Virchows Arch [Pathol Anat] (1984) 403:235–240

Virchows Archiv A

Immunocytochemical demonstration of cytokeratin in gastrointestinal carcinoids and their probable precursor cells

Heinz Höfler and Helmut Denk
Institute of Pathology, University of Graz School of Medicine, Auenbruggerplatz 25, A-8036 Graz, Austria

Summary. Occurrence and distribution of cytokeratin, neuron specific enolase (NSE) and actin were studied by the immunoperoxidase-antiperoxidase (PAP)-technique using specific antibodies in formalin-fixed, paraffin-embedded material from 6 cases of neurogenic appendicopathy with numerous endocrine cells in the mucosal stroma (SEC), 5 cases of microcarcinoidosis of the stomach, 12 gastrointestinal carcinoids and 4 bronchial carcinoids. Cytokeratin was detectable in all tumor cells. In addition, the epithelial endocrine cells (EEC) and the SEC of intestinal origin were cytokeratin positive. EEC, SEC and cells of microcarcinoids and carcinoids showed a positive immunoreactivity with antibodies to NSE, whereas actin antibodies did not reveal significant staining of these cells. These results strongly suggest that carcinoids of the gastrointestinal tract originate from SEC that have migrated downwards into the stroma from the epithelial layer ("Endophytie" according to Feyrter).

Key words: Carcinoid – Immunocytochemistry – Cytokeratin – Neuron specific enolase – Histogenesis

The histogenesis of carcinoids is still equivocal: apart from the hypothesis proposing the origin of carcinoids from undifferentiated epithelial stem cells (Capella et al. 1973; Soga et al. 1975; Warner and Seo 1979; Isaakson 1981; Lyss et al. 1981) another hypothesis, originally introduced by Masson (1924 and 1932) exists. This suggests that carcinoids develop from subepithelial stromal cells without connection with the epithelium (stromal endocrine cells – SEC). Auböck and Ratzenhofer (1982); Rode et al. (1982) and Auböck and Höfler (1983) have partly confirmed the latter hypothesis by light- and electron microscopic studies and have stressed the extraepithelial origin of carcinoids. In contrast to Masson (1932) and Feyrter (1934) the mother

Dedicated to Prof. Dr. H.G. Klingenberg on the occasion of his 65th birthday.

Offprint requests to: H. Höfler at the above address

cells of carcinoids, the SEC, were not related by these later authors to epithelial cells but to paraganglionic elements ("Endokrinoblasten") which originally develop within the mucosal stroma (for review see Höfler et al. 1982).

In the present study epithelial endocrine cells (EEC), SEC, microcarcinoids and carcinoids were investigated with regard to their immunoreactivity with antibodies to cytokeratin, a specific marker of epithelial cells (Franke et al. 1979; Moll et al. 1983), to neuron specific enolase (NSE) and to actin. We have shown that neuroendocrine cells and tumor tissues contain cytokeratin indicating their epithelial derivation.

Material and methods

Material. Tissue was fixed in 10% buffered formaldehyde solution immediately after surgical removal and embedded in paraffin by routine techniques. The material consisted of 6 appendices (3 of which showed intramucosal proliferation of SEC, 3 proliferation of SEC in central neuromas of neurogenic appendicopathy (Masson)), 5 cases of microcarcinoidosis of the stomach, 12 gastrointestinal carcinoids (4 derived from the stomach, 4 from the appendix including one goblet cell carcinoid, 3 from the small intestine and 1 from the rectum), and 4 bronchial carcinoids. Material from one stomach and 1 bronchial carcinoid was immediately frozen in isopentane at the temperature of liquid nitrogen after surgical removal for frozen sectioning.

Immunohistochemistry. The PAP-technique (Sternberger 1979) was used on 5 µm thick paraffin sections and cryostat sections. All reagents were diluted with phosphate buffered saline (PBS, 0.5 M). Peroxidase activity was visualized with 3,3 diaminobenzidine-tetrahydrochloride (0.05% w/v) and hydrogen peroxide (0.01% v/v), in Tris-HCL buffer (0.05 M, pH 7.2). Subsequently the sections were treated with osmium tetroxide (0.1% in water) to enhance colour intensity. For immunocytochemical demonstration of keratin the paraffin sections were pretreated with 0.1% pronase (type VII, Serva, Heidelberg, FRG) for 1 h at 37° C according to Denk et al. (1977). The following antibodies were used: anti-neuron specific enolase (NSE, Dako, Denmark; dilution 1:1,000), anti-cytokeratin (guinea pig antibody to cytokeratin component D derived from mouse liver as described previously, dilution 1:3,000), anti-actin (rabbit antibody to chicken gizzard actin, prepared against actin after electrophoretic separation and elution as described by Denk et al. 1981, dilution 1:3,000). Preimmune sera and non-immune sera from other animal species substituted for immune sera served as controls.

Results

The NSE-related immunoreactivity of EEC and SEC in the appendix was specific but weak, whereas microcarcinoids of the stomach and carcinoids were conspicously stained (see also Höfler and Heitz 1982). Antibodies to cytokeratin outlined EEC, SEC and cells of microcarcinoids of the stomach as well as carcinoids in all other localisations (Fig. 1 and 2). Keratin immunoreactivity was confined to epithelial cells and endocrine cells whereas non-epithelial elements were uniformly negative. In one case of microcarcinoidosis of the stomach and in one carcinoid of the stomach a direct continuity of intestinal metaplastic foveolar epithelium and carcinoid tumor cells was obvious (Fig. 2). The intensity of keratin-related immunostaining reaction was identical in epithelial and endocrine cells as well as in SEC and

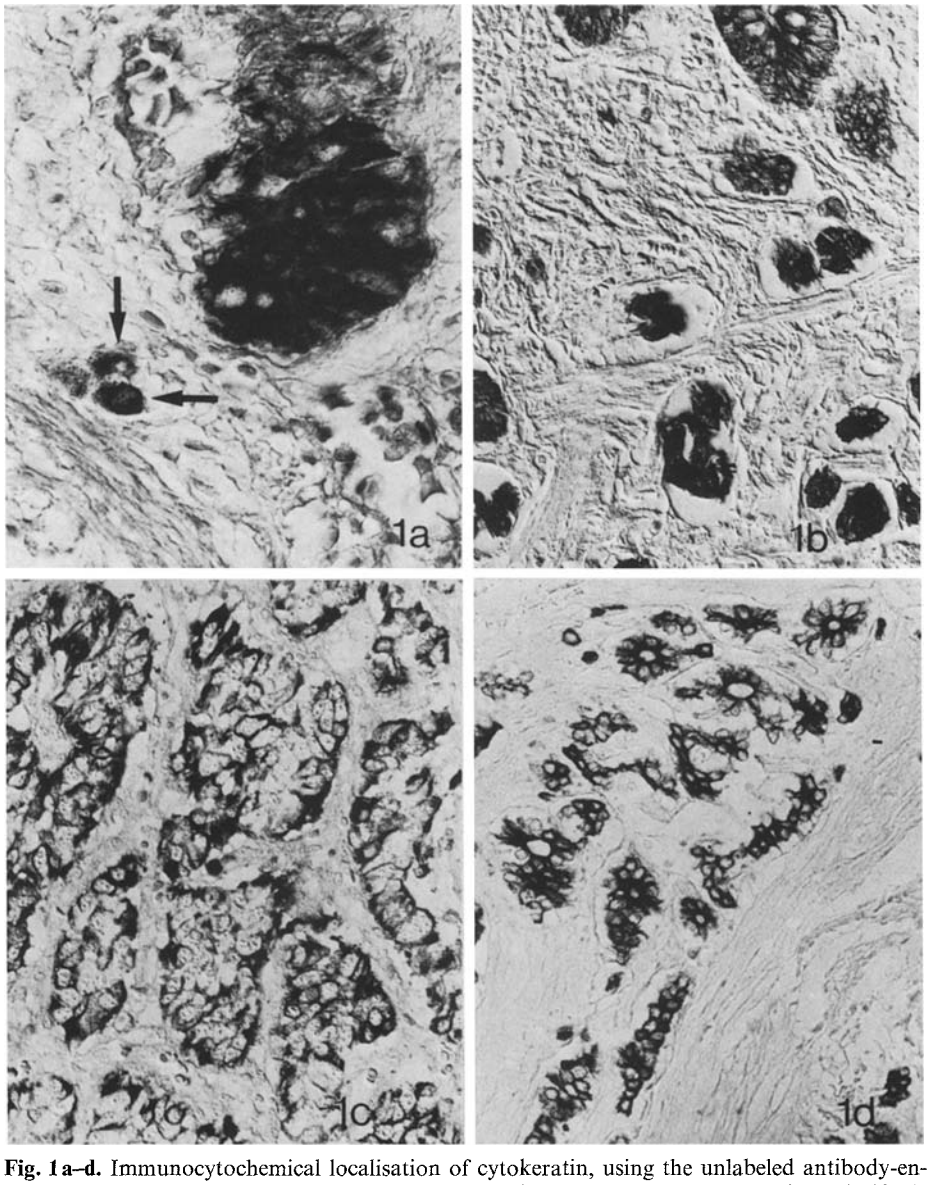

Fig. 1 a–d. Immunocytochemical localisation of cytokeratin, using the unlabeled antibody-enzyme method (antibodies to mouse liver cytokeratin component D, see Denk et al. 1981). **a** Neurogenic appendicopathy: strongly positive reaction in crypt cells and SEC (*arrows*), ×360. **b** Gastric microcarcinoidosis: cytokeratin-reactivity in intestinal metaplastic epithelium (*top*) and in nodular proliferations of endocrine cells in the lamina propria and muscularis mucosae, ×280; differential interference contrast optics. **c** Intestinal carcinoid (trabecular and glandular type), ×280. **d** Bronchial carcinoid, ×280

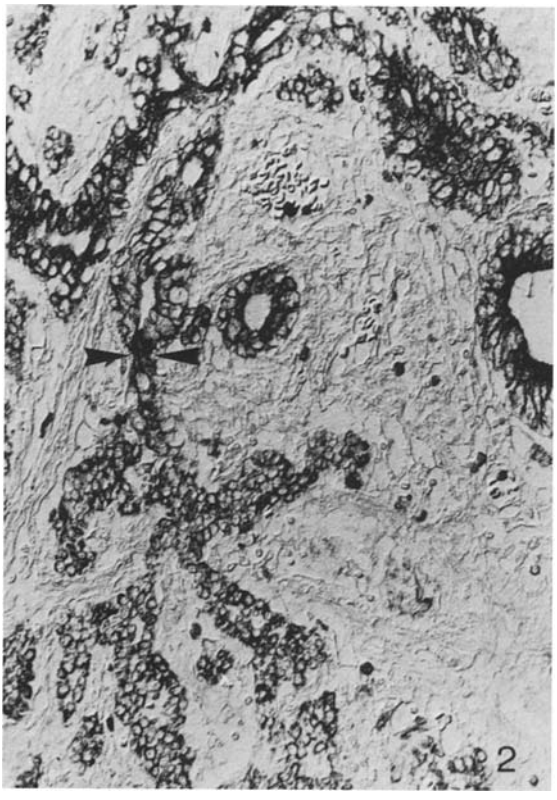

Fig. 2. Gastric carcinoid: strong cytokeratin reactivity of epithelium (*top*) and tumor cells. Note the connection of foveolar epithelium and tumor (*arrows*), ×280; differential interference contrast optics

Table 1

	EEC	SEC	Micro-carcinoids (stomach)	Carcinoids	Ganglion cells
Neuron specific enolase (NSE)	(+)*	(+)*	+	+	+
Cytokeratin	+	+	+	+	−
Actin	−	−	−	−	−

* Weak reaction

in cells of the carcinoids. However, within carcinoids the reactivity of the tumor cells was somewhat variable: most of the tumor cells showed moderate or strong positive reaction with regard to keratin antibody, but some keratin negative tumor cells were interspersed among tumour cells particularly at the periphery. In all cases studied, actin-antibodies failed to stain to a significant degree epithelial and tumor cells, SEC, and ganglion cells but reacted strongly with smooth muscle cells. The results are summarized in the Table 1.

Discussion

The presence of cytokeratin in EEC can be expected from their ultrastructure, i.e. the presence of desmosomes and bundles of intermediate sized filaments, and from their position within the epithelial layer. The conspicuous positive reaction of SEC with cytokeratin antibodies in appendices with neurogenic appendicopathy was a surprising finding and not compatible with our theory of carcinoid development (Höfler et al. 1982). SEC, precursors of carcinoids, were related to cells of (para-) neuronal origin without relationship to the epithelium; under these circumstances SEC should not contain cytokeratin. However, because of cytokeratin reactivity in SEC a close relationship between EEC and SEC has to be assumed: the results of this study suggest that SEC originate from EEC migrating (budding) into the stroma which agrees with electronmicroscopic observations of Osaka et al. (1976) and the hypothesis proposed by Masson (1932) and Feyrter (1934). Moreover the intensity of NSE-specific immunoreaction in the SEC corresponds to that of EEC and not of ganglion cells. One has therefore to assume that the SEC is a cell with epithelial characteristics despite its endocrine, paracrine and neurocrine functions and its position between nerve fibres (neuroendocrine complexes, Auböck and Ratzenhofer 1982).

Because of the immunohistochemical demonstration of cytoceratin and NSE in EEC, SEC, microcarcinoids and carcinoids we would like to propose the following hypothesis concerning the histogenesis of carcinoids in extension of the results published previously (Auböck and Höfler 1983). The endocrine cells, normally present in the mucosal stroma of the gastrointestinal tract (i.e. SEC), are the origin, at least of the majority, of carcinoids. These SEC do not arise, from paraganglionic elements as previously postulated ("Endocrinoblasten" = endocrinoblasts, Auböck and Ratzenhofer 1982) but from EEC which migrate into the stroma as suggested by Feyrter and Masson and designated "Endophytie" and "bourgeonnement", respectively. However, because of the continuity between non-neoplastic epithelium and tumour tissue, observed in two cases, carcinoids may also apparently arise from EEC directly without preceding budding.

References

Auböck L, Ratzenhofer M (1982) "Extraepithelial enterochromaffin cell-nerve-fibre Complexes" in the normal human appendix, and in neurogenic appendicopathy. J Pathol 136:217–226

Auböck L, Höfler H (1983) Extraepithelial intraneural endocrine cells as starting-points for gastrointestinal carcinoids. Virchows Archiv [Pathol Anat] 401:17–33

Capella C, Solcia E, Snell CK (1973) Ultrastructure of endocrine cells and argyrophil carcinoids of the stomach of Praomys (Mastomys) natalensis. J Nath Cancer Inst 50:1471–1485

Denk H, Radaszkiewicz T, Weirich E (1977) Pronase pretreatment of tissue sections enhances sensitivity of the unlabeled antibody (PAP) technique. J Immunol Methods 15:163–167

Denk H, Franke WW, Dragosics B, Zeiler I (1981) Pathology of cytoskeleton of liver cells: demonstration of mallory bodies (alcoholic hyalin) in murine and human hepatocytes by immunofluorescence microscopy using antibodies to cytokeratin polypeptides from hepatocytes. Hepatology 1:9–20

Feyrter F (1934) Carcinoid and Carcinom. Ergebn Allg Pathol Anat 29:305–489
Franke WW, Appelhans B, Schmid E, Freudenstein C, Osborn M, Weber K (1979) Identification and characterization of epithelial cells in mammalian tissue by immunofluorescence microscopy using antibodies to prekeratin. Differentiation 15:7–25
Höfler H, Auböck L, Ratzenhofer M (1982) Die neurogene Appendikopathie. Eine lichtmikroskopische Studie. Normale und pathologische Anatomie. G Thieme, Stuttgart New York, Bd 45
Höfler H, Heitz PHU (1982) Immuncytochemische Befunde bei neurogener Appendicopathie. Wien Klin Wochenschr 21:588–591
Isaacson P (1981) Crypt cell carcinoma of the appendix (so-called adenocarcinoid tumor). Am J Surg Pathol 5:213–224
Lyss AP, Thompson JJ, Glick JH (1981) Adenocarcinoid tumor of the colon arising in preexisting ulcerative colitis. Cancer 48:833–839
Masson P (1924) Appendicite neurogéne et carcinoids. Ann Anat Pathol 1:3–59
Masson P (1932) Neural proliferations in the vermiform appendix. In: Penfield W (ed) Cytology and cellular pathology of the nervous system. Hoeber, New York, pp 1095–1130
Moll R, Krepler R, Franke WW (1983) Complex cytokeratin polypeptide patterns observed in certain human carcinomas differentiation 23:256–269
Osaka M, Kobayashi S (1976) Duodenal basal-granulated cells in the human fetus with special reference to their relationship to nervous elements. In: Fujita T (ed) Endocrine gut and pancreas. Elsevier, Amsterdam, pp 145–158
Rode J, Dhillon AP, Papadaki L, Griffiths D (1982) Neurosecretory cells of the lamina propria of the appendix and their possible relationship to carcinoids. Histopathology 6:69–79
Soga J, Kohro T, Tazawa K, Kanahara H, Sano M, Sakashita T, Tajina K, Morooka H, Karaki Y (1975) Argyrophil cell microneoplasia in Mastomys' stomach – an observation on early carcinoid formation. Natl Can Inst 55:1001–1006
Sternberger LA (1979) The unlabeled antibody peroxidase-antiperoxidase (PAP) method. In: Sternberger LA (ed) Immunocytochemistry (2ed) John Wiley, New York, pp 104–169
Warner CS, Seo IS (1979) Goblet cell carcinoid of appendix. Ultrastructural features and histogenetic aspects. Cancer 44:1700–1706

Accepted March 7, 1984