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A Somatostatin-secreting Cell Line Established from a Human Pancreatic Islet Cell Carcinoma (Somatostatinoma): Release Experiment and Immunohistochemical Study[1]

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ABSTRACT

Production and secretion of somatostatin (SRIF) were studied using a carcinoembryonic antigen (CEA)-producing cell line (QGP-1) established from a human pancreatic islet cell carcinoma. High concentrations of SRIF (274 ± 51 ng/mg of protein, mean ± SD, $n = 5$) and CEA (3083 ± 347 ng/mg of protein, mean ± SD, $n = 5$) were present in QGP-1 cells, and the basal secretion rates of SRIF and CEA by the cells ($n = 5$) were 40.4 ± 4.8 and 1690 ± 78 pg/10^5 cells/h, respectively. Immunohistochemical studies revealed the presence of SRIF in xenografts of QGP-1 cells and colocalization of SRIF and CEA. Secretion of SRIF by QGP-1 cells was stimulated in the presence of high K^+ (50 mmol) and theophylline (10 mmol), but arginine (10 mmol) and glucose (300 mg/dl) had no effect on the SRIF secretion. The QGP-1 cell line may be useful for studying the regulation mechanism of SRIF secretion.

INTRODUCTION

SRIF[3] was initially isolated from ovine hypothalami as a tetradecapeptide that inhibits growth hormone release from rat cultured pituitary cells (1). SRIF is widely distributed throughout the body of various species and has a variety of biological actions, mostly inhibitory (2, 3). In the human pancreas, SRIF is localized in D-cells of pancreatic islets and believed to regulate the secretion of insulin and glucagon through a paracrine mechanism (2–4). SRIF has also been found in endocrine neoplasms, and about 20 cases of SRIF-secreting tumors, which mostly arose from pancreatic islets, have been reported to date (5, 6).

QGP-1 is a CEA-secreting cell line established from a human pancreatic islet cell carcinoma (7). Recently, we found that this cell line secretes SRIF. The original tumor seemed to be a somatostatinoma based on the clinical findings. In this study, we examined secretion of SRIF by QGP-1 cells and characterized the molecular form of SRIF present in the culture medium and cell extract of QGP-1. We also immunohistochemically examined paraffin sections of the original tumor and a transplanted tumor of QGP-1 cells in nude mice.

MATERIALS AND METHODS

Cell Culture. The QGP-1 cell line was maintained in 25-cm² T-flasks in RPMI 1640 medium (Flow Laboratories, Rockville, MD) containing 10% fetal bovine serum (Flow), 200 units/ml of penicillin, and 200 μg/ml of streptomycin (GIBCO, Grand Island, NY) in 5% CO_2-95% air at 37°C. To determine basal secretion of insulin, glucagon, SRIF, PP, and CEA, the cells were detached from T-flasks by trypsin treatment and plated into 35-mm dishes (5×10^5 cells/dish, $n = 5$). After incubation for 2 days, the medium was changed, and the cells were further incubated for 24 h. Then, the medium was taken and stored at −20°C.

Effects of high K^+ (50 mmol), theophylline (0.1, 1.0, and 10 mmol), arginine (0.1, 1.0, and 10 mmol), and glucose (300 mg/dl) on the release of SRIF from QGP-1 cells were determined. The cells were plated into 35-mm dishes (3×10^5 cells/dish) after trypsin treatment, and a release experiment was performed on the third day after plating. The cells were washed twice with PBS and incubated in the presence or absence of test substances in the medium for 60 min. Then, the medium was taken and stored at −20°C until analysis.

Cell Extraction. The cells in each dish were scraped into 1 ml of 1 M acetic acid, boiled in a water bath, and extracted by sonication. After centrifugation, an aliquot was dried using a centrifugal concentrator (Taiyo VC-36; Taiyo Scientific Industrial Co., Ltd., Tokyo, Japan) and reconstituted with an appropriate solution just before analysis. Protein concentrations were determined using a Bio-Rad protein assay kit (Richmond, CA) with bovine serum albumin as a standard.

Xenotransplantation. The cells in culture were trypsinized, and the cell suspension was centrifuged (700 rpm, 5 min). The pellet was washed with PBS twice and resuspended in 0.9% NaCl solution at a density of 1×10^8 cells/ml. One hundred μl of this cell suspension (1×10^7 cells) were injected s.c. into 6- to 8-wk-old nude mice (Clea Japan, Inc., Osaka, Japan). After 6 wk, the s.c. tumor was removed, fixed in formalin, and paraffin-embedded for immunohistochemical analysis.

Gel Permeation Chromatography. Gel permeation chromatography of the cell extract and the culture medium was performed on a Sephadex G-50 column (95 x 1.4 cm) equilibrated with 1 M acetic acid. Two ml of appropriately diluted samples were layered onto the column and eluted with 1 M acetic acid at 4°C. Fractions (1.3 ml) were collected, dried using a centrifugal concentrator (Taiyo VC-36), and reconstituted with an RIA buffer before assay for SRIF. The column was calibrated with protein markers (V_0, catalase; M_r 13,700, RNase; M_r 6,000, human insulin and SRIF 1-14).

Immunohistochemistry. Cryostat sections (5 μm) of the paraffin-embedded blocks from the original pancreatic islet carcinoma and the xenograft of QGP-1 cells were immunostained for SRIF, insulin, glucagon, PP, and CEA using the peroxidase-antiperoxidase technique (8). The antisera of SRIF, insulin, glucagon, and CEA were purchased from DAKO (Glostrup, Denmark) and the antiserum for PP was purchased from Peninsula Laboratories, Inc. (Belmont, CA).

Determination of SRIF, Insulin, Glucagon, PP, and CEA. SRIF was measured by RIA (9) using rabbit antisera raised against SRIF 1-14 (a gift from Dr. R. H. Unger, University of Texas, Dallas, TX). Synthetic cyclic SRIF 1-14 (Peptide Institute, Inc., Minoh, Japan) and ^{125}I-Tyr^{11}-SRIF 1-14 (Amersham Japan, Inc., Tokyo, Japan) were used as a standard and tracer, respectively.

PP was measured by RIA (10). Insulin and glucagon were measured by RIA using kits (Dainabot Co., Tokyo, Japan), and CEA was measured by EIA using an EIA kit (Boehringer-Mannheim-Yamanouchi Co., Tokyo, Japan).

Statistics. Differences between SRIF concentrations in the control medium and those in the medium with the test substances were assessed using Student's t test. A P value of less than 0.05 was considered to be statistically significant.

RESULTS

Effects of High K^+, Theophylline, Glucose, and Arginine on SRIF Secretion by QGP-1 Cells. Large amounts of SRIF and

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[3] The abbreviations used are: SRIF, somatostatin; CEA, carcinoembryonic antigen; PP, pancreatic polypeptide; PBS, phosphate-buffered saline; RIA, radioimmunoassay; EIA, enzyme immunoassay.

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CEA and a small amount of PP were present in QGP-1 cells and secreted, while insulin and glucagon were not detected in the cells (Table 1).

As shown in Fig. 1, high K^+ (50 mmol) caused a 7- to 8-fold increase of SRIF secretion by QGP-1 cells. Significant dose-responsive stimulation of SRIF secretion was seen in the presence of theophylline (Fig. 1), whereas stimulation of SRIF secretion was not found in the presence of arginine or glucose (data not shown).

Gel Permeation Chromatography of the Extract and the Culture Medium of QGP-1 Cells. Most of the SRIF immunoreactivity was eluted at the position corresponding to SRIF 1-14 in the cell extract and the culture medium (Fig. 2).

Immunohistochemistry. A xenograft using QGP-1 cells was established in nude mice. Histological examination of the xenograft by light microscopy revealed a solid nest and tubular pattern of small- to medium-sized cells with round to short spindle-shaped hyperchromatic nuclei and an eosinophilic cytoplasm separated by a thin fibrovascular stroma (Fig. 3), which resembled the original pancreatic islet cell carcinoma.

Immunohistochemistry revealed numerous SRIF and CEA-positive cells in the xenograft (Fig. 4). The staining of SRIF was seen in the cytoplasm, whereas the staining of the CEA was seen on the cell surface. SRIF and CEA seemed to be colocalized in these serial sections (Fig. 4). Only a few PP-positive cells were found, but insulin and glucagon were not positively stained (data not shown). Immunohistochemical examination of the original tumor yielded similar results (data not shown).

DISCUSSION

QGP-1 cells secrete CEA but not insulin or glucagon (7). In the present study, we demonstrated that these cells also secrete SRIF. An immunohistochemical study revealed the presence of SRIF in the xenograft of QGP-1 cells and the original tumor, as well as colocalization of SRIF and CEA. These findings, together with the lack of insulin and glucagon in QGP-1 cells, suggest that QGP-1 originates from pancreatic islet D-cells. Clinical observations of the patient with the original tumor did not reveal any characteristic symptoms, *i.e.*, cholelithiasis, gallbladder dilatation, or steatorrhea. Thus the patient was diagnosed as having nonfunctioning pancreatic islet cell carcinoma. However, impaired glucose tolerance during a 50-g oral glucose tolerance test and decreased bicarbonate output during a PS test was noted, although plasma SRIF levels and hydrochloride response to various stimuli were not examined. The presence of SRIF in the original tumor and the secretion of SRIF by QGP-1 cells strongly suggest that the original tumor was somatostatinoma.

Patel *et al.* (11) and Barden *et al.* (12) observed the stimulatory effect of dibutyryl cyclic AMP and theophylline in neonatal

Table 1 *Basal secretion rates and cell contents of pancreatic islet hormones and CEA in QGP-1 cells*

	Basal secretion rate (pg/10^5 cells/h)	Cell content (ng/mg of protein)
Insulin	ND[a]	ND
Glucagon	ND	ND
SRIF	40.4 ± 4.8[b]	274±51
PP	0.5 ± 0.2	23 ± 6.1
CEA	1690 ± 78	3083 ± 347

[a] ND, not detected.
[b] Mean ± SD of five replicate dishes in two independent experiments.

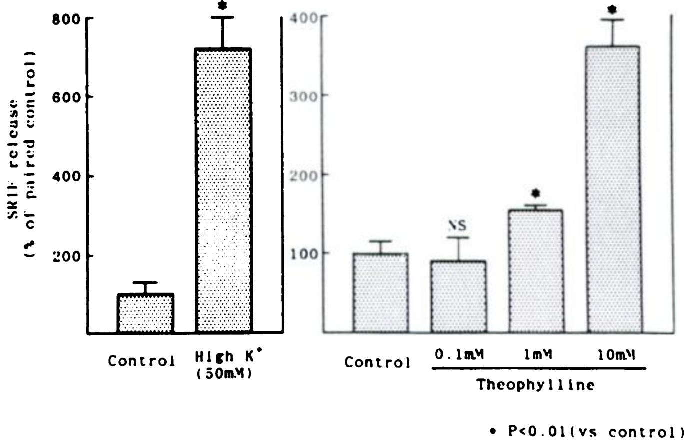

Fig. 1. Effect of high K^+ (*left*) and theophylline (*right*) on SRIF secretion by QGP-1 cells. Cells (3×10^5 cells/dish) were incubated for 60 min in the presence of KCl (50 mmol) and theophylline (0.1, 1.0, and 10 mmol). *Columns*, mean of 4 replicate dishes in two independent experiments; *bars*, SD. * $P < 0.01$ (*versus* control); NS, no significant.

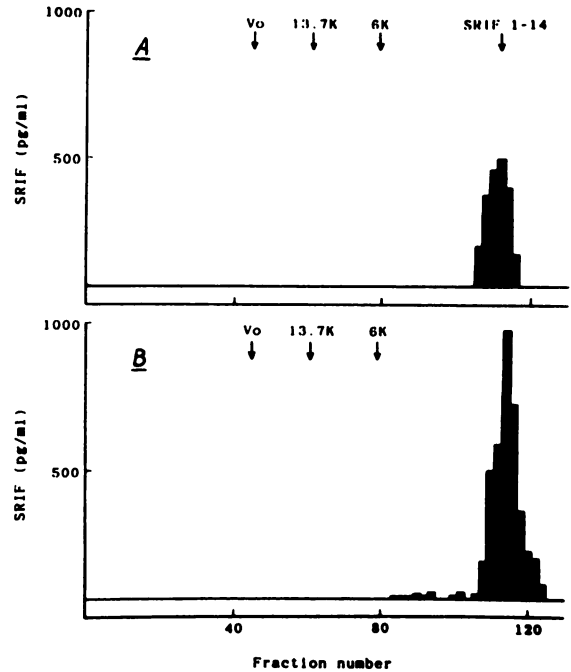

Fig. 2. Gel permeation chromatography of the extract (*A*) and culture medium (*B*) of QGP-1 cells on a Sephadex G-50 column (95 x 1.4 cm). Samples (2 ml) were layered and eluted with 1 M acetic acid at 4°C. Fractions were collected and assayed for SRIF. The column was calibrated with protein markers (V_0, catalase; M_r 13,700, RNase; M_r 6,000, human insulin and SRIF 1-14).

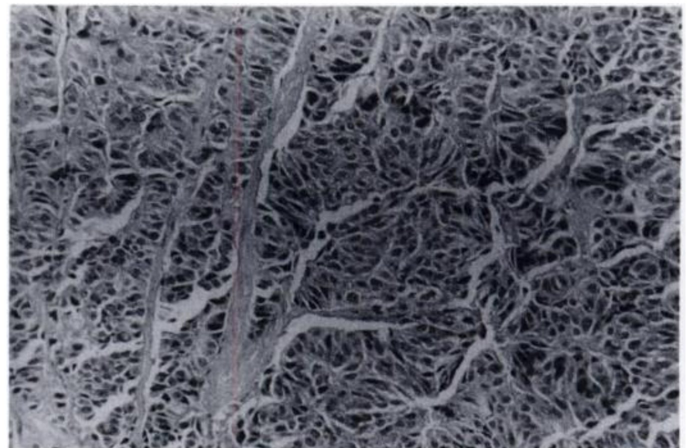

Fig. 3. Nude mouse xenograft of QGP-1 cells. The tumor cells grew in solid nests and tubulus separated by a fibrovascular stroma. H & E, × 200.

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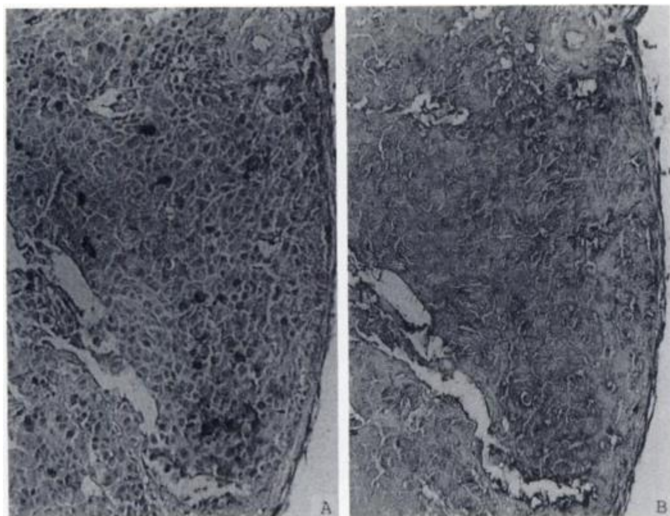

Fig. 4. Immunostaining of SRIF (*A*) and CEA (*B*) in the serial sections of the nude mouse xenograft of QGP-1 cells (× 50). Numerous cells were positively stained for SRIF and CEA. The staining of SRIF was seen in the cytoplasm, whereas that of CEA was seen on the cell surface.

rats and suggested the role of a cyclic AMP-dependent mechanism on SRIF secretion. However, reports concerning the regulation of SRIF secretion by human pancreatic islets are limited, possibly due to the difficulties in making preparations of human pancreatic islets or D-cells. In the present study, a marked increase in SRIF secretion by QGP-1 cells was noted in the presence of high K^+, suggesting that SRIF is localized in secretory granules and secreted by the cells through exocytosis in response to depolarizing stimuli. We also found a dose-responsive stimulation of SRIF secretion induced by theophylline, which is consistent with the findings using neonatal rats (11, 12). This suggests that the A-kinase transduction system is involved in SRIF secretion by QGP-1 cells. On the other hand, arginine and glucose, which are effective secretagogues on SRIF secretion by pancreatic islets (3), did not stimulate SRIF secretion by QGP-1 cells. This may be attributed to QGP-1 cells being derived from the pancreatic islet tumor.

Both SRIF 1-14 and SRIF 1-28 have been detected in the rat pancreas and a human medullary thyroid carcinoma cell line (13, 14). Both are generated from prosomatostatin after proteolytic cleavage, and such processing is different in various tissues or species (15). Analysis of the culture medium and cell extract of QGP-1 cells by gel permeation chromatography revealed only one peak corresponding to SRIF 1-14. This suggests that only SRIF 1-14 is processed from prosomatostatin and secreted by QGP-1 cells.

We also transplanted QGP-1 cells into nude mice and found that the tumor consisted of human pancreatic islet cells that produced SRIF and CEA. One of the characteristics of QGP-1 cells is its slow growth rate (7). SRIF possesses growth-inhibiting action on endocrine (16–18) and nonendocrine tumors (19–21), and SRIF receptors have been detected even on D-cells of the pancreatic islets (22). Further studies are needed to clarify whether SRIF secreted by QGP-1 cells affects their growth in an autocrine fashion.

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