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CLINICAL STUDY

The analysis of quantitative expression of somatostatin and dopamine receptors in gastro-entero-pancreatic tumours opens new therapeutic strategies

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Abstract

Objective: Somatostatin (sst) are present in the majority of gastro-entero-pancreatic (GEP) tumours. Effects of somatostatin receptor (sst) analogues are partial and of limited duration. Cell lines derived from GEP express dopaminergic receptors D_2. New chimeric analogues simultaneously recognising sst_2 and sst_5 or sst_2 and D_2 have additive effects in inhibition of GH and prolactin secretion in pituitary adenomas. Our aim was to quantify the expression of sst and D_2 mRNA in human GEP tumours.
Design and methods: mRNA expression of sst_1, sst_2, sst_3 and sst_5 as well as D_2, was analysed using real-time PCR (TaqMan probe) in a series of 35 patients with GEP tumours (pancreas ($n=19$) and intestinal ($n=16$)). Levels of expression were compared with a group of 13 somatotroph adenomas.
Results: All GEP tumours express sst_1, sst_2 and D_2. Expression of sst_3 and sst_5 was observed in 89 and 76% of tumours respectively with highly variable levels. sst_2 mRNA expression was higher in non-functional tumours ($P<0.009$) and sst5 was higher in pancreatic than in intestinal tumours ($P<0.02$). Whereas sst_2 levels were similar between GEP and somatotroph tumours, levels of sst_5 and D_2 were higher in the former ($394.9\pm156.1\times10^{-2}$ vs $69.7\pm19.5\times10^{-2}$ copy/copy β-Gus ($P<0.0036$) and $519.6\pm121.2\times10^{-2}$ vs $50.0\pm21.6\times10^{-2}$ copy/copy β-Gus ($P<0.0001$) respectively). In small tumours (<30 mm), sst_2 density appeared as a crucial parameter in somatostatin receptor scintigraphy results, whereas in big tumours, a consistent bias in SRS results was introduced by the size. In pancreatic GEP, high-level sst_3 expression was found in tumours with more active angiogenesis (higher microvessel density and vascular endothelial growth factor expression ($P<0.03$)).
Conclusions: GEP tumours co-express sst_2 and D_2 in 100% of cases and sst_5 in 89% thus supporting the testing of bi-specific agonists (sst_2/sst_5 or sst_2/D_2) in these tumours.

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Introduction

Somatostatin receptor (sst) subtype status has been characterised using various techniques in gastro-entero-pancreatic (GEP) tumours, however, results are heterogeneous and depend on tumour types (1–4). Relatively, few data are available concerning semi-quantitative sst expression from studies using densitometry from autoradiography, immunohistochemistry and RT-PCR analysis (3, 5, 6). Differential expression of sst subtypes, but especially sst_2, appears important in predicting results of somatostatin receptor scintigraphy (SRS) and therapeutic response to cold somatostatin analogues (7–13). A good correlation has been found to exist between RT-PCR and immunohistochemistry in GEP tumours indicating that the former method may indeed be sufficiently accurate in detecting sst subtypes (5). Real-time PCR adds a quantitative dimension to receptor mRNA detection.

Therapy using standard somatostatin analogues (either daily octreotide or slow-release depot preparations; Octreotide LAR and SR-Lanreotide, sst_2 agonist) in patients with functional GEP is not universally efficacious and the effects of treatment wane with time (14). Ligands with enhanced receptor binding or those recognising several receptor subtypes may improve clinical outcome and perhaps offer an effective anti-tumoural benefit. BIM-23 244, a somatostatin receptor subtypes 2 and 5

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selective analogue was found to have enhanced efficacy in suppressing growth hormone from octreotide-resistant human growth hormone-secreting pituitary adenomas (15). Recently, a multivalent ligand, SOM230, capable of binding to sst_1, sst_2, sst_3 and sst_5, has been developed with promising results in treatment of patients with acromegaly (16, 17) and trials are on-going in patients with symptomatic GEP tumours resistant to standard somatostatin analogue therapy. Better knowledge of sst receptor status in these patients may allow for a tailored approach using such compounds and indeed, quantitative receptor expression may correlate with clinical outcome. A further interesting concept stemmed from combined receptor targeting as demonstrated by the enhanced potency of a chimeric somatostatin–dopamine molecule, BIM-23A387, in suppressing growth hormone and prolactin secretion from human pituitary somatotroph adenoma cells *in vitro* (18, 19). Here, the chimeric compound was far more potent than standard somatostatin or dopamine analogues alone (18). Although D_2 receptors (D_2) have been found in the neuroendocrine tumour cell lines, SCAT and BON-1 (20), no data are available in patients with GEP tumours.

The purpose of this study was to quantify sst and D_2 receptors mRNA using real-time PCR in a group of patients with GEP tumours originating from the pancreas and small intestine with detailed clinical data available for comparative analysis. The results were compared with a group of patients with somatotroph adenomas.

Subjects and methods

The study was carried out in 35 patients with GEP tumours (19 men and 16 women), aged 51 ± 14.7 years. The present study was approved by the ethics committee of the university and was undertaken after informed consent was obtained from each patient and all participants. The analysis involved only the primary tumour, which had been surgically resected and detailed clinical and pathological characteristics were available in all patients. After surgery, a portion of each tumour tissue was analysed in terms of the quantitative expression of mRNA for the D_2 and for sst_1, sst_2, sst_3 and sst_5 receptor subtypes. The primary tumour location included: pancreas ($n=19$) and intestine ($n=16$): ileum, 13; jejunum, 2 and duodenum, 1. The following tumour characteristics were recorded: size (largest perpendicular diameter), functional status (presence or absence of symptoms from hormonal overproduction), SRS results, histological differentiation and WHO status (21). General clinical and tumour characteristics of patients are summarised in Table 1.

Quantitative expression of mRNA for receptors targeted by actual somatostatin and dopamine agonists (sst_2 and sst_5 and D_2) were compared in patients with GEP tumours to a group of 13 patients with somatotroph adenomas, characterised by immunocytochemistry.

VEGF and microvascular density estimation

The vascular endothelial growth factor (VEGF) protein was detected by the murine MAB VG1 (22) in pancreatic tumours using a methodology described recently (23) PBS was substituted for primary antibody as the negative control. Positive controls consisted of serum in blood vessels and of islets (detected with strong intensity) in non-tumoural pancreas (adjacent to the tumours). All sections were performed in the same run. The specimens were scanned at a low optical power (×40) to study the tissue distribution of staining and at a high optical power (×250) to study the cellular staining patterns. The percentage of cells with positive reactivity was scored. A cytoplasmic score was calculated by multiplication of the percentage of cytoplasmic-stained cells by their staining intensity (negative scored as 0, weak scored as 1, moderate scored as 2 and strong scored as 3) (23) *Microvessel counting* was also only available for pancreatic tumours

Table 1 Patient's and tumour characteristics.

	Pancreatic	Intestinal
Total number of cases	19	16
Gender, male/female	10/9	9/7
Age, median years	49	59
Tumour size, mean (range) (mm)	45.9 (9–160)	30 (10–60)
Tumour location	Body/tail: 11 head: 8	Ileum: 13 Jejunum: 2 Duodenum: 1
Functional status	Glucagon (2), VIP (1), gastrin (1), non-functional (15)	Serotonin-secreting (13), gastrin (1), non-functional (2)
Histology classification (WHO) (21)		
Well-differentiated tumour	1	0
Well-differentiated tumour of 'uncertain' behaviour	8	1
Well-differentiated carcinoma	9	15
Poorly differentiated carcinoma	1	0
SRS (Octreoscan)	Positive: 13, negative: 6	Positive: 14, negative: 1, not done: 1

VIP, vasoactive intestinal peptide.

and was performed on ×200 fields (area of a ×200 field: 0.442/mm^2) after CD34 staining. Two areas of high vascularisation were chosen for microvessel counting at a low optical power (×40). The final microvessel density (MVD) was the mean value of 3 appraised fields in each area (total area: 2.65/mm^2). Vessels with a clearly defined lumen or well-defined linear vessel shape were taken into consideration for counting. In addition, the cellular proliferation index, Ki-67 (%), was calculated using a murine monoclonal MIB-1 antibody (DAKO, Trappes, France) as described previously (23).

Detection of somatostatin receptor and D2 mRNAs

Total RNA was extracted from 30 to 60 mg tissue from each tumour using the RNAeasy isolation system (QIAGEN). Tissue sampling was carefully evaluated at microscopy to ensure that sampling was from tumoural tissue and not from adjacent tissues. One microgram of total RNA prepared from tumoural tissues was used for cDNA synthesis with 200 U Superscript II reverse transcriptase (Life Technologies, Inc.) primed with 300 ng random primer (18).

The 5′ exonuclease (TaqMan) assay, which produces a direct proportional readout for the progression of PCR, was used. Amplification of cDNA derived from 50 to 150 ng total RNA was performed in a 25 µl reaction volume with 300 nM of each primer, 200 nM probe and 12.5 µl MasterMix (PE Applied Biosystems, Paris, France). The synthetic sst$_1$, sst$_2$, sst$_3$, sst$_5$ and D$_2$ primers and TaqMan probes used in the PCR were described previously (18, 24) and were follows (forward (F), reverse (R) primers and probe (P)):

D2 F: CAAGACCATGAGCCGTAGGAAG, R: TGTGTGTGATGAAGAAGGGCAG, P: CCCAGCAGAAGGAGAAGAAAGCCACTCA; sst1 F: GCTAGGACACTGACAGCCTTTGA, R: GTAGCCTGAAAGCCTTCCCA, P: CCCAAGAAAGGCGCGCGACAAT; sst2 F: GCCTCCAGGGTCCATTAAGG, R: ATTGAGTGGCTCATCCGCC, P: AGAATAAGATCTCTGGGCTGGCTGGAA; sst3 F: TGGGCCTGCTGGGTAACT, R: GATGTAGACGTTGGTGACTGAAGG, P: CATCTATGTGGTCCTGCGGCACACG; sst5 F: CTGGTGCCAAGGACGCT, R: GCTGCCGGATCCTGTCTG, P: ACGCCACGGAGCCGCGT. Forty cycles of two-step PCR-annealing extension were performed on an ABI Prism 7700 sequence detection apparatus (PE Applied Biosystems, Paris, France). The sst and D$_2$ mRNA levels were normalised to the ß-Gus mRNA levels obtained in the same reaction. The ß-Gus primers and probe were purchased from PE Applied Biosystems (18). For each measurement, three independent RT-PCR analyses were performed. To produce standard curves for sst, D$_2$ and ß-Gus mRNA, cDNA constructs were produced for each parameter and verified by sequencing. The results were expressed as copy of sst or D$_2$/copy of ß-Gus.

Statistical analysis

The results are presented as the mean ± S.E.M. Statistical significance between two unpaired groups was determined by the Mann–Whitney *U* test. A *P* value less than 0.05 was considered significant for all tests.

Results

sst and D$_2$ mRNA expression in GEP tumours

All GEP tumours had constant but variable expression of sst$_1$, sst$_2$ and D$_2$ (Fig. 1; Table 2). Somatostatin receptor subtypes sst$_3$ and sst$_5$ were expressed in 76 and 89% of GEP tumours respectively (Table 2). mRNA level expression of sst5 was higher in pancreatic than in intestinal tumours ($P<0.02$); mRNA level expression of sst2 was also higher in pancreatic than in intestinal GEP; however, this did not achieve statistical significance (Table 2; Fig. 2).

The level of mRNA sst$_2$ expression was significantly higher in patients with non-functional tumours ($n=19$) compared with functional tumours ($n=16$, $91.7\pm 37.4\times10^{-2}$ vs $39.9\pm19.7\times10^{-2}$ copy/copy β-Gus, $P<0.009$).

Comparison with somatotroph adenomas

Somatostatin receptor subtypes sst$_2$, sst$_5$ and D$_2$ mRNA levels were compared with a group of 13 somatotroph adenomas (Fig. 3). Levels of sst$_5$ and D$_2$ mRNA were significantly higher in pituitary adenomas compared with GEP tumours ($P<0.0036$ and $P<0.0001$ respectively), whereas sst$_2$ mRNA levels were similar. Levels of sst$_5$ and D$_2$ mRNA were in the range of those observed in somatotroph adenomas in 43 and 17% of GEP tumours respectively.

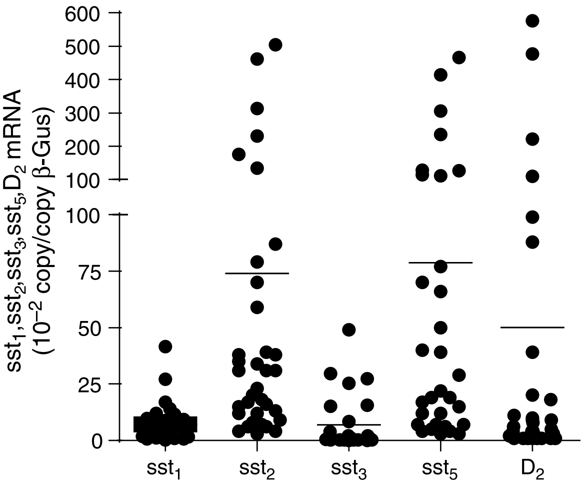

Figure 1 sst$_1$, sst$_2$, sst$_3$, sst$_5$ and D$_2$ mRNA levels in 35 GEP tumours (19 pancreatic and 16 intestinal). The quantification was performed by real-time PCR. Measurements were reported to the level of β-Gus.

Table 2 Expression levels of sst$_1$, sst$_2$, sst$_3$, sst$_5$ and D$_2$ in GEP tumours and in pituitary adenomas.

	Mean ± s.e.m. copy/copy β-Gus			
	GEP tumours (n=35)			
Receptor subtype	Pancreatic	Mid gut	GEP	Somatotroph adenomas (n=13)
sst$_1$	6.6±2.5 (100%)	7.3±1.1 (100%)	7.0±1.4 (100%)	ND
sst$_2$	102.4±33.8 (100%)	39±18.7 (100%)	73.8±20.7 (100%)	115.9±32.0 (100%)
sst$_3$	11.1±3.6 (84%)	1.2±0.1 (63%)	6.8±2.3 (76%)	ND
sst$_5$	128.5±39.8 (79%)	31.8±9.6 (100%)	69.7±19.5 (89%)	394.9±156.1 (100%)
D$_2$	65.9±37.8 (100%)	31±14.7 (100%)	50.0±21.6 (100%)	519.6±121.2 (100%)

The percentages of tumours expressing a subtype receptor are indicated in parenthesis. ND, not done.

sst expression compared with tumour stage and SRS status

Level of mRNA for sst$_2$ and sst$_3$ was significantly higher in WHO stages 1–2 compared with stage 3 ($115\pm48\times10^{-2}$ vs $60\pm22\times10^{-2}$ ($P<0.03$) and $17\pm7\times10^{-2}$ vs $4\pm2.4\times10^{-2}$ ($P<0.00673$) copy/copy β-Gus respectively). However, as the majority of intestinal tumours tested were stage 3, this analysis may be biased by the tumour type. These differences were not apparent when pancreatic tumours were considered alone ($n=8$, $115\pm48\times10^{-2}$ vs $n=9$, $91\pm43\times10^{-2}$ copy/copy β-Gus) thus confirming that these effects may be attributable to tumour type alone. mRNA levels of sst$_2$ and sst$_5$ were also compared with SRS results (available in 34 patients, Table 1): sst$_2$ levels were higher in SRS-positive ($n=27$) than in SRS-negative ($n=7$) tumours ($87.1\pm26.3\times10^{-2}$ and $28.4\pm10.6\times10^{-2}$ copy/copy β-Gus) without achieving statistical significance. However, when small tumours were considered (<30 mm; $n=16$) receptor density clearly influenced SRS results: ten SRS-positive patients had a mean sst2 density of $128.3\pm48.6\times10^{-2}$ vs $31.2\pm12.1\times10^{-2}$ copy/copy β-Gus ($P<0.05$) in six SRS-negative tumours (Fig. 4). Expression of other receptor subtypes had no influence on SRS results, in fact, mean sst$_5$ receptor levels were higher in SRS-negative than in SRS-positive tumours ($109.9\pm65.9\times10^{-2}$ vs $59.4\pm19.0\times10^{-2}$ copy/copy β-Gus) however, not statistically significant.

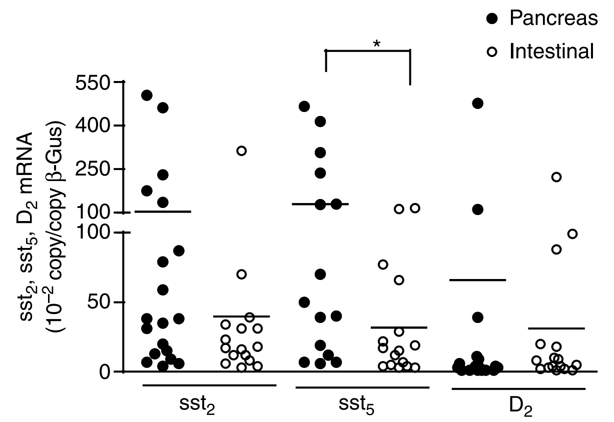

Figure 2 Comparison of mRNA expression levels of sst$_2$, sst$_5$ and D$_2$ mRNA between 19 pancreatic and 16 intestinal tumours. The quantification was performed by real-time PCR. Measurements were reported to the level of β-Gus. *$P<0.02$.

sst and D$_2$ expression compared with vascular markers and Ki-67 in pancreatic tumours

sst and D$_2$ were then analysed with respect to vascular markers, MVD and VEGF, in pancreatic tumours (Table 3). The MVD ranged from 80 to 674 vessels/mm^2 (329 ± 44). The VEGF score ranged from 0 to 180 (58.4 ± 14.3). No correlations were found between MVD and VEGF in relation to sst$_1$, sst$_2$, sst$_5$ and D$_2$ receptors. When pancreatic tumours were classified into two groups expressing high-level sst$_3$ ($n=7$, $24\pm5\times10^{-2}$ copy/copy β-Gus) and tumours not expressing or expressing low-level sst$_3$ ($n=12$, $0.6\pm0.3\times10^{-2}$ copy/copy β-Gus), MVD and VEGF expression were significantly higher in the high-level sst$_3$ group (428 ± 70 vs 236 ± 41 vessels/mm^2 and 94 ± 25.8 vs 24.5 ± 7.4 respectively, $P<0.03$; Fig. 5), although general low sst$_3$ expression.

When tumours were classified into two groups according to the Ki-67 level above 3% ($n=9$, 7.2 ± 0.6) or below 3% ($n=8$, 2.6 ± 0.3), sst5 levels were found to be higher in first group compared with the second group ($25\pm11.8\times10^{-2}$ vs $206\pm62\times10^{-2}$ copy/copy β-Gus, $P<0.005$; Fig. 6).

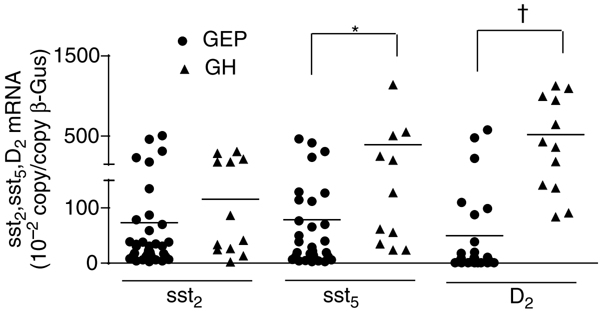

Figure 3 Comparison of mRNA expression level of sst$_2$, sst$_5$ and D$_2$ mRNA between 35 GEP tumours and 13 somatotroph adenomas (GH). Quantification was performed by real-time PCR. Measurements were reported to the level of β-Gus. *$P<0.0036$; $^{\dagger}P<0.0001$.

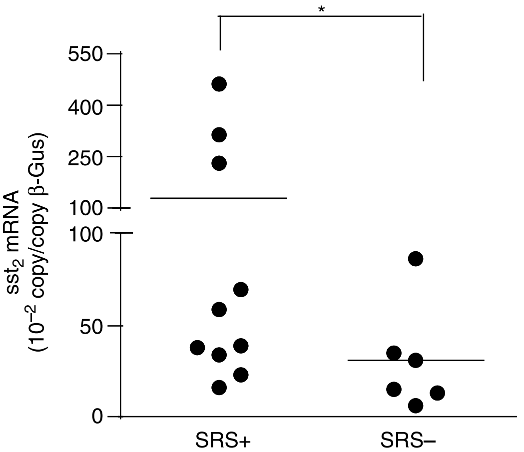

Figure 4 mRNA level expression of sst_2 in small GEP tumours (<30 mm) according to the SRS-positive ($n=10$) or SRS-negative patients ($n=6$). The quantification was performed by real-time PCR. Measurements were reported to the level of β-Gus. $^*P<0.05$.

Discussion

Somatostatin analogues are widely used in the treatment of GEP tumours and SRS is a useful tool in diagnosis. The general use of SRIF agonists is justified by the presence in these tumours of SRIF receptors detected by qualitative mRNA and protein methods. For the first time, using real-time PCR, we systematically quantified not only sst subtypes but also D_2 mRNA, in a large series of human GEP tumours. All GEP tumours expressed sst_1, sst_2 and D_2 while only 76 and 89% of tumours expressed sst_3 and sst_5 respectively. These results are close to those of Papotti *et al* using semi-quantitative PCR and immunohistochemistry with a good correlation between these two techniques (5). In our series, the expression levels were higher for sst_2 and sst_5 and markedly lower for sst_1 and sst_3. Note that non-functioning GEP tumours had a significantly higher level of sst_2 than functional ones ($P<0.009$); however, the significance of this remains to be determined. Similar results concerning sst_2 and sst_5 were also previously observed in 38 patients with GEP (mostly pancreatic) using semi-quantitative PCR, although sst_1 and sst_3 were less frequently observed (66 and 50% respectively) (3). Compared with a group of pituitary tumours, the level of sst_5 mRNA expression was less while sst_2 expression levels were almost similar (Table 2). In a group of 27 ileal carcinoids, Reubi and Wasser (6) found sst_2 to have the highest density (5.4 d.p.m./mg tissue) followed by sst_1, sst_5, sst_3 and sst_4 (3.4, 2.4, 1.6 and 1.5 d.p.m./mg tissue respectively). However, comparison of techniques using ligand binding and mRNA detection is difficult. Indeed, techniques focused on protein detection may reflect more accurate levels of sst receptors, whereas PCR may have overestimated quantities expressed. Moreover, the use of PCR does not allow for analysis of the percentage of cells expressing different receptor subtypes which requires *in situ* hybridisation or immunohistochemistry; indeed, the heterogeneity of sst expression has been previously described (5).

Although the dopamine receptors and the transmembrane dopamine transporter are known to play an important role in gastrointestinal physiology (25), they

Table 3 Expression levels of sst_1, sst_2, sst_3, sst_5 and D_2 receptors according to the microvascular density (MVD) as measured by microscopy using CD34 staining (vessels/mm^2), vascular endothelial growth factor (VEGF, measured as a score by multiplication of the percentage of stained cells by their staining intensity 0) by immunohistochemistry and cellular proliferation index (Ki-67) in 19 pancreatic tumours.

				10^{-2} Copy/copy/β-Gus				
Tumour	**VEGF score**	**MVD** (vessels/mm^2)	**Ki-67** (%)	sst_1	sst_2	sst_3	sst_5	D_2
C1	180	496	7	4.3	175.0	49.0	7.0	1.0
C2	10	95	3	0.9	7.0	0.3	236.0	4.0
C3	180	400	9	1.2	9.0	0.3	7.0	3.0
C4	50	209	2	0.7	6.0	0.3	414.0	1.0
C5	20	173	3	27.1	13.0	0.3	306.0	39.0
C6	50	674	5	1.3	31.0	27.3	0.0	1.0
C7	150	417	3	3.5	38.0	25.2	12.0	4.0
C8	160	100	8	3.4	79.0	15.2	0.0	1.0
C9	0	102	3	0.2	20.0	3.9	70.0	6.0
C10	40	276	4	0.6	135.0	0.0	127.0	110.0
C11	60	652	7	1.6	15.0	1.0	40.0	2.0
C12	0	337	5	41.6	35.0	0.1	0.0	576.0
C13	30	464	4	7.4	461.0	0.0	466.0	9.0
C14	60	563	1	4.5	38.0	15.5	19.0	3.0
C15	60	442	6	7.3	230.0	8.4	129.0	1.0
C16	0	307	9	2.7	59.0	29.7	0.0	1.0
C17	0	80	10	9.2	504.0	0.3	39.0	477.0
C18	0	357	4	1.9	87.0	0.5	6.0	11.0
C19	60	112	9	ND	4.0	0.3	50.0	3.0
Mean ± S.D.	58.4 ± 14.3	329 ± 44	5.3 (1–10)	6.6 ± 2.5	102 ± 33	11 ± 3.6	128 ± 40	65 ± 37

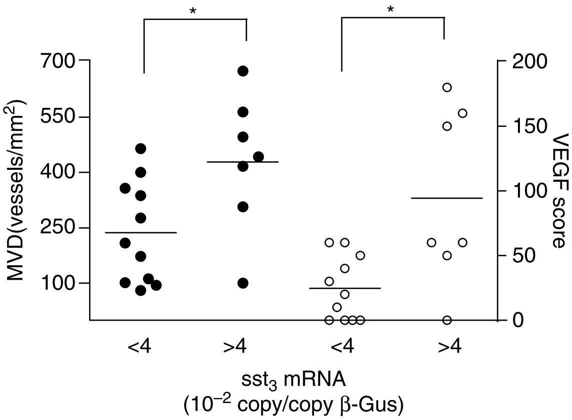

Figure 5 Microvascular density (MVD), VEGF score in 19 pancreatic tumours expressing a high level (>4) or a low level (<4) of sst3 mRNA. MVD were evaluated by microscopy, VEGF score by immunocytochemisty (measured as a score by multiplication of the percentage of stained cells by their staining intensity) and sst$_3$ by real-time PCR. *$P<0.03$.

have not been investigated in neuroendocrine gastrointestinal tumours. Interestingly, Lemmer *et al.* have showed the presence of D_2 in two cell lines, STC1 murine neuroendocrine gut tumours and BON human pancreatic neuroendocrine tumours (20). Here, we showed for the first time the presence of D_2 mRNA in all human GEP tumours. Moreover, the quantitative analysis showed that D_2 expression level was in the range of those observed in somatotroph adenomas in 17% of GEP tumours. The identification of D_2 in all GEP tumours of both intestinal and pancreatic origin opens the possibility of examining new chimeric analogues which simultaneously recognise sst$_2$ and D_2, such as BIM-23A387 or BIM-23A370, which have been shown to enhance the suppression of growth hormone and prolactin in pituitary adenomas compared with sst$_2$ and D_2 analogues used alone (18, 26). A direct antiproliferative effect of the somatostatin/dopamine chimeras, BIM-23A387 and BIM-23A370, was found in the lung carcinoma cell line (Calu-6) possible via sst/D_2 dimerisation (27). In addition, other analogues capable of recognising both sst$_2$ and sst$_5$ (BIM-23 244) or the multivalent agonist SOM230 (recognising sst$_1$, sst$_2$, sst$_3$ and sst$_5$) offer the possibility of increasing therapeutic efficacy by acting via more than one receptor. Although D_2 was found in all tumours, low levels were expressed in about 80% inferring that D_2 receptor targeting may only be relevant to subset of patients with GEP tumours. Moreover, cellular distribution of sst and D_2 receptors should also be an important parameter involved in the efficacy of such treatment. Examining the potential effect on tumour secretion or even proliferation in GEP tumours will require functional studies.

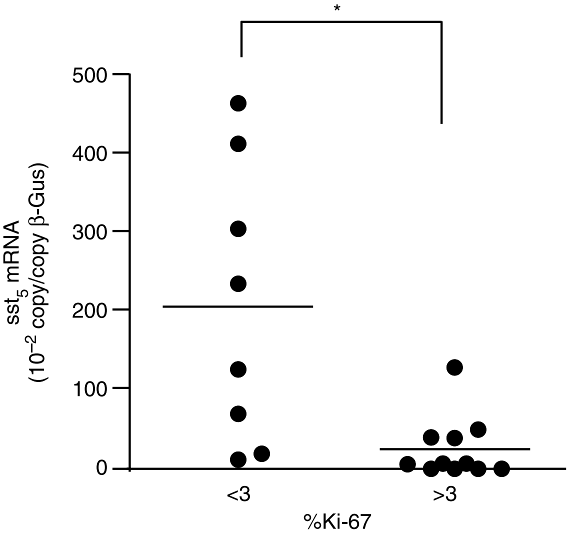

Figure 6 Nineteen pancreatic tumours were classified into two groups according to the level of % Ki-67 (above or below 3%). sst$_5$ were evaluated by real-time PCR. *$P<0.005$.

Somatostatin receptor scintigraphy is performed not only for tumoural localisation but also for predicting the efficacy of somatostatin agonist or modified radiopharmaceutical analogues (28–31). However, in some case, SRS results were not correlated with results of treatments (32–34). Actual SRS using ^{111}pentetreotide recognises mostly sst$_2$ subtype. Pentetreotide had tenfold higher affinity for sst$_2$ than for sst$_5$ or sst$_3$ (6). Globally, SRS is positive in approximately 80% of all patients with GEP tumours (35). Previously, semi-quantitative RT-PCR revealed most prominently sst$_2$ expression in scintigraphic positive tumours (36). Using sst$_2$ knockout mice, Hofland and collaborators confirmed the crucial role of sst$_2$ in determining the uptake of [^{111}In DTPA-D-Phe]octreotide (28). In the present study, we confirm sst$_2$ as the most important receptor subtype in case of positive SRS uptake as the levels of sst$_2$ in 27 SRS-positive tumours was markedly higher as compared with seven SRS-negative tumours. Levels of other sst did not differ between SRS-positive and SRS-negative tumours thus confirming that other sst have less influence on SRS outcome (36). Jais *et al* previously observed no difference in sst receptor distribution and SRS results although again numbers were small (17 positive SRS vs 4 negative) (3). In the present study, when only small tumours (<30 mm) were examined, sst$_2$ mRNA level expression was significantly higher in SRS-positive tumours than in SRS-negative ones (Fig. 4), thus confirming the crucial role of sst$_2$ receptor alone on scintigraphic results for small tumours. Moreover, compared with SRS-negative tumours, SRS-positive tumours were larger (44 ± 7 vs 21 ± 2.7 mm, $P<0.01$, data not shown) confirming the role of tumour size itself on scintigraphic results for the large tumours. Among all SRS-positive tumours, sst$_2$ mRNA levels were significantly higher in the group of small versus big tumours ($128\pm48\times10^{-2}$ vs $73\pm48\times10^{-2}$ copy/β-Gus copy, $P<0.05$, data not shown) confirming the bias introduced by the size in SRS results.

sst level expression and in particular sst2 may predict clinical response to somatostatin receptor analogues and could thus help in tailoring targeted therapy as shown in somatotroph adenomas (37, 24). As in patients with breast cancer where oestrogen receptor status and levels help in predicting therapeutic responses, quantitative receptor measurements may help in determining responses to cold and radiolabelled somatostatin (or somatostatin–dopamine) analogues to be used in treatment according to analogues binding characteristics (38, 39).

As well as being involved in regulation of hormonal release, sst are known to have direct and indirect effects on angiogenesis and cellular proliferation (review: (40)). Interestingly, sst3 was higher in pancreatic than in intestinal tumours (Table 2). Thus, when pancreatic tumours were separated into high and low sst_3 level groups, a positive correlation with both microvessel density and VEGF score ($P < 0.03$) was observed (Fig. 5). MVD and VEGF expression have been found to be increased in benign pancreatic tumours and their expression decreases as tumours dedifferentiate (23). sst_3 has previously been found to be the predominant somatostatin receptor subtype in endothelial cells (41). sst_3 may be expressed also in the microvessels of the tumours of our series. Nevertheless, the observation of co-expression of angiogenic factors with sst_3 is important as underlined by the recent observation that somatostatin inhibition of tumour angiogenesis in a Kaposi's sarcoma cell xenograft model occurred via sst_3-mediated inhibition of both nitric oxide synthetase and MAPK activities (42). In endothelial cells-expressing sst_3, addition of the sst_3 antagonist, BN81658, significantly reversed the anti-angiogenic effects of somatostatin (42). VEGF and somatostatin were also recently found to be co-expressed in the same tissue compartments in ovarian cancer suggesting a major role for somatostatin in angiogenesis (43).

Finally, low sst_5 mRNA expression was present in pancreatic tumours with high Ki-67 level. Overall, few data are available comparing sst receptors with cellular markers of proliferation. Interestingly, our results were contradictory to recent data in a series of 16 insulinomas, where sst_5 was positively correlated with aggressive tumour behaviours, such as large tumour size, tumours of uncertain behaviour and presence of nuclear atypia (44). In brain tumours (astrocytomas and meninginomas), no relation existed between Ki-67 and sst subtypes in two series (45, 46). In contrast, in breast cancer, sst_2 mRNA expression was significantly higher in low-proliferating (as measured using Ki-67) breast cancers (47). Note that in an endothelial cell line model (HUVEC) sst other than sst2 and sst3 appears important in controlling proliferation as demonstrated by the ability of the multivalent ligand SOM230, but not octreotide, to inhibit proliferation and this did not appear to be mediated via sst_3 as only sst_1, sst_2 and sst_5 were detected using RT-PCR and western blotting (48). SOM230 or other specific sst_2–sst_5 or sst_2–D_2 agonists may thus represent suitable candidates as potential anti-angiogenic and anti-proliferating drugs.

In conclusion, GEP tumours were found to co-express sst_2 and D_2 in all cases and sst_5 in 89%. SRS results were strongly correlated to the sst_2 mRNA expression level in small tumours (<30 mm), but were consistently distorted by the size in large tumours explaining some discordance between SRS and somatostatin treatment results. While mean level of receptor expression for sst_5 and D_2 is lower in GEP than in pituitary adenomas, comparative levels are observed in almost a half and a fifth of tumours respectively. These results argue for testing of bi-specific agonists (sst_2/sst_5 or sst_2/D_2) in the treatment of GEP tumours not only for inhibiting secretion but also angiogenesis and cell proliferation.

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