Langenbecks Arch Chir (1995) 380: 90–95

ORIGINAL ARTICLE

S. Jonas · M. John · J. Boese-Landgraf · R. Häring
G. Prevost · F. Thomas · S. Rosewicz · E.-O. Riecken
B. Wiedenmann · P. Neuhaus

Somatostatin receptor subtypes in neuroendocrine tumor cell lines and tumor tissues

Received: 12 September 1994

Subtypen des Somatostatinrezeptors in neuroendokrinen Tumorzellinien und Tumorgeweben

Abstract Somatostatin receptor scintigraphy (SRS) is positive in approximately 80% of all patients who have been found to have neuroendocrine (NE) gastroenteropancreatic (GEP) tumors. The reasons for negative results are unclear. The aim of the present study was identification of the specific somatostatin receptor (SSTR) subtypes that are responsible for the in vivo binding of the widely used somatostatin (SST) analogues octreotide and lanreotide in human neuroendocrine gastroenteropancreatic tumors. Ten patients were subjected to SRS with radiolabeled octreotide. Following surgical resection, tumor tissues were analyzed for SSTR subtype mRNA expression by the reverse transcription-polymerase chain reaction (RT-PCR). In addition, SSTR subtype transcripts were investigated by Northern blot analysis and RT-PCR in neuroendocrine tumor cell lines. Expression of SSTR at the protein level was studied by chemical cross-linking experiments. Three patients were negative by SRS. However, RT-PCR revealed most prominently SSTR 2 expression in all tumor specimens. In addition, all tumor tissues analyzed by chemical crosslinking exhibited SST-14 binding sites, indicating that at least some NE tumors were false-negative on SRS. The heterogeneous SSTR subtype pattern of NE tumor cell lines with either SSTR 1 (BON, RIN 38) or SSTR 2 (QGP 1, AR 42 J) as the predominant subtype suggests an additional role for other SSTR subtypes than SSTR 2.

Zusammenfassung In 80% der Fälle von neuroendokrinen (NE-) Tumoren des gastroenteropankreatischen (GEP-) Systems ergibt die Somatostatinrezeptorszintigraphie (SRS) positive Ergebnisse, wobei Ursachen negativer Befunde nicht bekannt sind. Ziel dieser Studie war die Identifizierung spezifischer Subtypen des Somatostatinrezeptors (SSTR), an die in NE-Tumoren des GEP-Systems die verbreiteten Somatostatin(SST-)analoge Oktreotid und Lanreotid in vivo binden. Bei 10 Patienten mit NE-Tumoren wurde eine SRS mit markiertem Oktreotid durchgeführt und anschließend eine chirurgische Resektion vorgenommen. Der Nachweis der mRNA-Expression von SSTR-Subtypen im Tumorgewebe erfolgte mittels reverser Transkription und Polymerasekettenreaktion (RT-PCR). Weiterhin wurden Transkripte von SSTR-Subtypen in NE-Tumorzellinien durch Northern-blot-Analyse und RT-PCR untersucht. Die Expression von SSTR-Subtypen auf der Proteinebene wurde anhand chemischer Quervernetzungsexperimente nachgewiesen. Ergebnisse der SRS waren in 3 von 10 Patienten mit einem NE-Tumor des GEP-Systems negativ. In allen Tumorgeweben wurde mittels RT-PCR hauptsächlich die Expression des SSTR 2-Subtyps nachgewiesen. Weiterhin konnten in allen untersuchten Tumorgeweben bei der chemischen Quervernetzung SST 14-Bindungsstellen dargestellt werden, so daß von falsch-negativen Ergebnissen der SRS ausgegangen werden muß. Das heterogene Muster von SSTR-Subtypen in NE-Tumorzellinien mit vornehmlicher SSTR 1- oder SSTR 2-Expression in BON- und RIN 38- bzw. in QGP 1- und AR 42 J-Zellinien könnte für eine zusätzliche Rolle anderer Subtypen als SSTR 2 sprechen.

Schlüsselwörter Somatostatinrezeptor-Subtypen
Neuroendokrine Tumorzellinien

S. Jonas · P. Neuhaus (✉)
Freie Universität Berlin, Universitätsklinikum Rudolf Virchow, Department of Surgery, Augustenburger Platz 1, D-13353 Berlin, Germany

J. Boese-Landgraf · R. Häring
Freie Universität Berlin, Universitätsklinikum Benjamin Franklin, Department of Surgery, Berlin, Germany

M. John · S. Rosewicz · E.-O. Riecken · B. Wiedenmann
Freie Universität Berlin, Universitätsklinikum Benjamin Franklin, Department of Gastroenterology, Berlin, Germany

G. Prevost
Institut d'Oncologie, Bobigny, France

F. Thomas

Introduction

Neuroendocrine (NE) tumor cells of the gastroenteropancreatic (GEP) system contain high-affinity binding sites for somatostatin (SST) and its analogues octreotide and lanreotide [23–26]. Stable somatostatin analogues (e.g., octreotide and lanreotide) are clinically used both to localize NE GEP tumors by somatostatin receptor scintigraphy (SRS) and, therapeutically, to control specific hypersecretion syndromes [9, 12, 28, 33]. Although most of these tumors are positive on SRS, approximately 20–25% of all NE GEP tumors are not detected by SRS when octreotide is used as ligand [28, 33]. Interestingly, some of the scintigraphically negative tumors exhibit in vitro binding sites for the "pan-ligand" somatostatin 14 (SST 14), but not for octreotide. This suggests that different somatostatin receptor subtypes must exist in NE tumor cells [11, 13].

Recently, five distinct somatostatin receptor (SSTR) subtypes have been identified and characterized by molecular cloning and expression studies [1, 2, 4, 5, 15–17, 22, 27, 29–32]. All SSTRs have been shown to be G-protein-coupled and to exhibit seven putative membrane-spanning domains [1, 17]. SSTR subtypes differ in their affinities for specific ligands, such as SST-14, SST-28, or stable SST analogues, such as octreotide [2, 22]. In vitro experiments showed that lanreotide and octreotide mainly bind SSTR 2 with high affinity. Thus, different SSTR subtypes expressed in NE GEP tumors cells may account for the positive or negative results obtained with SRS.

In the present study, we investigated the expression of SSTR 1–3 mRNA in both pancreatic NE cell lines and NE GEP tumor tissues. In addition, expression of SSTR at the protein level was studied by chemical crosslinking experiments using SST-14 as ligand.

Materials and methods

Cell culture

The NE pancreatic tumor cell lines AR 42 J (rat) [7], RIN 38 (rat) [18], BON (human) [6] and QGP 1 (human) [8] were cultured in plastic flasks or in 60-mm-plastic petri dishes as previously described. Cells were harvested at >90% confluence.

Tumor tissues

Tumor tissues of 10 patients with NE GEP tumors were obtained by surgery at the Universitätsklinikum Rudolf Virchow (UKRV) and Universitätsklinikum Benjamin Franklin (UKBJ), Berlin, Germany. Informed consent was obtained from all patients, and the study was performed in accordance with the standards set by the ethical committee of the UKRV and UKBJ. Tissue samples were quick-frozen in liquid nitrogen and stored at –80 °C. The NE tumor histology was verified by conventional and immunohistological methods prior to either RNA or protein preparation.

Somatostatin receptor scintigraphy (SRS) was performed preop-

RNA isolation

RNA extraction from reference tissue (rat brain) and cells (AR 42 J, RIN 38, BON, QGP 1) was performed by the guanidinium isothiocyanat/cesiumchloride method as previously described [14]. Subsequent mRNA preparation was done by the use of a PolyAtract kit (Promega, Serva, Heidelberg, FRG) according to the manufacturer's instructions.

Total RNA from minor amounts of tissue was purified following the method of Chomczynski and Sacchi [3] with minor modifications. Briefly, 10–20 mg tumor tissue were homogenized in guanidinium thiocyanate homogenization buffer. RNA purification was performed in an acidified solution by phenol/chloroform/isoamyl alcohol extraction. Aqueous phase was subsequently extracted twice by chloroform.

RNA samples were precipitated using isopropanol, the pelleted RNA was washed twice with 75% ethanol, stored in 75% ethanol at –80 °C and redissolved in RNase-free water prior to use for Northern blotting or reverse transcription.

Northern blotting

mRNA analysis was done by Northern blotting using 20 µg of denatured mRNA separated on a formaldehyde-agarose-gel and transferred to a nylon membrane (Hybond N, Amersham, Braunschweig, Germany) by standard capillary blotting methods [14].

pPUC 18 containing mouse SSTR 1 cDNA and pGEM 3 Z containing SSTR 2 cDNA were kind gifts from S. Seino, Chiba, Japan. pBluescript SK containing rat SSTR 3 cDNA was a kind gift of W. Meyerhof, Hamburg, FRG. A 0.95-kb Pstl/Sall fragment of the mouse SSTR 1, a 1.2-kb Xbal fragment of the mouse SSTR 2 and a 3.6-kb EcoRI fragment of the rat SSTR 3 were used as SSTR-subtype-specific cDNA probes. The respective cDNAs were labeled with ^{32}P-dCTP (NEN) using a random prime labeling kit (Amersham, Braunschweig, FRG). Hybridization using SSTR-specific radiolabeled cDNA probes was carried out overnight in buffer containing formamide at 42 °C, followed by high-stringency washes. Northern blots were exposed to X-ray films at –80 °C 24–72 h.

Reverse transcription-polymerase chain reaction (RT-PCR)

First-strand cDNA for PCR was generated from about 1 µg of total RNA or mRNA. Reverse transcription was carried out according to standard protocols of the manufacturer using M-MLV reverse transcriptase (Gibco-BRL, Berlin, Germany). Control experiments were carried out without M-MLV reverse transcriptase or with water instead of mRNA solution (control RT). Unless otherwise indicated, the RT mixture was used directly as template for PCR in a 1/500 dilution. Control amplifications were performed with control RT as template to exclude contamination of the reaction mixtures. No amplified cDNA was found in any of the control experiments. The identity of the amplified cDNA fragments was confirmed by Southern blotting and subsequent hybridization with SSTR-subtype-specific cDNA probes as described below.

For semiquantitative determination of SSTR subtype mRNA of NE tumor cell lines, RT mixtures were amplified at consecutive PCR cycles using subtype-specific primer pairs: human SSTR 1: 5′-TCCCAGAACGGGACCTTGAGC and 5′-AACCTGGGCGTGT GGGTGCTA, human SSTR 2: 5′-GGCTCTGTGGTGTCAAC-CAAC and 5′-TGCACCATCAACTGGCCAGGT, human SSTR 3: 5′-TCGGTGTCCACGACCTCAGAA and 5′-CGCTACCTGGCC GTGGTACAT, rat SSTR 1: 5′-GGTCAGGGTAGCGCCATTCTC and 5′-TGCGGTGCGTGAGAAGACCAC, rat SSTR 2: 5′-CTG-GCCTCCGGAGCAACCAGT and 5′-CGTGGTCTCATTCAG-CCGGGA, rat SSTR 3: 5′-CTGGACACGTCCCTGGGGAAT and 5′-ATGAAGGCTGTTCGCCAGGCA. The reaction was carried out in 10 mM Tris-HCl buffer pH 9.0 containing 50 mM KCl, 0.1% Triton X-100, 1.0 mM $MgCl_2$, 100 µM of each dNTP, 12.5 pmol of each primer and 2.5 U Taq DNA Polymerase (Promega-Serva, Heidel-

a Trio Thermocycler (Biometra, Göttingen, FRG). Following an initial denaturing step for 5 min at 95 °C, Taq DNA polymerase was added. The amplification program consists of 28–38 cycles with 30 s denaturation at 94 °C, 1 min annealing at 63 °C and 1.5 min extending at 72 °C.

Human and rat glyceraldehyde-3-phosphate dehydrogenase-specific primers (Clontech, ITC, Heidelberg, FRG) were used to amplify the respective cDNA fragment (about 1000 bp in size) as internal standard according to the manufacturer's instructions.

Amplificates from consecutive cycles were analyzed by agarose gel electrophoresis (2% agarose), DNA was visualized by ethidium bromide, and photographs of the respective gels were scanned and subsequently examined by densitometry. The PCR resulted in single bands of the expected size (human SSTR 1, 417 bp; human SSTR 2, 531 bP; human SSTR 3, 420 bp; rat SSTR 1, 428 bp, rat SSTR 2, 521 bp; rat SSTR 3, 551 bp).

RNA samples from tumor tissued were analyzed in RT-PCR using a pair of degenerated primers chosen from highly conserved regions of all known SSTR subtypes from rat, mice and humans (srcom 1: 5′-AYCGITAYSTGGCYGTRGTICAYC, srcom 2: 5′-VGG -GTTKGCRCAGCTRTTRGCRTA). The amplification was carried out in 10 mM Tris HCl buffer at pH 9.0, containing 50 mM KCl, 0.1% Triton X-100, 1.6 mM $MgCl_2$, 200 µM of each dNTP, 50 pmol of each primer and 2.5 U Taq DNA Polymerase (Promega-Serva, Heidelberg, FRG) in a final volume of 50 µl. Following an initial denaturing step for 3 min at 95 °C, Taq DNA polymerase was added. The amplification program consists of 35 cycles with 40 s denaturation at 95 °C, 1.5 min annealing at 55 °C and 1.5 min extending at 72 °C.

Aliquots of the PCR mixtures were analyzed by agarose gel electrophoresis using a 2% agarose gel. The PCR resulted in cDNA fragments of the expected size of about 500 kb. After electrophoresis, gels were denatured in buffer A (0.5 M NaOH, 1.5 M NaCl) about 30 min and subsequently blotted on positively charged nylon membranes (Amersham Hybond N+, Braunschweig, Germany) for a minimum of 2 h using a standard capillary blotting protocol with buffer A as blot buffer. After blotting, membranes were washed briefly in neutralization buffer and baked for 2 h at 80 °C.

Southern blot analysis

Southern blots of PCR amplificates were probed with fluorescin-labeled probes of the respective SSTR cDNA fragments. Labeling, hybridization and ECL detection were carried out according to the manufacturers protocol (Amersham, Braunschweig, Germany) with three high-stringency washes at 65 °C.

Chemical crosslinking of somatostatin receptors with SST-14

Chemical crosslinking of SSTR with radiolabeled SST-14 was performed as previously described [19, 20]. Briefly, frozen tumor tissue samples or frozen cell pellets from about 10^6 cells of each cell line (AR 42 J, RIN 38, BON) were homogenized by sonication in cold buffer containing 20 mM Tris-HCl pH 7.4, 1 mM PMSF, 20% glycerol, diluted at 1 g protein/ml and stored at –20 °C. The protein concentration was determined by a colorimetric method (Sigma, Paris, France). The solution was diluted at a protein concentration of 1 mg/ml in cold binding buffer containing 20 mM Tris-HCl pH 7.4, 5 mM $MgCl_2$, 1 mM PMSF. Then 100 µl of the respective protein solution was mixed with 50 µl of ^{125}I-labeled somatostatin-14 (10^5 dpm) solubilized in 100 mM Tris-HCl pH 7.4, 5 mM $MgCl_2$, 1 mM PMSF, 0.1% bovine serum albumin (BSA). The mixture was incubated at 4 °C for 30 min. Chemical crosslinking was achieved by the use of 0.1 mM ethylene-glycol-bis(succinimidyl-succinate) (Sigma, Paris, France), and crosslinking was done at 4 °C for 15 min. Crosslinking was stopped by the addition of 50 µl gel loading buffer (125 mM Tris-HCl pH 7.4, 10% SDS, 50% glycerol, 20% mercaptoethanol, 0.025% bromophenol blue). To confirm specific binding of SST-14, control binding experiments were performed with unlabeled SST-14 in excess. Samples were heated at 95 °C for 2 min and proteins were separated in 12.5% SDS polyacrylamide gel. Gels were dried and autoradiographed at –80 °C.

Results

SSTR subtype expression in NE tumor cell lines

The distribution of SSTR 1, SSTR 2, SSTR 3 was examined by Northern blotting in rat brain as reference tissue and in cell lines from rat (AR 42 J, RIN) and humans (QGP 1, BON). By Northern blotting, readily detectable levels of SSTR 1 mRNA (3.6 kb) were found only in rat brain and RIN insulinoma cells (Fig. 1, Table 1). After prolonged times of exposure, a weak signal for SSTR 1, mRNA was found in the case of AR 42 J cells. SSTR 2 mRNA was detected at high levels in rat brain and in AR 42 J cells, but only at a low signal strength in RIN cells (Fig. 1, Table 1). Transcripts of 2.2 kb hybridized with the SSTR 2 probe. The SSTR 3 mRNA transcripts of approximately 4.4 kb were only detected in rat brain and not in any of the cell lines studied (Fig. 1, Table 1).

SSTR subtype gene expression was also analyzed by RT-PCR technique using SSTR subtype specific primer pairs in the subsequent PCR step. In all PCR experiments, the amplification products were of the expected sizes

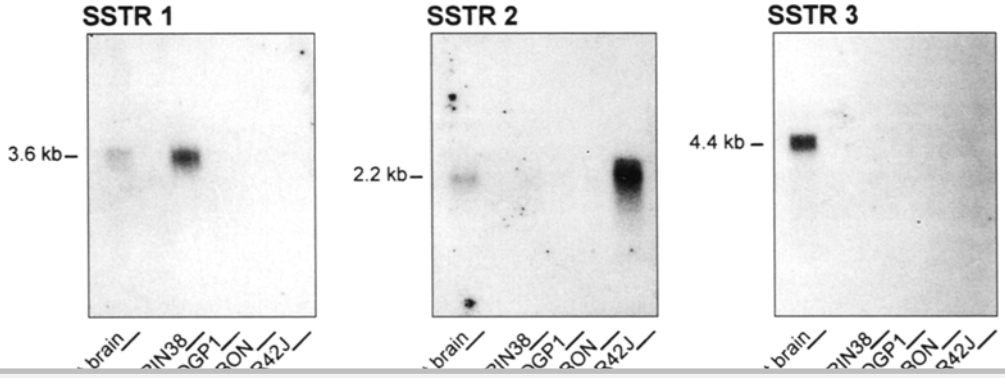

Fig. 1 SSTR subtype mRNA expression in NE pancreatic tumor cell lines as revealed by Northern blot analysis. Poly(A) RNA samples from cell lines (RIN 38, QGP 1, BON, AR 42 J) and rat brain (control) were denatured, electrophoresed, transferred to nylon membranes and hybridized with radiolabeled SSTR subtype-specific cDNA probes

Table 1 Specific somatostatin receptor (SSTR) subtype mRNA expression in neuroendocrine (NE) tumor lines as revealed by Northern blot analysis (+ hybridization signal; (+) low signal after prolonged exposure time; – negative)

	SSTR 1	SSTR 2	SSTR 3
Rat brain	+	+	+
QGP 1	–	–	–
BON	–	–	–
RIN 38	+	(+)	–
AR 42 J	(+)	+	–

Table 2 SSTR subtype mRNA levels and SST-14 binding in NE pancreatic tumor cell lines. Relative abundance of SSTR subtype transcripts was estimated from the signal strength of the electrophoresed amplifications yielded by consecutive PCR cycles in relation to glyceraldehyde-3-phosphate dehydrogenase mRNA expression (*n.d.* not detected; *n.t.* not tested; *RT-PCR:* (+), +, ++, +++ relative abundance; *chemical crosslinking:* + signal for specific SST-14 binding, i.e., specific binding of radiolabeled SST-14 to polypeptide of 42, 57, 70 or 90 kDa in SDS-PAGE)

	RT-PCR			Crosslinking
	SSTR 1	SSTR 2	SSTR 3	SST-14
QGP 1	++	+	n.d.	n.t.
BON	+++	+	(+)	+
AR 42 J	++	+++	(+)	+
RIN 38	+++	++	(+)	+

Table 3 SSTR subtype expression in human NE GEP tumor tissues. RT-PCR analysis was performed using degenerated primer pairs. PCR-generated DNA fragments were analyzed by Southern blot analysis using SSTR subtype-specific probes [–, (+), +, ++, intensity of the hybridization signal] (*SRS* somatostatin receptor scintigraphy (performed preoperatively in all patients: + positivity as described elsewhere [7, 8]); *chemical crosslinking:* + positive signal for specific SST-14 binding as described in Table 2, *p* primary tumor, *m* metastasis)

Tumor tissue	RT-PCR			SRS	Cross-linking
	SSTR 1	SSTR 2	SSTR 3		
Insulinoma (liver m.)	+	++	–	–	+
Atypical carcinoid (p.)	–	++	–	–	+
Carcinoid 1 (ovary, m.)	(+)	++	–	–	+
Carcinoid 2 (liver m.)	+	++	–	+	+
Carcinoid 3 (p.)	+	++	–	+	n.t.
Carcinoid 4 (p.)	–	++	–	+	+
Carcinoid 5 (liver m.)	–	++	–	+	+
Gastrinoma 1 (p.)	–	++	–	+	+
Gastrinoma 2 (liver m.)	+	++	–	+	+
Gastrinoma 3 (liver m.)	(+)	++	–	+	n.t.

(Fig. 2). In addition, the specificity of the primer pairs was confirmed by Southern blotting and subsequent hybridization of the respective amplified DNA fragments with SSTR subtype specific cDNA probes (data not shown).

Figure 1 shows the results of a representative RT-PCR experiment using mRNA of the indicated NE tumor cells

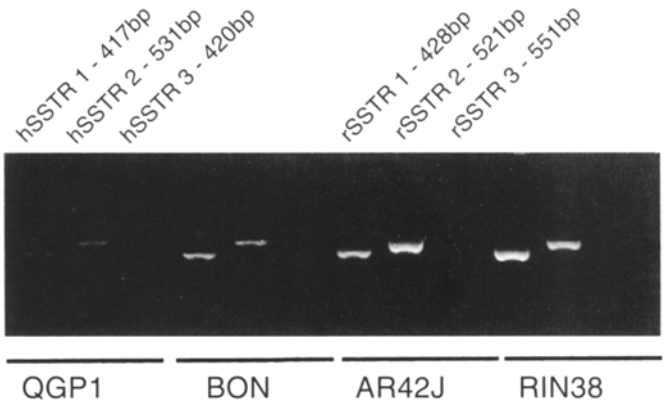

Fig. 2 SSTR subtype mRNA expression in NE pancreatic tumor cell lines as revealed by RT-PCR analysis. Poly(A) RNA samples from cell lines were reversely transcribed, subsequent PCR was performed using SSTR subtype specific primer pairs (h, human; r, rat). Reaction mixtures were analyzed by electrophoresis in ethidium bromide stained agarose gels. The PCR resulted in single bands of the expected size (hSSTR 1 – 417 bp, hSSTR 2 – 531 bp, hSSTR 3 – 420 bp, rSSTR 1 – 428 bp, rSSTR 2 – 521 bp, rSSTR3 – 551 bp)

the subtype transcripts (Table 2) was estimated from the signal strength of the electrophoresed amplificates yielded by consecutive cycles in relation to glyceraldehyde-3-phosphate dehydrogenase mRNA expression. SSTR 1 transcripts were detected most prominently in RIN 38 cells and in BON cells, and at lower levels in AR 42 J and QGP 1 cells. The SSTR 2 transcript was found to be the predominant SSTR mRNA in AR 42 J and QGP 1 cells and to occur at lower levels in BON and RIN cells. The expression levels of SSTR 3 mRNA were very low in both BON and AR 42 J cells, and not detectable in QGP 1 and RIN cells.

The expression of SSTR at the protein level was confirmed by chemical crosslinking experiments using SST-14 as ligand. As shown in Table 2, all cell lines tested exhibited positive signals for SST-14 binding. Cell lines positive by chemical crosslinking showed specific binding of radiolabeled SST-14 to polypeptides of 42, 57, 70 or 90 kDa in SDS-PAGE, owing to the molecular heterogeneity of the SSTR found in this analytical approach [21].

SSTR subtypes in human NE GEP tumors

Tumor tissue samples of ten patients preoperatively subjected to somatostatin receptor scintigraphy (SRS) were studied by RT-PCR and chemical crosslinking (Table 3). The NE GEP tumors of seven patients (4 with carcinoids, 3 with gastrinomas) were positive on SRS. In three patients with, variously, an insulinoma, an atypical carcinoid and an ileal carcinoid, the primary tumor or metastases failed to be detected by SRS. Interestingly, all tumor tissues investigated were positive by chemical crosslinking with SST-14 (Table 3). As mentioned above, tumor tissues positive by chemical crosslinking showed specific binding of

SDS-PAGE. Thus, all tumor tissues tested exhibited somatostatin-binding sites in vitro. RT-PCR analysis of the expression of the SSTR subtype transcripts using degenerated primer pairs revealed a heterogeneous pattern. SSTR 2 mRNA was found at high levels in all tumor tissues, whereas SSTR 1 subtype mRNA was detectable in only six of the ten tissue samples. SSTR 3 transcripts were not detected in any of the tumor tissues tested under the PCR conditions used.

Discussion

Binding sites for SST-14, SST-28 and the clinically relevant SST analogues octreotide or lanreotide have been found in a large number of NE tumors by in vitro radioligand binding techniques [23–26]. Using SRS, in vivo binding of the SST analogue octreotide to NE tumors was initially observed by Lamberts and co-workers [9, 12] and was later confirmed by others [28, 33]. To date, the expression of the known SSTR subtypes (for nomenclature see [2]) has only been examined in a small number of patients with NE tumors of the pancreas and intestine by a non-quantitative RT-PCR approach [10]. The authors suggested that only SSTR 2 was functionally relevant, but the study lacks data on the expression of SSTRs at the protein level. In addition, the data obtained were not correlated to in vivo binding conditions, i.e. SRS.

In this study, we have assessed the composition of SSTR subtypes in human NE tumor tissue by RT-PCR and by in vitro crosslinking using SST-14 as the SSTR ligand as well as by in vivo imaging using SRS. In addition, we studied SSTR subtypes in NE tumor cell lines by Northern blotting, RT-PCR and in vitro crosslinking using SST-14 as SSTR ligand.

SRS was performed in all ten patients and failed to detect tumors in three patients. As demonstrated both by RT-PCR and by crosslinking experiments, all tumor tissues exhibited at least one SSTR subtype transcript and all tumor tissues investigated contained SST-14-binding sites. At present, we cannot exclude the possibility that the expression of SSTRs was due to the presence of some normal tissue surrounding the tumors and expressing SSTR. However, our data indicate that at least some NE tumor lesions were false-negative by SRS.

The observed SSTR subtype pattern determined by RT-PCR of NE GEP tumor tissues and cell lines implies a central role for SSTR 2 as target for octreotide or lanreotide in tumor diagnostics (SRS) and therapy (control of hypersecretion syndromes). However, as SRS-negative tumor tissues showed somatostatin-binding sites in vitro, the development of additional SSTR-subtype-specific ligands may be proposed for these tumors to avoid false-negative results in SRS.

In addition, the heterogeneous SSTR subtype pattern of NE tumor cell lines with either SSTR 1 (BON, RIN) or SSTR 2 (QGP 1, AR 42 J) as the predominant subtype suggests that GEP tumor tissues and tumor cell lines should be also studied by semiquantitative PT-PCR to determine the complete SSTR subtype pattern, including the recently described subtypes 4 and 5.

Further studies, using SSTR subtype-specific ligands may help to clarify the role of SSTR subtypes other than SSTR 2. Such investigations may lead to improved diagnosis and treatment of NE GEP tumor disease.

Acknowledgements The authors thank I. Eichhorn for expert technical assistance. This study was supported in part by grants from the Deutsche Krebshilfe/Dr.-M.-Scheel-Stiftung (W31/91/Wi1), the Verum-Stiftung, and the Deutsche Forschungsgemeinschaft (SFB 366/A5) to B.W.

References

1. Bell GI, Reisine T (1993) Molecular biology of somatostatin receptors. Trends Neurosci 16:34–38
2. Bruns C, Weckbecker G, Raulf F, Kaupmann K, Schoeffter P, Hoyer D, Lübbert H (1994) Molecular pharmacology of somatostatin receptor subtypes. Ann NY Acad Sci (in press)
3. Chomczynski P, Sacchi N (1987) Single-step method of RNA isolation by acid guanidinium thiocyanata-phenol-chloroform extraction. Anal Biochem 162:156–159
4. Corness JD, Demchyshyn LL, Seeman P, Tol HHM van, Srikant CB, Kent G, Patel YC, Niznik HB (1993) A human somatostatin receptor (SSTR 3), located on chromosome 22, displays preferential affinity for somatostatin-14 like peptides. FEBS Lett 321:279–284
5. Demchyshyn LL, Srikant CB, Sunahara RK, Kent G, Seeman P, Tol HHM van, Panetta R, Patel YC, Niznik HB (1993) Cloning and expression of a human somatostatin-14-selective receptor variant (somatostatin receptor 4) located on chromosome 20. Mol Pharmacol 43:894–901
6. Evers BM, Townsend CM, Upp J, Allen E, Hurlbut S, Kim S, Rajaraman S, Singh P, Reubi JC, Thompson J (1991) Establishment and characterization of human carcinoid in nude mice and effect of various agents on tumor growth. Gastroenterology 101:303–311
7. Jessop NW, Hay RJ (1980) Characteristics of two rat pancreatic exocrine cell lines derived from transplantable tumors. In Vitro Cell Dev Biol 16:212
8. Kaku M, Nishiyama T, Yagawa K, Abe M (1980) Establishment of a carcinoembryonic antigen-producing cell line from human pancreatic carcinoma. Gann 71:596–601
9. Krenning EP, Breeman WAP, Kooij PPM, Lameris JS, Bakker WH, Koper JW, Ausema I, Reubi JC, Lamberts SWJ (1989) Localisation of endocrine-related tumours with radioiodinated analogue of somatostatin. Lancet I:242–244
10. Kubota A, Yamada Y, Kagimoto S, Shimatsu A, Imamura M, Tsuda K, Imura H, Seino S, Seino Y (1994) Identification of somatostatin receptor subtypes and an implication for the efficacy of somatostatin analogue SMS 201-995 in treatment of human endocrine tumors. J Clin Invest 93:1321–1325
11. Kvols LK, Brown ML, O'Connor MK, Hung JC, Hayostek RJ, Reubi JC, Lamberts SWJ (1993) Evaluation of a radiolabeled somatostatin analog (I-123 octreotide) in the detection and localisation of carcinoids and islet cell tumors. Radiology 187:129–133
12. Lamberts SWJ, Bakker WH, Reubi JC, Krenning EP (1990) Somatostatin-receptor imaging in the localization of endocrine tumors. N Engl J Med 323:1246–1249
13. Lamberts SWJ, Hofland LJ, van Koetsveld PM, Reubi JC, Bruining HA, Bakker WH, Krenning EP (1990) Parallel in vivo and in vitro detection of functional somatostatin receptors in human

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS
Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS
Sync your system to PACER to automate legal marketing.