Biological and Biochemical Characterization of a Somatostatin-producing Human Carcinoma Established by Heterotransplantation

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ABSTRACT

A somatostatin-producing human carcinoma cell line was established by heterotransplantation into athymic nude mice. The original material, which was derived from a colon tumor of a patient who had previously had bilateral ovarian tumors contained 66 ng extractable somatostatin/g tissue. Somatostatin-producing cells could be identified by immunohistochemistry within the first tumor transplants. Although initially the somatostatin concentration was low (14 ng/g) a progressive increase was observed with each successive transplantation so that after 10 passages it reached a level of 127 ng/g tissue. Analysis of tumor extracts by gel filtration and high-performance liquid chromatography indicated that somatostatin-14 was the only molecular form produced by the original and by the transplanted tumor after multiple passages. This result demonstrates that the tumor has the ability to constitutively express the prosomatostatin gene and to process the primary translation product to somatostatin-14.

INTRODUCTION

The structure of human prosomatostatin may be predicted from the sequence of cloned DNA complementary to preprosomatostatin mRNA isolated from a pancreatic tumor (1). Prosomatostatin is a peptide of 92 residues that is proteolytically processed in a tissue specific manner. In the pancreas and hypothalamus somatostatin-14 is the predominant molecular form but in the gut cleavage at the site of a single arginine residue generates somatostatin-28 as the major form (2). Production of somatostatin-related peptides by tumors has been extensively documented and includes carcinoid tumors of the gut clearance at the site of a single arginine residue generates somatostatin-28 as the major form (2). Production of somatostatin-related peptides by tumors has been extensively documented and includes carcinoid tumors of the fore- and hindgut (3), adenocarcinomas of the duodenum (4, 5, 6) and colon (7), medullary thyroid carcinoma (8, 9), small cell lung tumors (9, 10, 11), pancreatic tumors (12, 13, 14), infiltrating duct carcinomas of the breast (15), adrenal pheochromocytoma (16), thymic carcinoid tumor (11) and pulmonary metastases of melanoma (17). To what extent somatostatin or related peptides play a role as autocrine growth and/or differentiation signals in these tumors is of present not well understood. The investigation of this question has been hampered by the limited availability of stable, well defined tumor cell lines producing the peptide hormones in question. We recently succeeded in the establishment of a somatostatin-producing tumor line by heterotransplantation of a colon tumor. The data presented here provide evidence for the epithelial origin of this tumor and for the stable expression of the somatostatin gene and processing to somatostatin-14. This tumor line represents a model not only for the study of regulation of somatostatin biosynthesis but also for the analysis of the possible influence of somatostatin on the neoplastic properties of the tumor.

MATERIALS AND METHODS

Case Report. The patient was a 58-year-old female. Upon diagnosis of a tumor in both ovaries in 1980, further tumor masses were recognized in the colon ascendens and mesenterium in 1982. Tumor lesions of the skin, liver, colon sigmoideum, and terminal ileum occurred between 1983 and 1984. Total hysterectomy with bilateral salpingo-oophorectomy was performed and the colon ascendens and sigmoideum as well as the terminal ileum were removed. The patient died in August 1984. An autopsy could not be performed.

Determination in a fasted state of somatostatin levels in the patient's blood by radioimmunossay revealed 54 pg/ml. This level rose to 118 pg somatostatin/ml 90 min after ingestion of a 550-kcal mixed meal. This finding already indicated the possibility of an endocrine tumor because the plasma somatostatin levels in healthy fasted subjects range from 3 to 39 pg/ml and the rise after a mixed meal is less than 20 pg/ml (18).

Xenotransplantation. Tumor samples originating from two separate tumor resections were transplanted into athymic nude mice. Sample 1 was obtained in 1982 from a tumor of the colon ascendens and the other one in 1984 from the sigmoideum (sample 2). Fresh biopsy material was transferred in RPMI 1640 medium (Biochrom, Berlin, Federal Republic of Germany) and sliced into small pieces of approximately 50 mg, which were implanted s.c. into 6- to 8-week-old NMRI nu/nu mice of either sex. Locally growing solid tumors developed in all transplanted mice (6 of 6 for each sample). The tumor weight reached 1 to 3 g within 2 to 4 months for samples 1 and 2, respectively. After this period tumor bearing mice were sacrificed and grafts were removed aseptically and transplanted into new animals or processed for further examinations.

Preparation of Tumor Material for Histopathology and Electron Microscopy. For histopathological evaluation by light microscopy the surgical specimens from both ovaries and the intestine were fixed in 4% formalin and initially embedded in paraffin. All slides were routinely stained with hematoxylin and eosin; additionally Grimelius and Fontana staining was performed. After reembedding the remaining tumor tissue in methacrylate semithin sections were prepared and stained with Giemsa and Goldner trichrome. For electron microscopy small pieces of tissue were fixed in 3% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.4, for 3 h, washed twice in 0.1 M cacodylate buffer and postfixed in 1% OsO4 in 0.1 M cacodylate buffer, pH 7.4, and washed again twice in buffer. The tissue was dehydrated in ethanol and embedded in Epon 812.

Immunohistochemistry. After removal the tumor transplants were rapidly frozen-fixed. Frozen sections were stained with the peroxidase-antiperoxidase technique as described previously by Sternberger (19) using rabbit antisomatostatin as primary antibody (Immuno Nuclear Corp., Stillwater, MN). Either normal rabbit serum in place of the immune...
Serum was used as negative control or the sections were incubated with the peroxidase-antiperoxidase substrate without prior application of any sera. Sections were counterstained for 30 s with hematoxylin. Pancreas tissues were used as positive controls.

The intermediate filament type was determined by the method of Osborn and Weber (20) on frozen sections. Mouse anti-human keratin and fluorescein-isothiocyanate-conjugated goat-ant-mouse antibody were a generous gift of M. Osborn (Max-Planck-Institut für Biophysikalische Chemie, Göttingen, Federal Republic of Germany).

Staining for NSE2 was carried out at 4°C for 18 h on ethanol-fixed paraffin sections with rabbit antiserum to NSE (Dako Corp., Denmark) by the peroxidase-antiperoxidase technique.

Serum and tumor tissue homogenate of the patient and of the tumor bearing mice were investigated for presence of CEA by the Abbott-CEA enzyme immunoassay according to the Abbott instruction (Abbott Diagnostic Division, Wiesbaden, Federal Republic of Germany).

**Tumor Extraction.** Tumor tissues were extracted without thawing in 0.5 m acetic acid (5 min at 100°C) followed by Ultratruarx homogenization according to the method of McIntosh et al. (21). The homogenates were centrifuged (20,000 x g for 30 min) and the supernatants were lyophilized. The residue was redissolved in radioimmunoassay buffer and the somatostatin content was measured at appropriate dilution according to the method of Conlon et al. (18) with minor modifications.

Radioimmunoassay. Asomatostatin-like immunoreactivity was measured in tissue extracts and plasma samples after extraction with C18 Sep-pak cartridges by a previously described method (22). Blood (5 ml) was collected into chilled tubes containing EDTA (0.5 ml of a 500 mM solution) and Trasyrol (10,000 K.14) and centrifuged immediately at 4°C for 10 min at 1600 x g. Samples were taken in the fasted state and for 90 min following a 550-kcal hospital breakfast.

HPLC. Peptides were isolated from extracts of tumor tissue taken from the patient and from the nude mouse (passage 6) using C18 Sep-pak cartridges as described previously (22). The effluent from the cartridge (40% acetonitrile) was lyophilized and redissolved in 0.1% v/v trifluoroacetic acid. After filtration (0.4 μm membrane; Schleicher and Schüll, Dassel, Federal Republic of Germany), the solution was injected into a Waters Radial-PACK C18μBondapak cartridge (10 μm). The cartridge was eluted at a flow rate of 2 ml/min with a linear gradient (total volume, 120 ml) formed from water:trifluoroacetic acid (99.1:0.1) and acetonitrile:water:trifluoroacetic acid (50.9:50:0.1). After 60 min the acetonitrile concentration was raised to 40% v/v. Fractions (2 ml) were collected and assayed at a dilution of 1:50.

**Gel Filtration.** After purification by HPLC the tumor somatostatin was chromatographed on a column (90 x 1.6 cm) Bio-Gel P-10 equilibrated with 1 M formic acid. The column was eluted at 4°C and a flow ratio of 10 ml/h. Fractions (2.15 ml) were collected, lyophilized (Savant SpeedVac), and reconstituted in radioimmunoassay buffer.

**RESULTS**

**Histopathological, Electron Microscopic, and Immunohistochemical Findings.** On macroscopic examination of the removed ovaries both were enlarged (size of the left ovary, 9 x 8 x 5 cm; size of the right ovary, 3.5 x 2.5 x 1.0 cm) and exhibited a firm yellowish-white cut surface. The partially resected colon and ileum showed multifocal, well-circumscribed, nodular tumor infiltrations. Multiple tumor nodules (diameter up to 8 cm) were present within the mesentery.

Histologically the tumor represented a neuroendocrine carcinoma. The morphology of the tumor from both ovaries and of the intestinal tumors was largely identical. The polygonal tumor cells were arranged in broad sheets or cords as well as in nests of varying size with scanty intervening fibrous stroma (Fig. 1, a-
The immunoreactive material taken from both the patient and the nude mouse were each eluted from the Bio-Gel P-10 column as a single peak with the elution volume of somatostatin-14. Data from a quantitative analysis of the somatostatin content of tumor tissue derived from the patient and from various tumor passages after heterotransplantation into nude mice are summarized in Table 1. They show that the somatostatin content of extractable somatostatin in the tumor tissue increased about 2-fold over the concentration in the original tissue from the patient and about 9-fold from mouse passage 1 to 10, indicating positive selection of the somatostatin-producing cells. This result supports the suggestion (24) that the nude mouse system can be used for the selection of neoplastic subpopulations from heterogeneous xenogenic neoplasms.

Propagation of human endocrine tumors in the athymic nude mouse has been described previously and examples include renal cell carcinomas (25) and malignant melanomas (17). In the latter study four of eight melanomas passed in nude mice had hormonal activity as detected by qualitative immunostaining for serotonin, somatostatin, and leu-enkephalin. An increase in the intensity of immunostaining for somatostatin observed after seven passages in the mouse was taken as an indication for the selection of a producer cell type. The quantitative analysis of somatostatin production by the tumor cells described here now provide direct evidence for such selection processes. As another tumor sample obtained from the same patient at a later date of tumor resection showed high somatostatin levels already after the second animal passage, the question can be raised whether the elevated somatostatin production is the cause or consequence of the malignant properties of this particular tumor. The establishment of a transplantable tumor which stably produces one and probably more peptide hormones provides now a model for the analysis of the role of somatostatin as an autocrine regulator of growth and/or differentiation of tumor cells in vivo. Furthermore as the *in vitro* culture of this tumor has now been achieved*3 and adaptation to growth under serum-free culture conditions is in progress, it will soon be possible to use this cell line for studies on the regulation of somatostatin biosynthesis.

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REFERENCES


* K. Vehmeyer, unpublished observations.


Fig. 1. a, semithin section of the ovarian tumor showing broad sheets of tumor cells and nests of varying size with scanty intervening fibrous stroma. Interspersed are some multinucleated tumor giant cells. Goldner trichrome, × 75; b, higher magnification of the tumor shown in a. Note relatively uniform predominantly medium-sized nuclei with a coarse chromatin pattern and prominent nucleoli and the abundant cytoplasm. Semithin section; Goldner trichrome, × 190; c, strongly argyrophilic tumor cells around a small blood vessel. Paraffin section; Grimelius stain, × 400.
Fig. 2. a, semithin section of the intestinal tumor consisting of moderately atypical cells and some interspersed multinucleated giant cells. Goldner trichrome, × 190; b, semithin section of a colon tumor showing marked cellular atypia with numerous multinucleated tumor giant cells and atypical mitoses. Goldner trichrome, × 400; c, semithin section of a transplanted tumor exhibiting the original histological and cytological pattern. Goldner trichrome, × 400.
Fig. 3. a, electron micrograph of transplanted tumor showing irregularly shaped nuclei and a few cytoplasmic granules of medium density. Bar, 5 μ, x 3330; b, electron micrograph of transplanted tumor cells with a prominent endoplasmic reticulum and low density granules of maximum 600 nm diameter. Bar, 1 μ, x 18,000.
Fig. 4. a, transplanted tumor positively stained with antibodies to cytokeratin. Frozen section; left, × 190; right, × 400. b, positive immunostaining of the transplanted tumor with antibodies to neuron-specific enolase. Paraffin section; peroxidase-antiperoxidase, × 400. c, transplanted tumor showing positive immunostaining with antisomatostatin. Frozen section; peroxidase-antiperoxidase, × 400.
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