Loss of sst2 Somatostatin Receptor Gene Expression in Human Pancreatic and Colorectal Cancer

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ABSTRACT

Five somatostatin receptor subtypes (sst1 to sst5) have been cloned. We demonstrated previously that sst2 and sst5 mediate the antiproliferative effect of the somatostatin analogues octreotide and vapreotide. Using reverse transcription-PCR, we investigated gene expression of the five receptors in 47 human normal and cancerous tissues or cell lines from pancreatic and colorectal origin. mRNAs of somatostatin receptor subtypes were detected in 98% of samples, with more than two mRNA subtypes being expressed in 55% of cases. sst1, sst4, and sst5 were heterogeneously expressed in both normal and cancerous tissues; sst2 was rarely or not expressed. sst2 was present in normal pancreatic tissues but was absent in exocrine pancreatic carcinomas and their metastases. sst2 mRNAs were detected in normal colon, sporadic polyadenomas, and 50% of Dukes’ stage B and 20% of Dukes’ stage C carcinomas but were undetectable in Dukes’ stage D carcinomas, hepatic metastases, and adenomas from familial adenomatous polyposis. The loss of sst2 expression could represent a growth advantage in these tumors and provide an explanation for the lack of therapeutic effect of somatostatin analogues in such adenocarcinomas. A subtyping of somatostatin receptors should be carried out before considering a somatostatin analogue treatment in patients with colorectal or pancreatic cancer.

INTRODUCTION

Somatostatin is a tetradecapeptide that participates in a variety of biological processes including inhibition of hormonal secretion and cell proliferation (1). These properties are used for the treatment of hormone-producing pituitary or gastroenteropancreatic tumors by stable somatostatin analogues (2). Thus, hormonal suppression is produced in patients with acromegaly or with neuroendocrine tumors such as insulinoma, glucagonoma, gastrinoma, vipoma, or carcinoid syndrome (2). In some patients, analogue therapy leads to an inhibition of tumor growth (2—4). Somatostatin exerts an antiproliferative effect by either indirectly inhibiting hormone and growth factor release or angiogenesis or by acting directly on neoplastic cells (1, 2, 4). The actions of the peptide are mediated by specific receptors that have been detected using binding assay or autoradiography in various human normal and tumor tissues (1, 2). Scintigraphy by i.v. administration of radiolabeled analogue allowed the detection and localization of somatostatin receptor-positive tumors and their metastases (2).

Recently, five somatostatin receptor subtypes and one splice variant have been cloned from humans, mice, and rats (5—10). After expression of sst1 to sst5 genes in mammalian cell lines, we and others demonstrated a distinct profile for binding of clinically used somatostatin analogues, SMS 201—995 (octreotide), BIM 23014 (lanreotide), and RC-160 (vapreotide). These analogues bind with high affinity to sst2, sst3, and sst5 (IC50, 0.1 to 22 nm) and with low affinity to sst1 and sst4 (IC50, 200 to 1000 nm) (5—15). The biological functions mediated by the five sst(s) have not yet been established completely. Recently, after stable expression of sst(s), we demonstrated that only sst2 and sst5 mediated the antiproliferative effect of the somatostatin analogues octreotide and vapreotide (11, 12). Recent studies demonstrated that sst2 mRNA was expressed predominantly in somatroph pituitary adenomas and gastro-entero-neuroendocrine tumors (16—19). All of these results led some authors to postulate that sst2 could be a candidate for mediating the therapeutic effect of analogues in these patients (16, 18).

Although somatostatin analogue administration is now considered as an accepted treatment for neuroendocrine tumors of the gut (2, 4), it is still unclear whether analogue therapy is effective against non-endocrine carcinomas such as colorectal and pancreatic cancers. Numerous studies conducted on experimental neoplasms in vitro and in vivo demonstrated that somatostatin analogues inhibit growth of colorectal and exocrine pancreatic cancers (4, 20—22). In contrast, clinical studies revealed that somatostatin analogue therapy does not produce an adequate clinical response in patients suffering from an advanced tumor stage of pancreatic or colon carcinomas (21, 23—25). Somatostatin binding sites were poorly or not detected in these tumors (2, 26, 27); this could be one of the explanations for a lack of direct antiproliferative effect of analogue therapy. Moreover, considering the differences in the pharmacological profile and the biological function mediated by somatostatin receptor subtypes, the therapeutic response may depend on the subtype expression for a given tumor. Finally, it is not known whether the presence of somatostatin receptors in pancreatic or colorectal cancers may influence their growth behavior or their prognosis. To gain more insight into somatostatin receptor expression in such adenocarcinomas, we examined simultaneously the differential mRNA expression of the five receptor subtypes on specimens obtained from primary tumors or metastases from colorectal or exocrine pancreatic cancer patients.

MATERIALS AND METHODS

Tissue Samples. Tissue samples were obtained from 38 patients at surgery. None of these patients received somatostatin analogue or anti-tumor treatment before or during surgery. All tumors were histopathologically diagnosed. Individual colon carcinomas were staged according to the Dukes’ classification system modified by Astler and Coller (28). Six pancreatic cancer specimens were analyzed, and all were of ductal appearance. Five of them were analyzed after intrapancreatic xenografts in nude mice from three primary tumors and two metastases, obtained as described (29, 30). Samples analyzed from xenografts corresponded to the first passage after implantation. Specimens from normal colon or pancreas were obtained from organ donors. All samples were immediately frozen in liquid nitrogen and stored at −80°C until use. The study protocol was approved by the hospital ethical committees.

Cell Lines. The established human pancreatic cancer cell lines BxPC3, Capan-1, Capan-2, Mia Paca-2, PANC Tu II, 818—1, and 818—7 and the...
human colon carcinoma cell line HT29 were investigated for the expression of somatostatin mRNA.

RNA Isolation. Total RNA was extracted by the modified procedure of Chomczynski and Sacchi (31). Tissue was homogenized with a Polytron tissue homogenizer in RNAzoITMB (Bioprobe, Montreuil-sous-Bois, France). Cultured cells were grown in 5-cm-diameter dishes for 48 h and directly lysed in RNAzo. Homogenized tissue or lysed cells were then incubated in RNAzo for 10 min at 4°C, and RNA was extracted as described (32).

RT-PCR. Before reverse transcription, 1 µg of total RNA was first denatured at 94°C for 10 min and immediately chilled on ice. Reverse transcription was then carried out in 50 mM Tris-HCl buffer (pH 8.3) containing 26.5 µM oligo d(T), 1 µM deoxynucleotide triphosphates, 20 units RNasin (Promega, Charbonnieres, France), 10 mM DTT, 75 mM KCl, 3 mM MgCl₂, and 1 µg of total denatured RNA in a final volume of 20 µl. Prior to the addition of reverse transcriptase, the reverse transcription mixture received 1 to 5 Hi of RNAse free DNase I (Promega) and was incubated at 37°C during 30 min to eliminate genomic DNA. The DNase was inactivated by incubation at 94°C for 5 min. After a 10-min incubation at 4°C, 200 units of Moloney murine leukemia virus reverse transcriptase (Life Technologies, Inc.) was added to the reverse transcription mixture, which was incubated for 10 min at 23°C, followed by 2 h at 39°C for first-strand cDNA synthesis. The reverse transcription mixture was then chilled on ice and diluted 2-fold with sterile water. PCR was then performed as described (32) in a final volume of 50 µl containing 1.5 mM MgCl₂, 50 mM KCl, and 0.01% Triton X-100. After denaturation of the sample at 94°C for 10 min, the PCR was carried out on a DNA Thermal Cycler (Techne) for 25 cycles (β-actin), 32 cycles (hst5), or 35 cycles (sst1 to sst4) consisting of 1 min at 94°C (denaturation), annealing for 1 min (56°C for sst1, 62°C for sst2, 70°C for sst3, 63°C for sst4, 60°C for sst5, and 54°C for β-actin), and extension at 72°C for 90 s. The amplification was terminated by a final extension step at 72°C for 10 min. The conditions of amplification have been determined as described previously (33). The location of oligonucleotide primer pairs used to amplify each cDNA is given with respect to the translational start site in the DNA sequence of sst(s) (5–8) and external control β-actin (34) as follows: sst1 sense (nucleotide 622–636) and antisense (nucleotide 1022–1036) primers; sst2 sense (1–23) and antisense (1081–1104) primers; sst3 sense (721–741) and antisense (1148–1167) primers; sst4 sense (843–862) and antisense (1099–1120) primers; sst5 sense (539–557) and antisense (994–1011) primers; and β-actin sense (506–529) and antisense (998–1021) primers. PCR amplification of both target sample and β-actin were run simultaneously. Only samples positive for β-actin were considered for somatostatin receptor subtype mRNA expression. Amplified fragments were separated by 7% PAGE and stained with ethidium bromide. To confirm that PCR products resulted from cDNA templates rather than genomic DNA, parallel RT-PCR reactions were carried out for each sample in the absence of reverse transcriptase during the reverse transcription procedure. These procedures and PCR reactions on water were used as a negative control of reaction. Positive controls were also used if necessary using amplification of hst plasmids. To avoid cross contamination during PCR reaction, this procedure was only performed for the confirmation of negative results. Two separate runs of PCR reaction were performed for each tissue specimen and for the five subtypes.

RESULTS

Before RT-PCR study of tumor specimens, specificity of the primers was verified for each receptor by PCR amplification of the plasmids containing hst1 to hst3 CDNA(s) (5, 6). RT-PCR analysis was also performed on CHO cells stably expressing hst4 and hst5 (12, 14). The results of amplification are shown in Fig. 1. No PCR product was obtained when specific primers for a given subtype was used to amplify the other receptor subtypes (data not shown).

The comparative distribution of the five sst(s) mRNA in 39 human normal or tumor tissues from 38 patients or in 8 human cancer cell lines is given in Tables 1 and 2. Representative results are shown in Figs. 2 and 3 for pancreatic and colorectal specimens, respectively. Somatostatin receptor subtype mRNAs were present in 98% of cases, and all of the five subtypes were expressed simultaneously. In 55% of cases, more than two mRNA subtypes were present. All somatostatin receptor subtype mRNAs were expressed in normal human exocrine pancreas except sst3. In contrast, sst2 mRNA was not expressed in human pancreatic adenocarcinomas, and sst1, sst4, and sst5 mRNA was detected in 83, 100, and 66% of cases, respectively. As in normal pancreas, neither primary tumors nor metastases expressed sst3 mRNA. In pancreatic cancer cell lines, sst2 mRNA was present in only 14% of cases, and sst3 mRNA was not detected; the three other subtypes were detected in 71 to 100% of cases (Table 1; Fig. 2).

Normal colon mucosa and sporadic polyadenomas all expressed sst2, sst4, and sst5 mRNA but weakly expressed sst1 and not sst3. sst2 was not expressed in adenomas from FAP patients.

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Table 1 Somatostatin receptor subtype mRNA expression in normal or cancerous human exocrine pancreas and derived cell lines

Normal human exocrine pancreatic tissues were sampled from nonpathological organs. Primary human pancreatic carcinomas were and metastatic tumors from human pancreatic carcinomas were from peritoneal (case 1) and hepatic (case 2) origins, respectively, and were sampled after intrapancreatic xenograft in nude mice.
In the present study, RT-PCR was used to characterize simultaneously the gene expression of the five somatostatin receptor subtypes in human normal tissues and adenocarcinomas from exocrine pancreas and colon. Our results indicate that, in contrast to that observed in normal tissue or benign lesions, there is a loss of gene expression of sst2 in pancreatic adenocarcinomas, in advanced colorectal cancers, and their respective metastases.

Pancreatic cancer is the fourth leading cause of death from malignant disease in Western countries. Unfortunately, late diagnosis because of unspecific early symptoms is responsible for the fact that few patients can undergo curative surgical tumor resection. Numerous studies showed that the somatostatin analogues octreotide or vapreotide significantly inhibited growth of pancreatic cancer cell lines in vitro or in xenografts as well as in experimental neoplasms (4, 20–22). The mechanism of action of the analogues may implicate the inhibition of release of growth factors and a direct antiproliferative effect through high-affinity somatostatin receptors (4, 20, 21). Among the recently cloned somatostatin receptor subtypes, sst2 displays the highest affinity for analogues (5, 9–13, 15). We demonstrated that this subtype mediates the antiproliferative effect of the stable analogues octreotide and vapreotide through the stimulation of a tyrosine phosphatase activity, both in rat pancreatic cells that endogenously express sst2 and in stably transfected cells with sst2 cDNA (11, 12, 35).

Pilot clinical trials using octreotide or vapreotide to treat patients with advanced and/or unresectable pancreatic cancer failed to demonstrate any significant benefit of analogue therapy (21, 23, 24). In the present study, we did not observe sst2 mRNA expression in pancreatic adenocarcinomas and metastases; this subtype was present in all normal pancreatic tissue samples. In addition, sst2 was not expressed in the pancreatic cancer cell lines, except in the Mia Paca-2 cells. Interestingly, RC-160 inhibited growth of this latter cell line both in vitro and in vivo through binding of high-affinity sites and stimulation of a tyrosine phosphatase activity, both in rat pancreatic cells that endogenously expressed sst2 and in stably transfected cells with sst2 cDNA (11, 12, 33, 35).

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may provide a growth advantage for tumors and their metastases. Moreover, a direct antiproliferative effect of somatostatin and its analogues may thus be excluded in the absence of sst2. Future studies are necessary to determine if the loss of this negative regulation contributes to pancreatic tumor development and progression.

Experimental studies conducted in vitro and in vivo demonstrated that somatostatin analogue treatment effectively reduced the growth of colon cancers (4, 21, 22). Somatostatin receptors have been detected in normal colon mucosa as well as in colon adenomas or carcinomas (2). Clinical studies have been conducted using short- and long-term octreotide treatments in patients with advanced or metastatic colorectal cancer, and analogue administration poorly influenced tumor growth (24, 25). The mechanism of action of the analogue may implicate the inhibition of release of growth hormone, insulin, insulin-like growth factor I, or gastrin; the inhibition of autocrine secretion of growth factors; or a direct antiproliferative effect via the somatostatin receptors (2, 4, 22, 37). As we postulated for pancreatic cancer, the decrease of sst2 gene expression may be related to tumor local invasion and metastasis, and the loss of this receptor may provide a growth advantage in advanced colon carcinomas.

We found in this study that mRNAs of sst1, sst4, and sst5 subtypes are present both in normal and cancerous tissues from exocrine pancreas and colon. We found previously that among these three subtypes, sst5 also mediated the antiproliferative effect of somatostatin analogues (12). However, the mechanism implicated in the mediation of cell growth inhibition does not involve activation of tyrosine phosphatase activity (12). In these conditions, and contrary to sst2, activation of sst5 cannot counteract the effect of growth factors acting via tyrosine kinase-dependent receptors (12) such as epidermal growth factor, insulin-like growth factor, and insulin that are implicated in the growth of pancreatic and colon cancers (2, 4, 20, 35, 36). sst5 may not necessarily regulate growth of these carcinomas. However, it is noteworthy that in two of the three Duke’s D stage carcinomas and in three of the six liver metastasis specimens, both sst2 and sst5 were not expressed; the loss of sst5 could also confer additional growth advantage to tumor cells in these cases. In the present study, the two subtypes sst1 and sst4 are also heterogeneously expressed in both normal and tumor specimens; however, the role of these two subtypes remains unknown in human colon and pancreas. Finally, sst3 mRNA was little or not detected, especially in exocrine pancreas. In contrast, this subtype seems to be present in endocrine pancreas because we and others could detect sst3 mRNA in both functioning and nonfunctioning islet cell tumors (18, 19).

In conclusion, the sst2 subtype that displays a high affinity for somatostatin analogues and mediates their antiproliferative effect was present in normal tissues but was weakly or not expressed in pancreatic and colorectal carcinomas and their metastases. The loss of sst2 gene expression and thus the mediation of negative regulation of cell proliferation in neoplastic tissues may provide a growth advantage for these tumors and explain, in part, the lack of antiproliferative effect of somatostatin analogues on advanced stage carcinomas. Conversely, sst1, sst4, and sst5 were heterogeneously expressed in both normal and cancerous tissues tested. The possibility that these subtypes may act directly or indirectly as mediators of growth inhibition could not be ruled out. Nevertheless, with regard to the heterogeneous distribution of somatostatin mRNA, a rational receptor subtyping should be carried out before considering somatostatin analogues for the treatment of patients with colorectal or pancreatic carcinomas. In parallel, the development of new analogues highly selective for a given subtype could lead to a better diagnosis and treatment of somatostatin receptor-positive human entero-pancreatic tumors.

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