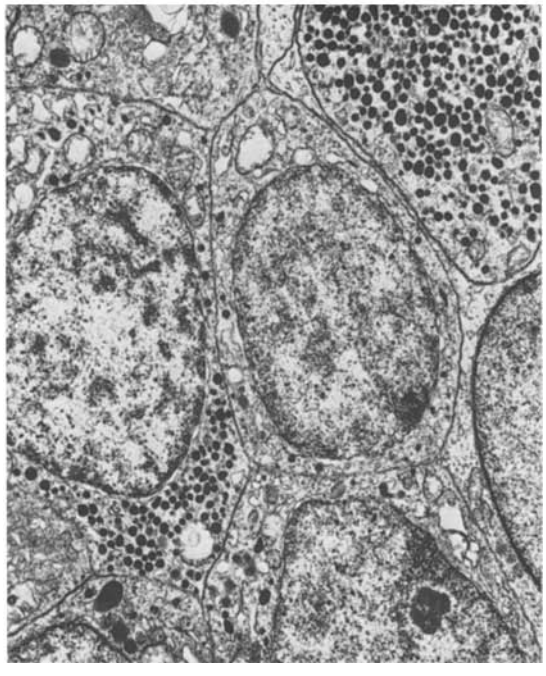

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Fig. 3. Group 1 cells (× 6,500)

Fig. 4. Group 2 cells (× 6,500)

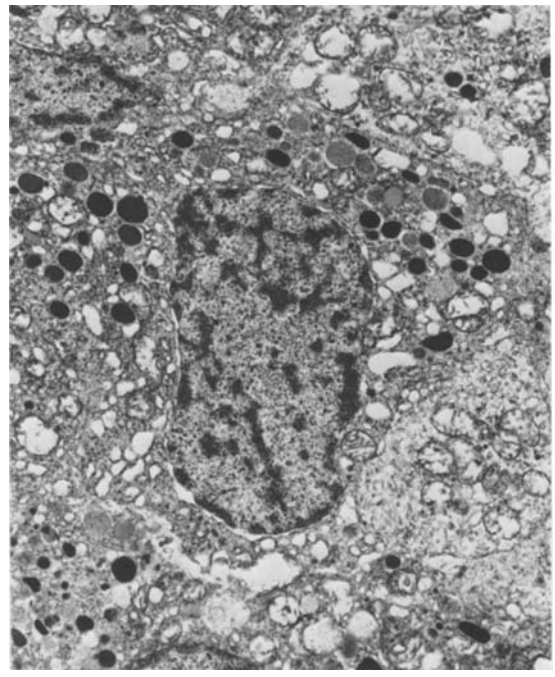

Fig. 5. Group 3 cells (× 6,500)

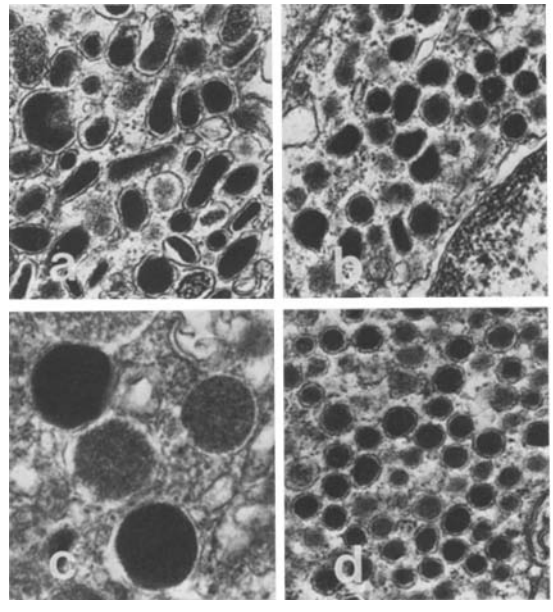

Fig. 6a–d. Characteristic membrane bound and electron dense granules associated with the cells of; group 1 (**a**), group 2 (**b**), group 3 (**c**), group 4 (**d**) (× 24,000)

Report

A 55 year old male caucasian presented with a four day history of acute rectal bleeding. Sigmoidoscopy revealed a small polypoid lesion at 17 cms from the anal margin, which was biopsied. The diagnosis of carcinoid tumor was made, and an anterior resection performed.

Grossly the tumor measured $1.2 \times 1.2 \times 0.7$ cms, and its smooth hemispherical contour protruded into the lumen of the bowel. On sectioning the tumor had a yellow/tan surface, and was confined to the mucosa and submucosa of the bowel wall. Microscopic examination of the lesion demonstrated regular columnar cells, arranged predominantly in a trabecular pattern separated by a thick collagenous stroma (Fig. 1). Perineural lymphatics in the submucosa were invaded, but there was no evidence of metastasis to the regional lymph nodes.

Table 1. Granule diameters of the four tumor cell types

Cell type	No. of cells	No. of granules	Range of mean diameter (±S.D.) per cell in nm
EC	7	456	185 (±44)–391 (±149)
F	7	825	164 (±40)–186 (±40)
L	6	55	356 (±92)–483 (±11)
H	1	147	149 (±24)

Table 2. Major endocrine cell types in the sigmoid colon – a summary from the literature

Cell type	EC	F	L	H
Frequency	++++	+++	++	+
Hormone	serotonin	pancreatic polypeptide	glucagon like	vasoactive intestinal polypeptide
Granules				
Diameter (nm)	200–400	200–320	195–400	145–205

The cells were uniformly strongly argyrophilic, as demonstrated with the Pascual stain (Fig. 2), and argentaffin negative. Immunoperoxidase stains for serotonin and neuron specific enolase were positive (in approximately 50% and 100% of the cells respectively), but stains for glucagon, pancreatic polypeptide, vasoactive intestinal polypeptide, somatostatin, insulin, and gastrin were all negative. This result was obtained by both PAP and Avidin-Biotin indirect labelling.

Electron microscopy was performed on tissue from the biopsy material. This was prepared routinely by glutaraldehyde/osmium fixation, uranyl acetate/lead staining, and embedding in Epon-Araldite. Several thin sections were examined from 4 blocks. Three major tumor cell populations, and possibly a fourth, were identified in the tumor. Each group could be distinguished most readily by the morphology of their electron dense, membrane bound, cytoplasmic granules. However, they also differed in their nuclear characteristics.

One group of cells, the most frequent, usually possessed a round to oval, regular nucleus, a fine chromatin pattern, and a well circumscribed nucleolus (Fig. 3). A second group, seen singly and in clusters less frequently than the first, had a very similar appearance, but the nuclear chromatin pattern was often more dense (Fig. 4). A third group of cells, seen again in clusters still less frequently, had irregular nuclear and nucleolar contours, and a coarse chromatin pattern (Fig. 5).

The granules of the first group of cells were variable in size but often large, round to rod shaped, and frequently angulated (Fig. 6a). In contrast, those of the second group were small, round to pear shaped, and uniformly electron dense (Fig. 6b), while those of the third group were generally very large and round, and variable in their electron density (Fig. 6c). A fourth type of cell was seen only very occasionally, and may possibly represent a distinct, minor group of cells, characterised by uniformly round and regular electron dense granules, smaller than those of the second group of cells (Fig. 6d). In addition to these cells there were also agranular cells which otherwise often resembled the most frequent group.

To further characterise these granules, photographs were taken of 6–7 cells of each group at ×20,000 magnification, and direct measurement of their granules carried out with the aid of a Zeiss MOP-3 semi-automated image analyser. The mean diameters of the four types of granule are shown in Table 1.

Discussion

The classification of carcinoid tumors has been related to the embryonic tissue of origin and the concept of an APUD cell system derived from the embryonic neural crest (Pearse 1974). This has required remodelling as it is now believed that most gut endocrine cells originate from an epithelial cell precursor (Cheng and Leblond 1974). Recent advances in diagnostic techniques have also expanded our knowledge of the variety of endocrine cells and hormone products distributed throughout the GI tract and its related tissues so that these cells, and their neoplasms, can now be classified by function (Solcia et al. 1980, Bensch 1983).

Although there is some variation in cell nomenclature, granule sizes, and products, a plausible summary of the major endocrine cell types described in the normal large bowel is shown in Table 2 (Cristina et al. 1978; Buffa et al. 1978; Henderson and Papadimitriou 1982). If the four neoplastic cell populations seen in this tumor are compared to the normal endocrine cells of the sigmoid colon, they appear to correspond to the EC, F, L, and H cell types, both with respect to their granule morphology and also in their relative proportions. While the nuclear characteristics of the tumor cells containing L type granules might fit the normal F cell type described in one report, the existence of normal cells containing both F and L type granules suggests that they may be closely related (Buffa et al. 1978).

Multiple cell types have been described quite frequently in endocrine cell tumors of the pituitary (Martinez and Barthe 1982) and pancreas (Capella et al. 1977), although the biological significance of this is uncertain. There is also evidence that cellular heterogeneity exists in many hindgut carcinoid tumors. Immunoperoxidase studies have shown that up to one half of hindgut tumors possess multiple hormone products (Alumets et al. 1981, O'Briain et al. 1982; Wilander et al. 1977; Yang et al. 1983). These hormones are usually demonstrated within different cells and often include serotonin, glucagon-like hormone, and pancreatic polypeptide. However granule heterogeneity has only been described in two previous case reports concerning

hindgut tumors (Williams 1979; Yoshida et al. 1981). In both instances two granule types have been described – a small regular dense granule and a large pleomorphic granule corresponding to that of the EC cell. The tumor reported here stains only for serotonin but is composed of a mixed population of cells with four distinct granule types.

The mixed composition of cells may reflect 'true heterogeneity' arising from the parallel differentiation of four distinct cell types from a single transformed precursor. Alternatively this pattern may represent a 'maturation' sequence within the life span of one cell type, with sequential genetic expression resulting in a series of secretory products and corresponding granules. This might be compared to the process of maturation of myeloid precursors in the evolution of the neutrophil, where sequential production of different types of granules is known to occur (Bainton et al. 1971).

Both of these processes, differentiation and maturation, have been considered to explain the origin of mixed endocrine cell tumors of the pancreas together with the possibility that the D_1 cell (which closely resembles the H cell of the large bowel) is a stem cell type (Creutzfeld 1975).

Further combined ultrastructural and histochemical study is needed to explain this phenomenon.

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