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Original Paper

Expression of cortistatin and MrgX2, a specific cortistatin receptor, in human neuroendocrine tissues and related tumours

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Abstract

Cortistatin (CST), a novel hormone originally described in the rat, mouse, and human cerebral cortex, displays structural and functional similarities to somatostatin (SRIF). CST binds to all five somatostatin receptors and, differently from SRIF, also binds to MrgX2, which has recently been identified as its specific receptor. Little is known about the distribution of CST and MrgX2 in peripheral non-tumour and neoplastic tissues. The aim of the present study was therefore to determine by immunohistochemistry and mRNA analysis (RT-PCR) the distribution of CST and MrgX2 in 56 human non-tumour and 108 tumour tissues, with special reference to neuroendocrine tissue types. Despite the high level of *CST* mRNA expression in non-tumour and tumour (both neuroendocrine and non-neuroendocrine) tissues, the presence of immunoreactive CST was confirmed in a subset of gastroenteropancreatic, parathyroid, and pituitary non-tumour cells only, and showed a predominantly focal pattern in most neuroendocrine tumours. Co-localization experiments in the gastroenteropancreatic system demonstrated that the normal CST-producing cells are δ cells, while in the adenohypophysis no preferential co-localization of CST with any of the pituitary hormones was observed. *MrgX2* mRNA was variably detected in the hypothalamus, pituitary, thyroid, lung, gastroenteropancreatic tract, testis, and ovary, and was negative in the cerebral cortex, parathyroid, and adrenal, as well as in a variety of tumour types. Conversely, immunolocalization of MrgX2 protein was restricted to neurohypophysis and testis, whilst all tumours analysed were negative. A possible explanation for the discrepancy between RT-PCR and immunohistochemistry is that MrgX2 protein was widely detected in blood vessels, scattered lymphocytes, and gastrointestinal ganglia in both normal and neoplastic samples. The findings demonstrate a selective distribution of CST in normal and neoplastic neuroendocrine tissues, suggesting that CST might have a broader functional role than previously assumed, whereas possible autocrine/paracrine actions via its recently described specific receptor MrgX2 are restricted to selected tissues.

Keywords: cortistatin; somatostatin; MrgX2 receptor; RT-PCR; immunohistochemistry; neuroendocrine tumours

Introduction

Cortistatin (CST) is a 17-amino acid neuropeptide recently discovered in rat, mouse, and humans that exhibits strong structural homology to somatostatin (SRIF) [1–4]. The human *CST* gene was mapped to chromosome 1p36.3–p36.2 and found to contain two exons separated by a 1 kb intron [3,5].

Two mature products, CST-17 and CST-29, are generated by a prohormone convertase, the former being equivalent to murine CST-14, a cyclic peptide that shares 11 of its 14 amino acid residues with SRIF-14, responsible for SRIF binding to its receptors [1,2].

CST-17 and CST-29 bind to all five SRIF receptors (subtypes 1–5) with an affinity comparable to that of SRIF-14 [6]. In addition, both CST-14 and CST-17, but not SRIF-14, are also able to bind the ghrelin receptor type 1a (growth hormone secretagogue receptor — GHS-R1a) [7–9]. SRIF receptors mediate multiple SRIF activities including neurotransmission, neuromodulation, regulation of endocrine and exocrine secretions, and also the inhibition of tumour growth [10,11]. Since CST mediates several of its effects

via activation of SRIF receptors, it was assumed to share the same biological activities as SRIF. However, CST also exerts a number of distinct biological activities (increases slow wave sleep, reduces locomotor activity) and this evidence led to the existence of a specific CST receptor being postulated [1].

Recently a novel receptor, named MrgX2 and belonging to a family of coding sequences related to the *Mas1* oncogene, has been identified and found to possess a high affinity for CST-14 *in vitro*. MrgX2 has been identified in the small sensory neurons of the dorsal root ganglia (with a possible role in nociception) and in rare hippocampal neurons, but not in the cerebral cortex [12], as opposed to CST, which is expressed by a distinct subset of gamma amino butyric acid (GABA)-containing cells in the cortex and hippocampus [13].

In tissues other than the central nervous system, CST has been localized in the human endocrine pancreas [14], a finding possibly related to the recently reported [9] capability of CST to inhibit basal insulin secretion (probably mediated via SRIF receptors), and in the immune system [15].

Concerning MrgX2 distribution in peripheral tissues, weak immunohistochemical reactivity was originally reported in endothelial cells and the testis [12], but no data are available on CST and MrgX2 distribution in other normal and neoplastic tissues to date. Since a possible role for CST in tumour growth control has been reported in thyroid [16] and liver [17] carcinoma cell lines, the aim of the present study was to analyse CST and MrgX2 distribution in a large series of human tissues and related tumours (with special reference to endocrine tumours), to create a map of tissues expressing this novel hormone and its target binding sites.

Materials and methods

Tissue samples

A total of 56 non-tumour autopsy or surgical samples included cerebral cortex (four cases), hypothalamus (three cases), pituitary gland (four cases), thyroid (five cases), parathyroid (two cases of hyperplasia), adrenal gland (two cases), lung (nine cases), stomach (six cases), ileum (four cases), colon (eight cases), pancreas (five cases), ovary (three cases), and testis (one case). Serial sections from formalin-fixed, paraffin-embedded material were used for conventional histology, immunohistochemistry, and immunofluorescence studies. An adjacent fragment was snap-frozen and kept at $-80\,^{\circ}C$ until it was processed for molecular analysis. In addition, a series of neuroendocrine tumours, for which both fresh frozen and paraffin-embedded material was available in most cases, were also studied. These included 73 benign and malignant neuroendocrine tumours from the pancreas (32 cases), lung (five cases), parathyroid (four cases), thyroid (seven cases of medullary carcinoma), adrenal medulla (five cases), pituitary gland (two cases), skin (ten cases), and gastrointestinal tract (eight cases). The hormonal status of the neuroendocrine tumours analysed was confirmed by clinical syndromes, hormonal measurements, and immunohistochemical findings. Finally, a total of 35 non-neuroendocrine tumours from the pancreas (five ductal adenocarcinomas), lung (three adenocarcinomas and two squamous cell carcinomas), thyroid (three follicular adenomas, three follicular carcinomas, three papillary carcinomas, and two anaplastic carcinomas), gastrointestinal tract (12 adenocarcinomas), and testis (two seminomas) were also analysed. The study was performed according to the standards of the Institutional Ethical Committee and to the Helsinki Declaration of 1975 as revised in 1983. The Institutional Review Board of our hospital approved the study. All tissue samples were de-identified and anonymized by staff not involved in the study, according to published procedures [18].

Immunohistochemistry (IHC)

A standard manual immunoperoxidase procedure for cortistatin-17 (CST-17) and MrgX2 was used on formalin-fixed and paraffin-embedded tissue sections after microwave heating and endogenous biotin activity blockade. CST immunostaining was performed using a commercial rabbit polyclonal antibody to human CST-17 (Phoenix Pharmaceutical, Belmont, CA, USA), diluted 1/10 000 and incubated for 1 h at room temperature. Rabbit polyclonal antibody to MrgX2 was purchased from LifeSpan (Seattle, WA, USA), diluted 1/750 and incubated overnight. The secondary antibody step, incubation with the streptavidin–peroxidase kit (Biogenex, San Ramon, CA, USA) and diaminobenzidine incubation, followed. For CST IHC reactions, further amplification with biotinylated tyramide (diluted 1/15; GenPoint kit, Dako, Glostrup, Denmark) and a second incubation with streptavidin were performed. Control experiments included the omission of the primary antibody in serial sections or, in the case of CST IHC, pre-absorption of the primary antibody with a 100-fold excess of CST-17 (Phoenix Pharmaceutical) or of SRIF-14 (Bachem Feinchemikalien, AG Bubendorf, Switzerland). Cerebral cortex or pancreatic islets [2,14] and human testis [12] served as positive controls for CST and MrgX2, respectively.

Co-localization experiments

In double immunofluorescence co-localization experiments on the same slides of human pancreas and gastrointestinal tissue, the same antibody to CST (diluted 1/15) used for IHC, a mouse monoclonal antibody to insulin (diluted 1/40; Biogenex, clone HB125), or rabbit polyclonal antibodies to glucagon (pre-diluted; Biogenex) and SRIF (diluted 1/30; Dako) were employed. A FITC-labelled secondary anti-rabbit antibody (Sigma Aldrich, Steinheim, Germany) was

used to reveal the CST immune reaction and TRITC-labelled anti-mouse secondary antibody (Southern Biotech Associates, Inc, Birmingham, USA) was used to reveal insulin. Glucagon and SRIF-producing cells were revealed by an anti-rabbit biotinylated secondary antibody (diluted 1/50; Multilink kit, Biogenex) followed by a TRITC–avidin incubation step (Sigma Aldrich). Double immunofluorescence slides were analysed using an Olympus FV300 confocal microscope (Hamburg, Germany) equipped with a green helium neon (543 nm) laser, a blue argon (488 nm) laser, and FluoView 300 software. In double-label experiments, the two channels were scanned alternately, using only one laser and one detector at any given time to avoid cross-talk. Cells were imaged using a 60× oil immersion objective. In the case of pituitary tissue, CST (diluted 1/10 000), and rabbit polyclonal antibodies to prolactin and ACTH (both diluted 1/100; Biogenex), and GH (1/2000; Dako) were used in a double immunohistochemical procedure, by means of an immunoalkaline phosphatase (LSAB plus kit, Dako)/Fast red (Biogenex) reaction in sequence to the standard streptavidin–peroxidase kit. To avoid interactions between the two immunological procedures, a treatment with 0.01 N HCl at 37 °C, followed by prolonged washing, was performed after the first reaction, as previously described [19].

Molecular analysis of CST and MrgX2 transcripts

The presence of *CST* and *MrgX2* mRNA was investigated by means of RT-PCR and dot Southern blot analysis. Total RNA extraction, cDNA transcription, and PCR amplification of the housekeeping gene (β_2 *microglobulin*) were performed as described elsewhere [20]. *CST* primer sequences and PCR conditions have been reported previously [14]. Primers and probes used to identify the specific *MrgX2* mRNA were selected according to recently published sequences [12]. Cranial nerve ganglion and cerebral cortex mRNA extracts served as positive controls for *MrgX2* and *CST*, respectively. Negative controls included omission of reverse transcriptase enzyme from the reverse mixture and of cDNA from the PCR mixture.

The SRIF receptors and GHS-R1a status of most cases of the current neuroendocrine tumour series have been reported previously [20–24]. Additional RT-PCR experiments for SRIF receptors and GHS-R1a were also performed in selected cases, with special reference to non-tumour and neoplastic parathyroid tissue, according to previously published protocols [20,21].

For both CST and MrgX2 experiments, dot Southern blot analysis was performed to test the specificity of the RT-PCR product further, as previously reported for CST [14].

Results

Cortistatin mRNA and protein expression in non-tumour tissues

CST mRNA and protein expression in non-tumour tissues, as revealed by RT-PCR and immunohistochemistry, is summarized in Table 1.

Concerning RT-PCR data, tissue samples from the cerebral cortex, hypothalamus, pituitary gland, thyroid, parathyroid, adrenal gland, ileum, and testis were positive in all cases investigated. Large intestine and pancreas were positive in the majority of cases, whereas a lower rate of expression was detected in the lung, stomach, and ovary.

Immunolocalization of CST protein was detected in the cytoplasm of neuronal cells of the cerebral cortex and hypothalamus, as well as in neuroendocrine

Table 1. Expression of CST and its receptor MrgX2 in human non-tumour tissues

	CST		MrgX2	
Tissue	**RT-PCR**	**IHC**	**RT-PCR**	**IHC***
Cerebral cortex	4/4	Positive in neuronal cells	0/4	Negative
Hypothalamus	3/3	Positive in neuronal cells	3/3	Negative
Pituitary gland	4/4	Positive in adenohypophysis	1/4	Negative in pituicytes Positive in single cells of neurohypophysis
Thyroid	5/5	Negative	3/5	Negative
Parathyroid	2/2	Positive in chief cells	0/2	Negative
Adrenal gland	2/2	Negative	0/2	Negative
Lung	6/9	Negative	3/9	Negative
Stomach	4/6	Positive in endocrine cells (δ)	3/6	Negative
Ileum	4/4	Positive in endocrine cells (δ)	2/4	Negative
Colon	7/8	Positive in endocrine cells (δ)	3/8	Negative
Pancreas	4/5	Positive in islet cells (δ)	3/5	Negative
Ovary	2/3	Negative	1/3	Negative
Testis	1/1	Negative	1/1	Positive in tubules

CST = cortistatin; MrgX2 = Mas-related gene X2; RT-PCR = reverse transcriptase-polymerase chain reaction; IHC = immunohistochemistry.

* In all tissues investigated, positive immunostaining was observed in endothelial cells and scattered lymphocytes; in the gastrointestinal tract, the myenteric plexus also stained positive.

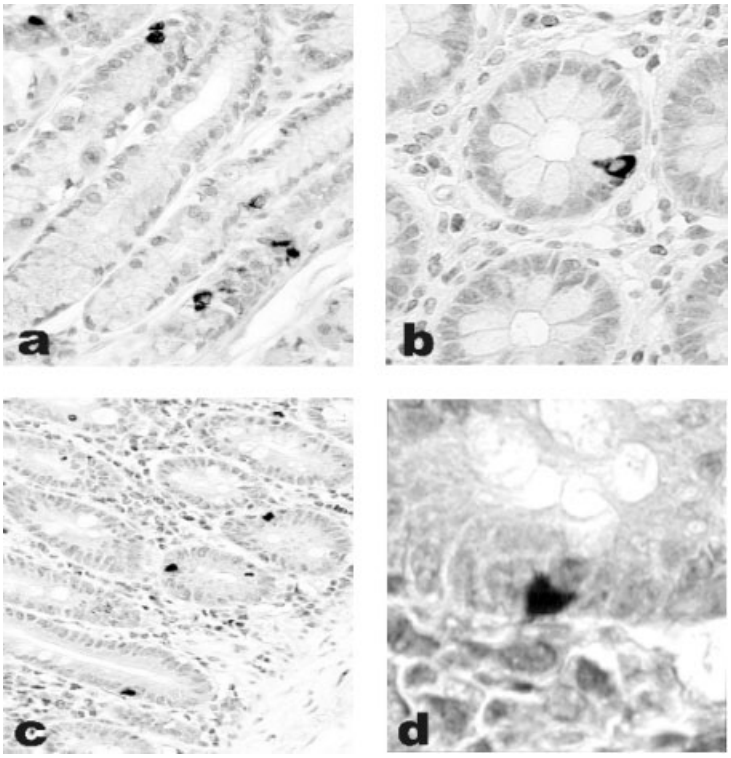

Figure 1. Single CST-immunoreactive cells in the mucosa of the gastric antrum (a), colon (b), and duodenum (c, d). Immunoperoxidase. Original magnification: (a) ×200; (b) ×400; (c) ×100; (d) ×600

pituitary cells. Strong positivity was detected in neuroendocrine cells of the pancreatic islets and of the gastrointestinal tract (Figure 1). Co-localization experiments demonstrated that pancreatic and gastrointestinal δ cells represent the main source of CST protein (Figure 2). In contrast, no preferential co-expression of CST with pituitary hormones was observed.

Despite positive RT-PCR results, no immunoreactivity for CST was found in the lung, ovary, thyroid, testis, and adrenal, neither in neuroendocrine nor in non-neuroendocrine cell types. Interestingly, CST peptide was diffusely present in chief cells of the parathyroid gland, oxyphilic cells being weakly immunoreactive (Figure 3). No SRIF-immunoreactive cells were detected in parathyroid tissue samples. In order to clarify the potential autocrine/paracrine role of CST in the parathyroid gland, the presence of *SRIF* receptor subtypes and *MrgX2* (see also below) and *GHS-R1a* mRNAs in non-tumour (hyperplastic) and neoplastic samples was tested. Neither non-neoplastic nor neoplastic tissues showed *MrgX2* and *GHS-R* mRNA expression, while *SRIF* receptor subtypes 1 and 2 (and 3 in a single case of parathyroid carcinoma) mRNA signal was demonstrated.

In control experiments, no immunoreactivity was found in parallel sections stained omitting the primary antibody or in pre-absorption experiments with an excess of CST-17 peptide, whereas CST reactivity was preserved after pre-absorption with an excess of SRIF-14.

MrgX2 mRNA and protein expression in non-tumour tissues

MrgX2 mRNA was distributed heterogeneously among different non-tumour tissues (see Table 1). No specific *MrgX2* mRNA was demonstrated in the cerebral cortex, parathyroid or adrenal gland. Variable amounts of *MrgX2* transcripts were demonstrated in all other tissues, the hypothalamus (3/3 cases), the thyroid (3/5 cases), and the pancreas (3/5 cases) being the most positive. Tissue localization analysis by immunohistochemistry (see Table 1) detected MrgX2 protein in scattered neurons of the neurohypophysis (adjacent to pituicytes in a single case) and in testicular tubules. No neuroendocrine cells in any site showed MrgX2 immunoreactivity. Conversely, MrgX2 protein was localized in blood vessels (endothelia), gastrointestinal ganglion cells, and scattered lymphocytes present in most tissues investigated (Figure 4).

Cortistatin mRNA and peptide expression in neuroendocrine and non-neuroendocrine tumours

CST peptide and mRNA expression data in neuroendocrine tumours are summarized in Table 2. Specific *CST* mRNA was detected in all phaeochromocytomas, parathyroid adenomas, and neuroendocrine tumours of the lung, as well as in non-functioning pancreatic endocrine tumours. A specific signal was found in the majority of the other neuroendocrine tumours tested, namely neuroendocrine tumours of the gastrointestinal tract; functioning neuroendocrine tumours

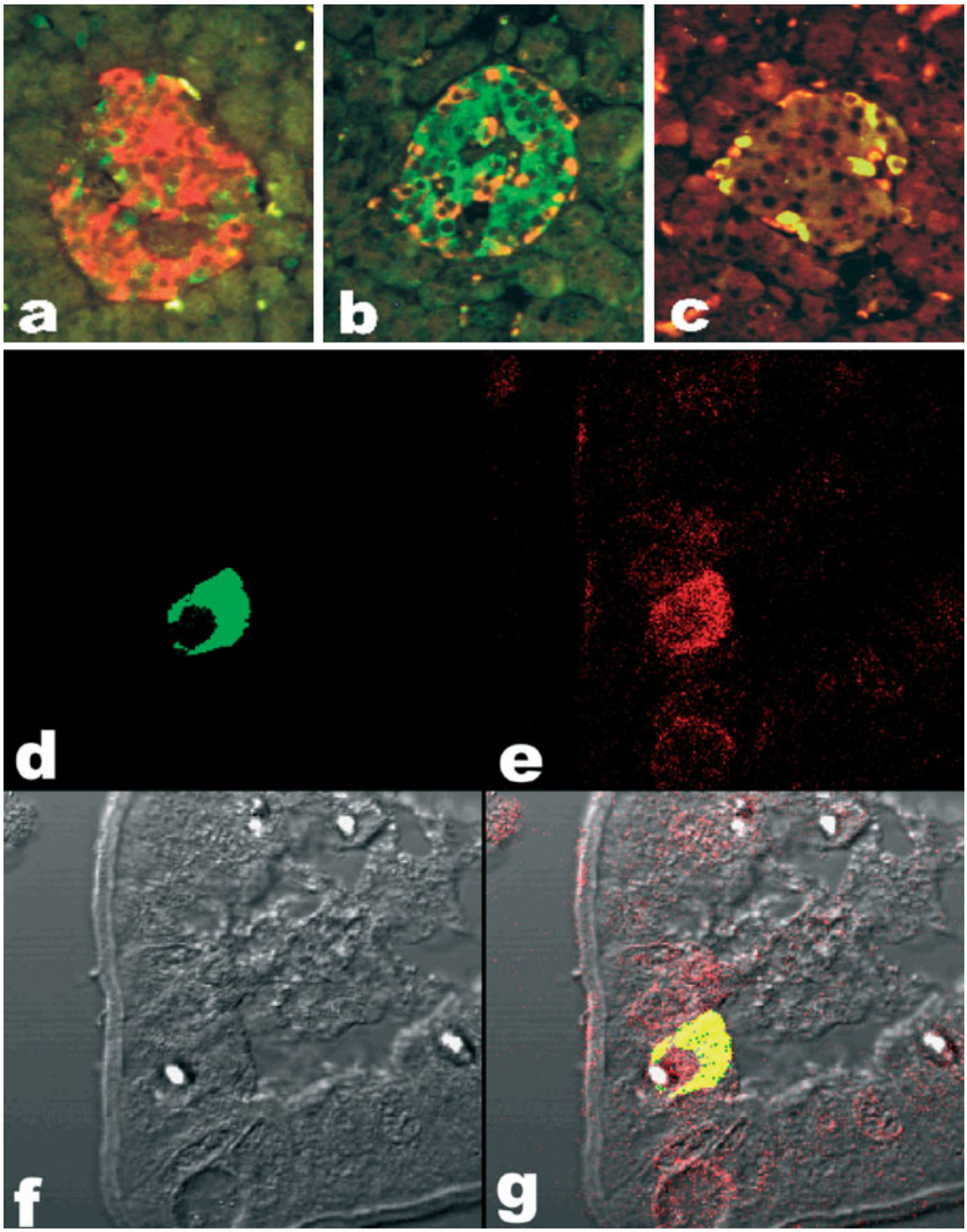

Figure 2. Co-localization of CST and other hormones in a pancreatic islet and duodenal mucosa by confocal laser microscopy. CST (labelled in green) does not co-localize with insulin and glucagon (labelled in red in a and b, respectively) but strongly co-localizes with SRIF, as revealed by the intense yellow colour (c), in serial sections of a pancreatic islet. Co-localization with SRIF is also demonstrated in the duodenal mucosa [same cell stained with green — CST (d), red — SRIF (e), and co-localization by differential interference contrast without (f) or with (g) fluorescence]

of the pancreas (Figure 5), where no specific association was observed in CST-positive tumours with respect to hormone production; medullary thyroid carcinomas; parathyroid carcinomas; and Merkel cell carcinomas.

The majority of the neuroendocrine tumours that contained *CST* mRNA by RT-PCR were also CST-positive by immunohistochemistry, with the exception of parathyroid adenoma and Merkel cell carcinoma, which showed no immunoreactivity. The immunohistochemical pattern of CST was granular cytoplasmic and the staining ranged from 5–10% (the predominant feature) to 90% (in a minority of cases) of tumour cells, irrespective of the histological pattern or the tissue of origin (Figure 6). No correlation was observed between CST expression and tumour behaviour.

Concerning putative endocrine precursor lesions, in two CST-negative medullary thyroid carcinoma

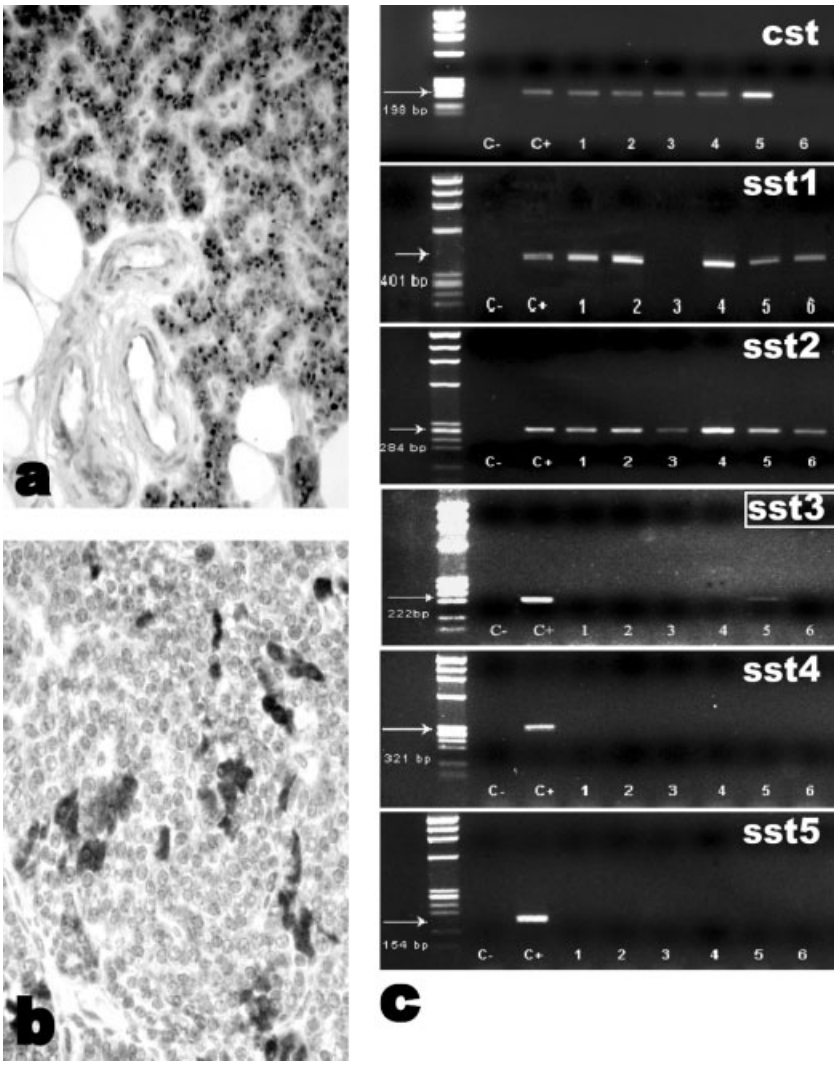

Figure 3. CST and SRIF receptor (sst) expression in parathyroid tissue. CST immunoreactivity in non-tumour hyperplastic parathyroid tissue (a) and a parathyroid carcinoma (b); mRNA analysis of *CST* and *sst* (c) in hyperplastic parathyroid tissue (lanes 1 and 2), parathyroid adenoma (lanes 3 and 4), and parathyroid carcinomas (lanes 5 and 6) determined by RT-PCR. (a, b) Immunoperoxidase; original magnification $\times 400$. (c) C$-$ = negative control consisting of the omission of cDNA from the PCR mixture; C$+$ = positive control for all PCR experiments consisting of pituitary tissue

cases (one associated with MEN2A syndrome and one sporadic), peritumoural C-cell hyperplasia was also negative by CST immunohistochemistry. Moreover, in a case of gastric carcinoid, both the tumour and the foci of adjacent nodular neuroendocrine cell hyperplasia were CST-immunoreactive (Figure 6). In the non-neuroendocrine tumour group, a weak mRNA signal was present in most cases, particularly in adenocarcinomas of the lung, stomach, and colon, and in follicular tumours of the thyroid. By contrast, CST immunohistochemistry failed to demonstrate immunoreactive cells in any of the non-neuroendocrine tumours.

MrgX2 mRNA and protein expression in neuroendocrine tumours

MrgX2 mRNA and protein expression was analysed in 24 selected neuroendocrine tumour samples (see Table 2) that showed immunoreactivity for CST, to correlate the presence of the hormone product with its putative receptor. *MrgX2* mRNA was detected in 8/24 tumours, generally with a weak signal. As observed in normal tissues (see above), MrgX2 protein localization was restricted to peritumoural blood vessels, ganglia, and lymphocytes, whereas the neoplastic component was negative in all 24 neuroendocrine tumour samples investigated, as well as in 12 control non-neuroendocrine tumours (three lung, three pancreatic, and three gastrointestinal adenocarcinomas; two seminomas; and one papillary thyroid carcinoma).

Discussion

In the present study, we have demonstrated that endocrine cells of the gastrointestinal tract, the pancreas, the pituitary, and parathyroid glands produce CST. Co-localization experiments showed that in both gastrointestinal tract and endocrine pancreas, the majority of neuroendocrine CST-producing cells co-express SRIF peptide, thus corresponding to δ cells. A

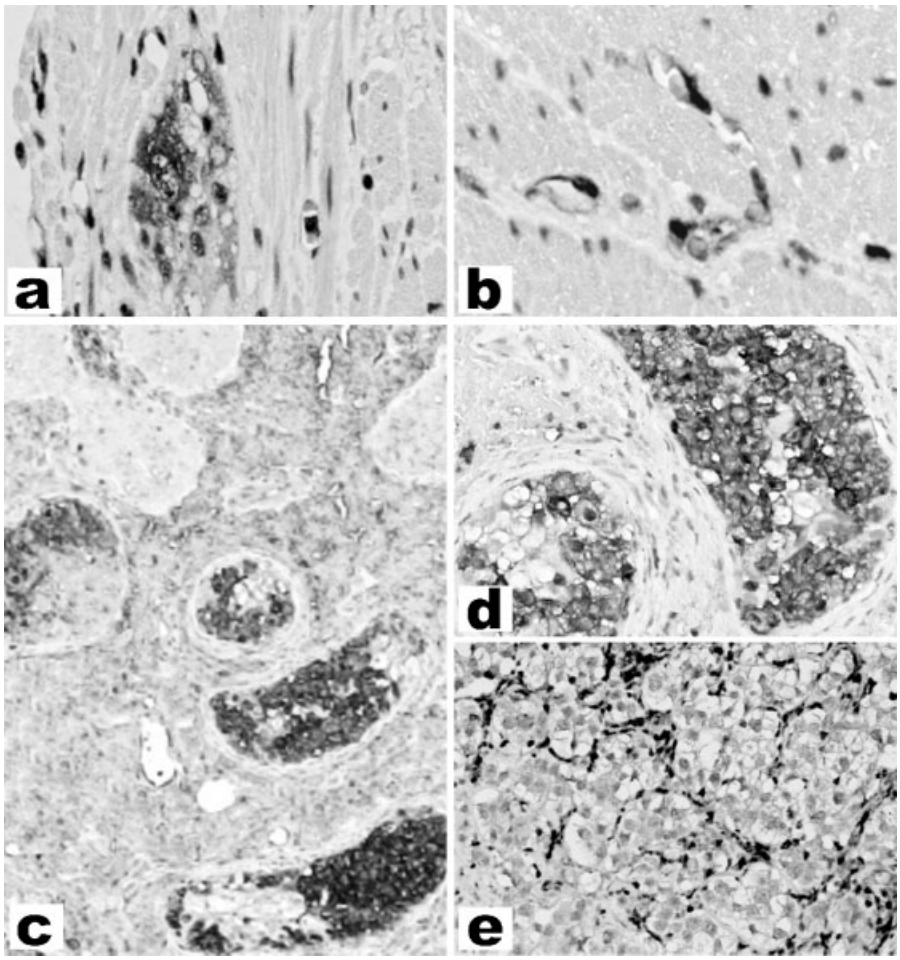

Figure 4. Immunohistochemical localization of MrgX2 protein in gastrointestinal ganglion cells (a) and small blood vessels (b). In a testicular seminoma (c–e), some peritumoural, partially sclerotic (c, bottom), as opposed to hyaline, tubules (c, top) show predominantly membrane (d) MrgX2 immunoreactivity. Conversely, in the seminomatous component (e), no tumour cell, but only intratumoural lymphocytes were CST-positive. Immunoperoxidase. Original magnification: (a, b, d, e) ×400; (c) ×100

Table 2. Expression of CST and its receptor MrgX2 in neuroendocrine tumours of various sites

			CST		MrgX2	
Location	**Diagnosis**	**Hormonal status**	**RT-PCR**	**IHC**	**RT-PCR**	**IHC**
Pancreas	NE tumour	Insulin	16/17	7/12	2/7	0/7
		Gastrin	0/1	0/1	ND	ND
		Glucagon	2/3	2/2	0/2	0/2
		VIP	1/1	1/1	0/1	0/1
		LH	1/1	1/1	0/1	0/1
		PP	1/1	1/1	1/1	0/1
		Somatostatin	1/1	0/1	ND	ND
		Not functioning	7/7	5/5	2/5	0/5
Stomach	NE tumour	—	1/1	1/1	0/1	0/1
Duodenum	NE tumour	Gastrin	0/1	ND	ND	ND
Ileum	NE tumour	—	4/4	1/3	1/1	0/1
Colon	NE tumour	—	1/1	ND	ND	ND
Appendix	NE tumour	—	1/1	0/1	ND	ND
Lung	NE tumour	—	5/5	2/3	1/2	0/2
Thyroid	Medullary carcinoma	Calcitonin	6/7	1/3	0/1	0/1
Parathyroid	Adenoma	Parathormone	2/2	0/2	ND	ND
	Carcinoma	Parathormone	1/2	1/1	0/1	0/1
Adrenal gland	Phaeo	Catecholamines	5/5	1/2	1/1	0/1
Skin	Merkel cell carcinoma	—	9/10	0/3	ND	ND

CST = cortistatin; NE = neuroendocrine; Phaeo = phaeochromocytoma; RT-PCR = reverse transcriptase-polymerase chain reaction; IHC = immunohistochemistry; MrgX2 = Mas-related gene X2; ND = not done.

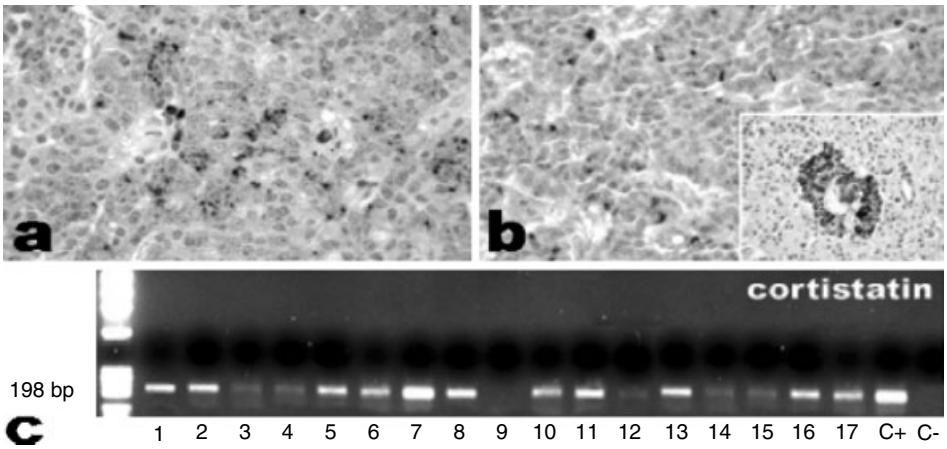

Figure 5. CST expression in pancreatic neuroendocrine tumours. Focal CST immunoreactivity in an insulinoma (a) and a PP-secreting tumour (b). CST-positive pancreatic islets served as an internal control (b, inset). *CST* mRNA analysis by RT-PCR in an insulinoma series (c) was positive in all but one case (lane 9). (a, b) Immunoperoxidase. Original magnification: (a, b) ×200; (b inset) ×100. (c) C− = negative control consisting of the omission of cDNA from the PCR mixture; C+ = positive control consisting of pituitary gland

slightly decreasing density of CST-positive cells was observed from the proximal to the distal gastrointestinal tract, in agreement with the distribution of δ cells [25].

The biological significance of dual expression in δ cells of CST and SRIF, which share both similarities (all SRIF receptor-mediated actions) and differences (CST actions mediated by non-SRIF receptors), remains unclear. The expression of SRIF receptors in neuroendocrine cells of the gastroenteropancreatic system has been demonstrated previously [20] and suggests puzzling circuits of autocrine/paracrine/endocrine regulation that would be exerted by either SRIF or CST, as demonstrated by insulin secretion and glucose metabolism at the pancreatic level [26]. However, as already mentioned, at variance with SRIF, CST also binds to the ghrelin receptor GHS-R1a that is widely expressed in the gastroenteropancreatic tract, where it mediates endocrine and non-endocrine actions. This would predict a functional interplay between the ghrelin system and CST, but not SRIF. Moreover, the relevance of functional differences between CST and SRIF has been emphasized by the recent cloning of *MrgX2*, the putative CST-specific receptor that is recognized by CST but not SRIF [12]. This discovery requires detailed mapping of its distribution in different tissues in order to define its potential biological properties and CST targets better. Here, we have demonstrated that although *MrgX2* mRNA is widely expressed in peripheral non-tumour and tumour tissues, its protein expression is selectively restricted to testicular tubules (as also reported in the original description by Robas *et al* [12]), to the widespread vascular and nerve structures, and to reactive lymphocytes. This latter observation deserves further studies on the role of the CST/MrgX2 axis in the immune system, since CST expression has also been reported in lymphocytes and other immune cell types [15].

A peculiar and unexpected finding was the presence of CST peptide (but not SRIF) in parathyroid chief cells. The possibility that the parathyroid gland could produce peptide hormones other than parathormone, namely calcitonin, has been recently described [27]. The presence of SRIF receptors (particularly subtype 2) correlates with the reported positivity of some parathyroid tumours on octreotide scintigraphy [28], although the absence of any biological effect of octreotide on parathormone secretion has been reported by others [29,30]. In any case, since neither MrgX2 nor GHS-R1a was found in normal and neoplastic parathyroid tissues, the possibility that CST plays a role in regulating parathyroid function via SRIF receptor subtypes (1–3), in either physiological or pathological conditions, should be considered.

With regard to tumours, scattered CST-immunoreactive cells (but not the corresponding MrgX2 receptor) were found in the majority of gastroenteropancreatic neuroendocrine tumours. No preferential association of CST with tumour behaviour or specific hormone secretion was observed. The possible existence of CST-secreting neuroendocrine tumours leading to chronic CST hypersecretion should be validated by detailed clinical and biochemical monitoring of hormonal parameters. Limited evidence for the role of CST in tumour growth control has already been reported [16,17]. Since all immunohistochemically CST-positive tumours did not contain MrgX2 protein, whereas the expression of SRIF receptors (particularly SRIF receptors 2, 3, and 5) and/or GHSR-1a had previously been detected [20–24] in all samples (at either protein or mRNA level), the actions of CST were probably mediated by pathways other than CST/MrgX2 in the tumours investigated.

Concerning *CST* and *MrgX2* mRNA analysis, our results are in agreement with recently published quantitative RT-PCR data showing widespread *CST* mRNA expression in peripheral tissues [31]. However, as

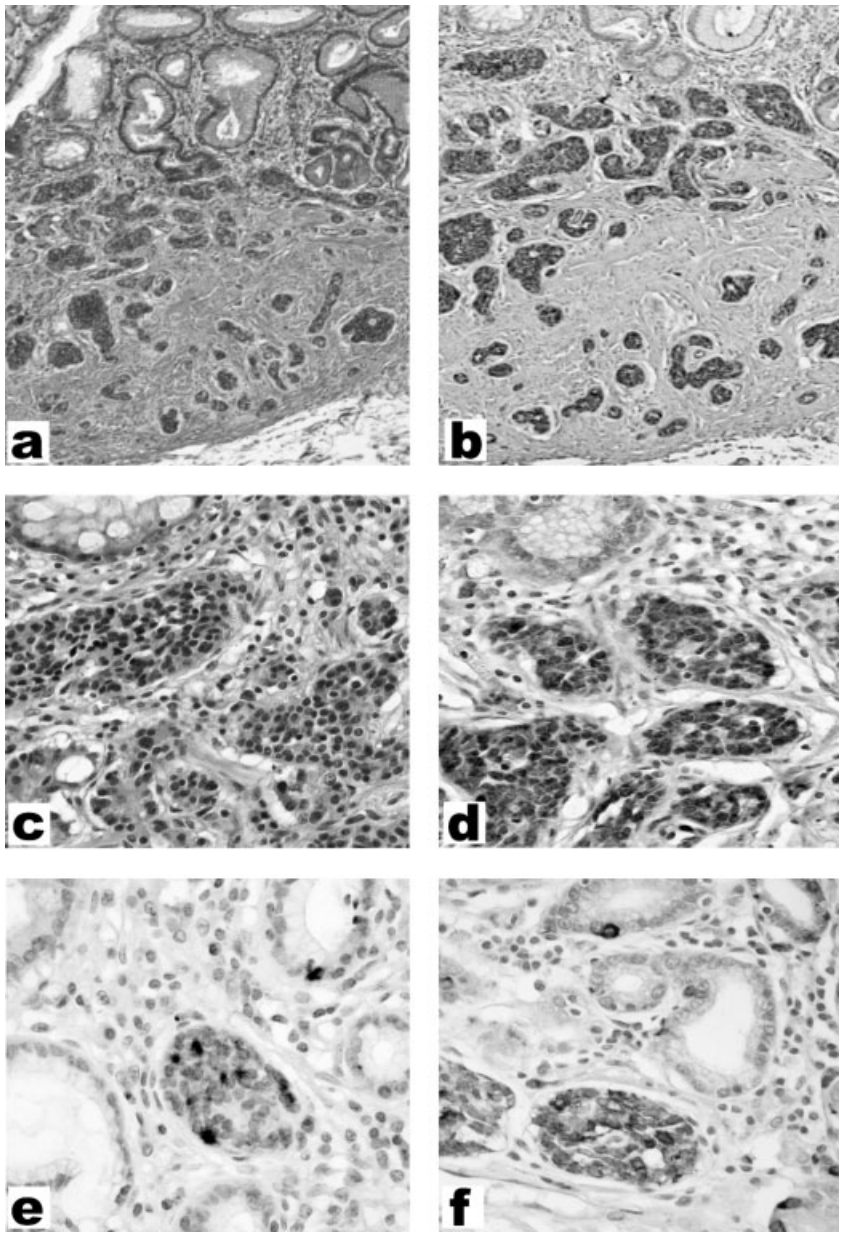

Figure 6. CST expression in a well-differentiated neuroendocrine tumour of the stomach associated with atrophic gastritis and enterochromaffin-like (ECL)-cell hyperplasia. Tumour cell nests located in the mucosa and deeply infiltrating the muscolaris mucosae (a, c) were immunoreactive for CST (b, d). At the periphery of the lesion, microscopic foci of nodular neuroendocrine cell hyperplasia, as revealed by chromogranin A immunohistochemistry (e), were also focally immunoreactive for CST (f). In the same pictures, adjacent normal glands show single cells reactive for both chromogranin A and CST. (a, c) H&E; (b, d–f) immunoperoxidase. Original magnification: (a, b) ×100; (c–f) ×400

observed, for example, for MrgX2 protein localization in blood vessels, pure mRNA data, especially when using very sensitive techniques such as quantitative PCR, should be interpreted carefully, and cannot stand alone without comparative analysis with tissue localization methods (immunohistochemistry or *in situ* hybridization). With regard to this, our heterogeneous *MrgX2* mRNA data on normal tissues may be related either to different detection thresholds in different samples (due to different amounts of interspersed positive cells — ie endothelia, lymphocytes or ganglion cells) or possibly to unrecognized mechanisms of modulation of the expression of the receptor.

In conclusion, we have demonstrated that, firstly, CST is produced by a subset of normal neuroendocrine cells, from the pancreas, gastrointestinal tract, pituitary, and parathyroid; secondly, in the gastroenteropancreatic system these cells appear to be predominantly δ cells; thirdly, the CST receptor, MrgX2, is selectively expressed by neurohypophyseal neurones, testis, blood vessels, reactive lymphocytes, and gastrointestinal ganglia; and fourthly, a consistent number of neuroendocrine tumours from different sites express CST focally, but not its receptor MrgX2: possible autocrine/paracrine CST actions in neoplastic conditions could therefore be mediated by SRIF or ghrelin receptors.

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References

1. Spier AD, de Lecea L. Cortistatin: a member of the somatostatin neuropeptide family with distinct physiological functions. *Brain Res Brain Res Rev* 2000;**33**:228–241.
2. de Lecea L, Criado JR, Prospero-Garcia O, Gautvik KM, Schweitzer P, Danielson PE, *et al*. A cortical neuropeptide with neuronal depressant and sleep-modulating properties. *Nature* 1996;**381**:242–245.
3. Fukusumi S, Kitada C, Takekawa S, Kizawa H, Sakamoto J, Miyamoto M, *et al*. Identification and characterization of a novel human cortistatin-like peptide. *Biochem Biophys Res Commun* 1997;**232**:157–163.
4. De Lecea L, Ruiz-Lozano P, Danielson PE, Peelle-Kirley J, Foye PE, Frankel WN, *et al*. Cloning, mRNA expression, and chromosomal mapping of mouse and human preprocortistatin. *Genomics* 1997;**42**:499–506.
5. Ejeskar K, Abel F, Sjoberg RM, Backstrom J, Kogner P, Martinsson T. Fine mapping of the human preprocortistatin gene (CORT) to neuroblastoma consensus deletion region 1p36.3–p36.2, but absence of mutation in primary tumours. *Cytogenet Cell Genet* 2000;**89**:62–66.
6. Siehler S, Seuwen K, Hoyer D. [^{125}I]Tyr^{10}-cortistatin-14 labels all five somatostatin receptors. *Naunyn-Schmiedebergs Arch Pharmakol* 1998;**357**:483–489.
7. Deghenghi R, Papotti M, Ghigo E, Muccioli G. Cortistatin, but not somatostatin, binds to growth hormone secretagogue (GHS) receptor of human pituitary gland. *J Endocrinol Invest* 2001;**24**:RC1–RC3.
8. Deghenghi R, Broglio F, Papotti M, Muccioli G, Ghigo E. Targeting the ghrelin receptor. Orally active GHS and cortistatin analogs. *Endocrine* 2003;**22**:13–18.
9. Broglio F, Arvat E, Benso A, Gottero C, Prodam F, Grottoli S, *et al*. Endocrine activities of cortistatin-14 and its interaction with GHRH and ghrelin in humans. *J Clin Endocrinol Metab* 2002;**87**:3783–3790.
10. Kreienkamp HJ. Molecular biology of the receptors for somatostatin and cortistatin. *Results Probl Cell Differ* 1999;**26**:215–237.
11. Reichlin S. Neuroendocrinology. In *Williams Textbook of Endocrinology* (9th edn), Wilson JD (ed). WB Saunders: Philadelphia, 1998; 165–248.
12. Robas N, Mead E, Fidock M. MrgX2 is a high potency cortistatin receptor expressed in dorsal root ganglion. *J Biol Chem* 2003;**278**:44 400–44 404.
13. De Lecea L, del Rio JA, Criado JR, Alcantara S, Morales M, Danielson PE, *et al*. Cortistatin is expressed in a distinct subset of cortical interneurons. *J Neurosci* 1997;**17**:5868–5880.
14. Papotti M, Tarabra E, Allia E, Bozzalla Cassione F, Broglio F, Deghenghi R, *et al*. Presence of cortistatin in the human pancreas. *J Endocrinol Invest* 2003;**26**:RC15–RC18.
15. Dalm VA, Van Hagen PM, Van Koetsveld PM, Langerak AW, Van Der Lely AJ, Lamberts SW, *et al*. Cortistatin rather than somatostatin as a potential endogenous ligand for somatostatin receptors in the human immune system. *J Clin Endocrinol Metab* 2003;**88**:270–276.
16. Cassoni P, Muccioli G, Marrocco T, Volante M, Allia E, Ghigo E, *et al*. Cortistatin-14 inhibits cell proliferation of human thyroid carcinoma cell lines of both follicular and parafollicular origin. *J Endocrinol Invest* 2002;**25**:362–368.
17. Notas G, Kolios G, Mastrodimou N, Kampa M, Visilaki A, Xidakis C, *et al*. Cortistatin production by HepG2 human hepatocellular carcinoma cell line and distribution of somatostatin receptors. *J Hepatol* 2004;**40**:792–798.
18. Merz JF, Sankar P, Taube SE, LiVolsi VA. Use of human tissues in research: clarifying clinician and researcher roles and information flows. *J Invest Med* 1997;**45**:252–257.
19. Bongiovanni M, Viberti L, Pecchioni C, Papotti M, Thonhofer R, Popper H, *et al*. Steroid hormone receptor in pleural solitary fibrous tumours and CD34+ progenitor stromal cells. *J Pathol* 2002;**198**:252–257.
20. Papotti M, Bongiovanni M, Volante M, Allia E, Landolfi S, Helboe L, *et al*. Expression of somatostatin receptor types 1–5 in 81 cases of gastrointestinal and pancreatic endocrine tumours. A correlative immunohistochemical and reverse-transcriptase polymerase chain reaction analysis. *Virchows Arch* 2002;**440**:461–475.
21. Volante M, Allia E, Gugliotta P, Funaro A, Broglio F, Deghenghi R, *et al*. Expression of ghrelin and of the GH secretagogue receptor by pancreatic islet cells and related endocrine tumours. *J Clin Endocrinol Metab* 2002;**87**:1300–1308.
22. Papotti M, Kumar U, Volante M, Pecchioni C, Patel YC. Immunohistochemical detection of somatostatin receptor types 1–5 in medullary carcinoma of the thyroid. *Clin Endocrinol* 2001;**54**:641–649.
23. Papotti M, Croce S, Bello M, Bongiovanni M, Allia E, Schindler M, *et al*. Expression of somatostatin receptor types 2, 3 and 5 in biopsies and surgical specimens of human lung tumours. Correlation with preoperative octreotide scintigraphy. *Virchows Arch* 2001;**439**:787–797.
24. Papotti M, Croce S, Macrì L, Funaro A, Pecchioni C, Schindler M, *et al*. Correlative immunohistochemical and reverse transcriptase-polymerase chain reaction analysis of somatostatin receptor 2 in neuroendocrine tumours of the lung. *Diagn Mol Pathol* 2000;**9**:47–57.
25. Dayal Y. Endocrine cells of the gut and their neoplasms. In *Pathology of the Colon, Small Intestine and Anus*, Norris HT (ed). Churchill Livingstone: New York, 1983; 267–300.
26. Broglio F, Koetsveld PvP, Benso A, Gottero C, Prodam F, Papotti M, *et al*. Ghrelin secretion is inhibited by either somatostatin or cortistatin in humans. *J Clin Endocrinol Metab* 2002;**87**:4829–4832.
27. Khan A, Tischler AS, Patwardhan NA, DeLellis RA. Calcitonin immunoreactivity in neoplastic and hyperplastic parathyroid glands: an immunohistochemical study. *Endocr Pathol* 2003;**14**:249–255.
28. Kaltsas GA, Besser GM, Grossman AB. The diagnosis and medical management of advanced neuroendocrine tumours. *Endocr Rev* 2004;**25**:458–511.
29. Sauter GP, Jones DL, Morgan JM, Neonakis E, Woodhead JS, Wheel MH. Role of octreotide on release of intact 1–84 parathyroid hormone from human parathyroid cells. *Br J Surg* 1998;**85**:1133–1137.
30. Zielke A, Hasse C, Bruns C, Sitter H, Rothmund M. Octreotide: effective treatment for hyperparathyroidism? A prospective, randomized, controlled clinical trial. *Surgery* 1997;**121**:606–610.
31. Dalm VA, Van Hagen PM, De Krijger RR, Kros JM, Van Koetsveld PM, Van Der Lely AJ, *et al*. Distribution pattern of somatostatin and cortistatin mRNA in human central and peripheral tissues. *Clin Endocrinol (Oxford)* 2004;**60**:625–629.