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Undifferentiated carcinoma of the colon containing exocrine, neuroendocrine and squamous cells

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Summary. The light microscopic, electron microscopic and histochemical features of a highly malignant colonic tumor resected from a 39 year old man are presented. The tumor was composed predominantly of undifferentiated cells with focally admixed neuroendocrine, exocrine and squamous cells, occasionally arranged in an organoid manner. Histochemically the tumor contained argyrophilic cells as well as cells that reacted positively with the antibodies to alpha-1-antitrypsin, alpha-1-antichymotrypsin, carcinoembryonic antigen and lysozyme. The term "stem cell carcinoma of the intestine" is proposed for this highly malignant tumor composed of undifferentiated cells exhibiting only focally their multidirectional developmental capacity.

Key words: Keratin – Alpha-1-antitrypsin – Lysozyme

It has been known for quite some time that gastrointestinal carcinomas may on occasion contain argentaffin or argyrophil cells (Hamperl 1927). In addition to these "carcinomas with included argentaffin or argyrophil cells" (Lillie and Glenner 1960) there are tumors composed of both neuroendocrine and mucin secreting cells (Hernandez and Reid 1969), neuroendocrine cell carcinomas containing foci of mucin rich cells (Tahara et al. 1982) and so called amphicrine cell tumors (Ratzenhofer 1977; Ratzenhofer and Auböck 1980). All these observations coupled with experimental data obtained from heterotransplantations of human gastrointestinal tumors (Goldenberg and Fisher 1970) and clonings of rat tumors of the colon (Cox and Pierce 1982) suggest that some gastrointestinal tumors arise from stem cells capable of differentiating into both exocrine and neuroendocrine cells. Other reports (Park and Reid 1980) indicate that the stem cells of some tumors may differentiate not only along the pathways which are normal

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for the gastrointestinal tract but also aberrantly, as evidenced by the appearance of choriocarcinoma in the metastasis of a colonic adenocarcinoma.

Petrelli et al. (1981) described recently a unique carcinoma of the colon composed of undifferentiated, carcinoid and squamous cells, suggesting that the stem cells of the tumor were capable of bidirectional differentiation. In the present paper, we further amplify the report of Petrelli et al. (1981). We describe another unique tumor of the colon which contained several distinct cell types. Unless this tumor was of polyclonal origin, which we cannot exclude, our findings provide further evidence for the developmental pluripotency of certain gastrointestinal tumor stem cells.

Clinical findings

C.B. was a 39 year old Black male who presented in the emergency room with crampy abdominal pain of 36 h duration and a six month history of weight loss. The pain was diffuse but most intense in the left lower quadrant radiating to the mid-epigastrium. He denied any nausea and vomiting but did also complain of multiple episodes of "yellow, watery diarrhea" occurring on the day prior to admission. The past medical history was unremarkable.

Physical examination revealed an emaciated Black male in marked distress with a temperature of 38° C. The abdomen was diffusely tender but especially so in the left lower quadrant. A palpable solid mass was identified in the epigastric area. Bowel sounds could be heard throughout the abdomen.

The abnormal laboratory results included a white blood count of 14,800 with 77% neutrophils and 23% lymphocytes, hemoglobin of 8.2 mg/dl and hematocrit of 26.3%, and LDH greater than 350 µl (normal 110–225 µl/l). No blood could be detected in the stool. All other laboratory data were within normal limits.

At surgery, a large mass occupying the transverse colon was identified. The tumor had apparently perforated the bowel and was extending into the transverse mesocolon. Metastatic lesions were seen in the liver and in the mesenteric lymph nodes. A transverse colectomy with a proximal colostomy was performed. The patient recovered from the operation but died 4 weeks thereafter.

Materials and methods

The tumor was extensively sampled at the time of the operation and fixed in 4% formalin for light microscopic studies and 4% glutaraldehyde for electron microscopic studies. The histochemical reactions were performed on 4 µm thick paraffin sections as described previously (Bosman and Louwerens 1981; Nieuwenhuijzen Kruseman et al. 1978). Briefly, the argyrophilic reaction was performed according to the method of Sevier and Munger, and the argentaffin reaction according to the method of Grimelius. Indirect immunohistochemical reactions were used for the demonstration of polypeptide hormones (gastrin, glucagon, somatostatin, calcitonin, pancreatic polypeptide), alpha-1-antitrypsin, alpha-1-antichymotrypsin, and carcinoembryonic antigen. In all these reactions the secondary antibody was a peroxidase labeled goat anti-rabbit IgG.

The antibody to epidermal keratin, prepared by immunizing rabbits with human sole keratin was a generous gift of Dr. T.T. Sun. This antibody reacts with cytokeratins of both stratified and simple epithelia (Sun et al. 1979). However on paraffin embedded tissues a short incubation (1 h) with this antibody will label only lightly the cells of simple epithelia, but will selectively bring out the markedly keratinized cells. The immunoreactivity of tissues exposed to anti-keratin antibody was visualized with a fluorescein-isothiocyanate labeled secondary goat anti-rabbit IgG antibody.

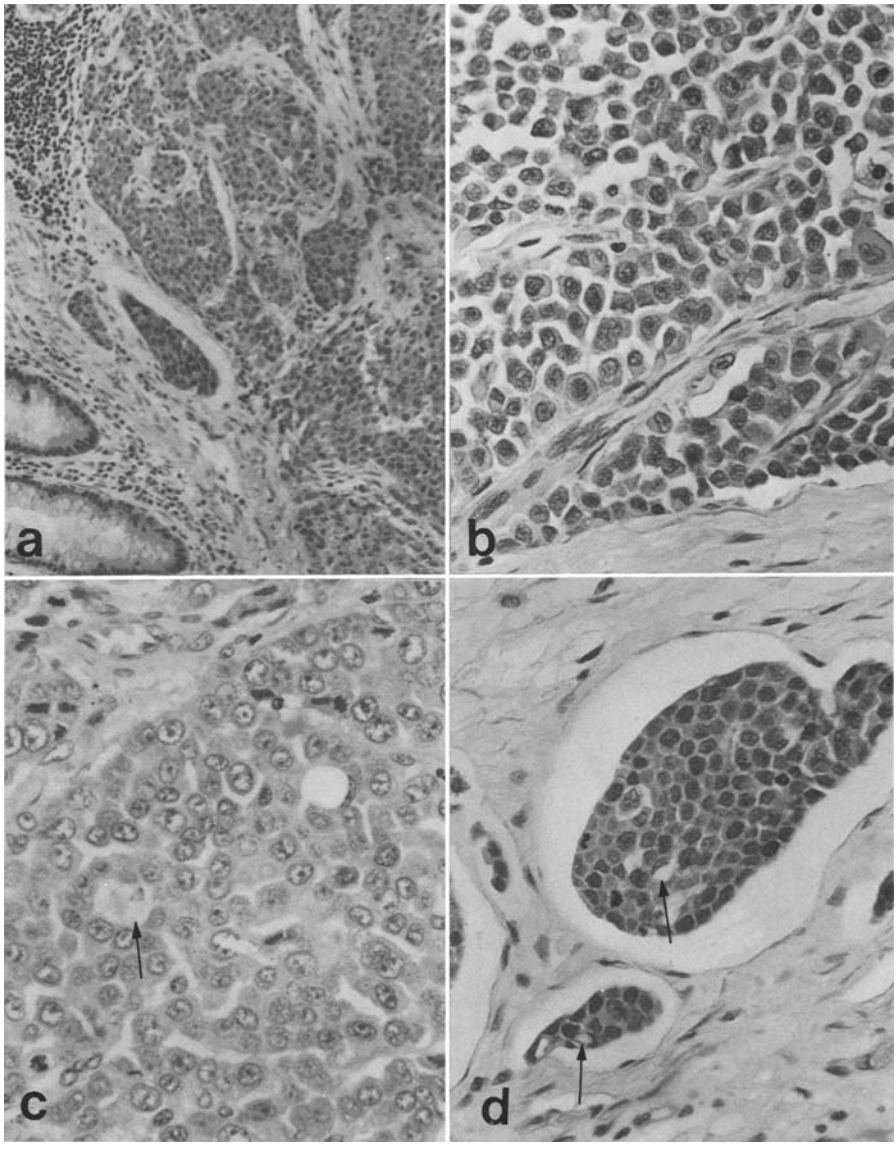

Fig. 1 a–d. Light microscopic appearance of the tumor stained with hematoxylin and eosin. **a** Submucosal location of the tumor and the permeation of the lymphatics is seen at low magnification (×90). **b** Loosely arranged groups of cells intersected with connective tissue septa (×200). **c** Solid sheets of tumor cells. These cells have vesicular nuclei and prominent nucleoli. Cells form abortive glands (*arrow*). Mitotic figures are prominent (×220). **d** Tumor cells in the lymphatics. Most of the cells have clumped chromatin. Some cells have vacuolated cytoplasm (*arrows*) (×220)

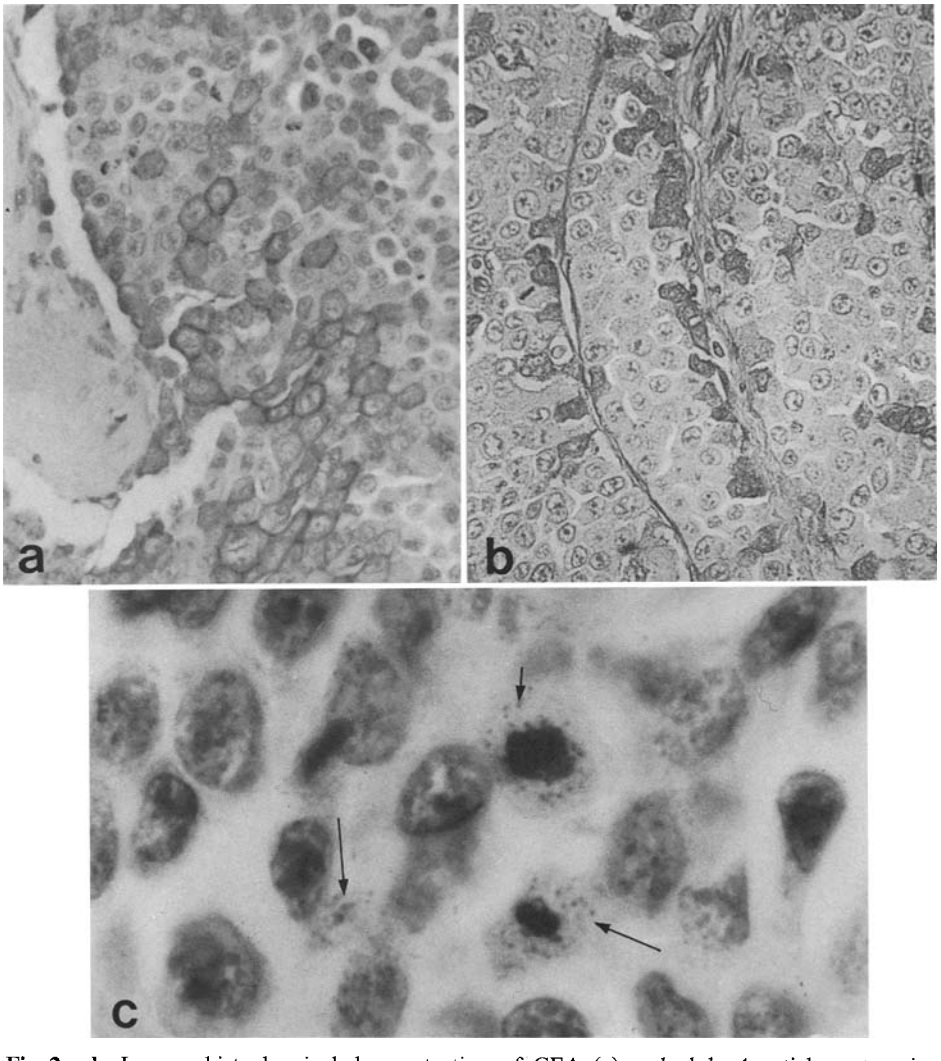

Fig. 2a, b. Immunohistochemical demonstration of CEA (**a**) and alpha-1-antichymotrypsin (**b**) in the tumor (× 220). In the silver impregnated slide (Sevier and Munger method) (**c**) there are argyrophilic cytoplasmic granules (*arrows*) (× 550)

Morphologic findings

The resected transverse colon contained a cauliflower-like mass measuring 7 cm in the larger diameter. The tumor occupied one half of the intestinal circumference and extended into the mesocolon. The mucosa overlying the central portion of the tumor was ulcerated in the central area but appeared intact peripherally.

Histologically, the tumor was composed of predominantly undifferentiated small cells forming sheets and nests intersected with bands of connective tissue stroma (Fig. 1). At lower power magnification, the submucosal location of the tumor cells arranged into sheets and nest and permeation of the lymphatics gave the impression of a carcinoid (Fig. 1a). However closer inspection at higher magnification disclosed considerable cellular pleomorphism and

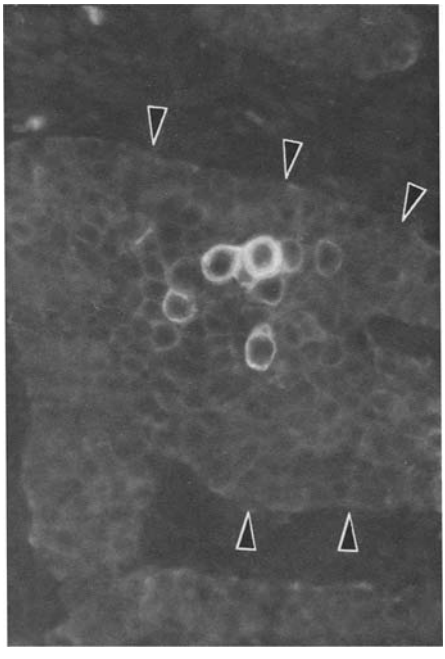

Fig. 3. Immunofluorescent microscopic demonstration of keratin in the tumor. The contours of the solid tumor nests are marked by weak immunoreactivity (*arrows*), whereas, the heavily keratinized cells in the center stand out prominently (× 220)

a large number of mitotic figures (Fig. 1 b, c) indicating that the tumor was a small cell undifferentiated carcinoma. Although many cells had rounded nuclei, there were also many cells with vesiculated nuclei, irregularly shaped nuclei and also cells with prominent nucleoli (Fig. 1 b, c). Most of these cells were loosely arranged (Fig. 1 b). In some areas the tumor cells had well developed cytoplasm, which was either finely granular, vacuolated or distinctly eosinophilic. Occasionally cells surrounded interstitial spaces reminiscent of abortive gland formation (Fig. 1 c). Occasionally cells formed tubular structures, surrounded by a basement membrane (Fig. 2 b). The cell aggregates permeating the lymphatics appeared more compacted than in the tumor proper, and although these cells were, in general, uniformly small with a high nucleocytoplasmic ratio, there were also occasional larger or even vacuolated cells (Fig. 1 d). The same histologic features were seen in the lymph node metastasis.

The histochemical examination of the tumor sections disclosed cells reacting with antibodies to CEA (Fig. 2 a), alpha-1-antitrypsin and alpha-1-antichymotrypsin (Fig. 2 b), and also, cells with argyrophilic cytoplasmic granules (Fig. 2 c). In serial sections it appeared that many argyrophilic cells reacted also with antibodies to alpha-1-antitrypsin and alpha-1-antichymotrypsin, whereas, the CEA positive cells were distinct from them. There were also scattered lysozyme positive cells. However, no argentaffin cells were seen. We could not demonstrate any immunoreactive HCG, somatostatin, ACTH nor calcitonin in the tumor. Antibody to human sole keratin outlined the contours of cells arranged in gland like structures and solid nests and intensely stained the cytoplasm of scattered heavily keratinized cells (Fig. 3). The loosely arranged cells forming the bulk of the tumor were unreactive with this antibody.

Electron microscopic findings. Most of the tumor cells were small averaging 10–12 μm in diameter, and had a high nucleocytoplasmic ratio (Fig. 4). The nuclei were plump, irregularly shaped, but not indented and contained predominantly euchromatin with scattered aggregates of heterochromatin and small single nucleolus. The cytoplasm contained free ribosomes, a few mitochondria and short profiles of rough endoplasmic reticulum. The cell surface was smooth or formed a few short microvilli. There were no intercellular junctions. Scattered among the undifferentiated cells there were also single cells or groups of cells, the cytoplasm of which

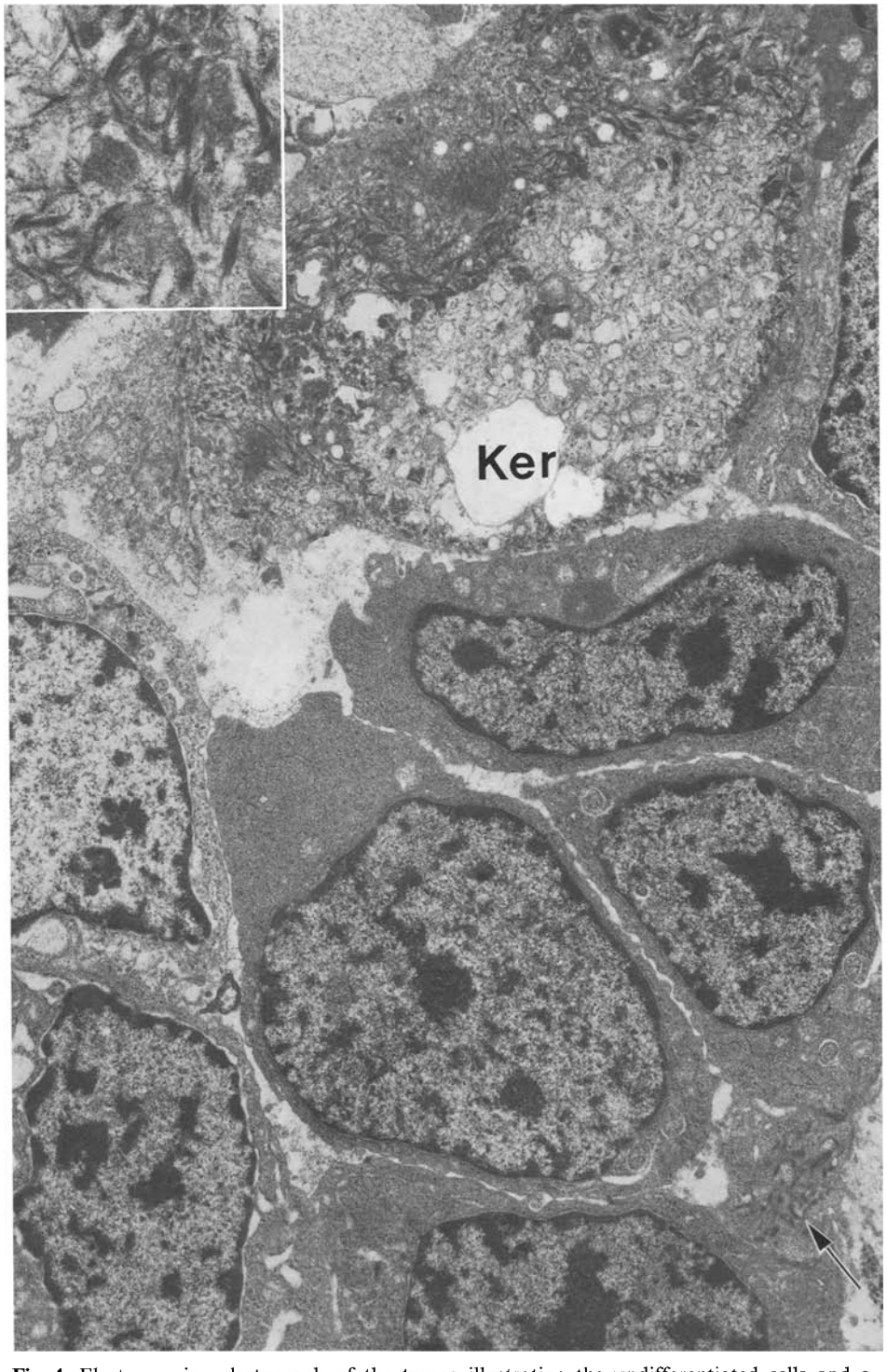

Fig. 4. Electron microphotograph of the tumor illustrating the undifferentiated cells and a keratinized cell (*Ker*). A cytoplasmic process of neuroendocrine cell can also be seen (*arrow*) (× 9,200). *Inset* shows the keratin bundles at higher magnification (× 18,000)

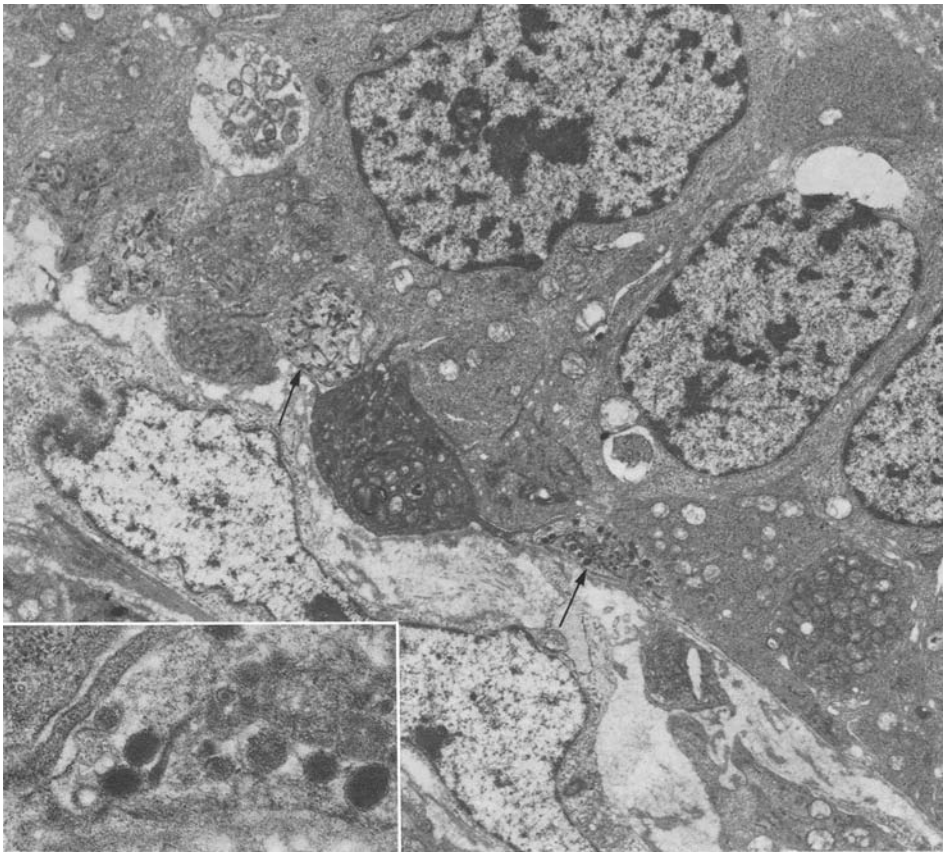

Fig. 5. "Organoid" arrangement of tumor cells. Cell group is surrounded on one side by a basement membrane. Along the basement membrane there are cytoplasmic processes of neuroendocrine cells filled with membrane bound granules (*arrows*). (×4,200). *Inset* shows the neuroendocrine granules (×28,000)

was more abundant and contained prominent aggregates of tonofilaments, indicative of keratinization (Fig. 4). There were also scattered cells forming cytoplasmic processes filled with 100–150 nm membrane bound granules. These granules were either round, oval or tear-shaped and had a dense central core separated from the membrane by a halo. In some areas the tumor cells were arranged in several cell thick tubular structures lined on one side by a basement membrane (Figs. 2a and 5). Along the basement membrane of these "organoid" portions of the tumor one could find the neuroendocrine granule containing cells, as well as their cytoplasmic processes, extending at a distance from the perikaryon (Fig. 5).

The tumor also contained cells with well developed cytoplasm devoid of neuroendocrine granules and showing no evidence of keratinization. Instead, the cytoplasm of these cells contained dilated profiles of rought endoplasmic reticulum (Fig. 6). The cells were often interconnected one to another with well developed desmosomes and surrounded intercellular gland like lumina (Fig. 6).

Discussion

The clinical course of the neoplastic disease in the present patient leaves no doubt that the tumor was highly malignant. The light microscopic and

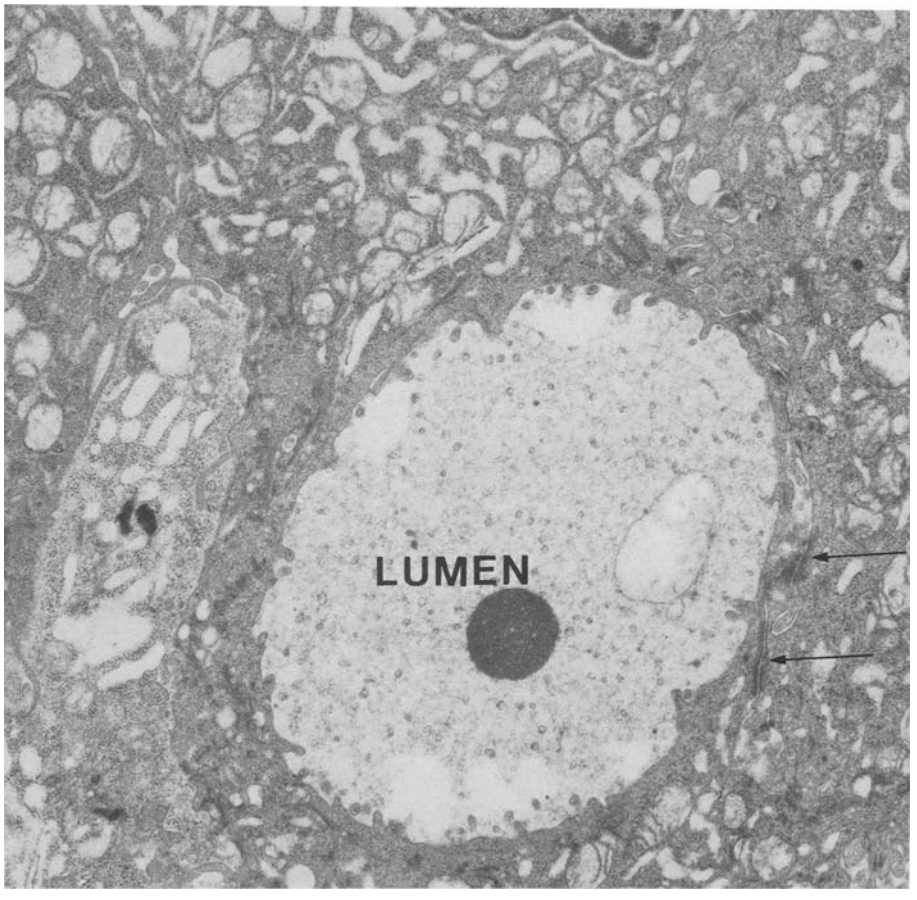

Fig. 6. Glandular lumen surrounded by tumor cells interconnected with desmosomes (*arrows*). (×18,000)

ultrastructural findings confirmed this notion by disclosing the undifferentiated appearance of the predominant tumor cell type. The tumor was thus labeled as stem cell carcinoma to denote its primitive nature, its malignancy and the poor prognosis.

Admixed with the undifferentiated cells were cells displaying ultrastructural or histochemical signs of differentiation. The cells containing the pleomorphic small granules, with or without cytoplasmic processes, and the argyrophilic cells were interpreted as neuroendocrine, although we could not determine their exact nature and could not prove definitely whether we are dealing with one or three cell types.

The cells containing immunoreactive alpha-1-antitrypsin could not be classified as endocrine or exocrine on the basis of current knowledge. The nature of alpha-1-antitrypsin positive cells in the normal intestine remains unclear (Geboes et al. 1982). The presence of this glycoprotein in carcinoids

and its abscence from adenocarcinomas of the intestinal or mucous type (Ray et al. 1982) suggests that it could serve as a marker or neuroendocrine intestinal cells. However, this designation is only tentative since alpha-1-antitrypsin may be found in other cell lines and tumors as well (Dictor 1982). Furthermore, since alpha-1-antitrypsin collocalized with alpha-1-antichymotrypsin in the present tumor, and since alpha-1-antichymotrypsin occurs in adenocarcinomas of the breast (Gendler et al. 1982) it is also possible that the cells immunoreactive with antibodies to this glycoprotein represent exocrine intestinal cells.

The cells containing prominent dilated profiles of rough endoplasmic reticulum and large membrane bound granules, interconnected with one another were considered to represent equivalents of exocrine intestinal cells. CEA and lysozyme immunoreactive cells were also considered most likely as adenocarcinomatous i.e. exocrine. CEA is a good marker of gastrointestinal adenocarcinomas, although it may occur in certain cells of the APUD series most notably thyroid C cells (Kodama et al. 1980). CEA has also been detected in other mixed exocrine-endocrine tumors of the gastrointestinal tract (Tahara et al. 1982a) although at the present time it is not clear whether it marks only exocrine, or both exocrine and endocrine cells. Lysozyme is found in many epithelial cells of the alimentary tract and was detected in 38% of gastric carcinomas (Tahara et al. 1982b).

The cells reacting with antibody to epidermal keratin and showing ultrastructural signs of keratinization were considered as squamous. Our data do not clearly show how these squamous cells arise in the tumor. It is possible that some of the squamous cells were actually exocrine intestinal cells which have undergone squamous metaplasia. On the other hand, it is also possible that these cells represent the end result of divergent differentiation, especially since they were frequently admixed to otherwise completely undifferentiated stem cells.

The present case was reported to illustrate the various cell types found in an otherwise undifferentiated tumor of the colon. There are several possible explanations for the occurrence of these divergently differentiated cells. However, the appearance of the same features in different parts of the tumor and even the intralymphatic tumor clumps and the metastatic foci suggest that the stem cells of this tumor were developmentally pluripotent and could differentiate under appropriate conditions into several cell types normally found in the alimentary tract. Tumors like the one reported in the present paper could thus serve as a source for isolation of malignant human gastrointestinal stem cell like populations. Further cloning of these cells and the study of their differentiation in vitro or in vivo could provide important, if not the definitive answers about the histogenesis of gastrointestinal neuroendocrine tumors and possibly also about the nature and development of the entire gastro-entero-pancreatic neuroendocrine or APUD system (Pearse 1969). Furthermore, tumors like the present one could provide new insight about the significance of the "multidirectional differentiation of human epithelial cancer" (Gould et al. 1981), a concept that deserves further studies because of its biological, as well as clinical implications.

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Note added in proof

As pointed out by Professor Doerr, the first report of a stem cell carcinoma of the intestine showing some of the features of the present case was published by Dietrich (1913). Dietrich A (1913) Kleine Darmcarcinome vom Typus der Carcinoide mit schwerer Lebercarcinose. Frankfurt Z Pathol 13:390–401