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Animal Model

A Transplantable Human Carcinoid as Model for Somatostatin Receptor-Mediated and Amine Transporter-Mediated Radionuclide Uptake

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A human midgut carcinoid tumor was successfully transplanted into nude mice and propagated for five consecutive generations (30 months) with well-preserved phenotype. Tumor cells in nude mice expressed identical neuroendocrine markers as the original tumor, including somatostatin receptors (somatostatin receptors 1 to 5) and vesicular monoamine transporters (VMAT1 and VMAT2). Because of the expression of somatostatin receptors and VMAT1 and VMAT2 the grafted tumors could be visualized scintigraphically using the somatostatin analogue ^{111}In-octreotide and the catecholamine analogue ^{123}I-metaiodobenzylguanidine. The biokinetics of the somatostatin analogue ^{111}In-octreotide in the tumors was studied and showed a high retention 7 days after administration. Cell cultures were re-established from transplanted tumors. Immunocytochemical and ultrastructural studies confirmed the neuroendocrine differentiation. The human origin of transplanted tumor cells was confirmed by cytogenetic and fluorescence *in situ* hybridization analyses. Spontaneous secretion of serotonin and its metabolite, 5-hydroxyindole acetic acid, from tumor cells was demonstrated. The tumor cells increased their $[Ca^{2+}]_i$ in response to β-adrenoceptor stimulation (isoproterenol) and K^+-depolarization. All somatostatin receptor subtypes could be demonstrated in cultured cells. This human transplantable carcinoid tumor, designated GOT1, grafted to nude mice, will give unique possibilities for studies of somatostatin receptor- and VMAT-mediated radionuclide uptake as well as for studies of secretory mechanisms. *(Am J Pathol 2001, 158:745–755)*

Midgut carcinoid tumors are derived from the enterochromaffin cells in the small intestine. In metastatic disease these tumors can give rise to severe hormonal symptoms because of excessive production of serotonin (5-HT) and tachykinins (substance P and neurokinins). Carcinoid tumors express numerous somatostatin receptors that can be used to alleviate hormonal symptoms by treatment with the long-acting somatostatin analogue octreotide. These receptors have also been used for visualization of tumors by scintigraphy and in receptor-guided surgery and may serve as targets for radionuclide therapy.[1] Studies of receptor mechanisms and secretory processes in these tumors have been hampered by the lack of suitable experimental models. Heterografting of human carcinoid tumors into privileged sites, eg, the anterior eye-chamber of immunosuppressed rats, has been tried but is restricted to the study of very small tissue pieces.[2] Midgut carcinoids can also be studied in primary cell cultures, but tumor cells proliferate slowly and survive only for limited periods of time.[3–6] Cell lines are ideal for studies of receptor mechanisms and secretory mechanisms, however, few carcinoid tumor cell lines are available today (ie, KRJ-I and BON).[7,8] The most widely used cell line, BON, was derived from a lymph node metastasis of a highly malignant pancreatic carcinoid. BON cells have been used to investigate responses to secretagogues and cytotoxic agents and express both epithelial markers (cytokeratin) and multiple amines and peptides [5-HT, chromogranin A (CgA), neurotensin, and pancreastatin].

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In this report we present a transplantable human midgut carcinoid tumor that can be propagated in nude mice for extended periods of time with well-preserved neuroendocrine phenotype, including expression of somatostatin receptors and VMAT1 and VMAT2.

Materials and Methods

Case Report

A 55-year old woman presented with a severe midgut carcinoid syndrome associated with elevated urinary levels of 5-HIAA (630 μmol/24 hours; reference <50). Computed tomography, octreotide scintigraphy, and bone scintigraphy demonstrated metastatic spread to the liver, paraaortic and mediastinal lymph nodes, and to the skeleton. At surgery, tumor biopsies were obtained. Because the tumor burden was large, hepatic embolization therapy was considered to be a high-risk procedure so this patient was given systemic radionuclide therapy with ^{111}In-labeled octreotide.[9]

Tumor Cell Culture

Tumor material from one liver metastasis was obtained at surgery. Primary cell cultures were prepared as previously described.[10] In one of these primary cultures a markedly increased proliferation rate was noted after 3 months. These cells (referred to as GOT1 cells) were subsequently cultured in quantities to enable heterotransplantation to nude mice. The studies of secretory mechanisms and Ca^{2+} channel signaling were performed in cell cultures identically prepared from a tumor of the second generation from nude mice.

Transplantation of Tumor Cells to Nude Mice

For transplantation of tumor cells we used male BALB/cABom-nu mice, 3 to 4 weeks of age (Bomholtgaard, Ry, Denmark). The animal experiments were approved by the Ethical Committee for Animal Experiments, University of Göteborg.

Tumor Generation I

Tumor cells from cultures were harvested and grafted to 10 nude mice. Approximately 20 million tumor cells were injected subcutaneously into the back of the neck of each animal. When tumors became visible their size was estimated once a week after measurement with a caliper. Eight animals could be followed 6 to 12 months. At sacrifice, blood was collected and the tumor tissues were harvested.

Tumor Generation II to V

From the first generation of tumors new tumors were propagated by subcutaneous transplantation of two pieces of tumor tissue (1 × 1 × 2 mm each) per animal into 20 to 100 animals per generation. To date, five consecutive generations of tumors have been generated. In the fifth generation, the tumor growth rate *in vivo* was again estimated during a period of 3.5 months.

Cytogenetic and Fluorescence in Situ *Hybridization (FISH) Analyses*

Chromosome preparations were made from tumor cells obtained from a tumor of generation V. Cells were harvested after overnight exposure to colcemid followed by hypotonic treatment and fixation in methanol:acetic acid.[11] Slides were subsequently G-banded and analyzed according to the guidelines of the International System for Human Cytogenetic Nomenclature.[12]

FISH analysis was performed on touch preparations prepared from a tumor of generation V as well as on unbanded metaphase chromosomes using whole chromosome painting probes for the human X chromosome and chromosome 1 (wcp X and 1; Vysis, Downers Grove, IL). The probes were labeled with Spectrum Green (X chromosome) and Spectrum Orange (chromosome 1) fluorophores. The conditions for hybridization and posthybridization washes were as recommended by the manufacturer. Nuclei and chromosomes were counterstained in blue with 4,6-diamino-2-phenylindole. Slides were examined in a Zeiss Axiophot epifluorescence microscope equipped with the appropriate filter sets. Fluorescence signals were digitalized, processed, and analyzed using the ProbeMaster FISH image analysis system (Perceptive Scientific International, Chester, England).

Binding and Scintigraphy of Radiolabeled Octreotide and ^{123}I-Metaiodobenzylguanidine (MIBG)

The binding of the radiolabeled somatostatin analogue octreotide was studied on nude mice transplanted with tumors of generation I, II, and IV. D-Phe^1-octreotide was labeled with ^{111}In mainly according to the manufacturer (Mallinckrodt Medical B.V., Petten, The Netherlands). The fraction of peptide-bound ^{111}In exceeded 99%. Each animal was injected intravenously with 0.25 to 4 MBq (0.1 μg) of ^{111}In-octreotide 4 hours before killing. Tumors and blood samples were collected and weighed, and the ^{111}In activity was measured in a γ counter. The activity concentration of the radionuclide was expressed as the fraction of injected activity per unit mass of the tissue (%IA/g) and the tumor-to-blood activity concentration ratio, T/B, was determined.[13] Before killing scintigraphy was performed with a γ camera.

The binding and scintigraphy of MIBG (10 to 12 MBq) was studied in eight nude mice transplanted with tumors of generation IV at 4 hours (n = 2), 24 hours (n = 3), and 48 hours (n = 3) after injection.

Biokinetics of Radiolabeled Octreotide

Each animal was injected into a tail vein with 2 MBq (0.1 μg) of ^{111}In-octreotide. The ^{111}In activity in the syringe

Table 1. Primary Antibodies Used for Immunocytochemistry

Antibody	Dilution (immuno-fluorescence)	Dilution (immuno-peroxidase, primary)	Dilution (immuno-peroxidase, nude mice)	Clone	Catalog no.	Company
Anti-human cytokeratin (mc)	1:50	1:50	1:50	MNF 116	M 821	DAKO, Glostrup, Denmark
Anti-E-Cadherin (mc)	1:20	1:20	—	36	C20820	Transduction Laboratories, Lexington, KY
Anti-E-Cadherin (human) (mc)	1:200	—	1:1000	HECD-1	13-1700	Zymed Laboratories, Inc., San Francisco, CA
NCAM (mc)	1:50	1:50	1:20	ERIC1	sc-106	Santa Cruz Biotechnology, Inc., Santa Cruz, CA
VMAT1 (pc)		—	1:500		sc-7718	Santa Cruz Biotechnology, Inc., Santa Cruz, CA
VMAT1 (pc)	1:100	1:10000				Gift from Jefferey D. Erickson, Ph.D., NIH, Maryland
VMAT2 (pc)	1:100	1:10000	—			Jefferey D. Erickson
VMAT2 (pc)	—		1:500		AB1767	Chemicon, Temecula, CA
CgA (mc)	1:1000	—	1:1000	LK2H10	1199021	Boehringer Mannheim, Mannheim, Germany
Synaptophysin (mc)	1:10	1:10	1:10	SY38	M0776	DAKO, Glostrup, Denmark
Vimentin (mc)	1:10	1:40	1:400	V9	M0725	DAKO, Glostrup, Denmark
Serotonin (mc)	1:20	—	1:20	H209	M758	DAKO, Glostrup, Denmark
SV2 (mc)	1:250	1:250	1:500			Gift from Reinhardt Jahn, Buckley Kelly
Substance P (pc)	1:200	1:200	—		RPN.1572	Amersham, Buckinghamshire, UK
Substance P (pc)	—	—	1:400		MAB356	Chemicon, Temecula, CA
Neurofilament	—	—	1:100	2F11	M0762	DAKO, Glostrup, Denmark

mc, monoclonal; pc, polyclonal; —, not incubated.

was measured before and after injection of the radiopharmaceutical in a well-type ionization chamber to obtain the injected activity for each animal. These animals were killed 0.5 hours ($n = 5$), 24 hours ($n = 5$), 3 days ($n = 5$), and 7 days ($n = 5$) after injection. The ^{111}In activity in tumor, blood, and whole body was measured using a Wallace 1480 γ counter (WIZARD 3"; Wallace, Oy, Finland). The ^{111}In-activity concentration in the tissue was expressed as the fraction of the injected activity per unit mass of the tissue (%IA/g).

Assays of 5-HTP, 5-HT, and 5-HIAA

The 5-HT precursor 5-hydroxytryptophan (5-HTP), serotonin (5-HT), and the main metabolite 5-hydroxyindole acetic acid (5-HIAA) in conditioned culture media were determined by reverse-phase HPLC with electrochemical detection.[6] For measurement of 5-HTP, 5-HT, and 5-HIAA in nude mice, whole blood was lysated in sterile water. Proteins were denatured with $ZnSO_4$ and NaOH. The blood samples were thereafter centrifuged at 3,500 $\times$ *g*

Table 2. Immunocytochemical Characterisation of the Transplantable Midgut Carcinoid

Antigen	Ileal primary	Primary cell culture*	Tumors transplanted to nude mice: Tumor generation I	Tumor generation II	Tumor generation III	Tumor generation IV
Serotonin	+++	+	+	+	++	+
Substance P	+++	+	+	−	++	+
Chromogranin A	+++	+	+++	+++	+++	+++
Synaptophysin	+++	+	+++	+++	++	++
SV2	+++	+	+++	+++	+++	+++
VMAT1	+++	+	+++	++	++	+++
VMAT2	++	+	+++	+++	+++	+++
NCAM(ERIC-1)	++	+	+++	+++	+++	+++
ECAD	+++	+	+++	+++	+++	+++
Cytokeratin (MNF116)	+++	+	+++	+++	+++	+++
Vimentin	−	−	−	−	−	−
Neurofilament	−	−	−	−	−	−

Findings on the original ileal carcinoid and primary cell culture, before transplantation, is given for comparison.
+, 1–25% of tumor cells positive; ++, 25–75% of tumor cells positive; +++, 75–100% of tumor cells positive.
*, Only +/− scale was applied.

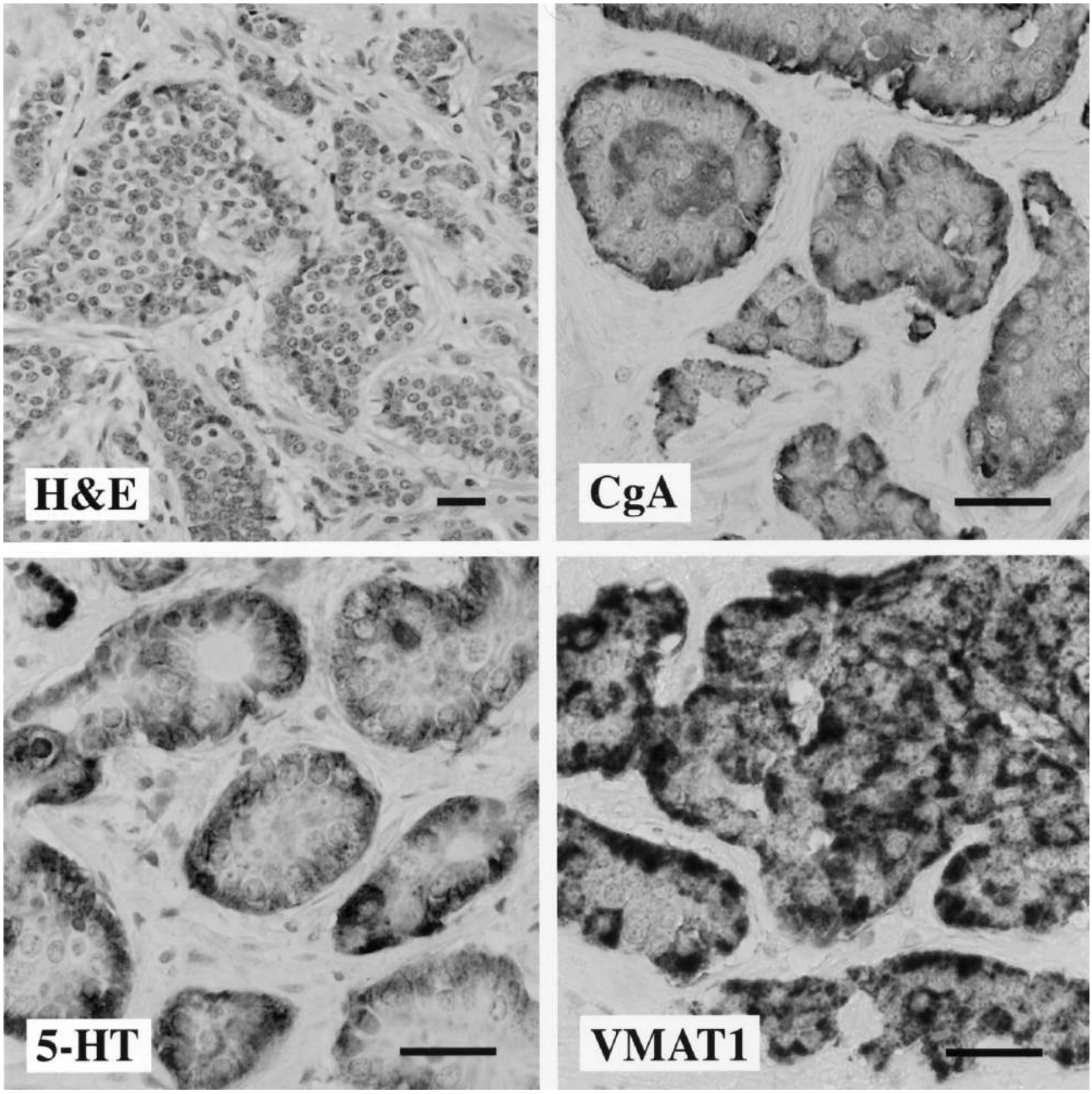

Figure 1. Morphology of the primary ileal carcinoid. Tumor cells grow in an insular pattern with abundant eosinophilic cytoplasm. A positive immunocytochemical reaction for CgA, serotonin (5-HT), and VMAT1 is observed in a majority of tumor cells. Scale bars, 30 μm.

for 30 minutes and aliquots (20 μl) of the supernatant were injected onto the column.

Immunocytochemistry

Tumor tissues (primary tumor and tumors from nude mice) were fixed in buffered formalin for 4 to 24 hours and embedded in paraffin wax. Sections were incubated with primary antibodies overnight (Table 1). Bound antibodies were visualized by an indirect immunoperoxidase technique. Cultures were fixed in 4% paraformaldehyde in phosphate-buffered saline at pH 7.4 for 4 hours and incubated for indirect immunofluorescence using primary antibodies listed in Table 1. Control cultures were incubated identically, except for the primary antibodies, which were replaced by normal rabbit serum/mouse IgG. Cultures were examined with confocal laser scanning microscopy using a Zeiss Laser Scanning Microscope (LSM 410).[5]

Electron Microscopy

Tumor tissue was fixed in 2.5% glutaraldehyde in 0.1 mol/L sodium cacodylate buffer (pH 7.2) for 24 to 48 hours and postfixed for 1 hour in 1% OsO_4 in the same buffer. After dehydration the specimens were embedded

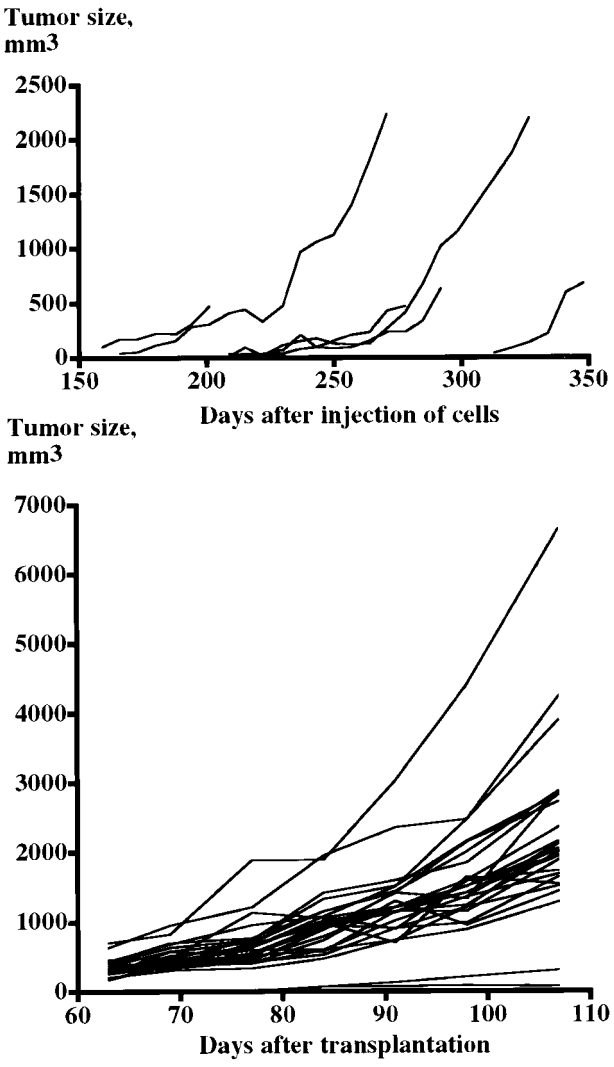

Figure 2. Diagrams showing the increase in tumor size throughout time in tumor generation I (**top**, $n = 6$) and tumor generation V (**bottom**, $n = 27$). The doubling times were 17.5 ± 2.2 days (range, 8.9 to 22.7 days) in tumor generation I and 15.6 ± 0.6 days (range, 5.4 to 21.5 days) in tumor generation V.

in epoxy resin (TAAB 812). Ultrathin sections were contrasted with uranyl acetate and lead citrate and examined in a Philips CM 12 electron microscope.

Northern Analysis

Cells from primary cultures and tumor tissues from nude mice were harvested and total RNA was prepared by acid guanidinum thiocyanate-phenol-chloroform extraction.[14] Electrophoresis and hybridization with ^{32}P-labeled antisense RNA probes for the five somatostatin receptor subtypes was performed as previously described.[15] As performed this analysis gave quantitative information of the expression of somatostatin receptor subtypes.

Intracellular Calcium Determinations

Cells attached to coverslips were washed and loaded for 30 minutes with FURA-2Am (1 μmol/L; Sigma) in the presence of 0.01% pluronic F-127 (Sigma) in a modified HEPES medium supplemented with 11.1 mmol/L glucose as previously described.[16] After loading, the cells were washed and the coverslips mounted in a temperature-controlled (37°C) chamber (volume, 110 μl) placed over a 100 X Fluor objective (Nikon, Tokyo, Japan) on the stage of an inverted Nikon microscope (DIAPHOT-TMD); 75 W xenon lamp. The cells were superinfused (flow rate of 1 ml/min) in the HEPES medium supplemented with 11.1 mol/L glucose and 0.5% bovine serum albumin to which was added isoproterenol, carbachol, or KCl (all from Sigma) according to the experimental protocol. The fluorescence of FURA-2 was recorded with dual wavelength excitation spectrophotofluorometry (emission wavelength, 510 nm; excitation wavelengths, 350 and 380 nm) and the $[Ca^{2+}]_i$ was calculated according to Grynkiewicz and colleagues.[17]

Statistical Analysis

The tumor size doubling time, 5-HT concentration in peripheral blood of nude mice, ^{111}In-octreotide and ^{123}I-MIBG concentration in tumor tissues, and $[Ca^{2+}]_i$ are given as mean ± SEM. For statistical analysis the Students *t*-test was used.

Results

Properties of the Primary Ileal Carcinoid

The primary tumor was a single lesion in the distal ileum measuring 2.5 cm in diameter. Light microscopic appearance was that of a typical ileal carcinoid with predominantly insular growth pattern. Immunocytochemical analysis demonstrated 5-HT- and substance P-production by tumor cells. The majority of tumor cells were positive for VMAT1 and VMAT2. General markers for neuroendocrine differentiation were also strongly positive (CgA, SV2, and NCAM) (Figure 1 and Table 2).

Properties of Tumor Cells before Transplantation

The epithelial phenotype was confirmed by positive immunocytochemical reactions for cytokeratin and E-cadherin, and negative reactions for vimentin and neurofilament. Immunocytochemical analysis demonstrated a strong expression of several neuroendocrine markers. A strong positive reaction was obtained for 5-HT, substance P, and for VMAT1 and VMAT2. In the cytoplasm of tumor cells SV2 immunoreactivity was abundant, whereas NCAM was located on the plasma membrane (Table 2). Electron microscopy showed numerous secretory granules in the cytoplasm of tumor cells. These granules had the appearance typical for midgut carcinoids, ie, pleiomorphic, dense-core granules, measuring 100 to 400 nm in diameter.

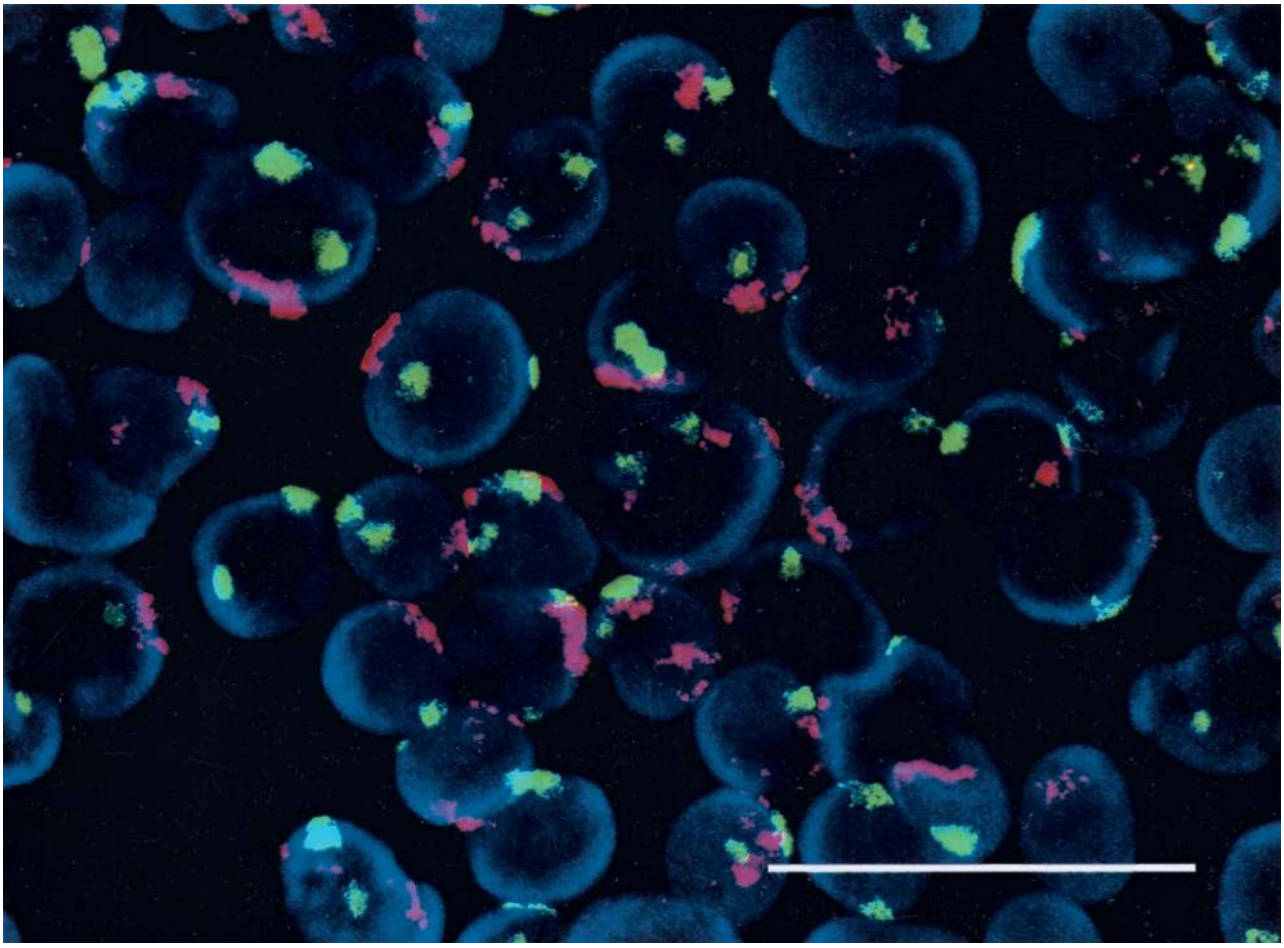

Figure 3. FISH analysis of a touch preparation made from a transplanted tumor of generation V. Hybridization with wcp probes specific for the X chromosome (green signal) and chromosome 1 (red signal) reveals human chromosome-specific signals in the vast majority of cell nuclei (blue staining). Scale bar, 30 μm.

Growth of Tumor Cells in Nude Mice

Tumor Generation I

Out of the 10 injected animals, eight were followed for 6 to 12 months after grafting of tumor cells. One animal was lost at transplantation and one was sacrificed before development of tumors, because of infectious complications. Gross tumors developed in 6 of 8 animals (take rate = 75%). The first tumors were observed 6 months after tumor cell injection. The doubling time for these tumors was estimated to 17.5 ± 2.2 days (n = 6; range, 8.9 to 22.7 days) (Figure 2).

Tumor Generations II to V

In these generations the first tumors were observed already after 4 to 6 weeks. The take rates were 67% in generation II, 89% in generation III, 98% in generation IV, and 100% in generation V. The estimated doubling time in generation V was 15.6 ± 0.6 days (n = 31; range, 5.4 to 21.5) (Figure 2). The tumors have now been continuously propagated for 30 months in nude mice.

The Transplanted GOT1 Tumor Cells Are of Human Origin

FISH analysis of the touch preparation revealed that >95% of the cells analyzed were of human origin. Hybridization with human painting probes showed that the majority of nuclei contained two X chromosome-specific and two chromosome 1-specific signals (Figure 3). The few cells that were negative for the human wcp probes had generally larger nuclei than did those that were positive for the probes.

Cytogenetic analysis of a 17-hour culture confirmed that >95% of the metaphases had a human chromosome complement. The chromosome counts of all but one of the 46 metaphases analyzed were in the hypodiploid region with a modal number of 44 chromosomes (range, 38 to 45 chromosomes). All metaphases had clonal structural rearrangements including several marker chromosomes. No cell with a normal human karyotype was observed. Only a small percentage (<5%) of cells had mouse karyotype.

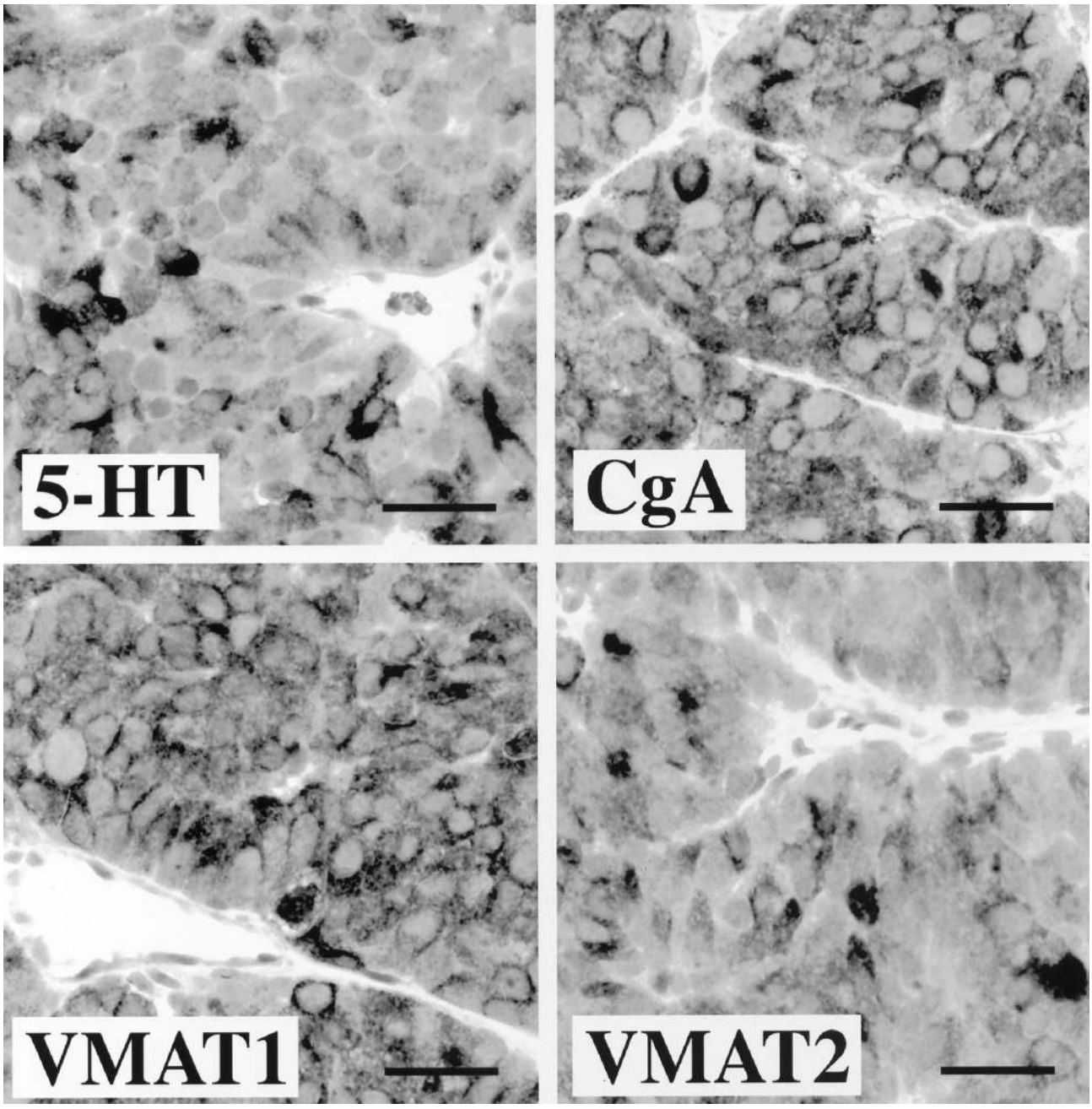

Figure 4. Immunocytochemical characterization of carcinoid tumors grown in nude mice. Tumor cells are positive for 5-HT, CgA, and VMAT1 and VMAT2. The immunohistochemical profile in grafted tumor cells was the same as in the primary midgut carcinoid tumor. Scale bars, 30 μm.

Immunocytochemical Properties and Amine Production of Transplanted Tumors

Microscopic examination of tumors demonstrated a characteristic pattern of insular growth with tumor cells positive for CgA, 5-HT, VMAT1, and VMAT2 (Figure 4). One tumor from each of the first four generations was examined. The neuroendocrine differentiation was well maintained over the four generations (Table 2). Electron microscopy confirmed presence of numerous electron-dense granules in the cytoplasm of the tumor cells.

The average 5-HT concentration in peripheral blood of nude mice with generation II tumors was 5,103 ± 795 μmol/L ($n = 7$) *versus* 1,784 ± 225 μmol/L ($n = 8$) in animals without tumor ($P < 0.001$).

Somatostatin Receptors in Transplanted Tumors

Northern analysis revealed expression of all five somatostatin receptor subtypes in tumor cells from the primary cell culture. The somatostatin receptor expression was also investigated in tumors from nude mice and all five somatostatin receptor subtypes were expressed in all tumor generations investigated (generations I, II, and III).

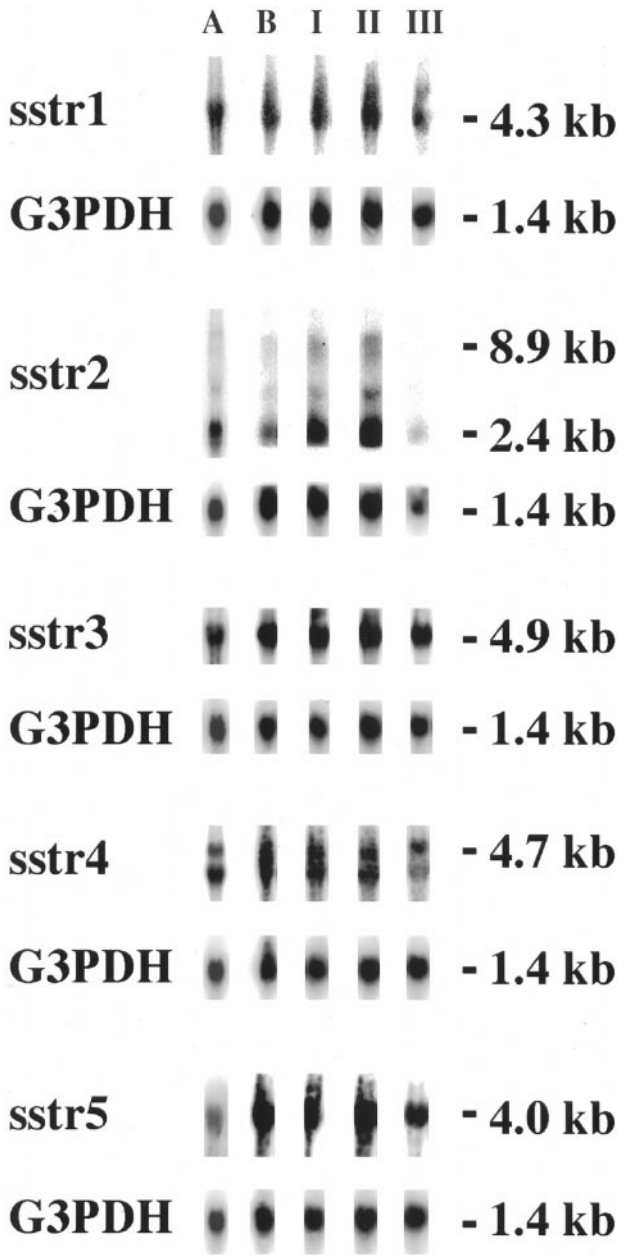

Figure 5. Quantitative analysis of somatostatin receptor expression. Northern analysis was performed on carcinoid tumor cells in primary culture before transplantation (**A**), tumor cells in cell culture derived from a transplanted tumor from nude mice (**B**) and tumors from generation I to III (I, II, and III). All five somatostatin receptor subtypes were expressed by the tumor cells in culture as well as tumors in nude mice. The expression of somatostatin receptors in the three generations was identical except for somatostatin receptor 2 in generation III, which was not detectable. G3PDH was used as housekeeping gene to confirm the integrity of the blotted mRNA.

The only exception was somatostatin receptor 2, which was not detectable in generation III. The transcript sizes were as expected (Figure 5).

Binding and Scintigraphy of Radiolabeled Octreotide and MIBG

The concentration of ^{111}In-octreotide in tumor at 4 hours after injection was very high for tumor generation I with 13% IA/g (n = 1), and lower values for generation II with 0.87 ± 0.32% IA/g (n = 3) and generation IV with 0.40 ± 0.08% IA/g (n = 10). The corresponding T/B values were 250, 63 ± 39, and 20 ± 4. Scintigraphy of the nude mice with tumor generation I showed high uptake in the tumor (Figure 6), but also uptake/distribution of the radionuclide in the kidneys and urinary bladder.

The concentration of ^{123}I-MIBG in tumor tissue of generation IV was 0.93 ± 0.06% IA/g (n = 2) at 4 hours, 1.1 ± 0.1% IA/g (n = 3) at 24 hours, and 0.54 ± 0.08% IA/g (n = 3) at 48 hours after injection. The corresponding T/B values were 12 ± 2, 28 ± 5, and 83 ± 13. Scintigraphy at 24 hours showed high uptake in the tumor, but also some uptake/distribution of the radionuclide in the salivary glands and urinary bladder (Figure 6).

Biokinetics of Radiolabeled Octreotide

The tumors accumulated ^{111}In after intravenous injection of ^{111}In-octreotide. After a rapid release during the first 24 hours the decline in ^{111}In-activity concentration was very slow for the tumor whereas it was considerably faster for blood and whole body. Seven days after the injection 17% of the initial ^{111}In-activity concentration still remained in the tumors *versus* 0.05% and 3.3% in blood and whole body, respectively (Figure 7). The concentration of ^{111}In-activity in tumor tissue was 1.25 ± 0.14% IA/g (n = 5) at 0.5 hours, 0.33 ± 0.09% IA/g (n = 5) at 24 hours, 0.29 ± 0.02% IA/g (n = 5) at 3 days, and 0.22 ± 0.06 (n = 5) at 7 days after injection.

Cell Cultures Generated from Transplanted Tumor (Generation II)

Spontaneous Secretion of 5-HTP, 5HT, and 5-HIAA by Tumor Cells

The spontaneous secretion of 5-HTP, 5HT, and 5-HIAA into culture medium was followed up to 72 hours after change of culture medium. 5-HT levels started to rise 4 hours after the change of medium and was elevated eightfold at 72 hours. The medium levels of 5-HTP and 5-HIAA started to rise at 24 hours after change of medium with very high levels of 5-HIAA (Figure 8).

Intracellular Calcium Determinations

The baseline $[Ca^{2+}]_i$ was 37 ± 4 μmol/L (n = 4). Isoproterenol (10 μmol/L) slightly increased $[Ca^{2+}]_i$, whereas carbachol (100 μmol/L) had no additional effect. Depolarization by KCl (20 mmol/L) rapidly increased $[Ca^{2+}]_i$ in a reversible manner (Figure 9).

Discussion

Increased understanding of the biology of carcinoid tumor cells and development of new approaches for diagnostic and therapeutic attempts require reliable human cell lines. In this study we present a human midgut car-

cinoid tumor that was transplanted to nude mice and successfully propagated for five consecutive generations with well-preserved neuroendocrine differentiation. The human origin of the cells was confirmed by cytogenetic and FISH analyses. Touch preparations from generation V showed that ≥95% of the cells were of human origin using painting probes for the human X chromosome and chromosome 1. Cytogenetic analysis revealed that the vast majority of metaphases had a human chromosome complement. The tumor had a hypodiploid stemline with several clonal structural and numerical abnormalities.

The neuroendocrine features of the transplanted tumors were those of a well-differentiated endocrine tumor, ie, strongly immunoreactive for general endocrine markers such as CgA and synaptophysin. Furthermore, the tumor cells expressed markers characteristic for midgut carcinoids, eg, 5-HT. Comparison of the immunohistochemical profiles and ultrastructural features confirmed a high degree of similarity between the original primary carcinoid and the transplanted tumors of all generations. ^{111}In-octreotide showed high uptake of the radionuclide and high T/B values in the tumors and Northern blot analysis showed expression of all five somatostatin receptor subtypes in the tumors. These tumors can therefore be used for diagnostic visualization by octreotide scintigraphy and also as a model for studies of somatostatin receptor-mediated radiation therapy. Quantitative analysis of the somatostatin receptor expression levels needs to be performed to give further information about the mechanism for ^{111}In-octreotide uptake. To date biokinetic studies of radiolabeled somatostatin analogues in animal models have been restricted to somatostatin receptor-expressing exocrine pancreatic tumors, ie, CA20948 and AR4-2J.[18,19] The strong accumulation and slow release of ^{111}In in the transplanted tumors of our model closely resembles the biokinetics of ^{111}In of the original midgut carcinoid tumor, demonstrating the relevance of this new model.[20] For therapeutic purposes it may be of interest to modulate somatostatin receptor expression, eg, selective up-regulation of somatostatin receptor 2 for optimal binding to octreotide, which today is the analogue most widely used for targeted radionuclide therapy. Such receptor up-regulation can probably be induced by steroid hormones.[21]

The uptake of radiolabeled MIBG has been shown to be high in pheochromocytomas, expressing both VMAT1 and VMAT2, but reduced to one third in midgut carcinoids, which often lack expression of VMAT2.[22] *In vitro* experiments have shown that MIBG may act as a substrate for chromaffin granules.[23] VMAT2 may thus be important for MIBG transport and tumor visualization. In this model we found that the transplanted tumors, cultured tumor cells and the original primary ileal carcinoid expressed both VMAT1 and VMAT2. ^{123}I-MIBG scintigraphy visualized the tumors in nude mice and determination of the radionuclide concentrations in tumor tissue and blood showed very high T/B, which clearly indicates that radiolabeled MIBG may be used therapeutically in GOT1 tumors.

In cell cultures very high levels of the main 5-HT metabolite, 5-HIAA, were found. Because midgut carcinoid tumor cells contain both the deaminating enzymes monoamine oxidase A and B, the high 5-HIAA/5-HT ratio indicates a high turnover of 5-HT and complete intracellular metabolism with rapid release of the metabolite into the culture medium.[6]

This study further shows that the tumor cells have mechanisms for homeostatic regulation of $[Ca^{2+}]_i$, which are similar to other neuroendocrine cells, eg, endocrine cells of the normal pancreatic islets[24] and chromaffin PC12 cells[25] in regard to baseline $[Ca^{2+}]_i$ and the $[Ca^{2+}]_i$ responses to isoproterenol, which is explained by opening of plasma membrane Ca^{2+} channels through activation of cyclic AMP and protein kinase A[25,26] and K^+ depolarization. In contrast, the

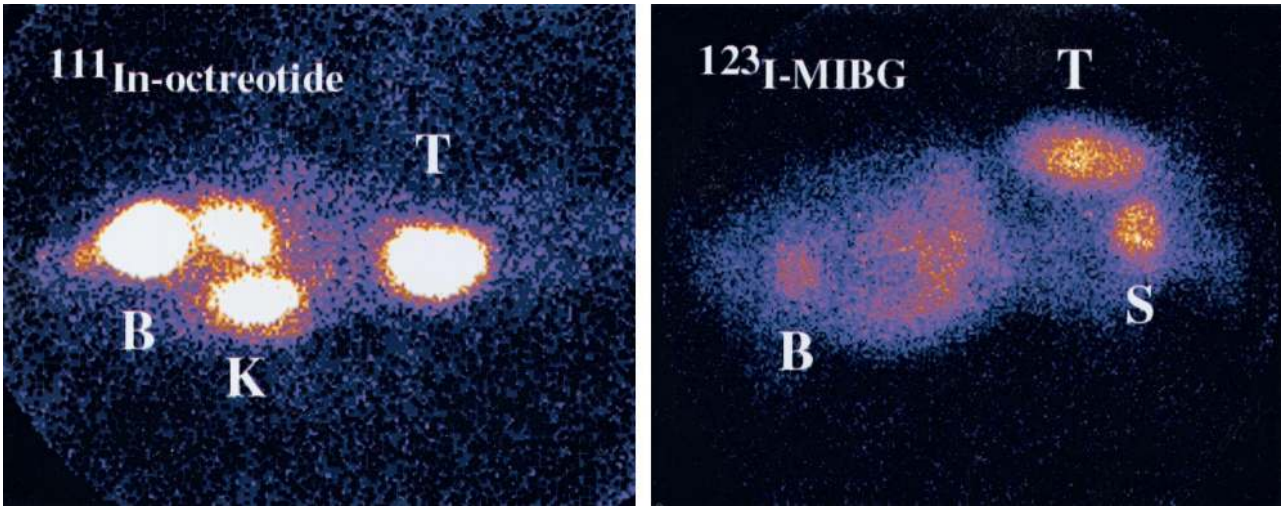

Figure 6. Octreotide scintigraphy (**left**) of a nude mouse with a carcinoid tumor located in the back of the neck (anteroposterior view). The mouse was examined in a γ camera 10 minutes after injection of ^{111}In-octreotide. Uptake in the tumor (T) is indicated as well as uptake in the kidneys (K) and the urinary bladder (B). MIBG scintigraphy (**right**) of a nude mouse with a carcinoid tumor growing in the back of the neck (lateral view). The mouse was examined in a γ camera for 30 minutes 24 hours after the injection of ^{123}I-MIBG. Uptake in the tumor (T) is indicated as well as uptake in the kidneys (K) and the urinary bladder (B).

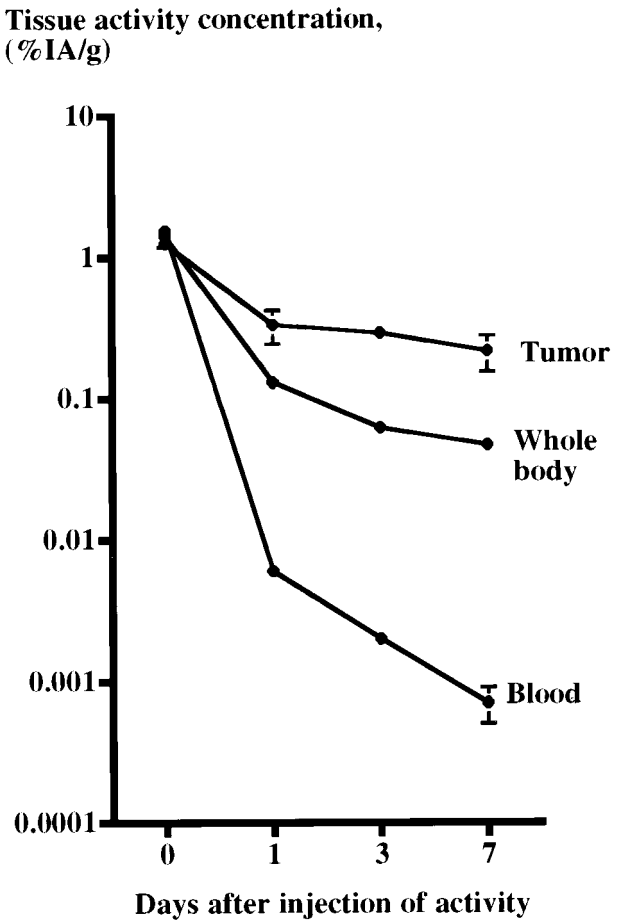

Figure 7. ^{111}In-activity concentration (%IA/g) in tumors transplanted to nude mice, blood, and whole body. After a rapid decline during the first 24 hours the decline in ^{111}In-activity concentration was very slow for the tumor. Still 7 days after the injection 17% of the ^{111}In-activity concentration at day 0 remained in the tumors whereas only 0.05% and 3.3% remained in the blood and whole body, respectively.

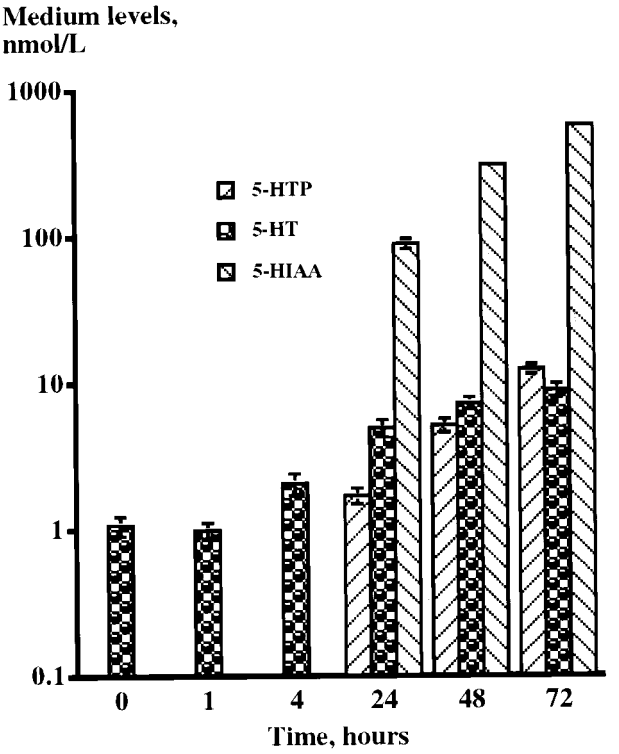

Figure 8. Spontaneous secretion of 5-HTP, 5HT, and 5-HIAA into medium of cell cultures established from a transplanted tumor of generation II. The medium levels of 5-HTP and 5-HIAA started to rise 24 hours after change of medium with very high levels of 5-HIAA indicating a rapid turnover of 5-HT by the tumor cells.

muscarinic agonist carbachol had no effect on $[Ca^{2+}]_i$, which is different from other cell systems[27] and suggests lack of functional muscarinic receptors in these cells or lack of a significant phospholipase C-dependent formation of inositol 1,4,5-trisphosphate. A previous study on human midgut carcinoid cells in a primary cell culture revealed the existence of voltage-gated Ca^{2+} currents through L-type Ca^{2+} channels studied by a patch-clamp technique.[28] Therefore, the GOT1 cells respond with changes in $[Ca^{2+}]_i$ in response to formation of cyclic AMP and depolarization as other neuroendocrine cells.

This study presents evidence that this transplantable human GOT1 carcinoid expresses all recognized somatostatin receptors, the amine transporters VMAT1 and VMAT2 both *in vitro* and *in vivo*, bind and internalize radiolabeled somatostatin analogue and MIBG and exhibits neuroendocrine characteristics of handling $[Ca^{2+}]_i$. This cell line is therefore suited to understand biological aspects of human midgut carcinoids and to evaluate new modalities for therapy.

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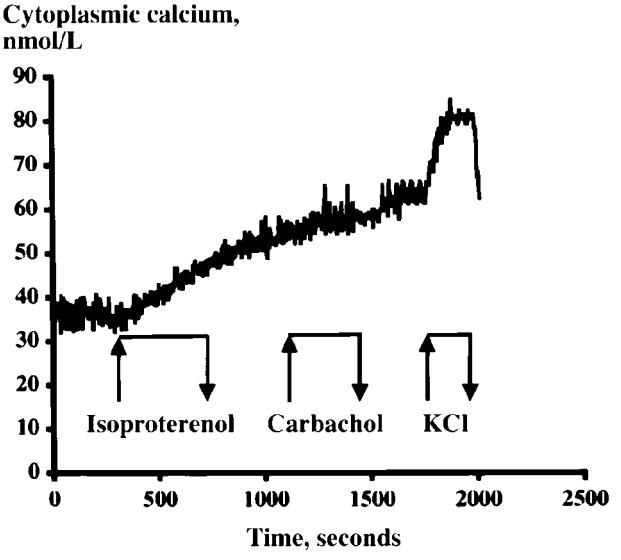

Figure 9. Effect of isoproterenol (10 μmol/L), carbachol (100 μmol/L), and depolarization by KCl (20 mmol/L), respectively, on $[Ca^{2+}]_i$ in single superinfused tumor cells prepared from heterografted tumors of generation II. The trace is representative of three different experiments.

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