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Expression of somatostatin receptor subtypes and growth inhibition in human exocrine pancreatic cancers

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Abstract The antiproliferative effects of somatostatin and its analogs on human pancreatic cancers were studied: (1) by evaluating the gene expression of somatostatin receptor (sstr) subtypes in human pancreatic cancer cell lines and cancer tissue specimens, (2) by evaluating the antiproliferative effects of somatostatin analogs, and (3) by evaluating the effect of sstr-2 cDNA transduction. Using a reverse transcriptase polymerase chain reaction (RT-PCR), the gene expression of five sstr subtypes (sstr-1 to -5) was examined in eight cell lines, and in ten pancreatic cancer tissues and in the normal surrounding pancreatic tissues. The antiproliferative effects of somatostatin (SS) -14 and its two analogs (SMS 201-995, RC-160) were examined by means of an MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (thiazolyl blue)) assay on three cell lines and Panc-1 transfectants with human sstr (hsstr)-2A cDNA. Sstr-2 was expressed in all samples tested. All examined cell lines simultaneously expressed sstr-2 and -5, while most of the examined pancreatic cancer tissues did not express both of these subtypes simultaneously. Somatostatin analogs inhibited epidermal growth factor (EGF)-stimulated pancreatic cancer cell proliferation. The cell proliferation was further and significantly inhibited by 14% in stable transfectants of Panc-1 cells with hsstr-2A. Based on these findings, it is concluded that somatostatin analogs with their antiproliferative effects mediated by sstr-2 could be potentially useful in the treatment of pancreatic cancers.

Key words Pancreatic cancer · Somatostatin · sstr · Octreotide

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Introduction

The prognosis of patients with exocrine pancreatic cancers remains very poor. Only 36.1% of patients are surgically treated, however, with a 5-year postoperative survival rate of less than 20%.[1] Therefore, new therapeutic approaches for the treatment of exocrine pancreatic cancers must be developed. In the past two decades, the employment of certain gastrointestinal hormones, growth factors, and steroids has been reported in new approaches to control exocrine pancreatic cancers.[2]

Somatostatin is a tetradecapeptide that is widely distributed in the body and inhibits hormonal secretion, cell proliferation, and other cellular processes.[3] These inhibitory effects of somatostatin are mediated by cell-surface somatostatin receptors (sstr), which consist of five subtypes, and form the sstr family.[4–6] All five sstr subtypes (sstr-1 to -5) differ in their tissue distribution,[4] pharmacological properties,[7] or affinity to somatostatin analogs.[8,9] Many kinds of somatostatin analogs bind selectively and more potently to sstr-2, -3, and -5 than endogenous ligands, SS-14/SS-28, but these analogs lose potency for sstr-1 and -4. Sstr-1 and -4 show strikingly low affinities for the octapeptide analogs, SMS 201-995 (octreotide), RC-160 (vapreotide), and BIM 23014 (lanreotide), which are already in clinical use as long-acting somatostatin analogs for the diagnosis and treatment of a variety of neuroendocrine tumors and gastrointestinal disorders. The antiproliferative effects of somatostatin and its analogs suggest their therapeutic potential for cancer treatment,[10] and these effects are suggested to be mainly mediated by sstr-1, -2, and -5.[11] Sstr-2 mediates the antiproliferative effects of the long-acting somatostatin analogs, SMS 201-995 and RC-160, in vivo through the stimulation of tyrosine phosphatase activity.[12] Paz-Bouza et al.[13] reported that RC-160 decreased the volume of experimentally induced tumors, and their colleagues also found regressive changes and

necrosis of the tumor by histopathological methods.[14] Although the potential usefulness of somatostatin analogs for the treatment of pancreatic cancers has been discussed previously,[15] the expression of sstr subtypes in human pancreatic cancer tissues has not been fully studied.

The aim of this study was to evaluate the differential mRNA expression of the five sstr subtypes in eight human pancreatic cancer cell lines, and in cancer tissues and matched normal surrounding pancreatic tissues that were surgically resected from ten patients with primary exocrine pancreatic cancers. We also evaluated the effects of somatostatin and its two analogs on the proliferation of three human pancreatic cancer cell lines, and investigated the effect of human sstr (hsstr)-2A cDNA transfection into Panc-1 cells to examine the possibility of enhancing the antiproliferative effects of somatostatin and its analogs.

Materials and methods

Cell lines

Eight human pancreatic adenocarcinoma cell lines were used. PSN-1 was a gift from Dr. Hiromi Sakamoto (National Cancer Center Research Institute, Tokyo, Japan), FA-6 was a gift from Dr. Naokazu Nagata (National Defense Medical College, Tokorozawa, Japan), SUIT-2 was a gift from Dr. Takeshi Iwamura (Miyazaki Medical College, Miyazaki, Japan), MIA PaCa-2 was purchased from the Japanese Cancer Research Resources Cell Bank (Tokyo, Japan), and BxPc-3 was purchased from the Riken Cell Bank (Ibaraki, Japan). The other three cell lines (Panc-1, Capan-1, and Capan-2) were purchased from the American Type Culture Collection (Rockville, MD, USA). MIA PaCa-2 was cultured in Eagle's minimal essential medium with 10% fetal bovine serum (FBS). Panc-1 and SUIT-2 were cultured in Dulbecco's modified Eagle's minimal essential medium (DMEM) with 10% FBS. FA-6 was cultured in alpha Eagle's minimal essential medium with 10% FBS. PSN-1 and BxPc-3 were grown in Roswell Park Memorial Institute (RPMI)-1640 medium with 10% FBS, Capan-1 was grown in RPMI-1640 medium with 20% FBS. Capan-2 was grown in McCoy's 5a medium with 10% FBS. The cells were incubated at 37°C under 5% CO_2 tension.

Tissue samples

Ten pancreatic cancer specimens and seven samples of matched normal surrounding pancreatic tissues were obtained from at operation our patients. No patients had received somatostatin analogs or antitumor treatment before or during surgery. All tumors were histopathologically diagnosed. All samples were immediately frozen in liquid nitrogen and stored at −80°C until use.

Reverse transcriptase polymerase chain reaction (RT-PCR)

Total RNA was extracted from cells or frozen tissue samples, using Isogen (Nippon Gene, Tokyo, Japan) according to the manufacturer's protocol. Cultured cells were grown in 9-cm-diameter dishes and directly lysed in Isogen. Tissue in Isogen was homogenized with a Polytron tissue homogenizer. Prior to the reverse transcriptase reaction, 50 μg of total RNA was mixed with 2 IU of RNase-free DNase I (GIBCO BRL, Gaithersburg, MD, USA) and incubated at 37°C for 30 min to eliminate genomic DNA. The reaction mixture was extracted with a solution of phenol-$CHCl_3$-isoamyl alcohol (25:24:1) and precipitated with ethanol. Ten micrograms of total RNA with 50 μM of random hexamers (Takara, Tokyo, Japan) were first denatured at 65°C for 10 min and immediately chilled on ice. Reverse transcription was performed at 37°C for 60 min in a volume of 30 μl, containing 10 μg of total RNA, 200 units of Moloney murine leukemia virus reverse transcriptase (Promega, Madison, WI) in 10 mM Tris-Cl (pH 8.3), 200 μM of each deoxynucleoside triphosphate (dNTP), 6.25 units of RNasin (Life Science, St. Petersburg, FL, USA), 10 mM dithiothreitol (DTT), 40 mM KCl, and 7 mM $MgCl_2$. Samples were incubated at 95°C for 5 min to terminate the reverse transcription. The PCR amplification, using 200 ng of cDNA as a template, was performed in 10 mM Tris-Cl buffer (pH 8.3), 50 mM KCl, 2 mM $MgCl_2$, 200 μM of each dNTP, 10 pmoles of each of two primers, and 0.25 U of AmpliTaq Gold (Perkin-Elmer, Foster City, CA, USA) in a final volume of 40 μl.

Following an initial denaturing step at 95°C for 10 min, the amplification program of 36 cycles, consisting of denaturation at 95°C for 1 min, annealing at 61°C for 1 min, and extension at 72°C for 1 min, was carried out by a Program Temp Control System PC-700 (Astec, Fukuoka, Japan). The amplification was terminated with the final extension step at 72°C for 8 min. Human glyceraldehyde-3-hosphate dehydrogenase (GAPDH)-specific primers were used to amplify the cDNA fragment as an internal standard. The sequences of oligonucleotide primers used for PCR and the sizes of the PCR products are listed in Table 1. Reactions without reverse transcriptase were performed simultaneously as negative controls (data not shown). The amplified products were analyzed by polyacrylamide gel electrophoresis. The PCR products on an 8% polyacrylamide gel were excised and extracted by the crush and soak method.[16] The identity of the PCR

Table 1. PCR primers and product sizes for hsstr subtypes mRNA and GAPDH mRNA as a positive control

Subtype		Primer sequence	Product size (bp)
sstr-1	Sense	5′-AAGGTAGTAAACCTGGGCGTG-3′	177
	Antisense	5′-CAGGAAGCCCATGAGAAATGT-3′	
sstr-2	Sense	5′-GCCGGGTGGTCATGACTGTG-3′	185
	Antisense	5′-GGGCAAGATGACCAGCAGAG-3′	
sstr-3	Sense	5′-CTTCATCCATCATCGGTGTCC-3′	198
	Antisense	5′-GACCACATAGATGACCAGCGA-3′	
sstr-4	Sense	5′-AAGCTCATCAACCTGGGCGTG-3′	204
	Antisense	5′-GTAGCACAGGCCAATGGCCAG-3′	
sstr-5	Sense	5′-CTGTCTCTGTGCATGTCGCTG-3′	251
	Antisense	5′-ATGCGCGTCACCTTCCGCTCC-3′	
GAPDH	Sense	5′-GCCAAATATGATGACATCAAGAAG-3′	230
	Antisense	5′-CATGTGGGCCATGAGGTCCACCAC-3′	

GAPDH, Glyceraldehyde-3-phosphate dehydrogenase; PCR, polymerase chain reaction; hsstr, human somatostatin receptor

products was confirmed by direct DNA sequencing with a dideoxynucleotide chain-termination procedure, using a Model 373A DNA sequencer (Perkin-Elmer).

Transfection into Panc-1 cells with human sstr-2A

Sstr-2 has two splice variants, sstr-2A and sstr-2B, which are generated by alternative splicing of a common primary transcript and differ only in their C-terminal amino acid sequence.[17] Both variants couple to adenylyl cyclase and mediate the antiproliferative actions of somatostatin and its analogs.[18] The full-length hsstr-2A cDNA in the pCMV-6b expression vector was provided by Dr. Susumu Seino (Division of Molecular Medicine, Center for Biomedical Science, Chiba University School of Medicine, Chiba, Japan). The *Sal*I-*Hind*III fragment (1.7 kb) including the coding region for the hsstr-2A in the pCMV-6b expression vector, was inserted into the same sites of the pBluescript SK(+). The *Xho*I-*Not*I fragment (1.7 kb), including the coding region for the hsstr-2A in the pBluescript SK(+), was inserted into the corresponding sites of expression vector pBCMGSneo.[19,20] The pBCMGSneo was provided by Dr. Hajime Karasuyama (Department of Immunology, University of Tokyo, Tokyo, Japan). Panc-1 cells cultured in DMEM with 10% FBS were transfected with the expression vector pBCMGSneo, using Lipofectin (GIBCO BRL). Wild-type Panc-1 cells were also transfected with a mock dicistronic vector devoid of hsstr-2A cDNA and used as the control clones. Each clone was selected and cultured in DMEM with 10% FBS and 0.6 mg/ml Geneticin (G418; GIBCO BRL).

In-vitro proliferation assay

Cells were trypsinized and plated out in 96-well plates (Sumilon Multi Well Plate; Sumitomo Bakelite, Tokyo, Japan) at 3×10^3 cells/well in medium with 10% FBS for all three cell lines. The plates were incubated at 37°C for 24 h. The medium containing serum was replaced with a serum-free medium to exclude all growth factors in the serum, and the cells were incubated for additional 24 h. After this incubation, the medium was supplemented with epidermal growth factor (EGF; Wako, Osaka, Japan) at a final concentration of 10^4 pM, with or without SMS 201-995 (Octreotide; Novartis, Basel, Switzerland) or RC-160 (Peninsula, Belmont, CA, USA) or SS-14 (Peptide Institute, Osaka, Japan) at the final concentrations of 1 to 10^4 pM. The cells were then incubated again at 37°C for 48 h. Analogs were added every 24 h. A single lot of FBS was used in all studies to exclude variability.

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (thiazolyl blue) (MTT) assay was performed as previously described.[21,22] Briefly, the cells were washed with phosphate-buffered saline (PBS), and 10 μl of 0.1 M sodium succinate, and 10 μl of 0.4% MTT (Sigma Chemical, St. Louis, MO, USA) were added. After incubation at 37°C for 3 h, the formazan produced from MTT by succinate dehydrogenase was dissolved in 150 μl of dimethyl sulfoxide, and quantified by measuring the optical density (OD) at 540 nm on a spectrophotometer (Easy Reader EAR-340; SLT Lab Instruments, Salzburg, Austria). The OD was found to accurately reflect the number of living cells in previous experiments when the cell count was over 10^4 cells per ml.[21,22] All tests were carried out in triplicate and repeated three times and the mean OD of the samples was used for further processing. Inhibition was expressed as the percentage change from the analog-untreated control.

All data values are expressed as means ± SE. Student's *t*-test was used for the statistical analyses. A difference was considered statistically significant when probability (P) was less than 0.05.

Results

Gene expression of sstr subtypes

Representative results for cell lines and tissue specimens are shown in Figs. 1 and 2, respectively. All cell lines, human pancreatic cancer tissues, and matched normal surrounding pancreatic tissues expressed the mRNAs of two or three sstr subtypes, but all five subtypes were not expressed simultaneously (Table 2). In most cancer cell lines, sstr-1 and -2 mRNAs were expressed, whereas sstr-3 and -4 mRNAs were not detected. In most pancreatic tissues, mRNAs of sstr-1, -2, and -3 were expressed, but sstr-4 mRNA was not detected. All cancer cell lines, pancreatic cancer tissues, and non-cancerous pancreatic tissues obviously expressed sstr-2 mRNA. Sstr-3 mRNA was not detected in cancer cell lines, in contrast to pancreatic tissues. In pancreatic cancer tissues, sstr-5 mRNA was not detected (except in one case), in contrast to the cancer cell lines. Sstr-4 mRNA was not detected in this study,

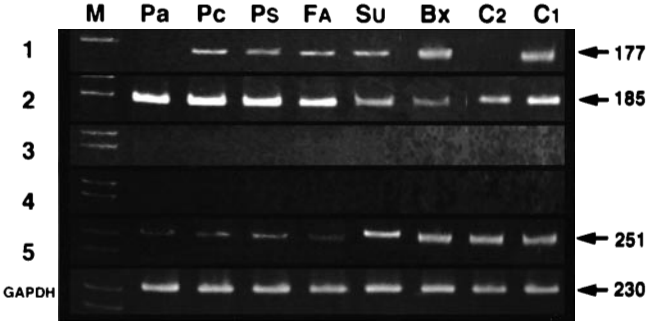

Fig. 1. Amplified products of exocrine pancreatic cancer cell lines. *Pa*, MIA PaCa-2; *Pc*, Panc-1; *Ps*, PSN-1; *FA*, FA-6; *Su*, SUIT-2; *Bx*, BxPc-3; *C2*, Capan-2; *C1*, Capan-1; *M*, lane containing *Hae*III digest of øX 174 DNA. The amplified product of exocrine pancreatic cancer cell lines in each gel is as follows: *1*, somatostatin receptor (sstr)-1; *2*, sstr-2, *3*, sstr-3; *4*, sstr-4; and *5*, sstr-5. *GAPDH* (Glyceraldehyde-3-phosphate dehydrogenase) is for positive controls

Table 2. Summary of the expression of sstr subtypes analyzed by reverse transcriptase (RT)-PCR

Gene	sstr-1	sstr-2	sstr-3	sstr-4	sstr-5
Exocrine pancreatic cancer cells					
MIA PaCa-2	−	+	−	−	+
Panc-1	+	+	−	−	+
PSN-1	+	+	−	−	+
FA-6	+	+	−	−	+
SUIT-2	+	+	−	−	+
BxPc-3	+	+	−	−	+
Capan-1	+	+	−	−	+
Capan-2	−	+	−	−	+
Exocrine pancreatic cancer tissues					
A	+	+	+	−	−
B	+	+	+	−	−
C	+	+	+	−	−
D	+	+	+	−	−
E	+	+	−	−	−
F	+	+	−	−	−
G	+	+	−	(+)[b]	−
H	−	+	+	−	−
I	−	+	+	−	−
J	−	+	−	−	+
Normal surrounding pancreatic tissues					
A-Normal[a]	+	+	+	−	−
B-Normal	+	+	+	−	−
C-Normal	+	+	+	−	−
D-Normal	+	+	+	−	−
G-Normal	+	+	+	−	−
H-Normal	+	+	+	−	−
J-Normal	−	+	+	−	−

A-J, patients from whom tissues were obtained
[a] Normal, Corresponding matched normal surrounding pancreatic tissue
[b] Faint band

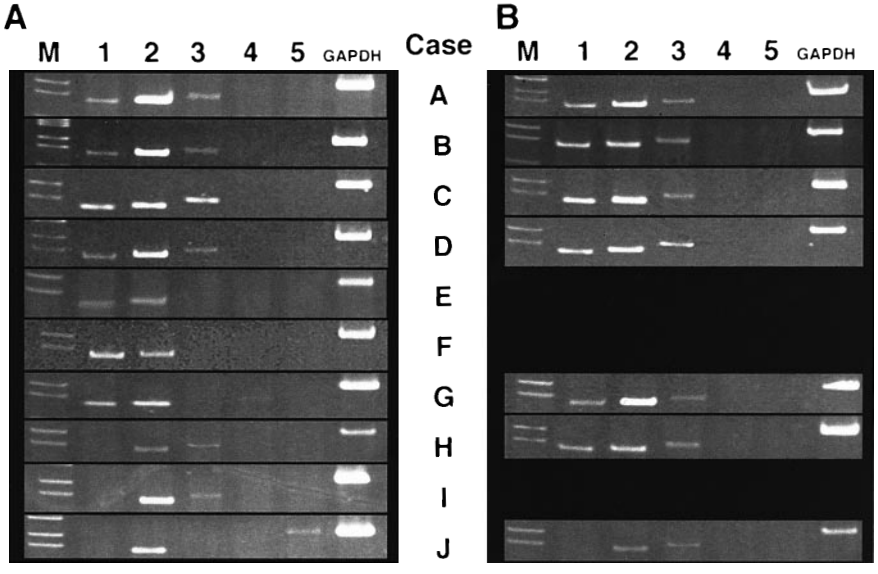

Fig. 2A,B. Amplified products of **A** ten exocrine pancreatic cancer tissues and **B** seven normal surrounding pancreatic tissues in each lane. *1*, sstr-1; *2*, sstr-2; *3*, sstr-3; *4*, sstr-4; and *5*, sstr-5. *M*, Lane containing *Hae*III digest of øX 174 DNA. GAPDH is for positive controls

except for patient G (see Table 2), whose tissue showed a faint band of sstr-4. Malfunction of the primers for the amplification of sstr-4 mRNA was ruled out by successful amplification of sstr-4 genomic DNA with the same primers.

Effect of EGF on proliferation of human exocrine pancreatic cancer cell lines

We tested the proliferative effect of EGF on human exocrine pancreatic cancer cell lines (BxPc-3, Panc-1, and MIA PcCa-2) under serum-free conditions. The addition of 10^4 pM EGF promoted the proliferation of the three human pancreatic cancer cell lines to 157.2 ± 2.9% for BxPc-3, 144.2 ± 7.4% for Panc-1, and 127.6 ± 2.1% for MIA PaCa-2 (Fig. 3).

Effect of somatostatin analogs on proliferation of human exocrine pancreatic cancer cell lines

To examine the effects of somatostatin analogs on the growth of the three human pancreatic cancer cell lines (BxPc-3, Panc-1, and MIA PaCa-2), cell proliferation was induced by 10^4 pM EGF after serum starvation. SMS 201-995, RC-160, or SS-14 inhibited the EGF-stimulated cell proliferation of the three human pancreatic cancer cell lines. Dose-dependent biphasic inhibition was observed in BxPc-3 and Panc-1. Maximal inhibition of the growth of BxPc-3 was observed at 10^3 pM for SMS 201-995, 10^3 pM for RC-160, and 10^3 pM for SS-14 (Fig. 4A). The maximal inhibition of the growth of Panc-1 was observed at 10^3 pM for SMS 201-995, 10^2 pM for RC-160, and 10^3 pM for SS-14 (Fig. 4B). The maximal inhibition of the growth of MIA PaCa-2 was observed at 10^3 pM for SMS 201-995, 10^3 pM for

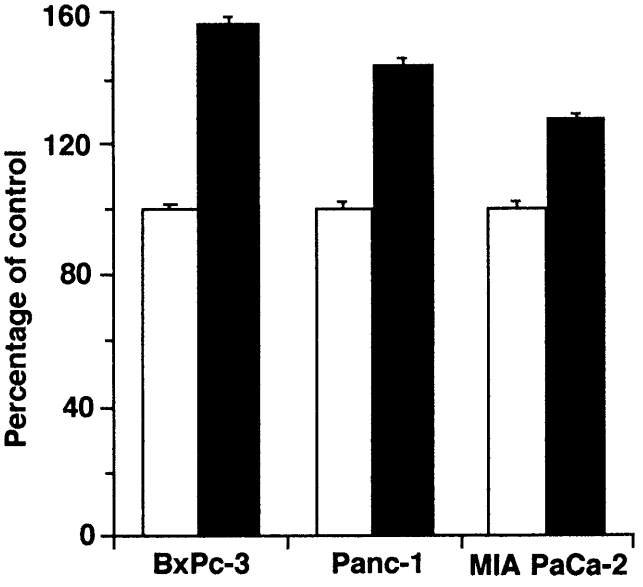

Fig. 3. Effect of epidermal growth factor (EGF) on cell proliferation of three human pancreatic cancer cell lines. Cells were treated with (*black bars*) or without (*white bars*) 10^4 pM of EGF for 48 h and then cell proliferation was determined as described under "Materials and methods." Each value is the mean ± SE (*vertical lines*) of three separate experiments performed in triplicate, and data values are expressed as percentages of the control values obtained from EGF-untreated cells

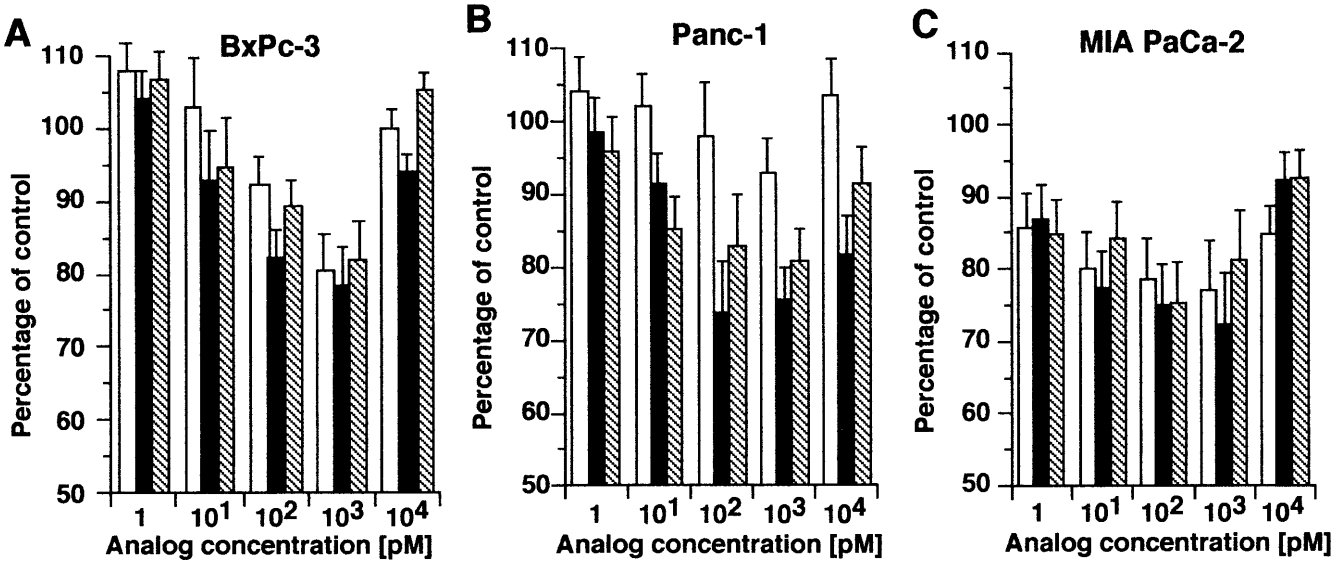

Fig. 4A–C. Effects of different doses of SMS 201-995, RC-160, and SS-14 on the EGF-stimulated proliferation of three human pancreatic cancer cell lines. **A** BxPc-3; **B** Panc-1; **C** MIA PaCa-2. Cells were treated with SMS 201-995 (*white bars*) or RC-160 (*black bars*), or SS-14 (*hatched bars*) at final concentrations of 1 to 10^4 pM. Each value is the mean ± SE (*vertical lines*) of three separate experiments performed in triplicate, and data values are expressed as percentages of control values obtained from somatostatin and its analogs-untreated cells

RC-160, and 10^2 pM for SS-14 (Fig. 4C). The order of inhibitory potency was RC-160, followed by SS-14, and SMS 201-995. Overall, the maximal inhibition of 27.6 ± 5.9% of EGF-stimulated cell proliferation was observed in MIA PaCa-2 by 10^3 pM of RC-160.

Inhibition of cell proliferation in stable transfectants of Panc-1 cells with hsstr-2A

The effect of somatostatin analogs on the proliferation of stable Panc-1 transfectants with hsstr-2A was ascertained. Panc-1 cells transfected with the vector plasmid alone (control) or with hsstr-2A (Panc-1/hsstr-2A) were stimulated by 10^4 pM EGF. RC-160 or SMS 201-995 was added at a final concentration of 10^3 pM. Under this condition, both RC-160 and SMS 201-995 significantly inhibited the EGF-stimulated proliferation of stable Panc-1 transfectants with hsstr-2A, by up to 12.8 ± 4.3% and 14.2 ± 3.9%, respectively (Fig. 5), compared with their control values.

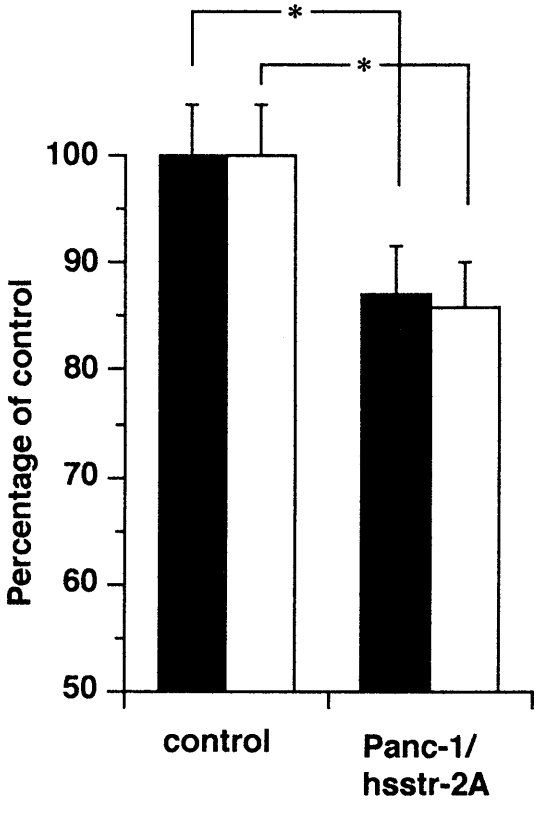

Fig. 5. Effect of RC-160 and SMS 201-995 on the EGF-stimulated proliferation of Panc-1 cells stably expressing human (*h*)sstr-2A. RC-160 (*black bars*) or SMS 201-995 (*white bars*) was added at a final concentration of 10^3 pM. Panc-1 cells containing the vector plasmid alone (*control*) or hsstr-2A stably transfected Panc-1 cells (*Panc-1/hsstr-2A*) are shown. Each result represents the mean ± SE (*vertical lines*) of three experiments for three independent clones in each group, and is expressed as a percentage of the control value. $^*P < 0.05$

Discussion

We clarified the expression pattern of mRNAs in human pancreatic cancer tissues. The presence of sstr-2 not only in normal surrounding pancreatic tissues but also in pancreatic cancer tissues in our study is contradictory to observations reported by two groups.[23,24] Buscail et al.[23] reported that sstr-2 was present in the normal human exocrine pancreas as well as in colon tissues, but that sstr-2 was not expressed in transplanted pancreatic and advanced colorectal carcinoma tissues. This discrepancy may be explained by differences in the culture environments, i.e., the monolayer culture in our study and the subcutaneous implant of tumor tissues in nude mice in their study. The expression pattern of the sstr subtype may be affected by the cellular environment, e.g., that of the monolayer culture or in xenografts.[25]

Somatostatin and its analogs alone did not significantly suppress the spontaneous in-vitro growth of three human pancreatic cancer cell lines. We used EGF to induce proliferation in the three pancreatic cancer cell lines after removing serum, which contains multiple growth factors. The addition of EGF mimics the in-vivo conditions, because the presence of immunoreactive EGF and overexpression of the EGF receptor have been reported in human pancreatic cancers.[26,27] Somatostatin was reported to dephosphorylate the EGF receptor and to suppress cell proliferation.[3] Under the condition of EGF-stimulated cell proliferation, SMS 201-995, RC-160, and SS-14 exerted antiproliferative effects on these three human pancreatic cancer cell lines in a dose-dependent manner, although the inhibition pattern was biphasic in the dose range from 1 to 10^4 pM. The dose-dependent biphasic pattern observed in our study is similar to those found by other researchers.[3,28]

In contrast to our and other promising in-vitro results, the published clinical data on octreotide in most pancreatic cancer patients have been disappointing.[29–32] The ineffectiveness of the somatostatin and its analogs in the clinical treatment of pancreatic cancer patients may be caused by the following factors.

(1) The differential expression of sstr subtypes in cultured cell lines and cancer tissues may be important. All examined cancer cell lines expressed both sstr-2 and -5, while most of the examined pancreatic cancer tissues expressed sstr-2, but not sstr-5. It is unclear why the expression pattern of sstr-5 in the cancer cell lines was different from that in the pancreatic cancer tissues. Sstr-1, -2, and -5 mediate the antiproliferative effects of somatostatin and its analogs.[11,33] Sstr-1 and -2 exert their antiproliferative effects by stimulating tyrosine phosphatase, while sstr-5 exerts its antiproliferative effect by modifying phospholipase C, inositol phospholipid metabolism, and calcium channel capacity, irrespective

of tyrosine phosphatase. The simultaneous expression of sstr-2 and -5 in the cancer cell lines, but the lack of simultaneous expression of these two subtypes in the cancer tissues may thus explain the stronger antiproliferative effect of somatostatin and its analogs on cancer cell lines than on cancer tissues.

(2) The dosage of somatostatin analogs may also be an important factor. Some researchers recommend a 10- to 30-fold higher dosage of octreotide than the usual dosage of 300 μg per day.[31,34] Alternatively, a method to raise the local concentration of somatostatin analogs should be designed. Because the expression of receptor subtypes is not different between human pancreatic cancer tissues and normal surrounding pancreatic tissues, localized intraarterial administration of somatostatin analogs for pancreatic tumors may be beneficial to raise the local drug concentration. Furthermore, Froidevaux et al.[35] reported that long-term continuous therapy with somatostatin analogs induced upregulation of functional sstr-2 on the cancer cell surface in vivo.

(3) The expression of functional receptors may be important. No data have been available to date about the expression levels of these proteins or their functional properties in human pancreatic cancer cells. There may be a post-transcriptional defect in the tumor cells that prevents the expression of functional sstr on the cell surface.[36] Even though such receptors exist, their amounts were reported to be trivial and were not detectable by receptor binding assay.[36] Delesque et al.[37] observed that several pancreatic cancer cell lines synthesized endogenous somatostatin-like peptides and lacked sstr expression. This is probably the reason why sstr-2 overexpression, by transfection itself, reduces cell growth without an exogenous ligand. During the passage of cultured pancreatic cancer cells and during the growth of human pancreatic tumors, the expression level of sstr may decrease gradually, because sstr expression is disadvantageous to cell proliferation, and cancer cells expressing sstr decrease under selective pressure. Some pancreatic cancers and substrains of cultured cell lines express sstr, and their growth is inhibited by somatostatin and its analogs, but others do not express sstr, and these substances are ineffective in suppressing growth. Although we did not determine the number of functional receptors on the cell surface, the level of sstr-2 mRNA assayed in our study was assumed to reasonably reflect the sstr-2 levels. The increased antiproliferative effect of somatostatin analogs in sstr-2A-transfected Panc-1 cell lines further supports an antiproliferative effect of somatostatin analogs on cancer tissues, as long as they express sstr-2.

In summary, the expression of the *sstr-2* gene in exocrine pancreatic cancer tissues and the significantly increased antiproliferative effects on stable Panc-1 transfectants with hsstr-2A provide a theoretical basis for using somatostatin and its analogs as therapeutic agents or as adjunctive drugs in pancreatic cancers.

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