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CYTOKERATIN EXPRESSION IN SMALL INTESTINAL AND APPENDICEAL CARCINOIDS

A basis for classification

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For a study of histogenesis of intestinal carcinoids a collection of 5 classical small intestinal carcinoids, 6 appendiceal carcinoids and 9 pheochromocytomas, were evaluated. The tumors were identified by routine morphology, silver staining and chromogranin immunocytochemistry and were then examined with regard to the expression of intermediate filaments of cytokeratin type. Eight different antisera identifying individual or combinations of cytokeratins were employed. All classical small intestinal carcinoids displayed cytokeratin immunoreactivity and an almost identical cytokeratin reaction was observed in the normal enterocytes of the small intestinal mucosa. Of the individual cytokeratin types, number 18 was most heavily expressed. The appendiceal carcinoids, like the pheochromocytomas, almost totally lacked a cytokeratin staining despite a positive reaction in the mucosa of the appendix. This, in agreement with some previous studies, indicates that the small intestinal carcinoids are histogenetically related to the epithelial cells of the intestinal mucosa, while the appendiceal carcinoids have a different histogenesis and are more like pheochromocytomas. The appendiceal carcinoid may represent a distinct type of intestinal paraganglioma. This offers one explanation for the different biological behavior of appendiceal carcinoids in comparison with the other intestinal carcinoids.

According to the classification by Williams & Sandler in 1983 the midgut carcinoids (1) represent a distinct tumor type. They are argentaffin, serotonin producing endocrine tumors and in the case of distant metastases they often give rise to the carcinoid syndrome. The term 'midgut' has an embryological basis and corresponds to the topographic area of the intestine receiving its blood supply from the superior mesenteric artery (the midgut artery of the fetus), namely the distal duodenum, the jejunum, the ileum, and the proximal colon.

However, one peculiarity within the group of midgut carcinoids is the different biological behavior of the appendiceal carcinoids. While small intestinal and colonic carcinoids can be considered as relatively slowly growing and malignant tumors, the appendiceal carcinoids mostly have a completely benign course. This discrepancy may reflect differences in histogenesis. Several previous studies indicate that the small intestinal carcinoids probably derive from the intestinal mucosa, while the appendiceal carincoids mostly, but not always, are regarded as histogenetically related to subepithelial endocrine cells of the intestinal wall (2–7). The present study is an attempt, by application of cytokeratin immunocytochemistry in small intestinal and appendiceal carcinoids and some pheochromocytomas, to put further light on the histogenesis of the intestinal carcinoids.

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Material and Methods

Paraffin blocks of 5 formalin-fixed classical small intestinal carcinoids were collected. From each block 12 serial sections were cut. The first 4 sections were stained with haematoxylin-eosin, an argentaffin reaction (8), an argy-

Table 1

Information of the monoclonal cytokeratin filament antibodies used in the study

Antibody No.	Antigen specificity Human cytokeratin No.	Source
1	8, 18, 19	Lab. Systems, Helsinki Finland
2	10, 17, 18	Dakopatts, Glostrup Denmark
3	1–8/9–19	Boehringer, Mannheim Biochemicals Indianapolis, In, USA
4	7	Sigma Chemicals, St. Louis, Mo, USA
5	13	Sigma Chemicals St. Louis, Mo, USA
6	14	Sigma Chemicals St. Louis, Mo, USA
7	18	Sigma Chemicals St. Louis, Mo, USA
8	19	ICN Immunobiologicals Caster Mesa, Ca, USA

rophil reaction (9, 10) and immunocytochemically with a chromogranin A + B (CAB, Milab, Malmö, Sweden) antiserum. In a second sequence, 8 additional sections from the tumors and the adjacent normal intestinal mucosa were stained immunocytochemically with a series of 8 different monoclonal cytokeratin antibodies. Details of the antibodies are given in Table 1. For the immunocytochemical reaction the ABC (Avidin, Biotin, Complex) technique was used (Vector Laboratories, Burlingame, Ca., USA). Endogenous peroxidase was inhibited applying =0.1% (v/v) hydrogen peroxide in methanol for 10 min. Predigestion with protease 0.05% (Sigma Chemicals, St. Louis, Ma., USA) in PBS buffer for 10 min was performed before the application of the primary antibody, which was dissolved 1 : 50–100 in a Tris-saline buffer 1 : 9, pH 7.6. All immunocytochemical stainings were performed in a moist chamber. Diaminobenzidine (DAB) 0.003% in the Tris-saline buffer was utilized to stain the sections. Negative controls were obtained by omission of the primary antibodies.

In a second study paraffin blocks of 6 formalin-fixed appendiceal carcinoids were collected. The diagnoses were verified, as for the small intestinal carcinoids, with an argentaffin and an argyrophil stain and by chromogranin A + B immunocytochemistry. Serial sections were then stained with those antibodies to cytokeratin filament (Nos. 2, 3, 4 and 7, see Table 1), that most prominently stained the mucosa and the corresponding carcinoid tumors of the small intestine. The immunocytochemical staining was performed as described above.

In a third study blocks of 9 adrenal medullary pheochromocytomas were collected and the diagnoses were verified by silver staining and chromogranin A + B immunocytochemistry. Sections from each tumor were then immunocytochemically stained with antibodies Nos. 1 and 2 (see Table 1).

Results

The mucosa of the small intestine and the appendix was stained by all the different cytokeratin antibodies applied. However, antibody No. 3 (pan-cytokeratin antibody) and No. 7 (cytokeratin No. 18) showed the strongest and most constant staining of the epithelial layer (Fig. 1), while antibody Nos. 5 and 6 stained the mucosal enterocytes inconstantly and weakly. In the normal adrenal medulla no cytokeratin immunoreactivity was present.

All tumors in the study fulfilled the criteria for exact diagnoses. They displayed an argyrophil reaction and chromogranin immunoreactivity in a majority of the tumor cells, indicating an obvious and prominent endocrine differentiation. Besides, all small intestinal and appendiceal carcinoids contained a population of argentaffin tumor cells.

The cytokeratin expression of small intestinal carcinoids is given in Table 2. As could be seen, the cytokeratin filament expression varied with the different antibodies applied. All tumors were stained with antibody Nos. 2, 3, 4 and 7. Of these antibodies Nos. 3 and 7 gave the most prominent immunoreaction in the tumor cells (Fig. 2). With antibody Nos. 5 and 6 no immunoreaction was recorded. Mostly the staining pattern of the mucosa and the carcinoids was concordant.

Among the appendiceal carcinoids a few weakly immunoreactive tumor cells were observed with cytokeratin antibodies Nos. 2, 3, 4 and 7 in one case and with antibody No. 3 in another two cases. The rest of the tumors were

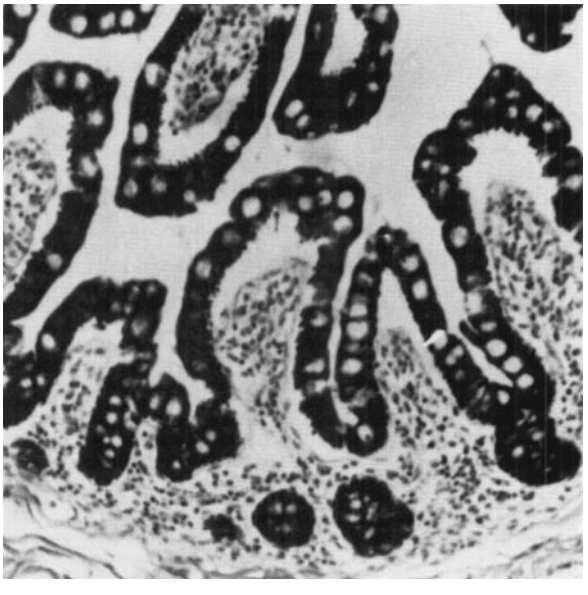

Fig. 1. Picture of normal small intestinal mucosa displaying a strong immunoreaction with cytokeratin antibody, No. 3 (pan-cytokeratin). ABC-staining. ×560.

Table 2

Expression of cytokeratin filament in classical small intestinal carcinoids (5 cases) and appendiceal carcinoids (6 cases)

Cytokeratin antibody No. (see Table 1)	Small intestinal carcinoids	Appendiceal carcinoids
1	2/5	–
2	5/5	1/6
3	5/5	2/6
4	5/5	1/6
5	0/5	–
6	0/5	–
7	5/5	1/6
8	3/5	–

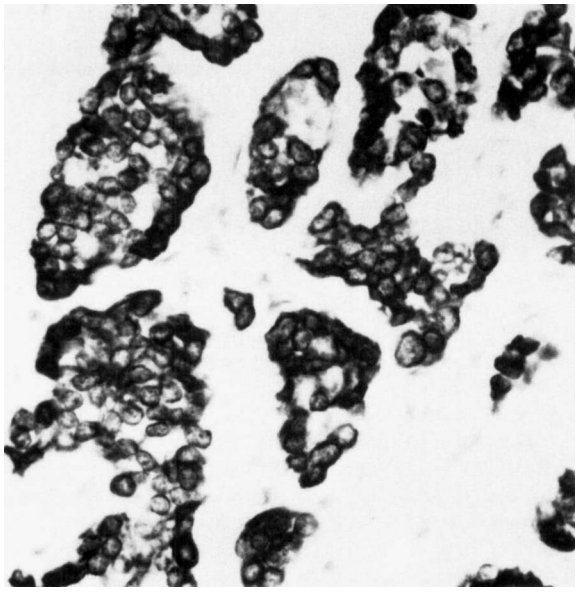

Fig. 2. Cytokeratin immunoreactivity (antibody No. 3) in the majority of the tumor cells of a small intestinal carcinoid. ABC-staining. × 560.

completely cytokeratin-negative despite a positive immunoreaction in the covering appendiceal mucosa (Table 2) (Fig. 3). Two of the 9 examined pheochromocytomas contained a few scattered cytokeratin immunoreactive tumor cells with antibody No. 1, but the rest of the tumors were negative.

Discussion

The present study was undertaken to obtain further information about histogenetic aspects of intestinal carcinoids. The basis for the interpretations is the tendency of tumors to display a fenotypic expression similar to that of the cells and tissues of origin. These properties are especially obvious in endocrine tumors that are highly differentiated and where deviations from normal features are not prominent (11).

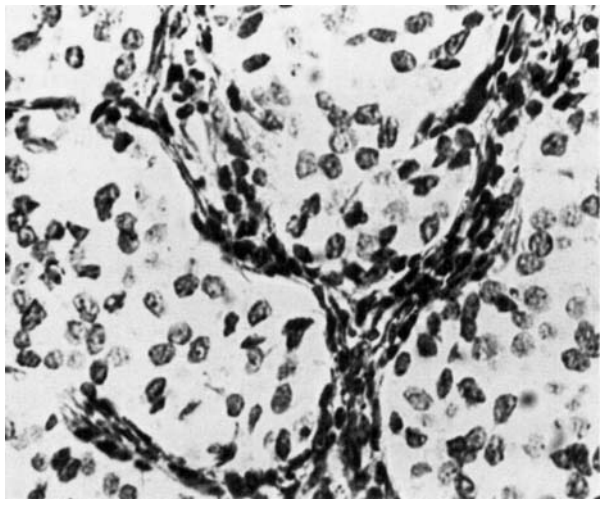

Fig. 3. Appendiceal carcinoid totally without cytokeratin (antibody No. 3) immunoreactivity. ABC-staining. × 800.

Tumor development may be preceded by premalignant alterations. In the MEN II syndrome, hyperplasia of the calcitonin-producing C-cells of the thyroid gland can sometimes be seen before or in conjunction with the growth of a medullary thyroid carcinoma. Further, the ECL-cell tumors of the gastric body in patients with atrophic gastritis, hypergastrinemia and pernicious anemia often arise towards a background of endocrine (ELC) cell hyperplasia (12–16). However, such features are not prominent in intestinal carcinoids. The classical small intestinal carcinoids displayed a cytokeratin immunoreaction similar to that of the normal mucosal enterocytes. The endocrine cells of the mucosa are considered to be specialised enterocytes and they are all presumed to be of endodermal origin. The classical carcinoids can thus be considered to be derived from the mucosal endocrine cells, since they express the same cytokeratin filaments as the mucosal enterocytes and contain hormonal products that are mostly present in the endocrine cells of the corresponding topographic region of the intestine. This opinion is supported by a previous study, in which serial sectioning of classical carcinoids were performed. It was observed that the tumors developed from the mucosal crypts in direct contact with the epithelial cell layer (7).

Of the appendiceal carcinoids, 4 out of 6 cases showed a negative cytokeratin expression and in the other 2 tumors only few cytokeratin immunoreactive cells occurred, despite a positive cytokeratin reaction in the epithelial cells of the appendix mucosa. Thus, the feature of the cytokeratin reaction was more like that obtained in pheochromocytomas than in the small intestinal carcinoids.

In the appendix, preferably in its caudal part, subepithelial cells are present. They are typically located in the lamina propria or in the upper part of the submucosa. Previously, these cells have been examined both at the light- and electro-microscopical level. Light-microscopi-

cally they are serotonin- and neuron-specific enolase immunoreactive and surrounded by cells with long cytoplasmic processes, presumably of Schwann cell origin because of a positive cytoplasmic S-100 protein reaction. Electron-microscopically the subepithelial cells contain pleomorphic neurosecretory granules similar to those seen in the mucosal enterochromaffin cells (3–7).

Since the appendiceal carcinoids are mostly serotonin immunoreactive they may be derived from either the mucosal endocrine (enterochromaffin) or the submucosal (subepithelial) neuroendocrine cells. However, the appendiceal carcinoids are frequently diphasic and contain a mixture of endocrine, serotonin immunoreactive cells and elongated S-100 protein immunoreactive cells of Schwann cell type—a picture also seen in pheochromocytomas. For that reason, and since the appendiceal carcinoids display a cytokeratin immunoreaction more similar to pheochromocytomas, it is suggested that they histogenetically are related to the peripheral nervous system and may represent a kind of intestinal paraganglioma opposite to the classical small intestinal carincoids.

Intestinal carcinoids and pheochromocytomas belong to the neuroendocrine tumors, all sharing some common features among which the presence of cytoplasmic granules is the most conspicuous. These secretory granules can, on the light microscopical level, be identified by argyrophil staining and by chromogranin immunostaining (11).

Small intestinal and appendiceal carcinoids are both argentaffin and serotonin immunoreactive. However, these properties do not give any information about histogenesis since both the mucosal (enterochromaffin) and the submucosal endocrine cells are serotonin-storing. The obvious difference between small intestinal and appendiceal carcinoids is their various biological behavior and ability to express cytokeratin, of which the latter is considered to reflect histogenesis. The characteristic tumor marker profile of small intestinal and appendix carcinoids and pheochromocytomas is given in Table 3 (11, 17, 18).

In conclusion, the present study as well as some previous ones indicates that the appendiceal carcinoid has derived from cells related to the peripheral nervous system, shows similarities to pheochromocytomas and could be regarded as a kind of intestinal paraganglioma.

Table 3

Tumor marker profile of small intestinal and appendix carcinoids and pheochromocytomas

	Small intestinal	Appendix	Pheochromocytoma
Argyrophil reaction	+	+	+
Chromogranin reaction	+	+	+
Serotonin reaction	+	+	(+)/−
S-100 protein reactive cells	−	+	+
Cytokeratin expression	+	−	−

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