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Secretin Receptors in Normal and Diseased Human Pancreas

Marked Reduction of Receptor Binding in Ductal Neoplasia

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Receptors for gut hormones, which are often overexpressed in cancer, are clinically relevant for receptor-targeted tumor imaging and therapy. Because the receptors for the gut hormone secretin are poorly characterized, we assessed secretin receptor expression in the main secretin target, the human pancreas. We investigated 58 non-neoplastic pancreases and 55 pancreatic tumors for receptor localization and density by *in vitro* receptor autoradiography using [^{125}I]Tyr^{10} rat secretin and for secretin receptor mRNA by reverse transcriptase-polymerase chain reaction. Secretin receptors were highly expressed in non-neoplastic ducts and lobuli and also in lower amounts in ductal neoplasias, including ductal adenocarcinoma, intraductal papillary mucinous tumors, and pancreatic intraepithelial neoplasia. Reverse transcriptase-polymerase chain reaction revealed wild-type receptor mRNA in the non-neoplastic pancreas and both wild-type and spliced variant receptor transcripts in ductal adenocarcinomas. Serous cystic tumors were highly positive for secretin receptors, whereas mucinous cystic tumors were negative. This study is the first to describe the precise secretin receptor distribution in human non-neoplastic pancreas and various pancreatic tumors. High secretin receptor expression in the non-neoplastic ducts reflects the major role of secretin in bicarbonate secretion. Reduced secretin binding in pancreatic ductal tumors may relate to (alternatively spliced) secretin receptor isoforms. Thus, secretin receptors in pancreatic tumors may represent potential clinical targets. *(Am J Pathol 2005, 167:959–968)*

Peptide hormones and their receptors are not only very important physiological regulators of digestion, but they also become increasingly relevant in tumor management: many malignant human tumors overexpress various peptide hormone receptors, which can be used for *in vivo* tumor targeting.[1] For instance, the high expression of somatostatin receptors in gastroenteropancreatic neuroendocrine tumors allows highly effective somatostatin receptor-targeted tumor imaging[2,3] and therapy[4] using radiolabeled somatostatin analogs. The identification of new peptide receptors overexpressed in malignant tumors is an important basis for the development of an effective *in vivo* targeting of these tumors. A novel such target may be the secretin receptor.

Secretin was the first hormone to be identified in history 100 years ago.[5] It is produced in the duodenal mucosa and released from there on food intake; it then acts in the pancreas, where it stimulates bicarbonate and water secretion.[5–7] Although this important role in digestive physiology has been long known, the receptor mediating secretin function was identified only recently.[8] This secretin receptor, together with the VIP receptors, glucagon receptor, and other peptide hormone receptors, belongs to the secretin receptor family, a subgroup of the G-protein-coupled receptor superfamily.[9] Presently, knowledge of secretin receptor expression in the pancreas is derived mainly from animal studies,[10–12] and little data exists on the human pancreas.[13–15] In particular, the precise secretin receptor distribution in the various parenchymal compartments of the human pancreas is unknown. Sparse

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evidence also exists on a possible secretin receptor expression in pancreatic tumors. Secretin receptor mRNA was identified in human pancreatic cancer cell lines,[16] and secretin binding sites were found in a small number of undefined human pancreatic "carcinomas."[15]

The aim of the present study was to assess the localization and the density of secretin receptors in the non-neoplastic human pancreas and various ductal, cystic, and neuroendocrine pancreatic tumors using *in vitro* secretin receptor autoradiography in correlation with morphology. In addition, reverse transcriptase-polymerase chain reaction (RT-PCR) was performed for the assessment of wild-type and spliced forms of the secretin receptor. Knowledge of secretin receptor expression in these tissues may give more insight into pancreatic physiology and tumor biology in humans as well as provide a basis for *in vivo* pancreatic tumor targeting. The development of new treatment strategies for pancreatic cancer is compelling, because these tumors are afflicted with a very poor prognosis.[17]

Materials and Methods

Tissues

For *in vitro* secretin receptor autoradiography, fresh frozen tissue samples obtained from a total of 110 surgical pancreatectomy and biopsy specimens were used. They comprised 21 samples of histologically normal pancreas mainly from transplant organ donors; 37 samples with chronic pancreatitis from pancreases with and without neoplastic disease; and 55 cases of primary pancreatic tumors. These included 31 ductal adenocarcinomas (grade 1, 3; grade 2, 17; and grade 3, 11), 5 intraductal papillary mucinous tumors (IPMTs), 7 serous microcystic adenomas, 3 mucinous cystic borderline tumors, and 9 gastrinomas. Tumor typing and grading was performed according to the World Health Organization guidelines.[18] In some of the carcinoma and chronic pancreatitis samples, noninvasive pancreatic intraepithelial neoplasia (PanIN) was present in the small-caliber ducts. PanIN-1A (mucinous cell hyperplasia) was found in 5, PanIN-1B in 13, PanIN-2 in 7, and PanIN-3 in 4 cases. For the classification of PanIN, the criteria published by the participants of the Pancreas Cancer Think Tank were used (http://pathology.jhu.edu/pancreas_panin).[19] Additionally, five samples of normal antral mucosa and two samples of normal duodenal mucosa were investigated by *in vitro* secretin receptor autoradiography; these tissues were selected as they represent the main source of neuroendocrine G cells, the non-neoplastic counterpart of gastrinomas. The tissue was stored at −80°C. The study conformed to the ethical guidelines of the Institute of Pathology, University of Bern, Switzerland, and the Department of Surgery, University of Heidelberg, Germany, and was reviewed by the respective institutional review boards.

In Vitro *Receptor Autoradiography*

Twenty-micrometer-thick cryostat sections were mounted on precleaned slides and stored at −20°C for several days to improve adhesion of the tissue to the slides. The slides were preincubated in 0.01 mol/L HEPES buffer (pH 7.4) for 5 minutes at room temperature. Afterward, they were incubated for 120 minutes at room temperature in the incubation solution containing HEPES buffer, 1% bovine serum albumin (BSA), 130 mmol/L NaCl, 4.7 mmol/L KCl, 5 mmol/L (Mangan(II)-chlorid)$4H_20$, 1 mmol/L EDTA, 1 mg/ml bacitracin, and 24,000 cpm/100 μl of the radioligand [^{125}I]Tyr^{10} rat secretin^{10} (2000 Ci/mmol; Anawa, Wangen, Switzerland). Nonspecific binding was evaluated by incubating tissue sections with the incubation solution containing additionally 100 nmol/L cold (nonlabeled) human secretin, which at this concentration displaces completely and specifically [^{125}I]Tyr^{10} rat secretin at the receptors. Because VIP and glucagon receptors have a low affinity for secretin,[6] competition experiments were performed to differentiate secretin receptors from these other receptors. For this purpose, serial tissue sections were incubated with [^{125}I]Tyr^{10} rat secretin and increasing concentrations of one of the following cold peptides: human secretin, VIP (Bachem, Bubendorf, Switzerland), and glucagon(1–29) (Bachem). After incubation, the slides were washed five times in ice-cold HEPES containing 1% BSA and twice in ice-cold HEPES without BSA. The slides were dried for 15 minutes under a stream of cold air at room temperature and then exposed to Kodak films Biomax MR for 7 days at 4°C. The resulting signals were analyzed in correlation with morphology using the corresponding tissue section stained with hematoxylin and eosin (H&E). In the antral mucosa, immunohistochemistry with an anti-synaptophysin antibody (BioGenex, San Ramon, CA) was additionally used to specifically identify neuroendocrine cells. The receptor density was quantitatively assessed using tissue standards for iodinated compounds (Amersham, Aylesbury, UK) and a computer-assisted image processing system (Analysis Imaging System; Interfocus, Mering, Germany). In all experiments, rat pancreas was included as positive control.[13]

RT-PCR Analysis of Secretin Receptor Transcripts

Selected cases were used for RT-PCR analysis of secretin receptor transcripts: four samples of normal pancreatic lobuli and six ductal adenocarcinomas with predominantly homogeneous secretin receptor expression. An area of either normal pancreatic tissue or tumor tissue without intervening normal pancreatic parenchyma was selected on an H&E-stained tissue section and cut out of the frozen tissue block with a sterile scalpel blade. Although this method allows specific dissection of a morphologically recognized tumor, we cannot completely exclude that some of the tumor samples were contaminated with residual normal pancreatic parenchyma present at a

deeper level in the tissue block. Human spleen was used as negative control.[13]

The RT-PCR procedure was carried out as follows. RNA was isolated from tissue samples, and RT-PCR was used to identify the secretin receptor spliceoforms present in each sample. Briefly, frozen pancreatic samples were microdissected and weighed before grinding in liquid nitrogen. Resulting tissue powders were decanted into microfuge tubes and resuspended in Trizol reagent (Invitrogen, Carlsbad, CA) to a final concentration of 50 μg tissue/1 ml Trizol. Total cellular RNA was isolated from 100 μl of each Trizol suspension using the RNEasy Mini kit (Qiagen, Valencia, CA). Purified RNA was digested with amplification grade deoxyribonuclease I (DNase I; Invitrogen) for 15 minutes at room temperature to remove any genomic DNA contamination. Subsequently, EDTA was added (2 mmol/L final; Invitrogen) and samples heated to 65°C for 15 minutes to inactivate the DNase I. The purified RNA was used to synthesize cDNA via the Reverse Transcription System (Promega, Madison, WI), and approximately 25 ng of resultant cDNA was used as template for the ensuing PCR reactions. Amplification of human secretin receptor transcripts was performed as previously described[20] using 5′ and 3′ primers corresponding to nucleotides 107 to 127 and 1434 to 1413 of GenBank accession number U28281, respectively (sense, 5′-CCA TGC GTC CCC ACC TGT CGC-3′; and antisense, 5′-CTC TCA GAT GAT GCT GGT CCT G-3′). Control reactions were included using previously described human secretin receptor isoforms (wild-type human secretin receptor and human secretin receptor-Δexon 3) cloned into the pBK-CMV vector[20] or with no template cDNA (water only). Actin PCR reactions were run using the primer pair: sense, 5′-CCA GCT CAC CAT GGA TGA TGA TAT CG-3′; and antisense, 5′-GGA GTT GAA GGT AGT TTC GTG GAT GC-3′. PCR reactions were run in 50-μl volume containing 0.2 μmol/L of both sense and antisense primers, 2 mmol/L $MgCl_2$ (Invitrogen), 0.2 mmol/L dNTPs (Stratagene, La Jolla, CA), and 0.5 unit of Platinum *Taq* Polymerase (Invitrogen). Reactions were performed in a DNA Engine (MJ Research, South San Francisco, CA) using optimized cycle conditions comprised of an initial denaturing step for 2 minutes at 94°C and subsequently 32 cycles of 94°C for 30 s, 64°C for 30 s, and 72°C for 2 minutes. Ten-microliter fractions of each amplification reaction were resolved on a 2% agarose/TAE gel along with the 1-kb Plus Ladder (Invitrogen) as marker.

Results

Secretin Receptor Expression in the Non-Neoplastic Human Pancreas and Antral and Duodenal Mucosa

In the non-neoplastic human pancreas, secretin receptors are highly expressed in all segments of the duct system. This is illustrated in Figure 1, which shows marked and homogeneous secretin binding to a large interlobular duct and surrounding tributary ducts (upper row), a small interlobular duct (middle row), and an intralobular ductule (lower row). Secretin binding is specific as shown by the complete displacement of ^{125}I-labeled secretin by cold secretin. The comparison of the autoradiographic images with the corresponding H&E-stained tissue sections at high magnification reveals that secretin receptors are located in the epithelial layer of the ducts. Table 1 shows the high secretin receptor density in the ducts, with comparable mean values in the various segments of the pancreatic ductal tree. In the pancreatic lobuli, secretin receptors are also highly expressed, basically in two tissue distribution patterns, as shown in Figure 1. Secretin receptor binding is found to be either predominantly homogeneous (Figure 1, lower row) or heterogeneous (Figure 1, middle row). The mean receptor density is as high as in pancreatic ducts (Table 1).

When comparing normal pancreas of transplant organ donors with non-neoplastic pancreatic parenchyma adjacent to tumors or chronic pancreatitis, no differences in secretin receptor expression can be observed in ducts or lobuli. In very rare cases, secretin binding cannot be detected in the pancreas; however, because we know the potential for extremely high enzymatic activity in this organ, it cannot be excluded that this negative result is due to rapid receptor destruction by pancreatic proteases. No secretin receptors are identified in pancreatic islets, blood vessels, or other connective tissue components.

In the antral and duodenal mucosa, cells expressing secretin receptors were observed in the deep portions of the mucosal glands, at a site corresponding to the localization of neuroendocrine cells (G cells), which were identified with immunohistochemistry for synaptophysin on adjacent cryostat sections (Figure 1, K–M).

Secretin Receptor Expression in Pancreatic Tumors

The results of secretin receptor expression in the various pancreatic tumors are summarized in Table 2. In ductal adenocarcinomas, secretin receptors are identified in 16 of 31 cases (52%). The receptor incidence decreases from well to poorly differentiated carcinomas (Table 2). Irrespective of tumor grade, the mean receptor density is moderate and therefore lower than in non-neoplastic pancreatic ducts and lobuli (Table 2). Figure 2 shows in the first row a typical ductal adenocarcinoma which expresses secretin receptors in moderate to high density. In this example, the secretin receptors are distributed homogeneously in the neoplastic ducts. However, in approximately one-half of the tested cases, the secretin receptor expression can be heterogeneous.

In IPMTs, the secretin receptor incidence amounts to 60%. The mean receptor density is high (Table 2). Like in ductal adenocarcinomas, the receptor distribution is often heterogeneous, as illustrated in Figure 2, second row, where secretin receptors are expressed predominantly in the neoplastic papilla in the center compared with the surrounding tumor parts. Correlation of the localization of

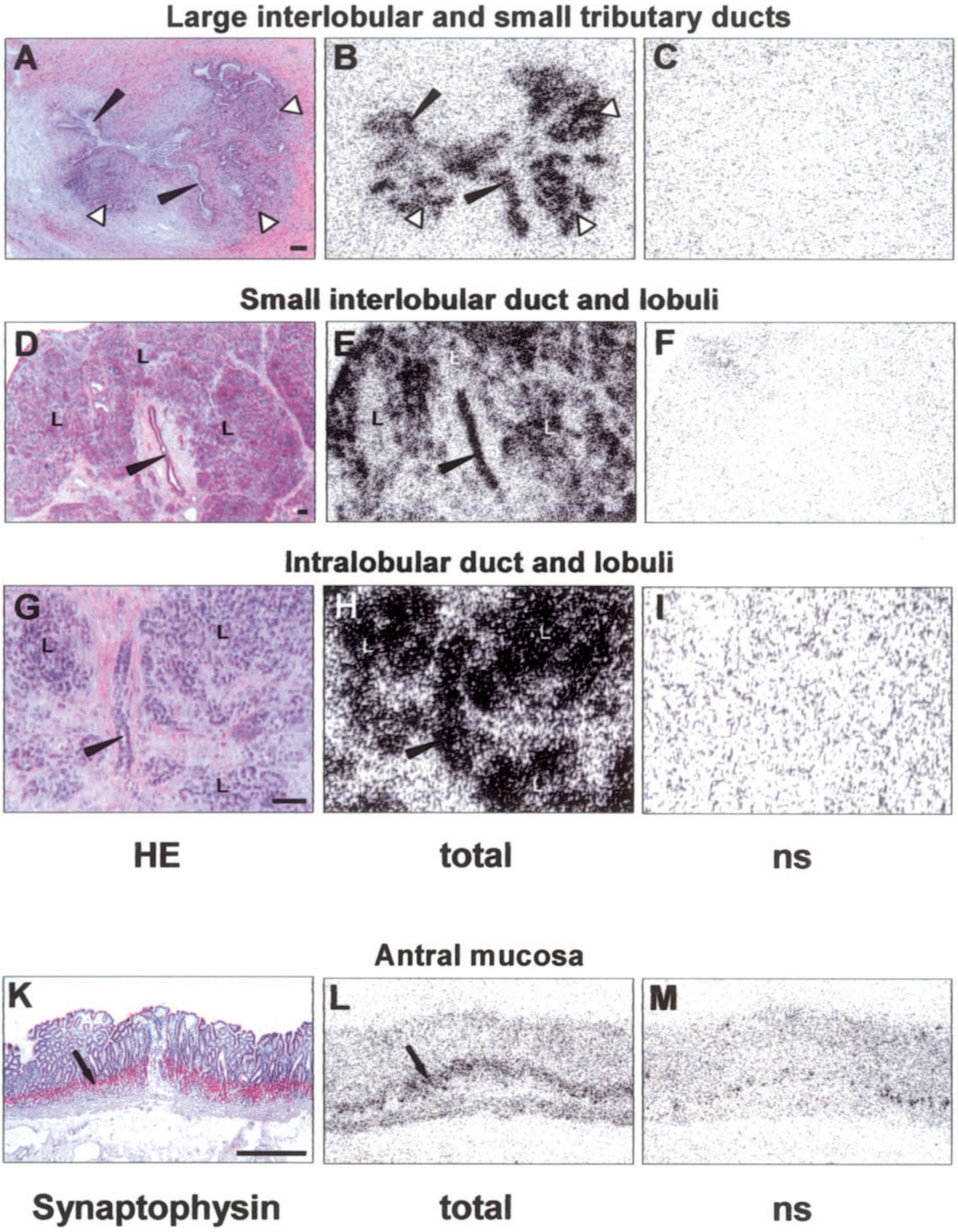

Figure 1. *In vitro* receptor autoradiography on serial tissue sections to assess secretin binding in non-neoplastic tissues, including pancreatic ducts and lobuli and antral mucosa. **A**, **D**, and **G:** H&E-stained tissue sections. **A:** Large interlobular duct (**black arrowheads**) surrounded by smaller tributary ducts (**white arrowheads**); **D:** small interlobular duct (**arrowhead**) and lobuli (L); **G:** intralobular duct (**arrowhead**) within a lobulus (L). **K:** Antral mucosa. The endocrine cells (G cells) in the deep portions of the glands are labeled red by immunohistochemistry for synaptophysin (**arrow**). Bars = 1 mm. **B**, **E**, **H**, and **L:** Autoradiograms showing total binding of [^{125}I]Tyr^{10} rat secretin. Strong and diffuse labeling of all ducts in **B**, **E**, and **H**; strong binding to lobuli, with either heterogeneous (**E**) or homogeneous distribution (**H**) throughout the lobulus; band-like labeling of the deep portion of the antral glands (**L**). **C**, **F**, **I**, and **M:** Autoradiograms assessing nonspecific (ns) binding of [^{125}I]Tyr^{10} rat secretin. Complete displacement of [^{125}I]Tyr^{10} rat secretin in the presence of 100 nmol/L unlabeled human secretin.

Table 1. Secretin Receptor Density in Non-Neoplastic Pancreatic Ducts and Lobuli (Mean Values in Donor Pancreases, Chronic Pancreatitis, and Surroundings of Tumors)

Tissue type	Number of cases	Density (mean ± SEM [dpm/mg tissue])
Interlobular ducts	21	2317 ± 436
Tributary ducts	28	3433 ± 387
Intralobular ducts	5	4502 ± 1858
Pancreatic lobuli	71	3648 ± 262

the receptor signal with the corresponding H&E-stained tissue section reveals that secretin receptors are situated in the tumor epithelium but not the fibrovascular papillary stalks.

All serous microcystic adenomas express secretin receptors homogeneously and in moderate density in the epithelium lining the cysts (Table 2; Figure 2, third row). In contrast, mucinous cystic borderline tumors do not express secretin receptors (Table 2; Figure 2, fourth row), except for a tiny focus of secretin receptors in the mucinous epithelium of an otherwise negative case.

Gastrinomas express secretin receptors in high incidence (Table 2). The receptor density is by far the highest of all pancreatic tumors (Table 2). In most cases, the receptors are distributed homogeneously throughout the tumor tissue (Figure 2, last row). Yet, in some cases, receptor-positive tumor nodules are present next to receptor-negative ones.

Secretin Receptor Expression in PanIN

Secretin receptors are also expressed in PanIN. Compared with non-neoplastic ducts, the secretin receptor expression is very heterogeneous in the various PanIN with respect to both receptor incidence (Table 3) and receptor distribution. This is also illustrated in Figure 3 showing a duct with PanIN-1 without secretin receptors on the right side and a duct with PanIN-2 with high amounts of secretin receptors on the left side.

Pharmacological Characterization of Secretin Receptors

Because other members of the secretin-receptor family, such as VIP and glucagon receptors, also bind secretin,[6,21] we had to demonstrate that the radioligand [^{125}I]Tyr^{10} rat secretin was identifying secretin but not VIP or glucagon receptors in the tissues. Knowing that the secretin receptor binds secretin with a much higher affinity than VIP or glucagon,[6,21] we performed competition experiments using increasing concentrations of secretin, VIP, and glucagon(1–29) to assess their rank orders of potencies at the receptors. Figure 4 shows the results obtained in pancreatic ducts (Figure 4A), pancreatic lobuli (Figure 4B), a ductal adenocarcinoma (Figure 4C), and a serous microcystic adenoma (Figure 4D). In all of these tissues, [^{125}I]Tyr^{10} rat secretin is displaced by cold human secretin with high affinity in the nanomolar range, whereas it is displaced by cold VIP and glucagon(1–29) with only low affinity. A similar rank order of potencies is found in control tissues, such as rat pancreas.[6] These results provide strong pharmacological evidence that secretin receptors are specifically identified.

RT-PCR for Secretin Receptor Transcripts

RT-PCR was performed to ascertain the expression of secretin receptor isoforms in selected samples using primers able to amplify full-length secretin receptor cDNAs. In the non-neoplastic pancreatic tissues containing lobuli and ducts, a single strong band corresponding to the wild-type human secretin receptor mRNA is present (Figure 5). Conversely, in all pancreatic carcinomas, multiple alternatively spliced secretin receptor transcripts are detected in addition to the larger wild-type human secretin receptor band. Of particular interest, a 1220-bp band is present in all carcinoma samples that was fully sequenced to confirm that it corresponds to the spliced variant of the human secretin receptor harboring an exon 3 deletion, described previously in a gastrinoma negative for the secretin provocative test,[22] as well as in human pancreatic cancer cell lines and primary pancreatic tumors.[20] Additional smaller molecular weight spliceoforms similarly seen only in the carcinoma samples are currently undergoing further characterization (G.M. Hayes, unpublished data). Heterogeneous expression of these mRNA variants is illustrated in the ductal adenocarcinomas where the spliced variant transcripts are identified as particularly strong bands compared with the wild-type band (Figure 5). No band is seen in the spleen, a tissue used as receptor-negative control.

Table 2. Secretin Receptor Incidence and Density in Pancreatic Tumors and Pancreatic Duct Lesions

Tumor type	Incidence (%)	Density (mean ± SEM [dpm/mg tissue])
Ductal adenocarcinomas		
Well differentiated (grade 1)	3/3 (100%)	1033 ± 464
Moderately differentiated (grade 2)	10/17 (53%)	1371 ± 279
Poorly differentiated (grade 3)	3/11 (27%)	1568 ± 207
Cystic tumors		
IPMT	3/5 (60%)	4063 ± 2921
Serous microcystic adenomas	7/7 (100%)	1254 ± 173
Mucinous cystic borderline tumors	0*/3 (0%)	
Neuroendocrine tumors		
Gastrinomas	8/9 (89%)	7565 ± 1381

*Including one largely negative case with only a very small receptor-positive focus.

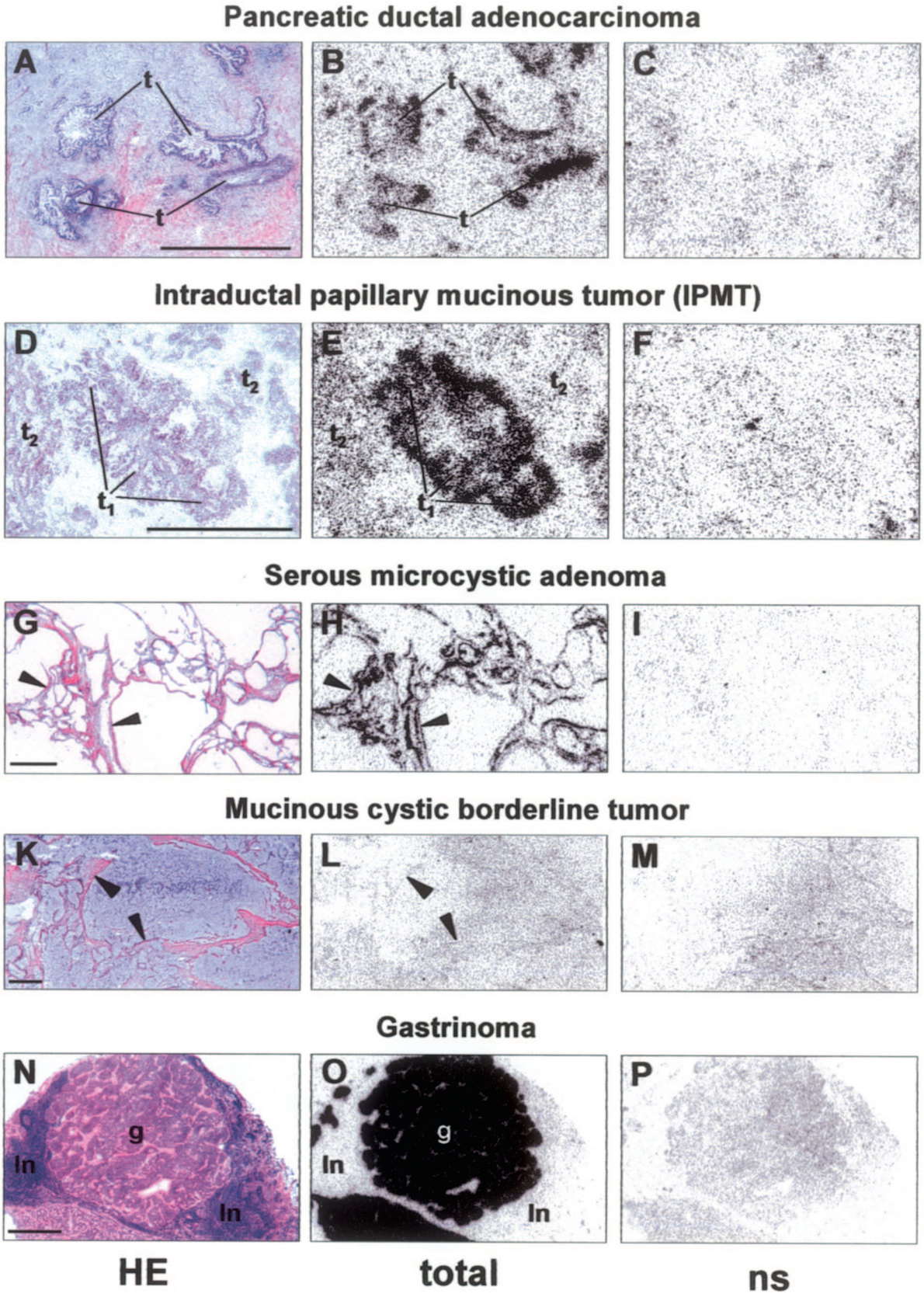
Pancreatic ductal adenocarcinoma
A
t
t
B
t
t
C
Intraductal papillary mucinous tumor (IPMT)
D
t2
t2
t1
E
t2
t2
t1
F
Serous microcystic adenoma
G
H
I
Mucinous cystic borderline tumor
K
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Table 3. Secretin Receptor Incidence and Density in PanIN Identified in the Samples with Chronic Pancreatitis and in Tissues Adjacent to Ductal Cancer

	Incidence (%)	Density (mean ± SEM [dpm/mg tissue])
PanIN-1A	1/5 (20%)	3362
PanIN-1B	9/13 (75%)	1738 ± 637
PanIN-2	4/7 (57%)	3450 ± 1062
PanIN-3	4/4 (100%)	2162 ± 869

Discussion

This study represents the first detailed investigation of the secretin receptor distribution and density in the human pancreas, including the various compartments of the non-neoplastic pancreas as well as various ductal, cystic, and neuroendocrine tumors. The study yields mainly three new messages. First, in the normal pancreas, secretin receptors are highly expressed along the entire ductal system and in the lobuli. Second, secretin receptors are also frequently expressed in pancreatic ductal neoplasia, including ductal adenocarcinomas, PanIN, and IPMTs, but often in reduced amounts compared with the non-neoplastic pancreas. Finally, the secretin receptor expression may permit differentiation of serous and mucinous cystic tumors, because secretin receptors are highly expressed in serous microcystic adenomas but virtually absent in mucinous cystic borderline tumors.

The present contribution represents a significant improvement over current knowledge in regard to the precise secretin receptor localization in the normal human pancreas. Previous studies dealing with human pancreatic secretin receptor expression have used methods that do not allow a correlation with morphology, such as Northern blot analysis[13] and secretin binding assays either performed on homogenized tissue[14] or with low-resolution methodologies.[15] The present investigation can, for the first time, attribute the secretin receptors to specific pancreatic structures and also quantify the data. Secretin receptors are present in high amounts in the epithelium of all segments of the ductal system. They are also highly expressed in the pancreatic lobuli. However, in this site, it is more difficult to link them with specific cell types, such as acinar cells or centroacinar cells, because of the limited resolution of receptor autoradiography. We have tried to compare the pattern obtained from immunohistochemistry for cytokeratin 19 (CK19), which labels centroacinar but not acinar cells,[23] with the pattern obtained from secretin receptor autoradiography on serial tissue sections. Although at the limit of the resolution possibilities of the latter method, this comparison suggests that secretin receptors are localized at least in part on CK19-positive centroacinar cells.

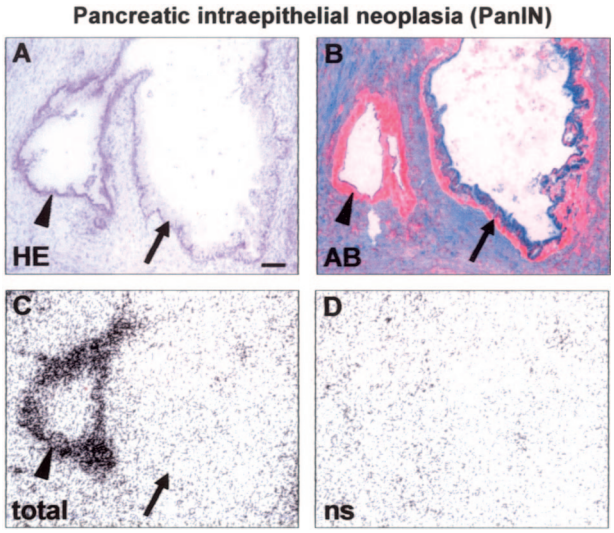

Figure 3. Secretin receptor expression in pancreatic intraepithelial neoplasia. **A:** H&E-stained tissue section showing two branches of a pancreatic duct. On the right (**arrow**), duct lined by an epithelium without cytological or architectural atypia, consistent with PanIN-1; on the left (**arrowhead**), duct lined by an atypical, hyperchromatic, papillary epithelium, corresponding to PanIN-2. Bar = 1 mm. **B:** Alcian blue stain showing prominent intraepithelial mucin in PanIN-1 (mucinous cell hyperplasia; **arrow**), but only traces of mucin on the apical cell portions in PanIN-2 (**arrowheads**). **C:** Autoradiogram showing total binding of [^{125}I]Tyr^{10} rat secretin. Strong and homogeneous labeling of the PanIN-2 duct (**arrowhead**), but no labeling of the PanIN-1 duct (**arrow**). **D:** Autoradiogram showing nonspecific (ns) [^{125}I]Tyr^{10} rat secretin binding (in presence of 100 nmol/L cold secretin).

The considerably high expression of the secretin receptor in the normal human pancreas may well reflect its physiological importance. In the pancreatic ducts and centroacinar cells, the secretin receptors colocalize with enzymes necessary for bicarbonate production and transcellular transport.[24–26] This strongly suggests that the secretin receptors are indeed the mediators for the well-established function of secretin to induce pancreatic bicarbonate and water secretion.[5–7] This statement is further corroborated by the expression of secretin receptors in bicarbonate producing serous cystic tumors but not in mucin-producing mucinous cystic tumors. Whether secretin also plays a role in stimulating enzyme secretion from acinar cells in humans, as it does in rats,[27] is presently unclear. Lack of evidence for secretin receptor expression in pancreatic islets suggests that secretin has no direct effect on pancreatic endocrine function in man.[10]

The pancreatic ducts are the presumed precursors of invasive ductal cancer.[28] It is therefore of interest to compare the incidence and density of secretin receptors in the normal ducts with those in ductal adenocarcinomas. From this point of view, the study suggests that the secretin receptor is underexpressed or lost in carcinoma. This conclusion is based mainly on four arguments: over-

Figure 2. *In vitro* secretin receptor autoradiography on five different pancreatic tumors. H&E-stained tissue sections showing a ductal adenocarcinoma (t; in **A**), an intraductal papillary mucinous tumor (t1 and t2; in **D**), a serous microcystic adenoma (in **G**), a mucinous cystic borderline tumor (in **K**), and a metastasis of a gastrinoma (g) in a lymph node (ln; in **N**). Bars = 1 mm. **B**, **E**, **H**, **L**, and **O:** Autoradiograms showing total binding of [^{125}I]Tyr^{10} rat secretin. **B:** In the ductal adenocarcinoma, strong [^{125}I]Tyr^{10} rat secretin binding to neoplastic ducts (t). **E:** In the IPMT, intense binding of [^{125}I]Tyr^{10} rat secretin to the tumor papilla in the center (t1), with very weak binding in the surrounding tumor portions (t2). **H:** In the serous microcystic adenoma, precise labeling of the epithelium lining the tumor cysts (**arrowheads**) with [^{125}I]Tyr^{10} rat secretin. **L:** In the mucinous cystic borderline tumor, no binding of [^{125}I]Tyr^{10} rat secretin (**arrowheads**). **O:** In the gastrinoma, extremely high [^{125}I]Tyr^{10} rat secretin binding in the tumor (g), but not in the lymph node (ln). **C**, **F**, **I**, **M**, and **P:** Autoradiograms showing nonspecific (ns) [^{125}I]Tyr^{10} rat secretin binding.

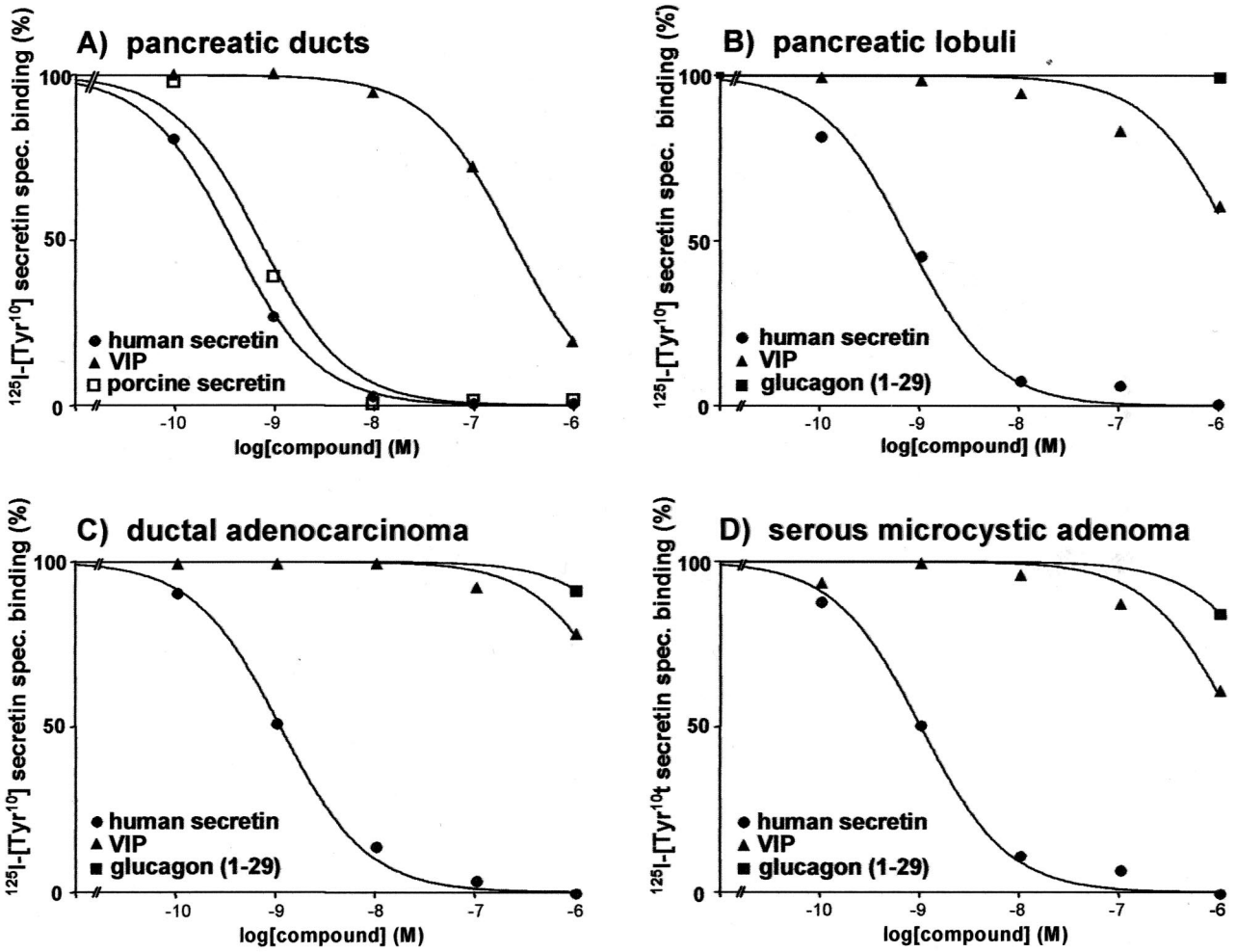

Figure 4. Representative competition experiments showing secretin receptors in pancreatic ducts (**A**), pancreatic lobuli (**B**), a ductal adenocarcinoma (**C**), and a serous microcystic adenoma (**D**). High-affinity displacement of [^{125}I]Tyr^{10} rat secretin by human secretin (●; **A–D**) and porcine secretin (□; **A**), but low-affinity displacement by VIP (▲; **A–D**) or glucagon(1–29) (■; **B–D**).

all, the receptor-positive carcinomas show a lower receptor density than normal pancreatic ducts; in the receptor-positive carcinomas, the receptor expression is often heterogeneous, with areas lacking the receptor; the receptor incidence decreases with higher tumor grade; and there are many receptor-negative carcinomas. These findings may very well be brought in conjunction with the demonstration of abundant secretin receptor spliceoforms in these cancers by RT-PCR. Secretin receptor spliced variants were shown *in vitro* to inactivate the wild-type secretin receptor, inducing loss of binding.[20] This may explain the reduced secretin receptor binding in carcinomas observed with receptor autoradiography.

In PanIN as well as in IPMT, an intraductal pancreatic tumor distinct from PanIN,[30] the same trend of a reduction of secretin receptor expression (decreased receptor incidence and heterogeneous receptor distribution) as in invasive ductal cancer is observed. This provides evidence that the loss of secretin receptor binding in ductal neoplasia not only occurs in invasive cancer but is already present in intraductal noninvasive neoplasia, and it may be speculated that the loss of secretin receptor expression occurs relatively early in ductal neoplastic transformation. Conversely, the gastrinomas display an extremely high secretin receptor binding that is, compared with their non-neoplastic counterparts, the antral and duodenal G cells, at least preserved or even up-regulated.

The biological significance of the secretin receptors in pancreatic cancer is most evident in gastrinomas: secretin receptors presumably mediate the secretin-induced gastrin release,[31] which is the basis for the diagnostic secretin-stimulation test.[32] Conversely, in ductal cancer, the role of secretin receptors in general and the consequences of a reduced secretin receptor expression in particular are yet unknown. At present, only scarce and conflicting experimental data exist regarding these issues. In human ductal adenocarcinoma cell lines expressing secretin receptors, no effect of secretin on cell proliferation or metabolism was observed,[33,34] whereas in a hamster pancreatic adenocarcinoma cell line, secretin led to an increased thymidine incorporation into these cells.[29] In pancreatic serous cystic tumors, the secretin receptors may substantially contribute to the intracystic fluid accumulation, analogous to the secretin-stimulated

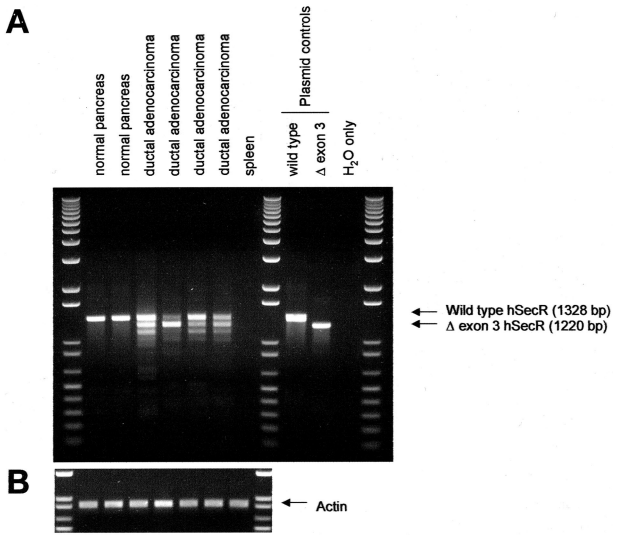

Figure 5. RT-PCR detection of secretin receptor mRNAs in pancreatic tissues. Total RNA was isolated from samples of two normal pancreases and four ductal adenocarcinomas, and RT-PCR was performed using primers able to amplify full-length secretin receptor transcripts (**A**) or actin (**B**). Control reactions were similarly run using wild-type and Δexon 3 human secretin receptor (hSecR) isoforms cloned into the pBK-CMV expression plasmid or with no template DNA added (negative control). The 1-kb Plus ladder from Invitrogen was included as a molecular weight marker.

bicarbonate and water secretion from non-neoplastic pancreatic ducts.

It is well known that other members of the secretin receptor family are expressed in the pancreas, including VIP receptors in the normal human pancreas and pancreatic cancer[35] or glucagon receptors in the rat pancreas.[36] Because these receptors can also bind secretin,[6,21] we performed competition experiments showing that the receptors present in the investigated tissues display a high affinity for secretin but a low affinity for VIP and glucagon. This rank order of potencies provides strong evidence that the identified receptors correspond to secretin receptors.[6,21] The identification of secretin receptors by *in vitro* receptor autoradiography was further substantiated by the results of the RT-PCR analysis. Wild-type secretin receptor mRNA is found in abundance in the non-neoplastic pancreas and invasive ductal cancer. It is absent in the spleen, a tissue used as negative control.

Whereas wild-type secretin receptor transcripts are found in both the non-neoplastic pancreas and ductal adenocarcinomas, transcripts for other forms of secretin receptors are identified in ductal cancer only. mRNA for the spliced variant of the receptor with exon 3 deletion[20,22] as well as for potentially new spliceoforms are consistently present in the investigated carcinomas but not in the normal pancreas. Transcripts for the spliceoform with exon 3 deletion were found previously in human pancreatic primary tumors and pancreatic cancer cell lines.[20] This spliceoform, which does not bind secretin, was shown to inhibit secretin binding when heterodimerized with the wild-type secretin receptor.[20] Potential novel spliceoforms identified in this study are currently undergoing further characterization. As mentioned above, the overall reduction of secretin binding in pancreatic cancer may be closely related to the expression of these spliced variants. The actual level of secretin binding in a particular tumor as assessed by receptor autoradiography may reflect the ratio of wild-type and spliced variant receptor forms in this tumor.

The expression of secretin receptors in pancreatic tumors may possibly lead to future clinical applications. New treatment strategies are needed for pancreatic ductal cancer, which is afflicted with a very poor prognosis.[17] The present analysis of secretin receptor incidence, density, and distribution *in vitro* in clearly defined tumors is a prerequisite for the development of effective *in vivo* receptor targeting of malignant tumors. Analogous to somatostatin receptor targeting of neuroendocrine gastroenteropancreatic tumors,[2–4] a secretin receptor targeting of pancreatic ductal adenocarcinomas using radiolabeled secretin analogs might be effective for therapeutic purposes, especially in combination with targeting of other peptide hormone receptors also expressed in ductal cancer, such as neurotensin receptors[37,38] or VIP receptors.[35]

Apart from a secretin receptor targeting with radiolabeled secretin analogs, another therapeutic option that can be derived from the present study may consist of the application of nonradioactive secretin receptor antagonists[39] for the treatment of serous cystic tumors. Such drugs could achieve a reduction in tumor size by a secretin receptor-mediated inhibition of fluid secretion, resulting in a reduction of symptoms and an easier surgical resection of the tumor.

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