Increased expression of the mRNA encoding the somatostatin receptor subtype five in human colorectal adenocarcinoma

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ABSTRACT

Numerous studies have suggested that the anti-proliferative potency of somatostatin (SS) analogues may be an efficient tool to improve the prognosis of colorectal cancer. In order to facilitate current efforts to design potent antitumour SS analogues, we studied the distribution of human SS receptors (hsst1–5) mRNAs in a large set of tumoural and normal colonic tissues. Localisation of hsst1–5 mRNAs in normal and tumoural tissues was performed by in situ hybridisation using radioactive antisense or sense riboprobes. Semi-quantitative analysis of hsst5 mRNA was performed using a computerised image analysis system. Hsst binding sites were characterised by studying the relative potency of SS14, SS28 or SS analogues in displacing [125I]Tyr²-d-Trp⁸-SS14 bound to HT29-D4 cells. Hsst5 mRNA was by far the most expressed subtype in both normal and transformed epithelial cells as well as in the HT29-D4 cell line.

INTRODUCTION

Colorectal cancer is one of the most common cancers in the Western world. The prognosis of patients with advanced colorectal cancer remains poor and new treatment modalities are needed. One of these treatments may involve the use of somatostatin (SS) analogues. SS is a pan-inhibitory peptide widely distributed in the central nervous system and in peripheral tissues. In the gastrointestinal tract it inhibits secretin, gastrin, histamine and gastric acid secretion and decreases gastric and intestinal motility, mesenteric blood flow and intestinal absorption (Lewin 1992). In addition, SS
inhibits cell proliferation by an inhibition of the release of various hormones and growth factors, an inhibition of angiogenesis, a direct inactivation of growth factors or an activation of programmed cell death (Lamberts et al. 1991, Patel & Srikant 1997). SS and its analogues such as octreotide or RC160 inhibit in vitro tumoural colon cell proliferation (Palmer Smith & Solomon 1988, Qin et al. 1991, Dy et al. 1992). In vivo, in murine colon cancer models and in human tumours grafted to nude mice, these substances reduce tumour volume and metastatic development and decrease tumoural markers (Dy et al. 1992, Qin et al. 1992, Dy & Morris 1993, Ruszniewski et al. 1993, Davies et al. 1995). However, clinical studies with SS analogues in patients with advanced colorectal cancer failed to demonstrate a significant benefit although a decrease of tumoural markers could be seen (Goldberg et al. 1995, Cascini et al. 1997); a 2-week treatment with octreotide decreased the Ki67 kinetic index and the carcinoembryonic antigen level in some patients with primary rectal cancer (Iftikhar et al. 1991). Similarly, octreotide treatment reduced the proliferating cell nuclear antigen-maximum proliferative index (Stewart et al. 1995).

Five human SS receptors (hsst) of seven transmembrane G protein-coupled types have been cloned (Patel & Srikant 1997). They are all negatively coupled to adenylate cyclase and can also modulate guanidylate cyclase and calcium or potassium channels, activate protein phosphatase, transmodulate tyrosine kinase type receptors, act on the mitogen-activated protein (MAP) kinase signalling pathway, and even induce apoptosis (Florio & Schettini 1996, Patel & Srikant 1997, Pollak & Schally 1998).

Binding assays and autoradiographic studies have shown the presence of hsst in normal and tumoural colon mucosa, nerve plexus, lymphoid tissue and the peritumoural veins (Iftikhar et al. 1992, Radulovic et al. 1992, Reubi et al. 1992, 1994, 1996). Few recent studies have analysed the expression of the different hsst subtypes in colonic tissue, giving controversial results. Buscail et al. (1996) found by RT-PCR a heterogeneous expression of hsst1–5 in colorectal cancer with a characteristic loss of hsst2 expression in the advanced stages; Laws et al. (1997), using RT-PCR and in situ hybridisation, found a retained expression of hsst2 but a decreased expression of hsst5 in late-stage tumours.

In order to get more insight into hsst in colorectal cancer, we studied, using semi-quantitative in situ hybridisation, the expression of hsst1–5 mRNAs in normal and tumoural colonic tissues. We also analysed on a human tumoural cell line (HT29-D4) the binding of \( ^{125}\text{I}\text{Tyr}^1\text{-D}-\text{Trp}^8\)-SS14 and its displacement by SS28 or by the newly described SS analogues BIM23268 and BIM23197 that show preferential specificity towards hsst5 and hsst2 respectively.

**MATERIALS AND METHODS**

**Materials**

Samples were obtained during surgery for colorectal cancer. All patients gave their written consent to participate in the study. The study was approved by the local Ethics Committee (Marseille I). One part of each specimen was used for histopathological classification and individual colon carcinomas were staged according to the Dukes’ classification system (Dukes & Bussey 1958). The other part was immediately frozen on dry ice and stored at \(-70{\degree}\text{C}\). The results of histopathological studies are summarised in Table 1.

**Peptides**

SS14 and SS28 were from Bachem (Voisins-le-Bretonneux, France). BIM23197 (N-hydroxyethylpiperezinyl-acethyl-d-Phe-c[Cys-Tyr-d-Trp-Lys-Abu-Cys]-Thr-NH\(_2\)) and BIM23268 (c[Cys-Phe-Phe-d-Trp-Lys-Thr-Phe-Cys]-NH\(_2\)) were provided by Biomeasure Inc. (Milford, MA, USA).

**Culture of HT29 cell line**

HT29-D4 human colon adenocarcinoma cell line was routinely cultured in Dulbecco’s Modified Eagle’s medium (Life Technologies, Cergy Pontoise, France) containing 25 mM glucose, 5 mM glutamine, antibiotics (streptomycin, penicillin) and 10% heat-inactivated foetal calf serum in 150 cm\(^2\) tissue culture flasks for binding assays. For in situ hybridisation studies, cells were cultured for 3 days on Labtek chamber slides (Nalge Nunc International, Roskilde, Denmark). The medium was then removed and cells were rinsed twice with PBS and processed immediately for in situ hybridisation assays.

**In situ hybridisation**

Consecutive sections (12 µm) were cut on a cryostat microtome at \(-20{\degree}\text{C}\). The sections were thaw-mounted onto twice gelatine-coated slides, dried on a slide warmer and kept at \(-70{\degree}\text{C}\). Adjacent sections were hybridised with probes recognising the five mRNAs encoding the different hsst subtypes. In situ hybridisation was performed as
previously described (Grino & Zamora 1998). Briefly, the sections were warmed at room temperature and fixed with 4% formaldehyde in PBS, pH 7.2. After two washes in PBS, they were placed in 0.25% acetic anhydride–0.9% NaCl, pH 8, for 10 min and delipidated in ethanol and chloroform. They were hybridised with 50 µl buffer containing 50% formamide, 600 mM NaCl, 10 mM Tris (pH 7.4), 1 mM EDTA, 10% (w/v) dextran sulphate, 0.2 mg/ml tRNA, 100 mM dithiothreitol, and 2×10^7 d.p.m./ml of antisense or sense (control) probe under a glass coverslip. After 20 h incubation in moist sealed chamber at 56°C, coverslips were removed in 2×SSC (1×SSC is 0.15 M NaCl, 0.015 M sodium citrate, pH 7.2). Slides were subsequently treated for 30 min at 30°C with 10 µg/ml Rnase A in 2×SSC and washed at room temperature with decreasing concentrations of SSC (2× to 0.1×) containing 10 mM β-mercaptoethanol (β-ME) and finally incubated for 1 h at 65°C in 0.1× SSC, 10 mM β-ME. Sections were exposed to X-ray films (Biomax-MR; Kodak, Rochester, NY, USA) for 24 h and subsequently dipped in nuclear emulsion (1:1 in water, K5; Ilford, Saint-Priest, France). In order to get a sufficient but not overexposed signal, antisense and sense slides were exposed for 3 days for hsst5, 9 days for hsst1, hsst3 or hsst4, and 3 weeks for hsst2, based on the intensity of the signal on the X-ray film. After development, sections were counterstained with eosin and haematoxylin.

Labelled riboprobes were prepared using [35S]UTP (New England Nuclear, Paris, France, SA: 1300 Ci/mmol) and T3 or T7 RNA polymerase (Stratagene, Paris, France) to synthesise sense or antisense transcripts from the hsst1–5 cDNAs cloned into Bluescript. The incorporation of [35S]UTP was comparable for all the antisense or sense probes. The hsst constructs used to generate the 35S-labelled cRNA probes were as follows: 228–1217 for hsst1, 320–1020 for hsst2, 232–1176 for hsst3, 201–1245 for hsst4 and 81–1016 for hsst5. The specificity of these probes has been described previously (Dutour et al. 1998).

Quantiﬁcation of hybridisation signal

Hsst5 mRNA hybridisation signal was quantiﬁed on emulsion-dipped tissue sections and on monolayers of HT29-D4 cultured cells. Silver grain density was measured under brightﬁeld microscopy (magniﬁcation ×650) using a computerised image analysis system (Unilog and Ware, Grenoble, France). For each case, three different areas were analysed: the tumoural tissue, morphologically normal tissue adjacent to the tumours and morphologically normal tissue taken at distance (>3 cm) from the

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R: rectal; LC: left colon; RC: right colon. WD: well differentiated; MD: moderately differentiated; UD: undifferentiated.
tumour. Silver grain density (grains/0.1 mm²) was measured on ten randomly chosen fields for each area analysed. The mean background level (measured for each tissue on serial sections hybridised with the hsst5 sense probe) was subtracted from each value.

Statistical analysis

Statistical analysis was performed by the Kolmogorov–Smirnov test, using a computerised program (Stat View 512; Brain Power, Inc., Calabasas, CA, USA).

[^125]I Tyr°-o-Trp°-SS14 binding assay

[^125]I-Labelled Tyr°-o-Trp°-SS14 (specific activity: 2200 Ci/mmol) was prepared in our laboratory by lactoperoxidase iodination and purified by reverse phase HPLC (Magnan et al. 1993). The iodinated peptide was diluted 1:2 in binding buffer, stored at 4°C and used within 3 days. For binding experiments, 10⁶ cells/tube were used. Tissue culture flasks were washed twice with 5 ml PBS followed by PBS containing 5 mM EDTA to detach the cells. Binding was performed for 2 h at 4°C in 20 mM Hepes pH 7.4, 5 mM MgCl₂, 100 µg/ml bacitracin, 230 µM phenylmethylsulphonyl fluoride, 100 µg/ml aprotinin, 0-1% BSA in a total volume of 0-1 ml containing 0-25 nM[^125]I Tyr°-o-Trp°-SS14 without (total binding) or with increasing amounts (10⁻¹¹ to 10⁻⁵ M) of SS14, SS28, BIM23268 or BIM23197. To test the specificity of the binding, gastrin was also used. The incubation was stopped by diluting the mixture with 5 ml ice-cold 50 mM Tris–HCl buffer containing 1% BSA. Separation of free and bound radioligand was obtained by rapid filtration in vacuo through Whatman GF/C glass-fiber circles (2.5 cm diameter), pre-soaked in 50 mm Tris–HCl containing 0-3% polyethyleneimine and 0-1% BSA. Filters were washed with 10 ml ice-cold buffer. All assays were performed in triplicate. Radioactivity was counted in a Cobra II (Packard, Warrenville, IL, USA) (99% efficiency for[^125]I).

Data analysis

Analysis of binding isotherms was performed by linear regression methods using the LIGAND program (Munson & Rodbard 1980). Curve fitting and IC₅₀ estimation were performed using the Allfit program (Delean et al. 1978).

RESULTS

After hybridisation of normal or tumoural tissues with the sense probes, silver grains were sparse and homogeneously distributed, demonstrating the specificity of the probes (Figs 1 and 2B, D, F, H and J).

Moderate levels (i.e. detected after 9 days of exposure) of hsst1 mRNA were found in the mucosa, mainly in lamina propria. The labelling was heterogeneous and distributed preferentially as foci in immune cells facing Lieberkühn glands. However, epithelial cells were also clearly labelled (Fig. 1A). Interestingly, no signal was detectable in lymphoid follicles as compared with immune cells in the mucosa. No signal was detected in endothelial, fibroblastic, nervous and muscle cells of the submucosa and in the three muscle layers (muscularis, longitudinal and circular). In tumour, hsst1 mRNA was also expressed by tumoural cells and its expression was heterogeneous within each tumour and between tumours. In many cases, immune cells of stroma close to epithelial cell clusters showed a strong signal (Fig. 2A).

The expression of hsst2 mRNA was very low (i.e. detected after 21 days of exposure) and sometimes barely detectable (Fig. 1C). Hsst2 mRNA was found in immune cells and in epithelial cells of the mucosa. In tumours, hsst2 subtype was also distributed homogeneously at a very low level in both tumoural epithelial and stromal cells (Fig. 2C).

Hsst3 mRNA was expressed at moderate levels (i.e. detected after 9 days of exposure) in the mucosa both in epithelial and immune cells (Fig. 1E). The lamina propria was heterogeneous labelled, the density of silver grains being marked in foci located below the cryptic epithelial cells. Hsst3 mRNA was also expressed in the submucosa. There was a quite marked expression in the three muscle layers. The tumoural distribution pattern of hsst3 mRNA was more diffuse without any foci (Fig. 2E). Hsst3 mRNA was homogeneously expressed in tumoural cells and immune cells of the stroma.

In the mucosa, hsst4 mRNA had the same distribution pattern as hsst1, the signal being preferentially distributed in immune cells (Fig. 1G). A weak signal was detected in the three muscle layers. In tumours, hsst4 expression was weak and heterogeneous and localised preferentially in immune cells (Fig. 2G).

In normal tissue, hsst5 mRNA was homogeneously expressed at a high level in epithelial cells, already highly detected after 3 days of exposure (Fig. 1I). Moderate levels of hsst5 mRNA were also detected in the three muscle layers, at the interface of the circular and longitudinal layers of the muscle and in endothelial cells. Interestingly, an increased labelling for hsst5 mRNA was found in all the tumours (Fig. 2I, Fig. 3A and B). In all patients analysed, silver grain density of the hsst5 mRNA
Localisation of hsst1–5 mRNA in normal colonic mucosa (patient no. 2) by in situ hybridisation. (A, C, E, G, I) Tissue sections were hybridised with hsst1–5 antisense riboprobes; (B, D, F, H, J) tissue sections were hybridised with hsst1–5 sense riboprobes (control). Tissue sections were exposed for 3 days for hsst5, 9 days for hsst1, hsst3 or hsst4 and 21 days for hsst2. Hsst1 mRNA is expressed in immune cells of the lamina propria as foci facing glandular epithelial cells and at low levels in epithelial cells (A). Hsst2 mRNA is expressed at very low levels in both immune and epithelial cells (C). Hsst3 mRNA is expressed at moderate levels in epithelial cells and in the lamina propria (E). Hsst4 mRNA expression pattern is identical to that of hsst1 (G). Hsst5 mRNA is expressed at high levels in normal epithelial cells and at lower levels in the lamina propria (I). Arrows indicate epithelial labelling and arrowheads indicate the labelling of immune cell of the lamina propria. CL, crypt of Lieberkühn; EP, epithelium; LP, lamina propria. Bar=10 µm.

Figure 1. 

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Localisation of hsst1–5 mRNA in tumoural colonic tissue (patient no. 2) by in situ hybridisation. (A, C, E, G, I) Tissue sections were hybridised with hsst1–5 antisense riboprobes; (B, D, F, H, J) tissue sections were hybridised with hsst1–5 sense riboprobes (control). Exposure time was as described in the legend of Fig. 1. Hsst1 mRNA is highly expressed as foci by stromal cells (A). Hsst2 mRNA is barely expressed in tumoural and stromal cells (C). Hsst3 is expressed at a moderate level by transformed epithelial cells and stromal cells (E). Hsst4 is preferentially expressed as foci by stromal cells (G). Hsst5 mRNA is very intensely expressed in tumoural cells and at a lower level in stromal cells (I). Arrows indicate tumoural labelling and arrowheads indicate the labelling of immune cell of the stroma. T, tumoural cells. Bar=10 µm.
hybridisation signal was 2- to 4.5-fold higher in tumoural tissues than in normal tissue (Fig. 4A). In 88% of the area analysed in normal tissue less than 30 grains/0.1 mm² were found whereas there was, in 66% of the area analysed in tumoural tissue, more than 45 grains/0.1 mm². Statistical comparison by the Kolmogorov–Smirnov test indicated a significant (P<0.001) shift towards higher values for hsst5 mRNA in tumours as compared with normal tissue (taken at a distance >3 cm from the tumour). Interestingly, a significant difference (P<0.001) was also found between morphologically normal tissues taken at distance from the tumour and morphologically normal tissue adjacent to the tumours (Fig. 4B).

The tumoural colonic epithelial cell line HT29-D4 showed the same expression pattern of hsst as in primary tumours with a high level of hsst5 mRNA, detected after 3 days of exposure (88–132 grains/0.1 mm²) (Fig. 5A) and a low level of expression of hsst2 mRNA, which could only be detected after 21 days of exposure (Fig. 5B).

**Receptor assays**

The binding of [125I]Tyr⁸-d-Trp⁸-SS14 to HT29-D4 cell line was found to be temperature- and time-dependent. The total binding was found to be 20% with 50% specific binding. No displacement was obtained with gastrin (not shown). Scatchard analysis of the displacement of [125I]Tyr⁸-d-Trp⁸-SS14 by native SS14 indicates that HT29-D4 possess a single class of SS binding sites (K_d=524 nM, B_max=1 fmol/10⁶ cells). In competition binding studies, SS28 and BIM23268 (an analogue that shows preferential specificity towards hsst5) effectively inhibited binding of [125I]Tyr⁸-d-Trp⁸-SS14 (IC₅₀: 15 and 157 nM respectively), while BIM23197 (an analogue that shows preferential specificity towards hsst2) was ineffective (IC₅₀ >1 µM) (Fig. 6).

**DISCUSSION**

In view of the many biological actions of SS, particularly in the gut, where SS inhibits endocrine and exocrine secretion, electrolyte and enzyme secretion, electrolyte transport, intestinal nutrient absorption and cell proliferation, an important question that arises is whether these effects are subtype selective. Because of the lack of good sst subtype-selective antagonist, studies on the expression pattern of sst in brain and peripheral tissue have been performed to approach this question. They have revealed an overlapping but characteristic pattern of expression that is subtype, tissue and also species specific (Patel & Srikant 1997). We present here the first study of the cellular localisation of the five hsst mRNAs in a large set of normal and tumoural human colonic tissue. We found a widespread expression of the five hsst mRNA subtypes in human colonic tissue, particularly expressed in both epithelial and in lamina propria cells. Interestingly, among the different subtypes, hsst5 was by far the most highly expressed and its expression was increased in tumoural cells.

Few studies have approached hsst distribution in normal human colonic tissue (Lewin 1992, Radulovic et al. 1992, Miller et al. 1993, Warhurst et al. 1995, Buscail et al. 1996, Laws et al. 1997). Binding studies have shown the presence of hsst in...
normal human mucosa (Radulovic et al. 1992, Miller et al. 1993). Buscail et al. (1996) have shown by RT-PCR the expression of hsst2, hsst4 and hsst5 in normal human mucosa. As shown by Laws et al. (1997), we demonstrated in a study performed by RT-PCR that all five hsst were detected in normal tissues, hsst5, hsst1 and hsst2 mRNA being the most frequently detectable (Vuaroqueaux et al. 1999). These non-quantitative results are in accord with our in situ hybridisation results showing a high expression of hsst5 mRNA in epithelial cells, and of hsst1 mRNA in stroma while hsst2 was found to be widely expressed in mucosa but at a low level. Using in situ hybridisation, Laws et al. (1997) found that hsst2 mRNA was widely distributed in normal mucosa and stroma in 90% of samples while hsst5 was present only in half of normal mucosal samples. The difference between our results and those of Laws is most probably related to the difference of sensitivity of the probes used. Several studies have been performed in rats. Using RT-PCR, Warhurst et al. (1996) have shown that crypt epithelium expressed sst1 and sst2 mRNA and, at lower levels, sst5 mRNA. However, in rat colon adenocarcinoma (DHD/K12) sst2, sst3 and sst5 were identified (Gouyon et al. 1995). A very detailed in situ hybridisation study in the rat gastrointestinal tract has been performed by Krempeles et al. (1997) showing a widespread expression of rat sst mRNAs and suggesting a large SS regulatory system in the gastrointestinal tract. Similar to what we found, they showed that all tissues contained mRNA for several sst subtypes. A clear expression of sst3 mRNA was demonstrated, rather comparable with what we found. There is also a close resemblance between sst1 and sst4 expression in rat and in human colonic tissue but sst5 expression was far higher in human tissue in our study than in rats. This difference is not surprising as species-specific distribution of sst has previously been shown, the difference of expression being particularly marked for sst5 (Hoefland & Lambert 1996, Thoss et al. 1996, Patel 1997).

Interestingly, we found expression of hsst1–4 in immune cells of both lamina propria and stroma, hsst1 being the most expressed. This underlines the
Cells display mononuclear cells of lamina propria and that these SS can inhibit immunoglobulin production by 1997). Furthermore, in the gut, it was shown that independent pathways CD2 and CD28 (Casini et al. 1989) can inhibit cell activation by interfering with the antigen-proliferation (Aguila et al. 1996), and to inhibit T cell activation by interfering with the antigen-independent pathways CD2 and CD28 (Casini et al. 1997). In the gut, it was shown that SS can inhibit immunoglobulin production by mononuclear cells of lamina propria and that these cells display specific high-affinity (Kd=2.1±0.34 nM) hstt, whose subtype was not characterised (Fais et al. 1991). Our data suggest that hstt1 may be a major actor.

Looking for therapeutic bases for SS analogue treatment of colorectal cancer, we studied hstt expression in 22 tumours. We show that the five hstt mRNAs were expressed in all the tumours. The tumoural status did not modify the expression of hstt1–4 although in some cases tumours seemed more labelled because of the higher cell density. By contrast, an enhanced labelling for hstt5 mRNA was found in tumoural cells, silver grain density being 2- to 4.5-fold higher in tumoural tissues than in normal ones. Of interest, hstt5 mRNA was retained in advanced tumours. Using a non-quantitative RT-PCR, Buscail et al. (1996) have previously noted a very frequent expression of hstt5 in tumours (in 71% of tumours whatever the stage of the tumours) while hstt2 was less frequently detected especially in advanced tumours (three out of six in Dukes’ B stage, one out of five in Dukes’ C stage, none out of three in Dukes’ D stage). Our work underlines the importance of hstt5 in tumours. The group of Primerose (Laws et al. 1997) found also an increased frequency of hstt5 expression in early-stage tumours as compared with normal mucosa (75 vs 45%) but this increase of frequency was lacking in late-stage tumours.

Although mRNA steady-state levels do not necessarily correlate with the amount of protein synthesised, the existence of large amounts of hstt5 mRNA was in favour of the presence of high levels of hstt5. The recent availability of SS analogues with preferential specificity for hstt5 allowed us to show the presence of hstt5 binding sites and a very low level of hstt2 receptor on HT29-D4. Indeed BIM23268, an SS analogue with a higher affinity for hstt5 than for hstt2 and hstt3 (40- and >10 000-fold respectively) (Shimon et al. 1997) was more effective than SS14 itself (IC50=157 nM vs 628 nM for SS14) in displacing [125I]Tyr3-Trp5-SS14 bound to HT29-D4 cells. Furthermore, BIM23197, an SS analogue with a higher affinity for hstt2 than for hstt5 and hstt3 (50- and 140-fold respectively) did not affect the binding of iodinated SS14 to HT29-D4 cells. Our results are in good agreement with the recently published immunohistochemical studies showing the presence of the hstt5 receptor protein in colonic tissue (Estève et al. 1998).

Our results show a high expression of hstt5 mRNA in human tumoural colonic tissue, while hstt5 protein is the predominant hstt protein subtype in a tumoural colonic cell line, and raise the question of the potential benefit of sst5 analogues in colonic cancer therapeutic approaches. Indeed, SS analogues such as octreotide and RC160 have been shown to decrease tumoural markers and the growth of human colonic tumours transplanted in rodents and to reduce metastatic development (Qin et al. 1991, 1992, Dy et al. 1992, Dy & Morris 1993, Ruszniewski et al. 1993, Davies et al. 1995). These antiproliferative effects of SS are mediated both indirectly through inhibition of secretion of hormones and growth factors involved in tumour growth and directly through sst located on tumour tissue (Lamberts et al. 1991, Patel & Srikant 1997).
Schematically, sst can activate protein tyrosine phosphatases inducing dephosphorylation of growth factor receptor kinase and then inhibit MAP kinase activity (Pan et al. 1992, Buscail et al. 1994, Patel & Srikant 1997, Pollak & Schally 1998). Several sst subtypes have been implicated in SS antiproliferative effects (Buscail et al. 1994, Sharma et al. 1996, 1999, Cordelier et al. 1997, Patel & Srikant 1997, Florio et al. 1999). Even though SS-induced apoptosis has been shown to occur via the hsst3 subtype in cycling cells (Sharma et al. 1996), a large body of experimental evidence suggests involvement of hsst5 in SS-induced antiproliferative effects (Cordelier et al. 1997, Sharma et al. 1999). A cytostatic effect of SS via hsst5 that occurred through induction of the retinoblastoma protein and G(1) cycle arrest was recently described (Sharma et al. 1999). Moreover, hsst5, the subtype overexpressed in colon cancer, can inhibit the proliferation induced by cholecystokinin (CCK) (Cordelier et al. 1997). Indeed, when stably expressed in CHO cells, hsst5 mediates the antiproliferative effect of SS analogues, via the successive inhibition of soluble guanylate cyclase, protein kinase G and p42 MAP kinase activities (Cordelier et al. 1997). It is interesting to note that we have previously shown in the tumours analysed in this study the presence of CCK-B long and short isoform and CCK-C receptors (Biagini et al. 1997).

In conclusion, our results suggest that studying the effect of hsst5 analogues in colorectal cancers may be an interesting approach.

Figure 6. Displacement of $[^{125}\text{I}]\text{Tyr}^7\cdot\text{Trp}^8\cdot\text{SS}14$ binding to epithelial colonic tumoural cell line HT29-D4. Cells were incubated for 2 h at 4°C with 0.25 nM iodinated peptide in the absence (total) or in the presence of increasing concentrations ($10^{-11}$–$10^{-5}$ M) of SS14, SS28, BIM23268 or BIM23197. Values are the mean ± s.e. of three determinations. Results are expressed as the percentage of total specific binding.
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