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SPECIFIC BINDING OF CHOLECYSTOKININ, ESTRADIOL AND SOMATOSTATIN TO HUMAN PANCREATIC CANCER XENOGRAFTS

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Summary—We recently reported that human pancreatic cancers differentially respond to the growth inhibitory effects of an estradiol (E_2) receptor antagonist, tamoxifen, and a long-acting analogue of somatostatin, Sandostatin. In the present study two human pancreatic cancers, established as xenografts in nude mice, were examined as representative of cancers that respond to either tamoxifen (PGER) or Sandostatin (SKI), for the presence of binding sites for various hormones. Male nude mice were inoculated with either SKI or PGER, by passage of tumor chunks (3 mm^2) to the interscapular region. Tumors, obtained from mice after ~30 days of *in vivo* growth, were analyzed for binding to cholecystokinin-octapeptide (CCK), somatostatin and E_2, by published procedures, using either crude tumor membranes (CCK), cytosol and nuclear fractions (E_2), or cryostat sections of whole tumors (somatostatin). SKI was highly positive for high-affinity ($K_d = \sim 1$ nM) CCK binding sites at the time of resection with a binding capacity of ~1000 fmol/mg protein. With increasing passages, the total number of high-affinity binding sites for CCK, were reduced to non-detectable levels in SKI tumors, while non-saturable binding ($K_d = > 10$ nM) became increasingly evident. Early passages of PGER tumors were similarly positive for high-affinity binding sites for CCK, that steeply declined with increasing passages. Specific binding sites for E_2, were observed only in the cytosolic fractions of PGER, with a high binding affinity ($K_d = \sim 0.05$ nM) and a low binding capacity (15 ± 3 fmol/mg cytosolic proteins), at all passages examined; E_2 binding sites were not detected in cytosolic and nuclear fractions of SKI and in the nuclei of PGER, at all passages. SKI and PGER at different passages were examined for somatostatin binding, and both the early and late passages of PGER were devoid of somatostatin binding sites, while SKI tumors were positive for them. Based on the above results, it appears likely that Sandostatin directly inhibited the growth of SKI tumors, since SKI was positive for somatostatin binding sites; it appears less likely that Sandostatin indirectly mediated its inhibition by attenuating possible stimulatory effects of CCK. Growth inhibitory effects of tamoxifen on PGER were apparently via E_2 binding sites, since only the tumors positive for E_2 binding sites (PGER) responded to tamoxifen; it remains to be determined if tamoxifen can exert additional effects independent of E_2 binding sites on pancreatic cancers. Screening of pancreatic cancers for specific binding sites for putative growth regulatory hormones/factors, such as E_2, somatostatin, and CCK, may thus help in future to determine a more appropriate treatment for patients, in a fashion analogous to treatment of breast cancer.

INTRODUCTION

Pancreatic adenocarcinoma is increasing in incidence and is currently the fifth leading cause of cancer deaths [1]. The number of new cases has tripled over the last four decades. Treatment of these cancers relies heavily on surgical resection, which is effective only in the unusual circumstance of localized tumor. Successful palliation of breast cancer is often achieved by endocrine manipulation when specific receptors for hormones that are known to stimulate growth of normal breast tissues are present in the cancer.

Several gastrointestinal hormones, including cholecystokinin (CCK), secretin, and bombesin are trophic for the normal rodent pancreas [2–4]. Some of these agents have similarly been reported to influence the growth of pancreatic cancer [5, 6]. In preliminary reports, we observed that caerulein, an analogue of CCK, and secretin, simulated the *in vivo* growth of a hamster pancreatic adenocarcinoma [5], and that specific high-affinity CCK receptors

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are present in normal and cancerous human pancreas [7].

On the other hand, somatostatin inhibits both the release and target organ response of a number of gut peptide hormones [8]. Chronic administration of somatostatin alone decreased the weight and DNA content of the normal rat pancreas [9], and inhibited the growth of some human pancreatic adenocarcinomas in nude mice [10, 11]. Somatostatin may inhibit the growth of these pancreatic cancers by a number of mechanisms [12]. It could inhibit the growth of tumors indirectly by preventing the growth stimulatory effects of an endogenous growth promoting hormone such as CCK [6], or inhibit the growth of pancreatic cancers directly by interacting with somatostatin receptors that may be present on these tumors. There is evidence that binding sites for somatostatin exist on a number of pancreatic cancer cell lines *in vitro* [13] and that the inhibition of cell division seen in these lines, *in vitro*, may be through activation of tyrosine phosphatase [14].

Steroid hormones have been shown to have dramatic effects on the function of normal pancreas (reviewed in Ref. [15]). Estradiol (E_2) receptors have been identified and characterized on normal pancreatic tissues from rodents [16–18], humans [19], and baboons [16]. E_2 receptors have also been identified in human pancreatic cancers [20] and in carcinogen-induced rat pancreatic adenocarcinomas [21], which suggests a role for estrogens in pancreatic cancer as well. In one study, no appreciable effect of the estrogen antagonist, tamoxifen, was observed on the growth of human pancreatic adenocarcinoma that had been xenografted into nude mice [22]. We, however, recently reported that some human pancreatic cancer xenografts respond to the growth inhibitory effects of tamoxifen, while the growth of others was not affected [11]. In some clinical studies [23–25], a possible beneficial effect of tamoxifen, especially on the prolongation of life [23], was reported. However, in one large-scale randomized study, these promising early results could not be confirmed [26]. This may be due to the use of a single dosage regimen of tamoxifen.

The present study was undertaken to determine possible factors that may explain why Sandostatin and tamoxifen inhibit the growth of only some human pancreatic cancers [11]. We examined two human pancreatic xenografts, as examples of cancers that responded to the inhibitory effect of either tamoxifen (PGER) or Sandostatin (SKI), for the presence of specific binding sites for the hormones E_2 and CCK (putative growth promoting agents for the pancreas) and somatostatin (a putative growth inhibitory agent for the pancreas).

MATERIALS AND METHODS

Transplantation of human pancreatic cancers

Freshly harvested samples of SKI (a human ductal pancreatic adenocarcinoma established as a xenograft in nude mice in our laboratory) and PGER [human ductal pancreatic adenocarcinoma xenografted in nude mice (a gift from Dr Frank Johnson, St Louis, MO, U.S.A.)] were transplanted as 3×3 mm chunks to the scapular region of male 20 g athymic Balb/c nude mice under ether anesthesia. The mice were maintained under specific pathogen-free conditions in a temperature-controlled isolation unit with a 12 h light–dark cycle. Diet consisted of standard chow (Autoclavable Rodent Chow No. 5010; Ralston Purina, St Louis, MO, U.S.A.) and autoclaved tap water *ad libitum*. After ~ 30 days of *in vivo* growth, the mice were killed and tumors harvested as published previously [11], and frozen at -70°C for measurement of specific binding sites for various hormones. The tumors had well-defined margins and were dissected from surrounding normal tissues without difficulty. We have previously reported [11] that tumors thus harvested are relatively free of host tissue and devoid of necrosis.

Measurement of CCK binding sites

Specific binding sites for CCK, were determined on crude membranes prepared from either the freshly resected frozen tumors (SKI), or from the xenografts (PGER and SKI) obtained from mice at increasing *in vivo* passages. Crude membranes were prepared from pancreatic tumors essentially by our published procedures [27]. Briefly, frozen pancreatic tumors were weighed and pulverized with a thermovac auto-pulverizer cooled in liquid nitrogen. The resulting powder was homogenized in 5 vol buffer A (10 mM Tris, 146 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 2.5 mM $MgCl_2$, 0.25 M sucrose, pH 7.4) + GSBT (0.1% gelatin [G], 1% soybean trypsin inhibitor [S], 1% bacitracin [B], 100 U Trasylol/ml [T]) using a pre-cooled polytron homogenizer (PG-10-ST; Brinkman Industries, Westbury, NY, U.S.A.). Crude

membranes (2000–30000 *g*) were prepared from the homogenate as previously described [27]. The membrane pellet was resuspended in binding buffer (25 mM HEPES, 5 mM $MgCl_2$) containing 0.1% BSA + GSBT. Protein concentration was measured in an aliquot by the method of Lowry *et al.* [28]. Aliquots of crude membranes containing 50–100 μg protein were used/binding assay point, as previously published [29]. Briefly, a multi-point saturation (7–12 point assay) was performed using increasing concentrations (0.05–0.5 nM) of [^{125}I]BH-CCK-8-sulfate (New England Nuclear, Boston, MA, U.S.A.) in the presence (non-specific) or absence (total binding) of 1000-fold excess radioinert homologous ligand in triplicate. The specific activity of the supplied radioactive ligand was reduced to 200 dpm/fmol by the addition of the required amount of radioinert ligand before using it for binding assays. The binding assay was performed at 30°C in a waterbath for 45–60 min, which was found to be optimal for estimating the maximum number of CCK binding sites. At the end of the incubation, the binding assay tubes were chilled on ice and 100 ml of ice-cold binding buffer added. The membrane bound peptide hormone was separated from excess unbound hormone by filtration over cellulose acetate filters (EHWPO550; Millipore, Bedford, MA, U.S.A.) followed by three 1-ml buffer washes, using two multi-filtration units (1225 Sampling Manifold, Millipore). The cellulose filters were placed in glass tubes and counted in a γ-counter (Minaxi 5000; Packard Instruments, Downers Grove, IL, U.S.A.) with 80% efficiency for ^{125}I. The results were analyzed by Scatchard plot of the specific binding data [30].

Specific binding to E_2

Specific binding sites for E_2 were measured in the cytosol and nuclear fractions of the xenografts (PGER and SKI) removed at increasing *in vivo* passages from mice, essentially by our published procedures [31]. Briefly the tumors were collected in TE buffer (0.01 M Tris–HCl buffer, pH 7.4, + 1 mM Na_2 EDTA) containing SBT and frozen immediately on dry ice. Tumors were weighed and pulverized with a thermovac auto-pulverizer and cooled in liquid nitrogen. The resulting powder was homogenized in 5 vol TEMG buffer (TE buffer + 5 mM $MgCl_2$ + 10% glycerol v/v, pH 7.4) containing SBT, using a pre-cooled polytron homogenizer (PG-10-ST; Brinkman Industries). The homogenate was filtered through a double layer of cheesecloth and centrifuged at 800 *g* for 10 min to yield a crude nuclear pellet. The supernatant was centrifuged at 135,000 *g* for 30 min in a Beckman L8-80 ultracentrifuge, and the resulting supernatant used as cytosol. The crude nuclear pellet was washed once with TEMG buffer containing 0.25% Triton X-100 and 3 times with TEMG buffer alone. The final pellet was suspended in TEGM buffer at a concentration of 100 mg tissue equiv/ml. Radiolabeled E_2 ([2,4,6,7-^{3}H]17β-E_2; New England Nuclear) (sp. act. = 91.8 μCi/nmol) and 17β-E_2 were used for measuring E_2 binding sites. Aliquots of cytosol, containing ~300–500 μg cytosolic protein, were incubated with increasing concentrations (10 pM–10 nM) of ^{3}H-labeled steroid in the presence (non-specific) or absence (total binding) of a 1000-fold molar excess of radioinert E_2. The incubation was carried out for 18 h at 4°C, in order to measure unoccupied binding sites and at 26°C for 60 min to measure total unoccupied sites. To stop the reaction, 0.5 ml of dextran-coated charcoal (dextran 0.05%, charcoal 0.5% in TE buffer) was added on ice, and the bound radioactivity separated by centrifugation and counted. Binding affinities were calculted by Scatchard plots of specific binding data. Aliquots of nuclear suspension were incubated with increasing concentrations of [^{3}H]E_2 with or without a 1000-fold molar excess of radioinert E_2. Samples were incubated either at 4°C for 18 h (to measure free, unoccupied sites) or at 37°C for 30 min (to measure total, occupied + unoccupied nuclear binding sites). Tubes were then cooled at 4°C. The nuclei were pelleted by centrifugation and washed 5 times with TEMG buffer. Radioactivity remaining bound in the particulate pellet was extracted with absolute ethyl alcohol and the extract counted for ^{3}H using 10 ml scintillation fluid, consisting of 5 g permablend 2 (Packard Instruments) dissolved in 1 l toluene, and counted in a Beckman LS-6000-IC scintillation counter, (Beckman Instruments) at $<2\%$ counting error with conversion to dpm by external standard ratio methods.

Specific binding of somatostatin

Binding sites for somatostatin were measured by autoradiography on 10 m thick sections, cut with a cryostat, from frozen samples of SKI and PGER tumors, as described before in detail for various types of tumoral and non-tumoral tissues [32, 33]. The ^{125}I-[Tyr^3]analogue of SMS 201-995 (Sandostatin, octreotide), code named

Table 1. Concentration of E_2 binding sites in pancreatic cancer xenografts

Tumor	Passage No.	Cytosol 4°C fmol[a]	Cytosol 4°C K_d (nM)	Cytosol 26°C fmol[a]	Cytosol 26°C K_d (nM)	Nuclei 4°C fmol[a]	Nuclei 4°C K_d (nM)	Nuclei 26°C fmol[a]	Nuclei 26°C K_d (nM)
SKI	P_1	<1.0	—	<1.0	—	<1.0	—	<1.0	—
	P_3	<1.0	—	<1.0	—	<1.0	—	<1.0	—
	P_7	<1.0	—	<1.0	—	<1.0	—	<1.0	—
	P_{11}	<1.0	—	<1.0	—	<1.0	—	<1.0	—
PGER	P_0	13.8 ± 3.2	0.04 ± 0.01	11.5 ± 2.9	0.03 ± 0.008	<1.0	—	<1.0	—
	P_5	6.6 ± 0.8	0.08 ± 0.03	8.3 ± 1.2	0.04 ± 0.001	<1.0	—	<1.0	—
	P_8	11.1 ± 2.1	0.05 ± 0.02	10.3 ± 1.9	0.06 ± 0.01	<1.0	—	<1.0	—

[a]Values are in fmol/mg cytosol protein, and are mean ± SEM of 4–12 determinations from 2–6 separate tumors, harvested at the given passage number; K_d = equilibrium dissociation constant.

204-090, was used as radioligand. It has been shown previously to specifically label somatostatin receptors [32, 33].

RESULTS

E_2 binding sites in cytosol and nuclei of SKI and PGER

SKI and PGER tumors at increasing *in vivo* passages, were examined for the presence of cytosolic and nuclear E_2 binding sites, as described under Materials and Methods. Specific binding sites for E_2 were consistently measured in the cytosolic fraction of PGER tumors, both at 4 and 26°C, at all the passages examined (Table 1). The total number of E_2 binding sites measured at 4 and 26°C in the cytosolic fractions were not significantly different (Table 1), indicating that almost all the E_2 binding proteins in the cytosol fractions of PGER were unoccupied, and available for binding to E_2. The binding affinity of the sites was determined from a Scatchard plot of the specific binding data (Fig. 1). The binding affinity of E_2 binding proteins in terms of equilibrium dissociation constant (K_d) ranged from ~0.04 to 0.08 nM, in tumors removed at different passages (Table 1). No specific binding of E_2 was detected to the nuclear fractions prepared from PGER tumors, at both the low and high temperatures (Table 1). Saturable specific binding sites for E_2 were not detected in the cytosol (Fig. 1) and nuclear fractions of SKI tumors, measured at both the low and high temperatures, in any of the passages examined (Table 1). The above results thus demonstrated that only the PGER tumors were positive for high-affinity cytosolic E_2 binding protein, and that SKI was devoid of E_2 binding protein; there was no evidence for the presence of specific binding sites for E_2 in the nuclei of either PGER or SKI tumors, examined in the present studies.

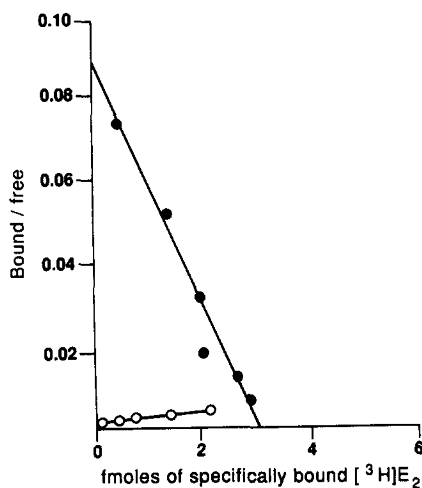

Fig. 1. Scatchard plot of specific binding of [^{3}H]E_2 to aliquots of cytosolic fraction (containing ~300 mg protein/assay point/ml), from PGER (P_5) (●—●) and SKI (P_2) (○—○). The binding data shown is representative of >9 separate measurements on tumors obtained at different passage numbers as detailed in Table 1. Each point is the mean of duplicate determinations from a single tumor.

CCK binding sites on pancreatic cancers

Crude membranes prepared from SKI and PGER, from different passages, were examined for specific binding to CCK, as described under Materials and Methods. At the time of resection from the patient, SKI pancreatic cancers were determined to be highly positive (800–900 fmol/mg protein, n = 2) for high-affinity CCK binding protein (K_d = ~0.1 nM). SKI xenografts from nude mice were examined at increasing *in vivo* passages for the presence of CCK binding sites. Saturable, high-affinity, Type I, binding sites for CCK (K_d = ~0.1 nM) were present in the early passages (Fig. 2), and declined rapidly with increasing passage numbers (Table 2). Non-saturable CCK binding proteins (low-affinity sites?), on the other hand, became increasingly evident with increasing passages,

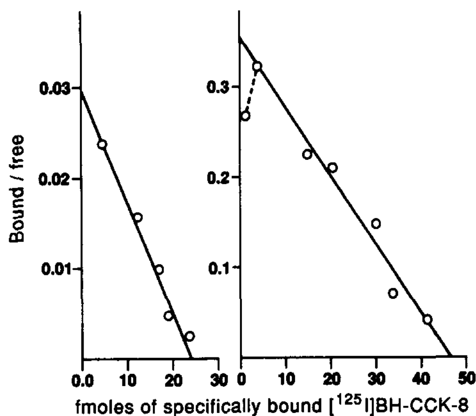

Fig. 2. Scatchard plot of specific binding of [^{125}I]BH-CCK to aliquots of crude membranes (containing ~50 μg protein/assay point/ml) prepared from PGER (P_0) (left) and SKI (P_0) (right) tumors. The results shown are representative of several measurements made on tumors at various passage numbers as detailed in Table 2. Each data point is a mean of duplicate determinations from a single tumor.

which we have arbitrarily termed Type II sites (Table 2); the significance of the low-affinity sites is not understood. PGER tumors were similarly positive for saturable, high-affinity CCK binding sites at very early passage numbers (P_0–P_2) (Fig. 2), which were reduced to non-detectable levels in later passages ($>P_6$) (Table 2). Non-saturable, specific binding sites for CCK, were once again predominately present in PGER tumors, removed at passages higher than 6 (Table 2).

Specific binding of somatostatin to pancreatic cancers

As seen in Fig. 3, somatostatin binding sites were found autoradiographically to be present in moderate density on tumor cells of SKI tumors (passage numbers P_6, P_9, P_{11} and P_{16}); somatostatin binding proteins were specific for somatostatin since unrelated peptides such as luteinizing-hormone-releasing-hormone at 1 μM concentration did not displace the radioligand (data not presented). Interestingly, the autoradiographic distribution of somatostatin binding sites was non-homogenous, with some tumor areas lacking the binding sites (Fig. 3). A sample of SKI tumor from later passage number (P_{20}) was also investigated and found to be lacking somatostatin binding sites. It cannot be evaluated retrospectively whether the whole tumor was completely negative for somatostatin binding sites (due perhaps to the later passage number) or whether a negative sample from a tumor with non-homogenous distribution of binding sites was analyzed. All PGER tumors analyzed (from passage numbers P_3, P_6 and P_9) were found to be negative for binding somatostatin (Fig. 3).

DISCUSSION

In previous studies, we reported that human pancreatic cancers differentially responded to the growth inhibitory effects of an E_2 receptor antagonist, tamoxifen, and a long-acting analogue of somatostatin, Sandostatin [11]. In the present study we observed that the human pancreatic cancer, that responded to the growth inhibitory effects of tamoxifen (PGER), was positive for a significant number of high-affinity E_2 binding sites. On the other hand, the pancreatic cancer xenograft, SKI, that in our previous studies [11] had not responded to the growth inhibitory effects of tamoxifen was negative for specific binding to E_2. These studies thus indicate the possibility that tamoxifen was perhaps

Table 2. Concentration of CCK binding sites on pancreatic cancer xenografts

	PGER			SKI	
Passage No.	Type I[a] K_d (nM)	Type II[b] K_d (nM)	Passage No.	Type I[a] K_d (nM)	Type II[b] K_d (nM)
P_0	440 ± 97 (0.82 ± 0.1)	ND	P_0,P_2	756 ± 240 (0.16 ± 0.08)	ND
P_2	240 ± 83 (0.35 ± 0.15)	>1000 (7–10 nM)	P_3,P_7 P_9	78 ± 18 (0.09 ± 0.03)	>200 (1–2 nM)
P_5	53 ± 31 (0.98 ± 0.3)	>1000 (~10 nM)	P_{11}	46 ± 19 (0.11 ± 0.05)	>1000 (2–3 nM)
P_8	<1	>2000 (>10 nM)	P_{12}	<1	>2000 (>10 nM)

[a]Values are in fmol/mg membrane protein, and are mean ± SEM of 6–18 determinations from 3–9 separate tumors, harvested at the given passage number; the equilibrium dissociation constant (K_d) values measured for these sites are given in parentheses. For SKI tumors, the concentration of binding sites on tumors from different passages are presented together, if the measurements between the passages were not significantly different from each other.

[b]Type II sites were either not detected (ND) at early passages, or a high number were present with significantly lower affinities (K_d = < 1–10 nM) at medium passages, or a non-saturable number of specific binding sites, with very low affinities (K_d = > 10 nM), were present at later passages, so we were unable to saturate with the concentrations of CCK used in the present studies.

mediating its inhibitory effects on PGER, by interacting with the E_2 receptors present in these tumors. In other words, pancreatic cancers that do not possess E_2 binding sites (SKI), will be less likely to respond to the growth inhibitory effects of tamoxifen. The present studies also indicate that only some pancreatic cancers are likely to be positive for E_2 binding protein; this may explain the discrepancy in literature regarding the growth inhibitory or beneficial effects of tamoxifen [22–26]. In one study, no appreciable effects of tamoxifen were observed on growth of human pancreatic adenocarcinomas that had been xenografted into nude mice [22]. In several clinical studies [23–25], on the other hand, a possible beneficial effect of tamoxifen, especially towards the elongation of life [23], were reported, indicating that tamoxifen may indeed be beneficial for treating at least some pancreatic cancers, and especially those that are positive for the presence of high-affinity E_2 binding sites.

We and others [19, 34], have demonstrated that E_2 binding sites in the rodent and human pancreas are antigenically different from those present in the typical target tissues of E_2 (mammary gland, uterus), and the commercially available E_2 receptor antibodies cannot be used for purposes of detecting E_2 binding sites in pancreatic tissues. In the present study, we therefore used ligand binding assays to sub-cellular fractions of pancreatic cancer xenografts, in order to measure binding sites for E_2. We

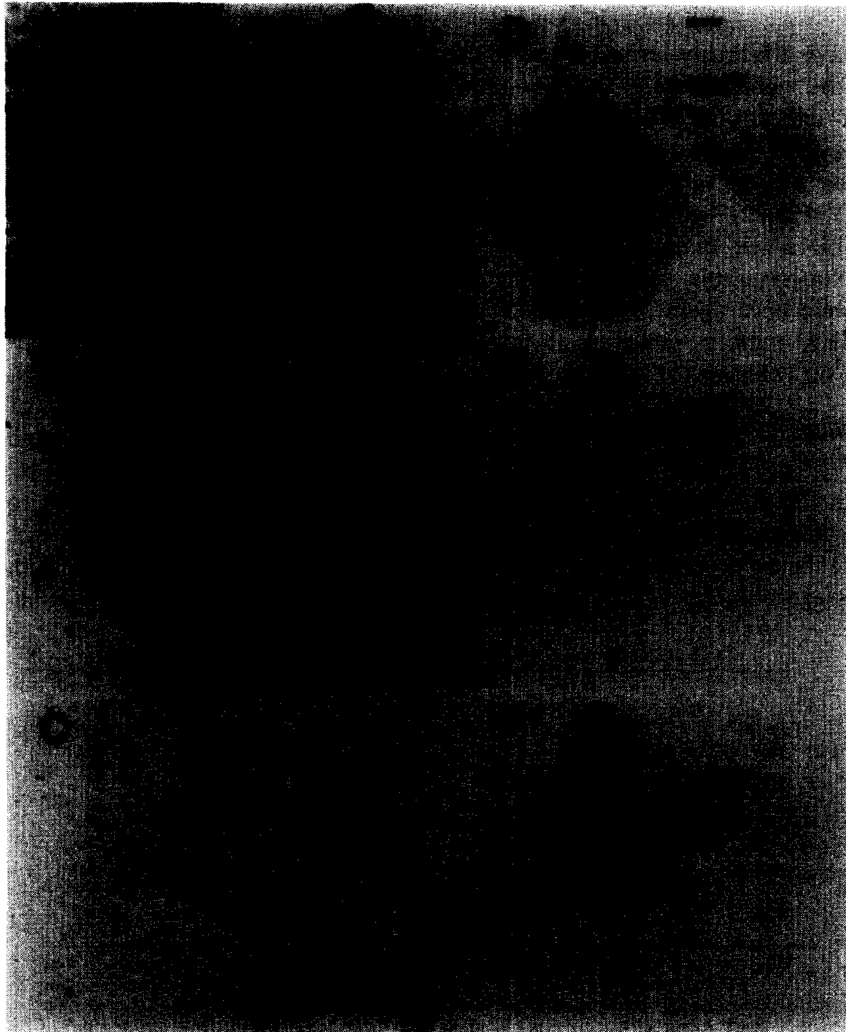

Fig. 3. Autoradiographic distribution of somatostatin binding sites in SKI (A–C) and PGER (D–F) tumor samples. A,D: hematoxylin-eosin stained sections. B,E: autoradiograms showing total binding of ^{125}I-[Tyr^3]-SMS 201-995. C,F: Autoradiograms showing non-specific binding of ^{125}I-[Tyr^3]-SMS 201-995 (in the presence of 10^{-6} M [Tyr^3]-SMS 201-995). Note the non-homogenous distribution of somatostatin binding sites in SKI tumors and the absence of binding in PGER tumors. Bars = 1 mm.

observed specific binding proteins for E_2 only in the cytosolic fractions of the receptor positive tumors, with no indication of the presence of either unoccupied or bound E_2 binding sites in the nuclear fractions. In studies with normal pancreatic acinar cells, we and others [17, 18, 34], have reported the absence of translocatable E_2 binding sites in the pancreatic acinar cells, with the possibility that the majority of the binding sites are microsomal in origin [35]. The pancreatic cancers we investigated in the present study were ductal carcinomas. At the present time there is no information regarding the intracellular localization of E_2 binding sites in the normal pancreatic ductal cells. Therefore we cannot definitely state whether the E_2 binding sites measured in the cytosol are localized in cytosol itself or are localized in some other particulate fraction *in situ*.

In a preliminary report we had observed the presence of high-affinity specific binding sites for CCK on normal human pancreas and pancreatic adenocarcinomas from patients [7]. More recently, the presence of high-affinity binding sites for CCK have been demonstrated on established pancreatic cancer cell lines [36]. In our hands, however, we have not been able to measure the presence of high-affinity binding sites for CCK in established human pancreatic cancer cell lines (MIA, PACA, H_2T) from tissue culture. In contrast, in the present study we observed that, while the freshly resected human pancreatic cancers were likely to be positive for the presence of a large number of high-affinity binding sites for CCK, there was a steep decline in the concentration of the high-affinity sites with increasing passages *in vivo*, indicating the possibility that the pancreatic adenocarcinoma cells rapidly lose their ability to express CCK receptors with increasing *in vivo* passages, the underlying mechanism for which remains to be investigated. An important point to be noted here is that despite the fact that both the pancreatic cancers were highly positive for the high-affinity CCK receptor-like binding sites at initial passages, with both becoming increasingly negative for the presence of these sites, only SKI responded to the growth inhibitory effects of Sandostatin while PGER did not. The latter finding indicates to us the possibility that the primary mechanism by which Sandostatin inhibited the growth of SKI, but not PGER [11], did not involve the indirect route of inhibiting possible growth stimulatory effects of CCK on the pancreatic cancer cells. The present findings, however, do not imply that Sandostatin cannot negate possible growth effects of CCK on human pancreatic cancers, but only that in the present studies with SKI and PGER, this pathway was probably not involved as the major pathway.

A curious finding of the present studies was the observation of low-affinity binding sites for CCK in both PGER and SKI tumors with increasing *in vivo* passages. At this point it would be highly speculative to suggest any relationship between the loss of high-affinity sites and the increasing presence of the low-affinity sites, in the pancreatic cancer xenografts with increasing *in vivo* passages. The presence of the low affinity to almost non-saturable binding sites for CCK, however, is not unique to pancreatic cancers, since we and others have similarly observed the presence of low affinity ($K_d = < 1\ \mu M$) binding sites for gastrin (a closely related peptide), on colonic cancers [37, 38]. The biologic significance of these low-affinity sites has yet to be determined.

Based on the results of the present studies, wherein specific binding sites for somatostatin were measured only in the SKI tumors, while PGER tumors were negative for binding somatostatin, it seems likely that the growth inhibitory effects of Sandostatin on SKI, was in all probability directly mediated via interaction of Sandostatin with the specific somatostatin binding sites measured on the pancreatic cancer cells. In previous studies, utilizing normal pancreatic acinar cells, we and others have demonstrated significant inhibitory effects of somatostatin on stimulated intracellular functions, using *in vitro* bioassay methods [39, 40], indicating direct, receptor mediated, effects of somatostatin on pancreatic acini. Somatostatin receptors have been described in experimental animals on endocrine and exocrine pancreatic cells by several investigators [41, 42], confirming the possibility that somatostatin may indeed inhibit the growth of somatostatin-receptor-positive pancreatic cancers, directly [12]. Whereas somatostatin receptors are present in human endocrine pancreatic tumors [32, 33], somatostatin receptors were not detected on several human exocrine pancreatic adenocarcinomas [43]; the growth inhibitory effects of Sandostatin on these adenocarcinomas, however, was not examined. Furthermore, preliminary clinical studies with Sandostatin treatment in patients with exocrine pancreatic adenocarcinomas did not reveal any

significant beneficial effects on the patient's survival [43, 44]. Early clinical trials using the somatostatin analogue RC-160 in patients with advanced ductal adenocarcinoma of the pancreas, on the other hand, are encouraging [45]. There may be a discrepancy regarding somatostatin receptor status and growth inhibitory properties of Sandostatin depending on the type of pancreatic cancer. At the present time, it is therefore difficult to know what percentage of human pancreatic cancers may respond to growth inhibitory effects of somatostatin or its analogs. Screening of pancreatic cancers for specific binding sites for putative growth regulatory hormones/factors, such as E_2, somatostatin and CCK, may, however, help in future to determine a more appropriate treatment for the patients, in a fashion analogous to the treatment of breast cancer. Moreover, the evaluation of the receptor status in the tumors may allow to develop optimal radioligands for the *in vivo* localization of these tumors [46].

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