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Molecular mechanisms of the antiproliferative activity of somatostatin receptors (SSTRs) in neuroendocrine tumors

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1. ABSTRACT

The current treatment of neuroendocrine tumors include the use of somatostatin (SST) agonists. These compounds are able to control most of the symptoms caused by the hypersecretory activity of the tumor cells, and for this reason, they provide a significant improvement in the well-being of the patients. Although, several reports also showed a possible direct antiproliferative activity of SST agonists in different neuroendocrine tumors, the therapeutic potential of an *in vivo* antiproliferative activity mediated by SST receptors is still debated. In recent years, there has been great insights on understaning the molecular basis of the antitumoral activity of SST that appears to be exerted via both direct and indirect mechanisms. Direct mechanisms require the activation of SST receptors in tumor cells and the induction of cell cycle arrest or apoptosis, mainly through the regulation of phosphotyrosine phosphatase (PTP) and MAP kinase activities. The indirect mechanisms involve the inhibition of tumor angiogenesis and the inhibition of the secretion of factors which are required for tumor growth. Here, we will review the molecular mechanisms which are implicated in the antiproliferaitve activity of SST. Such an understanding is necessary for improving the antitumoral efficacy of SSTR agonists as well as for the development of novel therapeutic strategies.

2. INTRODUCTION

Neuroendocrine tumors are a heterogeneous group of neoplasms derived from the diffuse neuroendocrine system. The definition of neuroendocrine was originally proposed in light of the similarity between these cells and neurons, although it is now clear that only few of the neuroendocrine cells are neuroetodermic in origin (for example the adrenal medulla and the paraganglia cells)(1). Independently from the embryologic origin, the definition of neuroendocrine cell has to meet the following requirements: i) the secretory activity of a neurotransmitter or a neuropeptide hormone; ii) the presence of dense core secretory granules that are exocytosed in response to extracellular stimuli; iii) the absence of neurites and synapses, as a morphological discrimination with neurons; iv) the expression of chromogranin A, that represent one of the most useful molecular marker of neuroendocrine cells (2).

Being derived form such a wide spectrum of different cell population, neuroendocrine tumors are necessarily heterogeneous as far as localization, hormonal secretory pattern, clinical and prognostic features. Neuroendocrine tumors include carcinoids, non-carcinoids gastro-entero-pancreatic tumors such as insulinomas, gastrinomas and VIPomas, pheochromocytomas, paragangliomas, ganglioneuromas, neuroblastomas and other catecholamine-secreting tumors, medullary thyroid

carcinomas, chromophobe pituitary adenomas, small cell lung cancers, etc. (2).

The incidence of these tumors is increased in the last years, accounting for about the 1% of all human tumors. The gastrointestinal localization accounts for more than 70% of all the neuroendocrine tumors. Clinically, beside the proliferative and invasive behaviours that may vary among the different tumor localizations, neuroendocrine tumors are characterized by hypersecretion of peptide hormones or catecholamines that, ultimately, are responsible of the characteristic syndromes of each tumor histotype. For example, patients with insulinomas will display severe hypoglicemia crisis while, in the case of carcinoids or VIPomas, watery diarrhoea, hypokaliemia and achlorhydria are observed, due to the hyperincretion of serotonin or VIP (3-5). Different therapeutic approaches have been used for these kind of tumors, although in most cases, non curative, including surgery, cytotoxic drug treatment (streptozotocin, dacarbazine adriamycin and 5-fluorouracil) that represent a common way of management mainly for the pancreatic tumors (6), and hepatic artery embolization or chemoembolization for the treatment of liver metastases (7).

The therapeutic protocol of neuroendocrine tumors considers also the use of long-acting somatostatin (SST) analogues, mainly octreotide and lanreotide, to take advantage of the powerful antihormonal activity of somatostatin receptors (SSTRs) to induce the palliation of syndromes related to the tumor-dependent hormone hypersecretion (8). In fact, in a large series of neuroendocrine tumors a high prevalence of SSTRs was observed in both primary tumors and metastases, showing a continuous expression even after long term treatment (9). In particular, although differences according to the tumor histotype were observed as far as frequency and amount, neuroendocrine tumors express all the SSTR subtypes (SSTR1-5) (10-12).

Presently, the responses to SSTR analogues in the therapy of neuroendocrine tumors can be defined according to three categories: a) symptomatic, b) biochemical and c) objective.

a) Symptomatic responses represent the reduction of the symptoms related to the hypersecretion in the functional neuroendocrine tumors and the inhibition of the tumor bulk-related symptoms, such as upper abdominal pain, causing an improvement of the quality of life and performance status in patients with non functional neuroendocrine tumors. The reported efficacy of the treatment with SST analogues may reach 50-70% of the treated patients (13-16).

b) Biochemical responses are defined as a reduction larger than 50% in the serum or urine levels of tumor markers. In particular although the relevance of this parameter is still debated, a marked early decrease in markers level can be considered of prognostic value for the therapy con SST analogues.

c) Although there are still contradictory reports, some studies observed that SST analogues (octreotide and lanreotide) may possess also a direct antiproliferative activity in neuroendocrine tumors. In fact, a temporary stabilization of gastroenteropancreatic tumor growth (from a minimum of 3 months to 5 years) was observed in 30-70% of the patients treated with SST analogues and a partial response in less than 10% of the cases (5, 15, 17-21). Furthermore, few studies showed tumor shrinkage in selected patients treated with ultra-high doses of lanreotide (13) or after a synergistic treatment with interferon alpha (22).

However, to date, although only a limited number of patients have been analyzed to provide a definitive response (23), these observations, altogether with the much more consolidated preclinical data demonstrating antiproliferative and pro-apoptotic activity of SSTRs, prompted the pharmacological research to identify novel molecules with potential somatostatinergic antitumoral activity.

In this perspective, here we report the state of the art of the intracellular mechanisms regulated by SSTR to induce antiproliferative activity as possible innovative targets of novel SSTR agonists.

3. MOLECULAR AND BIOCHEMICAL CHARACTERIZATION OF SSTRs

Since the synthesis of octreotide, the first somatostatin analogue [for rev see (24)], it was postulated the existence of multiple SSTRs since it was demonstrated a differential pattern of binding of SST and octreotide to rat brain slices. These observations were indeed confirmed in the early ‘90s when an entire family of five SSTRs was molecularly cloned, being named SSTR1 through 5 (25). These receptors, whose sequence is highly conserved through the species (2-14% divergence between human, rat and mice)(26), display a common structure, all belonging to the G protein coupled receptors (GPCR). SSTRs are encoded by five non-allelic genes located, in humans, on chromosomes 14, 17, 22, 20 and 16 for SSTR1-5, respectively (see Table 1). Although subsequent pharmacological studies proposed the possible existence of additional components of this receptor family (27), to date no further SSTRs have been cloned. Interestingly, while SSTR1, SSTR3, SSTR4 and SSTR5 are intronless genes, in humans, mice and rats, SSTR2 gene can produce two splice variants of the receptor, named SSTR2A and B, characterized by a longer or shorter carboxyl terminus, respectively (13 amino acids in humans and 23 in mice)(28, 29). It was reported that SSTR2A and B isoforms may differ in their sensitivity for intracellular signalling (inhibition of cAMP production)(30) but the real biological meaning of SSTR2B it is not well understood and SSTR2A represents the largely dominant isoform in humans.

All SSTRs possess seven alpha-helical putative transmembrane domains, with the N-terminus extracellular and the C-terminus intracellular, as expected for all GPCRs. The transmembrane domains show the highest

Table 1. Biochemical and biological features of the human somatostatin receptor

	SSTR1	SSTR2	SSTR3	SSTR4	SSTR5
Chromosomal localization	14q13	17q24	22q13.1	20p11.2	16p13.3
mRNA (kb)	4.3	8.5	5.0	4.0	4.0
Amino acids	391	SSTR2A: 369 SSTR2B: 356	418	388	364
Protein size (kDa)	42.7	41.3	45.9	41.9	39.2
Glycosilation sites	3	4	2	1	3
G protein coupling	+	+	+	+	+
Antiproliferative activity					
Cell cycle progression	↓	↓	↓	↑↓	↓
Apoptosis	–	↑	↑	–	–

↑ = activation, ↓ = inhibition, – = no effect.

sequence similarity (55-70%) among the SSTR family, with the N- and C-termini more divergent (31). Overall there is a 39-57% identity in the sequences of these receptors that allow the subdivision of SSTRs in two subfamilies named SRIF-1 (from the original name of SST, Somatotropin Release Inhibiting Factor) that consists of SSTR2, SSTR3 and SSTR5, and SRIF-2 that includes SSTR1 and SSTR4 (31). Importantly, beside different homology sequences, SRIF-1 and SRIF-2 receptor subfamilies differ also for their pharmacological features and, in particular, for their binding properties to SST synthetic agonists. In fact, while all the SSTRs bind with similar high affinity (nanomolar range) the natural SST isoforms (SST14 and SST28) and the related peptide cortistatin (Table 2)[only a slight preference of SSTR5 for SST28 was reported (32)], a more selective binding profile was identified for the synthetic agonists (Table 2). Octreotide, lanreotide, vapreotide and seglitide selectively bind SRIF-1 receptors with a high affinity for SSTR2 and SSTR5 and intermediate affinity for SSTR3, but do not interact with the SRIF-2 subfamily. In consideration of the widespread distribution of all the five SSTRs, often overlapping in the same cell types, more recently both peptidic and non peptidic compounds able to selectively modulate the activity of each SSTR subtype were developed, as well as pan-SSTR agonists that should better mimic the activity of the natural SST (Table 2)(33).

SSTRs are expressed in discrete or overlapping distribution in multiple target organs. Importantly, the differences in signal transduction among SSTRs are not only related to the specific subtypes, but also to the cellular environment, where these receptors are expressed. All SSTRs are coupled to G proteins (Table 1) and different members of these transducing molecules were identified to be coupled to components of the SSTR family. In particular, the three isoforms of the inhibitory G proteins ($G\alpha_{i1-3}$) were all coupled to the different SSTRs (26), while, using a mRNA antisense strategy, it was reported the coupling of SSTR2 to the $G\alpha_{o2}/\beta_2/\gamma_3$ complex to control Ca^{++} channel activity in pituitary cells (34-36). SSTR3 was also identified to couple to $G\alpha_o$, $G\alpha_{14}$ and $G\alpha_{16}$ (37). It is important to note, however, that all the biological effects of SSTRs are sensitive to pertussis toxin, with the only exception of the regulation of the Na^+/H^+ exchanger, NHE1, by SSTR1 (38).

Recently, the ligand-dependent homodimerization was discovered as a novel mechanism for SSTR signalling. All SSTRs can homodimerize and more importantly, it was demonstrated that SSTRs can also oligomerize with either other SSTR subtypes or different families of GPCR, such as the μ opioid receptor 1 (MOR1) and the dopamine D2 receptor (D2R) (39). In particular, SSTR2 can dimerize with SSTR3 resulting in a reduced receptor internalization and thus, changing the pattern of receptor desensitization observed in the individual receptor subtypes (40). SSTR5 and SSTR1 (but not SSTR4) can also form heterocomplexes, with altered internalization properties (41). In fact, while SSTR1, when expressed alone, exhibits a lack of ligand-induced cell internalization, a significant endocytosis occurs when co-expressed with SSTR5. SSTR2 was reported to heterodimerize with MOR1 and the formation of such complexes, while not affecting the receptors signalling, induces a cross-modulation of the desensitization and internalization of both receptors (42). Conversely, the heterodimers formed by SSTR5 and D2R display pharmacological properties that are distinct from those of the two individual receptors with an enhanced inhibition of cAMP formation (41). The dynamic interaction between SSTR5 and D2R can be induced independently by both somatostatin and dopamine (41).

Thus, these receptor interactions may allow the activation of intracellular pathways not regulated by the individual receptors or modify their binding and desensitization responses that may be useful for therapeutic purposes. Recently, in light of these studies, chimeric molecules with high affinity for both SSTR2/5 and D2R (Dopastatins) have been preclinically developed (43-46) and will be soon in clinical trials for the treatment of pituitary adenomas.

4. PHYSIOLOGICAL ROLES OF SSTRs

The physiological key role of SST, through the activation of SSTRs, is represented by the inhibition of many different endocrine and exocrine secretory activities. At pituitary level SSTR1 was reported to control the secretion of GH and prolactin secretion; SSTR2 is the main regulator of the secretion of GH, and ACTH; SSTR5 controls GH and prolactin release (47).

In the endocrine pancreas SSTR1 and, at higher levels, SSTR5 are expressed in insulin secreting beta-cell, SSTR2 mainly in glucagon secreting alpha-cells and SSTR5 in the SST-releasing delta-cells, while SSTR3 and SSTR4 are poorly expressed (48, 49). In intestinal cells, SSTR5 controls the release of glucagon like peptide-1 (50).

The role of SST is not restricted to the endocrine system. SSTR2 is the predominant subtype controlling the acid gastric secretion (51), SSTR3, expressed in gastrointestinal smooth muscle cells (52), controls their

Table 2. Affinity of natural and synthetic somatostatin agonists for the individual human sstr subtypes

		IC_{50} (nM)				
		SSTR1	**SSTR2**	**SSTR3**	**SSTR4**	**SSTR5**
Endogenous peptides	SST-14	0.1-2.26	0,2-1.3	0.3-1.6	0.3-1.8	0.2-0.9
	SST-28	0.1-2.2	0.2-4.1	0.3-6.1	0.3-7.2	0.05-0.4
	CST-17	7	0.6	0.6	0.5	0.4
Short synthetic peptides	Octreotide	>1000	0.4-2.1	4.4-34.5	>1000	5.6-32
	Lanreotide	>1000	0.5-1.8	43-107	>1000	0.6-14
	Vapreotide	>1000	0.2-5.4	31	45	0.7
	Seglitide	>1000	0.1-1.5	27-36	>1000	2-23
	BIM23268	18.4	15.1	61.6	16.3	0.37
	BIM23926	4	>1000	>1000	>1000	>1000
	BIM23120	>1000	0.34	412	>1000	213,5
	BIM23206	>1000	166	>1000	>1000	2.4
	BIM23704	6.3	1.4	43.2	>1000	115
	BIM23190	>1000	0.35	215	>1000	11.2
	SOM-230	9.3	1	15	100	0.16
	KE108	2.6	0.9	1.5	1.6	0.65
Non peptide agonists	L-797,591	1.4	1875	2240	170	3600
	L-779,976	2760	0.05	729	310	4260
	L-796,778	1255	>10000	24	8650	1200
	L-803,087	199	4720	1280	0.7	3880
	L-817,818	3.3	52	64	82	0.4
Antagonists	Cyn154806	>1000	3.6	150	650	20
	ODN-8	>10000	>10000	6.7	>10000	>10000
	BN81658	>1000	>1000	1.58	>1000	>1000

Table 3. Principal transducing systems regulated by the activation of SSTRs

		SSTR1	**SSTR2**	**SSTR3**	**SSTR4**	**SSTR5**
cAMP production	Adenylyl cyclase	↓	↓	↓	↓	↓
Ion currents	Voltage sensitive Ca^{++} channels (L and N)	↓	↓	–	–	↓
	K^+ current	↑	↑	↑	↑	↑
	GIRK activity	–	↑↑	↑	↑	↑
	Delayed rectifier K^+ channel	↑	↑↑	–	↑↑	↑
Tyrosine phosphatases	Tyrosine phosphatase activity	↑	↑	↑	↑	↑
	SHP-1		↑			↑
	SHP-2	↑	↑	↑	↑	↑
	PTPeta	↑				
MAP kinases	ERK1/2	↓/↑	↓/↑	↓	↑	↓
	p38		↑	–	↑	
	JNK	–	↓	–	–	↑
Tyrosine kinases	Src	↑	↑			
	Jak2	↑	↑			
Phospholipid kinase	PI3K	↓/↑	↓/↑			
Nitric oxide synthases	nNOS	–	↑			↓
	eNOS	↓	↓	↓	–	
Na^+/H^+ exchanger	NEH1	↓	↓/↑	–	↑	–

↑ = activation, ↓ = inhibition, – = no effect.

contractile activity. Furthermore, in studies using SSTR2-deficient mice, SSTR2 was identified in nitric oxide synthase (NOS)-expressing myenteric neurons to modulate gut motility (53) as well as it was involved in ductal bile absorption and secretion (54).

SST is also an important neuromodulator within the central nervous system, with SSTR expression widespread throughout the brain (55). In particular, while SSTR1 and SSTR2 expression is diffuse in all the brain, SSTR4 is mainly localized in the hippocampus and SSTR5 in the hypothalamus. Although SSTR1 is also expressed in different brain areas its subcellular localization is peculiar, being also presynaptic and thus controlling SST release from somatostatinergic neurons (56). Behavioural studied assessed a role for SST in the modulation of the learning and memory processes in different animal models, (57-59) suggesting that, in humans, an impairment of the somatostatinergic neuronal system may be involved in the development of neurodegenerative diseases. In fact, although in Alzheimer disease a reduction of SST content and SST neurons was known by many years (60-62), the alteration of its activity was recently directly associated to the generation of the beta-amyloid, one of the pathogenetic molecules in this kind of dementia (63).

In the peripheral nervous system, SST was reported to mediate analgesia, through the activity of receptors localized in proximity of the pain terminal and inhibiting the firing of dorsal horn neurons (64, 65).

SST has also been implicated in the regulation of the immune system, and the chronic inflammatory diseases were recently proposed as potential new target pathologies

for SST analogues (66). Different SSTRs were identified in thymus (SSTR1, SSTR2 and SSTR3) with a higher expression of SSTR2 in immature thymocytes (CD2+/CD3-) and SSTR3 mainly in more mature cells (CD3+). Thymic epithelial cells express both SSTR2 and, in lower amounts, SSTR1. It was proposed that SSTR3 was the main determinant of apoptosis in thymic cells, playing relevant physiological functions in thymus (67, 68). SSTR3 was also expressed by peripheral human T lymphocytes (directly derived from mature thymocytes) and its activation by SST induces apoptosis in these cells. Both B and T lymphocytes, as well as monocytes but not granulocytes, express SSTRs. In resting cells SSTR3 is the subtype with the highest expression but, upon activation, also SSTR2 and SSTR5 are detected, largely increasing the sensitivity of these cells to SST analogues (66). In peripheral blood immune cells SSTR activation causes: a) the regulation of monocyte/macrophage activity reducing the secretion of cytokines [IL-1, IL-6, tumor necrosis factor alpha(TNFalpha)] and interferon gamma; b) the inhibition of the activity of T lymphocytes (cytokine release, proliferation), that was synergic with that of tacrolimus, leading to a substantial immunosuppression; c) the inhibition of the Ig secretion from B lymphocytes (69).

Thus, SST activity, through its specific receptors, is involved in a large number of biological functions. However, one of the effects that attracted much of the interest of the researcher is its prominent role as endogenous regulator of cell proliferation.

5. SSTRs AND TUMOR CELL PROLIFERATION

SST activity as endogenous antiproliferative agent is now recognized in many different experimental tumor models *in vivo* and *in vitro*. However, these effects, highly significative in preclinical studies, become much more questionable when the laboratory data are translated to clinical trials. Indeed, although SST analogues have been successfully used in few specific tumor histotypes, the impressive bulk of preclinical data generated in the past years is still far to find a convincing general clinical application. Notwithstanding the research is still moving further and a significant progress in the understanding the mechanisms by which SSTR activation may lead to cytostatic or apoptotic effects, has been obtained. In particular, it is now commonly accepted that, among all the different second messengers regulated by SSTRs, the main transduction system involved in the antiproliferative activity of SST is represented by the activation of a subset of phosphotyrosine phosphatases (PTP)(25, 70). Moreover, the developing of a large number of compounds (agonists or antagonists) for each SSTR subtype, as well as molecules with high affinity for multiple SSTRs (Table 2), is further increasing the potential efficacy of SST analogue-based therapy in oncology. Importantly, beside the direct antiproliferative effects of SST, an indirect antitumor activity was also identified. In fact, early evidences showed that SST analogues are able to inhibit the proliferation of SSTR negative tumors, such as the Swarm chondrosarcoma (71). It was shown that these tumor cells express high levels of IGF-1 receptors and that the effects of octreotide were mediated by the inhibition of the GH-IGF-1 axis, identifying a role for the antisecretory activity of SST for its antiproliferative effects. More recently, in an experimental model of Kaposi sarcoma, in which the tumoral cells do not express SSTR mRNA, it was demonstrated that SST and its analogues powerfully blocked the *in vivo* tumor growth through a pure antiangiogenic mechanism (72).

Thus, the effects of SST on tumor cell growth may take place at different levels (73): directly blocking the cell cycle progression through the binding to SSTRs expressed on tumor cell membranes and the activation of PTPs, and through the indirect modulation of tumor growth mediated by the inhibition of the production of growth factors that sustain the tumor development (antisecretory activity) or *via* an antiangiogenic effect that involve the regulation of the activity of both endothelial cells and monocytes.

5.1. Direct antiproliferative effects

The activation of tumor-expressed SSTRs is responsible for the direct antiproliferative effects of SST. SSTR expression has been detected in most tumors cells. Beside pituitary adenomas and other neuroendocrine tumors (11, 12), SSTRs have been detected in all the brain tumors (gliomas, meningiomas, schwannomas, oligodendrogliomas), neuroblastomas, lung, hepatic, breast, ovarian and prostatic carcinomas and even in gastrointestinal stromal tumors (GIST) and soft tissue sarcomas (74-81). Interestingly, as far as receptor subtypes and intracellular signalling, the antiproliferative effects of SST are dissociated by the ability of the peptide to control hormone secretion, as demonstrated in both pituitary adenoma cells and acromegalic patients (82, 83).

In most cases the activation of SSTRs causes cytostatic effects, with cell cycle arrest in the G1 phase. However, delving deeper in the SST effects, it is now evident that, in some instances, also apoptosis may be induced.

5.1.1. Cytostatic effects.

The role of SST as an endogenous regulator of cell cycle is now a largely recognised activity and, using different *in vitro* and *in vivo* experimental models, all the five SSTR subtypes were reported to induce arrest of cell proliferation (33).

Although many different intracellular pathways were recognised to mediate these antiproliferative effects, differing not only regarding the SSTR subtype studied but also the cellular model analyzed, there is now a large consensus about the notion that most of these effects are mediated by the activation of phosphotyrosine phosphatases (PTP). In turn, the different PTPs, activated by SSTRs, control the activity of a number of downstream signalling molecules (in particular the MAP kinases ERK1 and 2) and, ultimately, induce an up-regulation of cyclin dependent kinase inhibitors (CDKI), such as $p21^{cip1/waf1}$ and $p27^{kip1}$. Conversely, a role for the reduction of cAMP production or Ca^{++} currents in the inhibitory effects of SST on cell proliferation was observed only in few specific cell types.

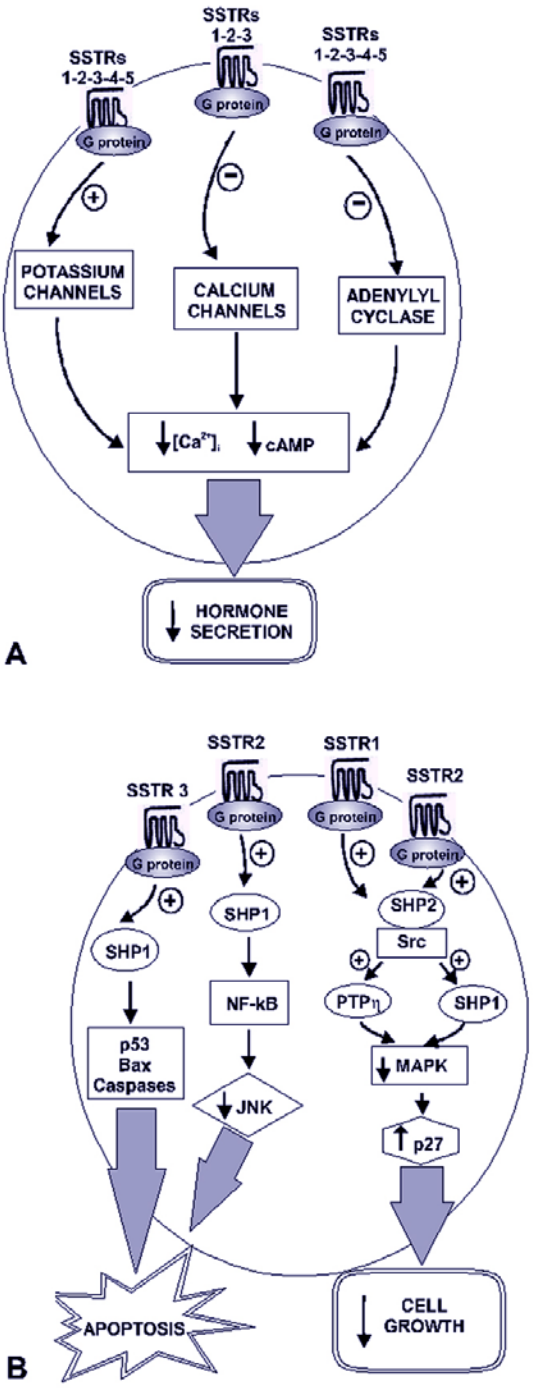

Figure 1. Diagrammatic representation of the intracellular pathways activated by individual SSTRs to elicit inhibition of hormone secretion (A) and cell growth arrest or apoptosis (B). Only the best characterized pathways are reported. See the text for a detailed description.

An overview of the identified intracellular pathways activated by the individual SSTR is reported in Table 3 and Figure 1.

5.1.1.1. Phosphotyrosine PHOSPHATASES

A possible effect of SST on tyrosine phosphorylation signalling was initially postulated since it was observed that SST inhibited the proliferation induced by tyrosine kinase receptors, *in vitro* (84). A SST-sensitive PTP activity was first described in the MIA-PaCa pancreatic tumor cell line (85) in which the treatment with SST caused an inhibition of epidermal growth factor (EGF) receptor tyrosine autophosphorylation. The regulation of PTP activity by SSTRs was directly shown in tumor cell lines, demonstrating that this activity was membrane-bound and regulated by pertussis toxin-dependent G proteins, whose activation by SSTR was required to induce both PTP activity and the dephosphorylation of EGFR (86, 87). More recently, an increased PTP activity following lanreotide treatment was observed also in primary cell cultures of human tumors, including pituitary adenomas (88, 89).

Interestingly, also the dopamine receptor D2R (90), the LHRH receptor (91) and the angiotensin II receptor (92, 93) were shown to induce antiproliferative effects through the regulation of PTP activity. Thus, the regulation of PTP activity is now regarded as a major transducing system for G protein coupled receptors to induce cell growth arrest.

Studies using cells transfected with individual SSTR subtypes have demonstrated that all the 5 receptor subtypes are able to induce PTP activity (94-98). This activity was identified to be associated with the cell membrane (86)(87) and ascribed to a couple of SH2 domain-containing PTPs, named SHP-1 and SHP-2 (97, 99-101). Indeed, these PTPs are localized in the cytosol in resting conditions but translocate to the cell membrane after SST treatment (99). However, the capability of SST to induce PTP activity also when added to partially purified membrane preparations (86, 102, 103) led to the hypothesis that also other members of the PTP superfamily may be involved in the antiproliferative activity of SST.

Arrest of cell proliferation, dependent on SSTR activation of SHP-1, was reported in different tumor cell lines derived from breast carcinomas (MCF-7), pancreatic cancers (MIA-PaCa, PANC-2, PC-1, PC-3), thyroid medullary carcinoma (TT) and pituitary adenomas (GH3), among others (104-109). Co-immunoprecipitation studies showed that, in CHO cells tranfected with SSTR2 and SHP-1, these molecules form a multiprotein complex regulated by $G_{i3\alpha}$ (110). In this cell model, the activation of SSTR2 by octreotide promoted the activation of SHP-1 and its dissociation from the receptor. SST-activated SHP-1 rapidly associated to the insulin receptor causing a tyrosine dephosphorylation of both the receptor itself and its substrates (i.e. IRS-1, Shc) leading to a negative modulation of insulin mitogenic signalling (100). The antiproliferative effects mediated by SSTR2 were related to an inhibition of the S phase entry and accumulation of the cells in the G1 phase of the cell cycle. This effect was mediated by an induction of $p27^{kip1}$ expression (but not $p21^{cip1/waf1}$) that, increasing its association with cdk2, prevents the recruitment of cyclin E by cdk2 and induces the accumulation of hypophosphorylated retinoblastoma

gene product (Rb)(111). In these studies, the activation of SHP-1 by SST analogues was related to changes in its own phosphorylation state. Thus, it was proposed that this effect may involve tyrosine kinase activation. Indeed, the activity of the cytosolic tyrosine kinase Jak2 resulted to be necessary for both SSTR2-mediated activation of SHP-1 and the inhibition of AR4-2J pancreatic cancer cell proliferation induced by high molecular weight acid FGF (112). In this model, SSTR2, Jak2 and SHP-1 are associated in a common signalling complex in resting conditions: upon SST analogue treatment JAK2 is activated inducing SHP-1 phosphorylation and activation, followed by a rapid dissociation of both molecules from the receptor (112). Moreover, also other PTPs (SHP-2) and cytosolic tyrosine kinases (Src) were subsequently detected in a multi-effector complex associated to SSTR2 in AR4-2J (113). Thus, it was proposed that the cytostatic effects of SST analogues, *via* SSTR2 activation, are the result of the sequential activation of kinases and phosphatases with SHP-2 activation by Src an absolute requirement for SHP-1 association to the receptor and activation (113).

SHP-2 was also involved in the antiproliferative activity of SST following SSTR1 activation (97, 101, 114). In particular, using SSTR1 expressing CHO-K1 cells, it was reported that SST induced cytostatic effects through a rapid activation of SHP-2 and Src (114). SHP-2 was identified in different cell types including glioma, neuroblastoma and thyroid cells. Its activation by SSTRs was reported to induce antiproliferative effects *via* the dephosphorylation and inactivation of the tyrosine kinase receptors for EGF, platelet derived growth factor (PDGF) and insulin (115-117) and, in consequence, inhibiting the growth factor-dependent activation of ras and ERK1/2 (118).

However, in SSTR1-expressing CHO-K1 cells, beside SHP-2 activity, also another delayed and long lasting PTP activity was observed (101) characterized by activation kinetics similar to that observed in membrane preparations from MIA-PaCa cells in the initial studies in which the activation of PTP by SST was reported (86). Thus, it was proposed that other PTPs, likely anchored to the cell membrane, could be involved in the SST cytostatic effects. One of these PTPs was identified in the receptor-like PTP named PTPeta (or DEP-1 in humans). In the thyroid cell line PC Cl3, a G1 cell cycle arrest *via* the overexpression of the CDKI $p27^{kip1}$, mediated by the activation of PTPs, was observed in response to SST (103, 117). However, when these cells were transformed by the overexpression of different oncogenes (E1A, middle T, mos) SST was ineffective (103, 117, 119). Interestingly, in mos-transfected cells, the loss of the SST effects on cell proliferation occurred in the presence of a SST-activated SHP-2 that caused the dephosphorylation and inactivation of the insulin receptor (117). It was observed that, in PC Cl3 cells, the oncogene-induced cell transformation caused the selective loss of the expression of PTPeta as potential mechanism of resistance to the antiproliferative effects of SST (117, 119). Indeed the re-expression of PTPeta completely restored the SST dependent up-regulation of $p27^{kip1}$ (117). The discrepancy in the effects of SHP-2 and PTPeta in the regulation of cell proliferation was explained according the experimental model used. Indeed, the oncogene mos, used to induce the transformation of PC Cl3 cells, is a direct MEK activator, thus causing the activation of ERK1/2 MAP kinase and cell proliferation, also in the presence of SHP-2-induced inhibition of the tyrosine phosphorylation of growth factor receptors. More importantly, these experiments suggested that PTPeta may act down-stream of MEK, and thus, directly on ERK1/2 (117). These results were confirmed studying glioma cell lines in which it was shown that SST-activated PTPeta was directly associated to ERK1/2 causing the dephosphorylation/inactivation of this MAP kinase (80) and the up-regulation of $p27^{kip1}$ (120). In PC Cl3 cells the up-regulation of this CDKI was induced through the inhibition of its phosphorylation by ERK1/2, preventing its ubiquitination and degradation by the proteasome (117). Importantly, in glioma cell lines and primary cultures from human glioblastomas, the responsivity to the cytostatic activity of SST was strictly related to the expression of PTPeta. Since PTPeta expression was observed only in about 1/3 of the 22 human glioblastomas analyzed (80), it was proposed that the commonly contradictory results obtained *in vivo* using SST analogs as antitumoral agents, may be related to the heterogeneous expression of down-stream effectors (for example PTPeta) rather than SSTRs that, on the contrary, are almost constantly detected. Further studies will be necessary to confirm this hypothesis.

Thus, in both glioma and thyroid cells, SST caused the activation of two PTPs: SHP-2 that is active on tyrosine kinase receptors and PTPeta that directly dephosphorylate ERK1/2. However, in the same way described for SSTR2 and SHP-1, the activation of PTPeta by SSTR1 involved a multi-effector complex, comprising both kinases and PTPs. In CHO-K1 cells expressing SSTR1 (or in C6 glioma cells treated with the SSTR1 selective agonists, BIM23926) it was shown that in resting conditions a large multimeric protein aggregation occurred in proximity of SSTR1 that, beside the receptor, included, the G protein, Jak2, SHP-2, Src and PTPeta (121). PTPeta activation required the sequential activation of Jak2 (G protein-mediated), that phosphoryated SHP-2. Upon phosphorylation, SHP-2 increases its activity, dissociates from the receptor and dephosphorylates the inhibitory tyrosine on Src C-terminus. Active Src, in turn, phosphorylate PTPeta causing the sustained activity of this PTP, to inactivate ERK1/2 (121).

The identification in different cell models of similar multieffector cascades activated by different SSTRs (SSTR1 and SSTR2) that, involving a similar interplay of kinases and PTPs (Jak2, SHP-2, Src), lead to the activation of a final effector PTP (SHP-1 or PTPeta)(113, 121), suggests that this multieffector pathway may represent a common modular mechanism by which cytostatic mechanisms are induced by SST.

SSTR3 was also reported to activate SHP-2 and to detemine Raf-1 inactivation (97, 122) in NIH3T3 cells. Similarly, in endothelial cells SSTR3 caused a PTP-

dependent inhibition of cell proliferation associated to the blockade of ERK1/2 activation (123).

5.1.1.2. MAP kinase activity

As described above, one of the main final effector of the SST-activated PTPs is the inhibition of the activity of the MAP kinase ERK1/2, either *via* an inhibition of the growth factor tyrosine kinase receptors or directly dephosphorylating ERK1/2 (see above). However, SST can induce inhibition of MAP kinase also through different mechanisms, namely a SSTR5-dependent inhibition of cGMP production and PKG activity (124).

Interestingly, in some instances, SST can also induce cell cycle arrest also through the hyperactivation of ERK1/2. It is known that the effects of ERK1/2 on cell proliferation are related to also to the duration and intensity of ERK1/2 activation (125, 126). Both SSTR1 and SSTR2 were reported to induce cell cycle arrest *via* the activation of MAP kinases, resulting in an up-regulation of $p21^{cip1/waf1}$ and $p27^{kip1}$, respectively (114, 127). Again, only partially overlapping intracellular pathways were used by these receptors to up-regulate MAP kinase activity. In particular, the activation of SSTR1 induced ERK1/2 activity regulating, *via* the beta/gamma subunits of a pertussis toxin-sensitive G protein, Src/SHP-2/phosphatidyl inositol 3 kinase (PI3K)/ras/Raf-1/MEK, while the SSTR2-regulated pathway involved SHP-1/SHP-2/PI3K/rap1 and ras/B-Raf/MEK (114, 127).

Moreover, also other MAP kinases, more frequently associated to the induction of cell growth arrest, are regulated by SST: p38 is activated by SSTR2 and SSTR4 (but not SSTR3) in CHO-K1 cells (128) and JNK is activated by SSTR5 through $G_{\alpha 12}$ (129).

In few cases, SST was reported to induce cell proliferation. This effect was identified following SSTR4 activation that increases ERK1/2 activity and, in turn, regulates PLA2 phosphorylation and activation (130). In transfected CHO cells, the sustained activation of p38 by SSTR2A was reported to induce the overexpression of $p21^{cip1/waf1}$ and growth arrest, while in SSTR2B-expressing cells caused a transient activation of Akt and increased cell proliferation. The differences in the biological responses induced by the two SSTR2 isoforms were ascribed to possible differences in the beta/gamma subunits activated (131).

5.1.1.3. Phosphatidyl inositol 3 kinase (PI3K)

More recently, an inhibitory effect of SSTR2 on PI3K, one of the main intracellular signals that mediate cell survival, was reported in different cell systems. In particular, in CHO-DG44 cells, the p85 subunit of PI3K is associated to the first intracellular loop of SSTR2 and its dissociation and dephosphorylation, upon SSTR2 activation, induces inhibition of PI3K activity (132). In pituitary cells, the inhibition of PI3K activity was mediated by SHP-1 that dephosphorylates p85 (109). The inhibition of PI3K causes reduction of the activities of PDK1 and Akt and the consequent activation of glycogen synthase kinase 3β (GSK3β). The increase of GSK3β activity up-regulated the expression of the onco-suppressor gene Zac1 that ultimately induced growth arrest and apoptosis (109).

5.1.1.4. Nitric oxide synthases

SST was reported to regulate nitric oxide (NO) generation through the activation of both the endothelial and neuronal nitric oxide synthases (eNOS and nNOS, respectively). The effects of SSTRs on eNOS are related to the antiangiogenic activity of SST and are detailed in the section 5.2.2.

The activity of nNOS was dually regulated by SSTR2 and SSTR5: the former receptor caused activation while the latter inhibition of NO generation. However, according to the cells analyzed, both effects were reported to cause inhibition of cell proliferation.

SSTR2 caused a SHP-1-dependent dephosphorylation and activation of nNOS (133). The increased NO production in these cells, resulted in activation of cGMP levels and growth arrest (133).

Opposite effects were reported following SSTR5 activation that, instead, caused an inhibition of nNOS activity (134). SSTR5 activation caused the recruitment of nNOS to the receptor and its phosphorylation by Src. The phosphorylation prevented nNOS homodimerization and activity, an effect that was essential for pancreatic cancer cell growth arrest, induced by SST (134).

5.1.1.5. Na^+-H^+ exchanger (NHE1)

The inhibition of NHE1 activity is responsible for antiproliferative effects of SST in enteric endocrine cells and hepatic cells. These effects are mediated by SSTR1, SSTR3 and SSTR4, but not by SSTR2 and SSTR5. Interestingly the inhibition of NHE1 is the only intracellular signalling regulated by SSTRs that was reported to be pertussis toxin insensitive (38).

5.1.1.6. Restoration of GAP junctions

Gap junctions, mainly composed of connexins, are intercellular structures critical for the maintenance of the differentiate state. In fact in most tumor cells connexin expression is impaired, causing the loss of the density-dependent cell growth arrest. SSTR2 expression in pancreatic cancer cells was reported to induce an over-expression of the connexins 26 and 43 and to restore the contact inhibition of cell proliferation (135).

5.1.2. Apoptosis

The induction of apoptosis upon SST treatment was detected in both normal and tumoral cells. The first identification of the possible pro-apoptotic activity of SSTRs was observed in breast and pituitary tumor cell lines (136, 137) and resulted to be mediated by PTPs (since it was reverted by vanadate). To date the SSTR subtypes involved in the induction of apoptosis are SSTR2 and SSTR3, acting through the modulation of both the intrinsic and the extrinsic intracellular apoptotic pathways.

The first pathway leads to apoptosis *via* the activation of pro-apoptotic genes (i.e. p53, members of the

Bcl2 superfamily) in responses to alteration of mitochondrial activity, DNA damage or loss of survival factors. The extrinsic pathway is related to the activation or sensitization of "death receptors" such as TNFalpha receptor 1 (TNFR1).

The first SSTR subtype reported to induce apoptosis was SSTR3. In CHO cells transfected with individual, recombinant SSTR subtypes it was shown that, among all the SSTRs, only SSTR3 was able to trigger apoptosis (96). This effect, mediated by PTPs, was dependent on the activation of p53 that preceded the induction of the pro-apoptotic protein Bax and the triggering of the apoptotic process (136). SSTR3 apoptosis was mediated by intracellular acidification (an event involving the SST-mediated activation of SHP-1 that, in turn, regulates caspase 8 activity)(138, 139) and the activation of cation insensitive acid endonucleases (140). In these studies, the apoptotic effects, mediated by SSTR3, are induced in all the cell cycle phases, and thus independent from the cytostatic effects of SST. Interestingly, the induction of apoptosis by SSTR3 was an event dependent on the cell type analyzed, since no apoptotic effects were observed in endothelial cells, when this receptor subtype was activated to block angiogenesis (123).

Subsequently, also SSTR2 activation was identified as a mechanism to induce apoptosis. This effect, differently from what observed for SSTR3, was independent from p53 activation (141) although, in both pancreatic and pituitary tumor cells, required the activation of the cytosolic PTP SHP-1 (109, 142). SSTR2-mediated apoptotic effects were, more recently, identified also in somatotroph tumor cells, again *via* a PTP-dependent and p53-, Bcl2/Bax- and death receptor-independent pathway (143), and in hepatocarcinoma cells (144). In NIH3T3 cells, SSTR2 was reported to activate NFkB in a SHP-1-dependent manner. In this way SSTR2 activation causes an inhibition of the anti-apoptotic effects of the MAP kinase JNK, whose activity plays an inhibitory role on caspases and apoptosis (145). However, differently from pituitary cells, in these studies, SSTR2 was shown to affect also the apoptosis induced by death receptors, sensitizing the cells to TNFalpha- and TRAIL-mediated responses, through the up-regulation of their receptors: death receptor 4 (DR4) and TNFR1 (142). Moreover, SSTR2 was reported to regulate TNFalpha signalling also inducing a synergistic activation of NFkB, that caused a higher inhibition of JNK activity and, in turn, an hyperactivation of caspase 8 (145).

5.2 INDIRECT ANTIPROLIFERATIVE EFFECTS

5.2.1. Antisecretory activity

Decrease of tumor growth may be related to the suppression of the secretion or the synthesis and thus the activity of growth factors [insulin-like growth factor-1 (IGF-1), EGF, transforming growth factor alpha] and hormones (GH, insulin, prolactin)(146). For example many neoplasms display receptors for IGF-1 and their activation causes tumor cell proliferation (147). Octreotide was reported to suppress IGF-1 serum levels through a direct inhibition of its gene expression or through the inhibition of GH secretion from pituitary and the consequent reduction of the GH-stimulated IGF-1 production in liver (148). In a model of atherosclerosis, lanreotide caused a significant inhibition of different growth factor secretion (EGF, IGF-1, PDGF) that caused inhibition of myocyte proliferation *in vivo* but not *in vitro* (149), clearly demonstrating the occurrence of an exclusive indirect antiproliferatve mechanism dependent on the antisecretory properties of SSTRs.

The inhibitory effects on GH secretion are mediated by the activation of the pituitary SSTR2 and SSTR5 through the inhibition of cAMP formation (150, 151) the reduction of the Ca^{++} influx through the voltage sensitive channels (152) and the activation of K^+ channels (153, 154).

While all SSTR subtypes are able to inhibit cAMP production (31), only SSTR1, SSTR2 and SSTR5 were reported to inhibit both L- and N- type voltage sensitive Ca^{++} channels (155-157). On the other hand, SSTR2 (more efficiently than the other subtypes), SSTR3, SSTR4 and SSTR5 (but not SSTR1) were shown to activate the G protein gated inward-rectifier K^+ channel (GIRK) (158). Conversely, in GH3 cells, SSTR2 and SSTR4, and less potently SSTR1 and SSTR5 (but not SSTR3) activated the transient outward (I_A) and delayed rectifier (I_K) K^+ currents (159)(see Table 3 and Figure 1).

In different cell types, it was also reported that SST induces also the inhibition of the secretion of other growth factors (146), cytokines and chemokines (160, 161), all of them possibly involved in the regulation of cell proliferation.

Conversely, the direct inhibition of IGF-1 production in the liver, depends on the activation of a PTP, *via* SSTR2 and SSTR3, that leads to the dephosphorylation of STATb5, impairment of its nuclear localization and a decrease in IGF-1 gene transcription (162).

5.2.2. Antiangiogenic activity

Angiogenesis is a biological activity in which the growth of new blood vessels occurs. It is a complex multistep process that involves endothelial cell proliferation, invasion, adhesion, chemotactic migration, morphogenic differentiation in tubular structures and, at the end, the production of a basement membrane that surrounds the neo-formed vessels (163, 164). In adults, in the absence of pathological conditions, angiogenesis is quiescent, with a very slow turnover of the endothelial cells (even years). Angiogenesis becomes highly active in some important physiological conditions such as wound/healing, the menstruation and, of course, during the embryogenesis. On the other hand, angiogenesis is at the basis of many disease-related conditions: proliferative retinopathy, reumathoid arthritis, cardiovascular and brain ischemia (164). In the past years, the possible pharmacological regulation of angiogenesis was under the spot of the preclinical and clinical research, being one of the most relevant biological processes at the basis of the growth of almost every kind of human tumors. Indeed, the inhibition

of tumor neo-vascularization may result, in combination with cytotoxic therapy in an efficacious and selective targeting of most of the tumors that and a more complete inhibition of proliferation.

The first evidence that SST may control angiogenesis was in 1991 by Woltering *et al.* that showed that the SST analogues octreotide and vapreotide displayed a significant *in vitro* antiangiogenic activity using the chicken corioallanthoic membrane model (CAM)(165). This observation was supported by the identification, in human primary colorectal carcinomas, breast cancers, renal carcinomas and small cell lung carcinomas, of an overexpression of SSTRs (in particular SSTR2) in the peritumoral vasculature (166), independently from the level of expression of this receptor in the tumor cells. Thus, it was hypothesized that the regulation of the activity of these receptors may represent a novel antitumoral approach. Subsequent studies extended the initial observations and an antiangiogenic activity of SST and its analogues was demonstrated using a number of *in vitro* and *in vivo* experimental models (CAM, matrigel sponge assay, etc.)(72, 123, 167-173). Cumulatively, three signal pathways were identified as responsible of the antiangiogenic activity of SSTRs: 1) inhibition of endothelial cell activity (proliferation, migration and invasion); 2) inhibition of the synthesis and secretion of pro-angiogenic factors such as vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF); 3) inhibition of monocyte activation.

5.2.2.1. Inhibition of endothelial cell proliferation, migration and invasion

The antiproliferative activity of SST analogues on endothelial cells was demonstrated in many different *in vitro* cell models including human umbilical vein endothelial cells (HUVEC), bovine artery endothelial cells (BAEC) and established endothelial cell lines (ECV304, EAhy926)(72, 123, 164, 174).

It was reported that octreotide, through the activation of SSTR2 and/or SSTR5, inhibits HUVEC proliferation (175, 176) and that these receptor subtypes are up-regulated during the "angiogenic switch" from resting to proliferating endothelia, *in vivo* (164, 177). However, other studies reported, in endothelial cell models, an antiproliferative effect of the SSTR pan-agonist SOM230 (effective on SSTR2, SSTR3, SSTR5 and with slightly lower affinity to SSTR1) but not of octreotide, implicating the involvement of also other subtypes in such effect (178). Indeed, in the immortalized endothelial cell line EAhy926, that express mRNA only for SSTR3, SST or the selective peptidomimetic SSTR3 agonist L-796,778, induced a significant inhibition of the growth factor-induced proliferation, an effect that was reverted in the presence of the SSTR3 antagonist BN81685 (72, 123). Importantly, the latter results were reproduced *in vivo* in mice, evaluating angiogenesis (matrigel sponge assay) and tumor growth (xenotransplantation of the Kaposi sarcoma derived cells, KSImm)(72). Particularly relevant were the experiments using the KSImm cells, since the KSImm tumor growth in mice was inhibited by SST and its analogues *in vivo*, with an efficacy comparable to the cytotoxic drug adriamicyn, despite these cells do not express any SSTR mRNA and their proliferation is not affected *in vitro* (72, 123). Thus, in this case, a pure antiangiogenic mechanism was proposed for the antitumoral effects of SST.

The antiproliferative effect of SST on endothelial cells through the activation of SSTR3 was mediated by the inhibition of the activities of ERK1/2 MAP kinase and endothelial nitric oxide synthase (eNOS) (123). Both pathways are relevant during angiogenesis, since the vasodilatation induced by nitric oxide (NO) is a prerequisite for the subsequent ERK1/2-mediated cell cycle activation in endothelial cells (179). Subsequently, it was demonstrated that also other SSTRs (SSTR1, SSTR2 and SSTR3, but not SSTR4) can control eNOS activity (180). In these studies, using CHO-K1 cells, it was demonstrated that independent pathways control NO production when activated by different receptors: the modulation of the release of Ca^{++} from the intracellular stores, upon colecystokinin treatment, or the sphyngomyelinase-dependent generation of ceramide, induced by bFGF (181, 182). Expressing individual SSTRs in CHO-K1 cells it was demonstrated that the former effect was inhibited *via* the activation of SSTR2 and SSTR3, while SSTR1, SSTR2 and SSTR3 were all effective in reverting the NO production induced by bFGF (180). Conversely, SSTR4 was not able to control the activation of eNOS, independently from the intracellular pathway activated (180).

SSTRs were also able to interfere with endothelial cell adhesion, migration and invasion, all functions required for the developing of new vessels. For example, this inhibitory activity was induced *in vitro,* using vapreotide or a derivative with enhanced lipophylic properties (164, 169). Octreotide also inhibited HUVEC invasion elicited by VEGF (175). In tumor cell lines, the anti-invasive effects of SST were dependent on the inhibition of MAP kinase and the small G protein Rac (183) and involved the inhibition of the expression of metalloproteases such as MMP2 (184).

5.2.2.2. Inhibition of the synthesis and secretion of pro-angiogenic factors

SST can also control angiogenesis through its antisecretory properties. Indeed, it is now well documented that beside hormones (GH, ACTH, insulin, etc) the activation of SSTRs may inhibit also the release of growth factors or cytokines. As far as the inhibition of pro-angiogenic factors release, this effect was reported to involve mainly the synthesis and/or the secretion of VEGF and bFGF (146, 185). These factors are indeed produced by different tumor cells and by infiltrating inflammatory cells, to promote endothelial and smooth muscle cell proliferation and migration, being these effects important for the tumoral vascularization when the developing tumor mass becomes hypoxic (186). In glioma cell lines and in human colorectal and pancreatic cancer cells, SSTR2 agonists were reported to inhibit VEGF and bFGF secretion acting at mRNA level (184, 187, 188). In particular in glioma cells, the SSTR2 selective agonist L-054,552 abrogated the secretion of VEGF induced by EGF, bFGF or hypoxia (187). Similar

effects on VEGF release were also observed in retinal pigment epithelial cells (189), where this growth factor plays a relevant role in the diabetic retinopathy through an angiogenesis-related mechanism (190). The inhibition of pro-angiogenic factor release was observed also *in vivo*, since it was reported that administration of octreotide significantly reduced VEGF and bFGF serum levels in patients with gastric carcinoma (191). Similarly to what observed for the antisecretory activity on hormones, the inhibition of angiogenic growth factors release involves mainly the regulation of cAMP production and ion fluxes (25).

5.2.2.3. Inhibition of monocyte activation

Monocytes represent other important mediators of the tumoral angiogenenic process. These cells, infiltrating the peritumoral area in response to VEGF (192), produce pro-survival and pro-angiogenic factors resulting in the activation of endothelial cells and the induction of neo-vascularization. SST was reported to inhibit monocyte migration and their recruitment in the areas where new vessels are formed (172, 193). In a model of Kaposi sarcoma, SST, through the interaction with SSTR2, SSTR3 and SSTR5, was reported to inhibit monocyte activation (evaluated as morphological changes, such as cell polarization) to impair both *in vivo* angiogenesis and tumor growth (72).

6. PERSPECTIVE

The use of SSTR agonists as antiproliferative agents represents a powerful therapeutic possibility in oncology. This approach in the past years was supported by the widespread expression of SSTRs in most tumor cells and by the strong antitumor activity of SST synthetic analogues observed *in vitro*. However to date, beside few cases (mainly pituitary adenomas and some neuroendocrine tumors), the promises raised by the large bulk of preclinical data has not been fulfilled. The reason for this partially unexpected failure it is not clear. Indeed, SSTRs controls tumor proliferation through both direct and indirect mechanisms, showing simultaneously both antiproliferative and antiangiogenic properties that in theory should represent the gold standard for an antineoplastic agent. However, in the last years the preclinical research greatly increased our knowledge concerning the antiproliferative mechanisms activated by SSTR and the discovery of the possible activation of PTP by G protein coupled receptors represents one of the more exciting biological discoveries in the past decades. Thus, it is now accepted that, among the plethora of intracellular second messengers regulated by SSTR, the antiproliferative activity and the apoptosis induced by SST are mediated by the activation of specific PTPs. It has been shown that the expression of PTPs in tumor cells can dictate the responsivity to SST, and thus a more consistent antiproliferative response could be obtained targeting specifically these intracellular effectors. Moreover, the identification of a more precise expression pattern for SSTRs, showing an almost constant presence of multiple subtypes in the same cell, changed also the research of novel agonist molecules from highly selective to multi-receptor agonists or even pan SSTR agonists that are presently tested in clinical trials. Finally, the identification of the agonist-dependent heterodimerization of SSTRs and D2R allowed the development of completely innovative agonists that bind and activate both receptors. Thus, it is possible that more satisfying results will be obtained in the next years. Moreover, it is clear that a closer interaction between preclinical and clinical research will be necessary to further develop these results.

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8. REFERENCES

1. N. M. Le Douarin: On the origin of pancreatic endocrine cells. *Cell*, 53(2), 169-171 (1988)
2. M. T. Barakat, K. Meeran and S. R. Bloom: Neuroendocrine tumours. *Endocr Relat Cancer*, 11(1), 1-18 (2004)
3. R. G. Long, M. G. Bryant, S. J. Mitchell, T. E. Adrian, J. M. Polak and S. R. Bloom: Clinicopathological study of pancreatic and ganglioneuroblastoma tumours secreting vasoactive intestinal polypeptide (vipomas). *Br Med J (Clin Res Ed)*, 282(6278), 1767-1771 (1981)
4. S. S. Fajans and A. I. Vinik: Insulin-producing islet cell tumors. *Endocrinol Metab Clin North Am*, 18(1), 45-74 (1989)
5. S. W. Lamberts, W. W. de Herder and L. J. Hofland: Somatostatin analogs in the diagnosis and treatment of cancer. *Trends Endocrinol Metab*, 13(10), 451-457 (2002)
6. K. Oberg: Carcinoid tumors: molecular genetics, tumor biology, and update of diagnosis and treatment. *Curr Opin Oncol*, 14(1), 38-45 (2002)
7. J. R. Strosberg and L. K. Kvols: A review of the current clinical trials for gastroenteropancreatic neuroendocrine tumours. *Expert Opin Investig Drugs*, 16(2), 219-224 (2007)
8. D. O'Toole, M. Ducreux, G. Bommelaer, J. L. Wemeau, O. Bouche, F. Catus, J. Blumberg and P. Ruszniewski: Treatment of carcinoid syndrome: a prospective crossover evaluation of lanreotide versus octreotide in terms of efficacy, patient acceptability, and tolerance. *Cancer*, 88(4), 770-776 (2000)
9. J. C. Reubi, L. K. Kvols, B. Waser, D. M. Nagorney, P. U. Heitz, J. W. Charboneau, C. C. Reading and C. Moertel: Detection of somatostatin receptors in surgical and percutaneous needle biopsy samples of carcinoids and islet cell carcinomas. *Cancer Res*, 50(18), 5969-5977 (1990)
10. K. Oberg, L. Kvols, M. Caplin, G. Delle Fave, W. de Herder, G. Rindi, P. Ruszniewski, E. A. Woltering and B. Wiedenmann: Consensus report on the use of somatostatin analogs for the management of neuroendocrine tumors of the gastroenteropancreatic system. *Ann Oncol*, 15(6), 966-973 (2004)
11. V. Panteris and D. G. Karamanolis: The puzzle of somatostatin: action, receptors, analogues and therapy. *Hepatogastroenterology*, 52(66), 1771-1781 (2005)
12. D. O'Toole, A. Saveanu, A. Couvelard, G. Gunz, A. Enjalbert, P. Jaquet, P. Ruszniewski and A. Barlier: The analysis of quantitative expression of somatostatin and

dopamine receptors in gastro-entero-pancreatic tumours opens new therapeutic strategies. *Eur J Endocrinol*, 155(6), 849-857 (2006)
13. B. Eriksson, J. Renstrup, H. Imam and K. Oberg: High-dose treatment with lanreotide of patients with advanced neuroendocrine gastrointestinal tumors: clinical and biological effects. *Ann Oncol*, 8(10), 1041-1044 (1997)
14. B. Eriksson, E. T. Janson, N. D. Bax, M. Mignon, R. Morant, P. Opolon, P. Rougier and K. E. Oberg: The use of new somatostatin analogues, lanreotide and octastatin, in neuroendocrine gastro-intestinal tumours. *Digestion*, 57 Suppl 1, 77-80 (1996)
15. L. Saltz, B. Trochanowski, M. Buckley, B. Heffernan, D. Niedzwiecki, Y. Tao and D. Kelsen: Octreotide as an antineoplastic agent in the treatment of functional and nonfunctional neuroendocrine tumors. *Cancer*, 72(1), 244-248 (1993)
16. L. Degen and C. Beglinger: The role of octreotide in the treatment of gastroenteropancreatic endocrine tumors. *Digestion*, 60 Suppl 2, 9-14 (1999)
17. R. Arnold, M. E. Trautmann, W. Creutzfeldt, R. Benning, M. Benning, C. Neuhaus, R. Jurgensen, K. Stein, H. Schafer, C. Bruns and H. J. Dennler: Somatostatin analogue octreotide and inhibition of tumour growth in metastatic endocrine gastroenteropancreatic tumours. *Gut*, 38(3), 430-438 (1996)
18. M. Ducreux, P. Ruszniewski, J. A. Chayvialle, J. Blumberg, D. Cloarec, H. Michel, J. M. Raymond, J. L. Dupas, H. Gouerou, R. Jian, E. Genestin, P. Hammel and P. Rougier: The antitumoral effect of the long-acting somatostatin analog lanreotide in neuroendocrine tumors. *Am J Gastroenterol*, 95(11), 3276-3281 (2000)
19. T. Aparicio, M. Ducreux, E. Baudin, J. C. Sabourin, T. De Baere, E. Mitry, M. Schlumberger and P. Rougier: Antitumour activity of somatostatin analogues in progressive metastatic neuroendocrine tumours. *Eur J Cancer*, 37, 1014-1019 (2001)
20. H. Shojamanesh, F. Gibril, A. Louie, J. V. Ojeaburu, S. Bashir, A. Abou-Saif and R. T. Jensen: Prospective study of the antitumor efficacy of long-term octreotide treatment in patients with progressive metastatic gastrinoma. *Cancer*, 94(2), 331-343 (2002)
21. W. W. de Herder, L. J. Hofland, A. J. van der Lely and S. W. Lamberts: Somatostatin receptors in gastroenteropancreatic neuroendocrine tumours. *Endocr Relat Cancer*, 10(4), 451-458 (2003)
22. S. Faiss, U. F. Pape, M. Bohmig, Y. Dorffel, U. Mansmann, W. Golder, E. O. Riecken and B. Wiedenmann: Prospective, randomized, multicenter trial on the antiproliferative effect of lanreotide, interferon alfa, and their combination for therapy of metastatic neuroendocrine gastroenteropancreatic tumors--the International Lanreotide and Interferon Alfa Study Group. *J Clin Oncol*, 21(14), 2689-2696 (2003)
23. W. W. de Herder, E. P. Krenning, C. H. Van Eijck and S. W. Lamberts: Considerations concerning a tailored, individualized therapeutic management of patients with (neuro)endocrine tumours of the gastrointestinal tract and pancreas. *Endocr Relat Cancer*, 11(1), 19-34 (2004)
24. S. W. Lamberts, A. J. van der Lely, W. W. de Herder and L. J. Hofland: Octreotide. *N Engl J Med*, 334, 246-254 (1996)
25. Y. C. Patel: Somatostatin and its receptor family. *Front Neuroendocrinol*, 20(3), 157-198 (1999)
26. W. Meyerhof: The elucidation of somatostatin receptor functions: a current view. *Rev Physiol Biochem Pharmacol*, 133, 55-108 (1998)
27. Y. C. Patel, R. Panetta, E. Escher, M. Greenwood and C. B. Srikant: Expression of multiple somatostatin receptor genes in AtT-20 cells. Evidence for a novel somatostatin-28 selective receptor subtype. *J Biol Chem*, 269(2), 1506-1509 (1994)
28. M. Vanetti, M. Kouba, X. Wang, G. Vogt and V. Hollt: Cloning and expression of a novel mouse somatostatin receptor (SSTR2B). *FEBS Lett*, 311(3), 290-294 (1992)
29. Y. C. Patel, M. Greenwood, G. Kent, R. Panetta and C. B. Srikant: Multiple gene transcripts of the somatostatin receptor SSTR2: tissue selective distribution and cAMP regulation. *Biochem Biophys Res Commun*, 192(1), 288-294 (1993)
30. M. Vanetti, G. Vogt and V. Hollt: The two isoforms of the mouse somatostatin receptor (mSSTR2A and mSSTR2B) differ in coupling efficiency to adenylate cyclase and in agonist-induced receptor desensitization. *FEBS Lett*, 331, 260-266 (1993)
31. L. N. Moller, C. E. Stidsen, B. Hartmann and J. J. Holst: Somatostatin receptors. *Biochim Biophys Acta*, 1616, 1-84 (2003)
32. R. Panetta, M. T. Greenwood, A. Warszynska, L. L. Demchyshyn, R. Day, H. B. Niznik, C. B. Srikant and Y. C. Patel: Molecular cloning, functional characterization, and chromosomal localization of a human somatostatin receptor (somatostatin receptor type 5) with preferential affinity for somatostatin-28. *Mol Pharmacol*, 45(3), 417-427 (1994)
33. G. Weckbecker, I. Lewis, R. Albert, H. A. Schmid, D. Hoyer and C. Bruns: Opportunities in somatostatin research: biological, chemical and therapeutic aspects. *Nat Rev Drug Discov*, 2(12), 999-1017 (2003)
34. C. Kleuss, H. Scherubl, J. Hescheler, G. Schultz and B. Wittig: Selectivity in signal transduction determined by gamma subunits of heterotrimeric G proteins. *Science*, 259(5096), 832-834 (1993)
35. C. Kleuss, H. Scherubl, J. Hescheler, G. Schultz and B. Wittig: Different beta-subunits determine G-protein interaction with transmembrane receptors. *Nature*, 358(6385), 424-426 (1992)
36. C. Kleuss, J. Hescheler, C. Ewel, W. Rosenthal, G. Schultz and B. Wittig: Assignment of G-protein subtypes to specific receptors inducing inhibition of calcium currents. *Nature*, 353(6339), 43-48 (1991)
37. K. Komatsuzaki, Y. Murayama, U. Giambarella, E. Ogata, S. Seino and I. Nishimoto: A novel system that reports the G-proteins linked to a given receptor: a study of type 3 somatostatin receptor. *FEBS Lett*, 406(1-2), 165-170 (1997)
38. C. Hou, R. L. Gilbert and D. L. Barber: Subtype-specific signaling mechanisms of somatostatin receptors SSTR1 and SSTR2. *J Biol Chem*, 269(14), 10357-10362 (1994)
39. M. Rocheville, D. C. Lange, U. Kumar, R. Sasi, R. C. Patel and Y. C. Patel: Subtypes of the somatostatin receptor assemble as functional homo- and heterodimers. *J Biol Chem*, 275(11), 7862-7869 (2000)

40. M. Pfeiffer, T. Koch, H. Schroder, M. Klutzny, S. Kirscht, H. J. Kreienkamp, V. Hollt and S. Schulz: Homo- and heterodimerization of somatostatin receptor subtypes. Inactivation of sst(3) receptor function by heterodimerization with sst(2A). *J Biol Chem*, 276(17), 14027-14036 (2001)
41. M. Rocheville, D. C. Lange, U. Kumar, S. C. Patel, R. C. Patel and Y. C. Patel: Receptors for dopamine and somatostatin: formation of hetero-oligomers with enhanced functional activity. *Science*, 288(5463), 154-157 (2000)
42. M. Pfeiffer, T. Koch, H. Schroder, M. Laugsch, V. Hollt and S. Schulz: Heterodimerization of somatostatin and opioid receptors cross-modulates phosphorylation, internalization, and desensitization. *J Biol Chem*, 277(22), 19762-19772 (2002)
43. P. Jaquet, G. Gunz, A. Saveanu, A. Barlier, H. Dufour, J. Taylor, J. Dong, S. Kim, J. P. Moreau and M. D. Culler: BIM-23A760, a chimeric molecule directed towards somatostatin and dopamine receptors, vs universal somatostatin receptors ligands in GH-secreting pituitary adenomas partial responders to octreotide. *J Endocrinol Invest*, 28(11 Sup), 21-27 (2005)
44. P. Jaquet, G. Gunz, A. Saveanu, H. Dufour, J. Taylor, J. Dong, S. Kim, J. P. Moreau, A. Enjalbert and M. D. Culler: Efficacy of chimeric molecules directed towards multiple somatostatin and dopamine receptors on inhibition of GH and prolactin secretion from GH-secreting pituitary adenomas classified as partially responsive to somatostatin analog therapy. *Eur J Endocrinol*, 153(1), 135-141 (2005)
45. A. Saveanu, E. Lavaque, G. Gunz, A. Barlier, S. Kim, J. E. Taylor, M. D. Culler, A. Enjalbert and P. Jaquet: Demonstration of enhanced potency of a chimeric somatostatin-dopamine molecule, BIM-23A387, in suppressing growth hormone and prolactin secretion from human pituitary somatotroph adenoma cells. *J Clin Endocrinol Metab*, 87(12), 5545-5552 (2002)
46. D. Ferone, A. Saveanu, M. D. Culler, M. Arvigo, A. Rebora, F. Gatto, F. Minuto and P. Jaquet: Novel chimeric somatostatin analogs: facts and perspectives. *Eur J Endocrinol*, 156 Suppl 1, S23-S28 (2007)
47. G. Olias, C. Viollet, H. Kusserow, J. Epelbaum and W. Meyerhof: Regulation and function of somatostatin receptors. *J Neurochem*, 89(5), 1057-1091 (2004)
48. U. Kumar, R. Sasi, S. Suresh, A. Patel, M. Thangaraju, P. Metrakos, S. C. Patel and Y. C. Patel: Subtype-selective expression of the five somatostatin receptors (hSSTR1-5) in human pancreatic islet cells: a quantitative double-label immunohistochemical analysis. *Diabetes*, 48(1), 77-85 (1999)
49. M. Z. Strowski, M. Kohler, H. Y. Chen, M. E. Trumbauer, Z. Li, D. Szalkowski, S. Gopal-Truter, J. K. Fisher, J. M. Schaeffer, A. D. Blake, B. B. Zhang and H. A. Wilkinson: Somatostatin receptor subtype 5 regulates insulin secretion and glucose homeostasis. *Mol Endocrinol*, 17(1), 93-106 (2003)
50. M. J. Low: Clinical endocrinology and metabolism. The somatostatin neuroendocrine system: physiology and clinical relevance in gastrointestinal and pancreatic disorders. *Best Pract Res Clin Endocrinol Metab*, 18(4), 607-622 (2004)
51. V. Martinez, A. P. Curi, B. Torkian, J. M. Schaeffer, H. A. Wilkinson, J. H. Walsh and Y. Tache: High basal gastric acid secretion in somatostatin receptor subtype 2 knockout mice. *Gastroenterology*, 114(6), 1125-1132 (1998)
52. K. S. Murthy, D. H. Coy and G. M. Makhlouf: Somatostatin receptor-mediated signaling in smooth muscle. Activation of phospholipase C-beta3 by Gbetagamma and inhibition of adenylyl cyclase by Galphai1 and Galphao. *J Biol Chem*, 271(38), 23458-23463 (1996)
53. J. P. Allen, A. J. Canty, S. Schulz, P. P. Humphrey, P. C. Emson and H. M. Young: Identification of cells expressing somatostatin receptor 2 in the gastrointestinal tract of Sstr2 knockout/lacZ knockin mice. *J Comp Neurol*, 454(3), 329-340 (2002)
54. A. Y. Gong, P. S. Tietz, M. A. Muff, P. L. Splinter, R. C. Huebert, M. Z. Strowski, X. M. Chen and N. F. LaRusso: Somatostatin stimulates ductal bile absorption and inhibits ductal bile secretion in mice via SSTR2 on cholangiocytes. *Am J Physiol Cell Physiol*, 284(5), C1205-C1214 (2003)
55. T. Florio, S. Thellung and G. Schettini: Intracellular transducing mechanisms coupled to brain somatostatin receptors. *Pharmacol Res*, 33(6), 297-305 (1996)
56. A. Vasilaki, D. Papasava, D. Hoyer and K. Thermos: The somatostatin receptor (sst1) modulates the release of somatostatin in the nucleus accumbens of the rat. *Neuropharmacology*, 47(4), 612-618 (2004)
57. G. Schettini, T. Florio, G. Magri, M. Grimaldi, O. Meucci, E. Landolfi and A. Marino: Somatostatin and SMS 201-995 reverse the impairment of cognitive functions induced by cysteamine depletion of brain somatostatin. *Eur J Pharmacol*, 151(3), 399-407 (1988)
58. L. Vecsei, I. Pavo, J. Zsigo, B. Penke and E. Widerlov: Comparative studies of somatostatin-14 and some of its fragments on passive avoidance behavior, open field activity and on barrel rotation phenomenon in rats. *Peptides*, 10(6), 1153-1157 (1989)
59. L. Vecsei and E. Widerlov: Effects of intracerebroventricularly administered somatostatin on passive avoidance, shuttle-box behaviour and open-field activity in rats. *Neuropeptides*, 12(4), 237-242 (1988)
60. P. Davies and R. D. Terry: Cortical somatostatin-like immunoreactivity in cases of Alzheimer's disease and senile dementia of the Alzheimer type. *Neurobiol Aging*, 2(1), 9-14 (1981)
61. P. Davies, R. Katzman and R. D. Terry: Reduced somatostatin-like immunoreactivity in cerebral cortex from cases of Alzheimer disease and Alzheimer senile dementa. *Nature*, 288(5788), 279-80 (1980)
62. L. Vecsei and P. Klivenyi: Somatostatin and Alzheimer's disease. *Arch Gerontol Geriatr*, 21(1), 35-41 (1995)
63. T. Saito, N. Iwata, S. Tsubuki, Y. Takaki, J. Takano, S. M. Huang, T. Suemoto, M. Higuchi and T. C. Saido: Somatostatin regulates brain amyloid beta peptide Abeta42 through modulation of proteolytic degradation. *Nat Med*, 11, 434-439 (2005)
64. J. Chrubasik, J. Meynadier, P. Scherpereel and E. Wunsch: The effect of epidural somatostatin on postoperative pain. *Anesth Analg*, 64(11), 1085-1088 (1985)
65. M. Schindler, S. Holloway, G. Hathway, C. J. Woolf, P. P. Humphrey and P. C. Emson: Identification of

somatostatin sst2(a) receptor expressing neurones in central regions involved in nociception. *Brain Res*, 798(1-2), 25-35 (1998)
66. S. Krantic: Peptides as regulators of the immune system: emphasis on somatostatin. *Peptides*, 21(12), 1941-1964 (2000)
67. D. Ferone, R. Pivonello, P. M. Van Hagen, M. Waaijers, J. Zuijderwijk, A. Colao, G. Lombardi, A. J. Bogers, S. W. Lamberts and L. J. Hofland: Age-related decrease of somatostatin receptor number in the normal human thymus. *Am J Physiol Endocrinol Metab*, 279(4), E791-E978 (2000)
68. D. Ferone, M. P. van Hagen, D. J. Kwekkeboom, P. M. van Koetsveld, D. M. Mooy, E. Lichtenauer-Kaligis, A. Schonbrunn, A. Colao, S. W. Lamberts and L. J. Hofland: Somatostatin receptor subtypes in human thymoma and inhibition of cell proliferation by octreotide in vitro. *J Clin Endocrinol Metab*, 85(4), 1719-1726 (2000)
69. A. M. ten Bokum, L. J. Hofland and P. M. van Hagen: Somatostatin and somatostatin receptors in the immune system: a review. *Eur Cytokine Netw*, 11(2), 161-176 (2000)
70. T. Florio and G. Schettini: Multiple intracellular effectors modulate physiological functions of the cloned somatostatin receptors. *J Mol Endocrinol*, 17(2), 89-100 (1996)
71. J. C. Reubi: A somatostatin analogue inhibits chondrosarcoma and insulinoma tumour growth. *Acta Endocrinol (Copenh)*, 109(1), 108-114 (1985)
72. A. Albini, T. Florio, D. Giunciuglio, L. Masiello, S. Carlone, A. Corsaro, S. Thellung, T. Cai, D. M. Noonan, G. Schettini: Somatostatin controls Kaposi's sarcoma tumor growth through inhibition of angiogenesis. *Faseb J*, 13, 647-655 (1999)
73. J. C. Reubi and J. A. Laissue: Multiple actions of somatostatin in neoplastic disease. *Trends Pharmacol Sci*, 16, 110-115 (1995)
74. J. C. Reubi, J. C. Schaer, B. Waser and G. Mengod: Expression and localization of somatostatin receptor SSTR1, SSTR2, and SSTR3 messenger RNAs in primary human tumors using in situ hybridization. *Cancer Res*, 54(13), 3455-3459 (1994)
75. J. C. Reubi, B. Waser, J. C. Schaer and J. A. Laissue: Somatostatin receptor sst1-sst5 expression in normal and neoplastic human tissues using receptor autoradiography with subtype-selective ligands. *Eur J Nucl Med*, 28(7), 836-846 (2001)
76. J. C. Schaer, B. Waser, G. Mengod and J. C. Reubi: Somatostatin receptor subtypes sst1, sst2, sst3 and sst5 expression in human pituitary, gastroentero-pancreatic and mammary tumors: comparison of mRNA analysis with receptor autoradiography. *Int J Cancer*, 70(5), 530-537 (1997)
77. J. C. Reubi, B. Waser, J. C. Schaer and R. Markwalder: Somatostatin receptors in human prostate and prostate cancer. *J Clin Endocrinol Metab*, 80(9), 2806-2814 (1995)
78. T. Florio, L. Montella, A. Corsaro, A. De Chiara, G. Apice, F. Fazioli, S. Lastoria, G. Schettini and G. Palmieri: In vitro and in vivo expression of somatostatin receptors in intermediate and malignant soft tissue tumors. *Anticancer Res*, 23(3B), 2465-2471 (2003)
79. S. Arena, F. Barbieri, S. Thellung, P. Pirani, A. Corsaro, V. Villa, P. Dadati, A. Dorcaratto, G. Lapertosa, J. L. Ravetti, R. Spaziante, G. Schettini and T. Florio: Expression of somatostatin receptor mRNA in human meningiomas and their implication in in vitro antiproliferative activity. *J Neurooncol*, 66(1-2), 155-166 (2004)
80. A. Massa, F. Barbieri, C. Aiello, S. Arena, A. Pattarozzi, P. Pirani, A. Corsaro, R. Iuliano, A. Fusco, G. Zona, R. Spaziante, T. Florio and G. Schettini: The expression of the phosphotyrosine phosphatase DEP-1/PTPeta dictates the responsivity of glioma cells to somatostatin inhibition of cell proliferation. *J Biol Chem*, 279(28), 29004-29012 (2004)
81. G. Palmieri, L. Montella, C. Aiello, F. Barbieri, D. Di Vizio, S. Schulz, S. Beninati, A. Budillon, M. Caraglia, L. Insabato and T. Florio: Somatostatin analogues, a series of tissue transglutaminase inducers, as a new tool for therapy of mesenchimal tumors of the gastrointestinal tract. *Amino Acids*, 32(3), 395-400 (2007)
82. D. C. Danila, J. N. Haidar, X. Zhang, L. Katznelson, M. D. Culler and A. Klibanski: Somatostatin receptor-specific analogs: effects on cell proliferation and growth hormone secretion in human somatotroph tumors. *J Clin Endocrinol Metab*, 86(7), 2976-2981 (2001)
83. E. Resmini, P. Dadati, J. L. Ravetti, G. Zona, R. Spaziante, A. Saveanu, P. Jaquet, M. D. Culler, F. Bianchi, A. Rebora, F. Minuto and D. Ferone: Rapid pituitary tumor shrinkage with dissociation between anti-proliferative and anti-secretory effects of a long-acting octreotide in an acromegalic patient. *J Clin Endocrinol Metab* 92(5), 1592-1599 (2007)
84. S. Tsuzaki and A. C. Moses: Somatostatin inhibits deoxyribonucleic acid synthesis induced by both thyrotropin and insulin-like growth factor-I in FRTL5 cells. *Endocrinology*, 126(6), 3131-3138 (1990)
85. M. T. Hierowski, C. Liebow, K. du Sapin and A. V. Schally: Stimulation by somatostatin of dephosphorylation of membrane proteins in pancreatic cancer MIA PaCa-2 cell line. *FEBS Lett*, 179(2), 252-256 (1985)
86. M. G. Pan, T. Florio and P. J. Stork: G protein activation of a hormone-stimulated phosphatase in human tumor cells. *Science*, 256(5060), 1215-1217 (1992)
87. B. Colas, C. Cambillau, L. Buscail, M. Zeggari, J. P. Esteve, V. Lautre, F. Thomas, N. Vaysse and C. Susini: Stimulation of a membrane tyrosine phosphatase activity by somatostatin analogues in rat pancreatic acinar cells. *Eur J Biochem*, 207(3), 1017-1024 (1992)
88. T. Florio, S. Thellung, S. Arena, A. Corsaro, R. Spaziante, G. Gussoni, G. Acuto, M. Giusti, G. Giordano and G. Schettini: Somatostatin and its analog lanreotide inhibit the proliferation of dispersed human non-functioning pituitary adenoma cells in vitro. *Eur J Endocrinol*, 141(4), 396-408 (1999)
89. T. Florio, S. Thellung, A. Corsaro, L. Bocca, S. Arena, A. Pattarozzi, V. Villa, A. Massa, F. Diana, D. Schettini, F. Barbieri, J. L. Ravetti, R. Spaziante, M. Giusti and G. Schettini: Characterization of the intracellular mechanisms mediating somatostatin and lanreotide inhibition of DNA synthesis and growth hormone release from dispersed human GH-secreting pituitary adenoma cells in vitro. *Clin Endocrinol (Oxf)*, 59(1), 115-128 (2003)

90. T. Florio, M. G. Pan, B. Newman, R. E. Hershberger, O. Civelli and P. J. Stork: Dopaminergic inhibition of DNA synthesis in pituitary tumor cells is associated with phosphotyrosine phosphatase activity. *J Biol Chem*, 267(34), 24169-24172 (1992)
91. A. Imai, H. Takagi, T. Furui, S. Horibe, T. Fuseya and T. Tamaya: Evidence for coupling of phosphotyrosine phosphatase to gonadotropin-releasing hormone receptor in ovarian carcinoma membrane. *Cancer*, 77(1), 132-137 (1996)
92. K. Bedecs, N. Elbaz, M. Sutren, M. Masson, C. Susini, A. D. Strosberg and C. Nahmias: Angiotensin II type 2 receptors mediate inhibition of mitogen-activated protein kinase cascade and functional activation of SHP-1 tyrosine phosphatase. *Biochem J*, 325 (Pt 2), 449-454 (1997)
93. M. B. Marrero, V. J. Venema, H. Ju, D. C. Eaton and R. C. Venema: Regulation of angiotensin II-induced JAK2 tyrosine phosphorylation: roles of SHP-1 and SHP-2. *Am J Physiol*, 275(5 Pt 1), C1216-C1223 (1998)
94. T. Florio, C. Rim, R. E. Hershberger, M. Loda and P. J. Stork: The somatostatin receptor SSTR1 is coupled to phosphotyrosine phosphatase activity in CHO-K1 cells. *Mol Endocrinol*, 8(10), 1289-1297 (1994)
95. L. Buscail, N. Delesque, J. P. Esteve, N. Saint-Laurent, H. Prats, P. Clerc, P. Robberecht, G. I. Bell, C. Liebow, A. V. Schally and et al.: Stimulation of tyrosine phosphatase and inhibition of cell proliferation by somatostatin analogues: mediation by human somatostatin receptor subtypes SSTR1 and SSTR2. *Proc Natl Acad Sci U S A*, 91, 2315-2319 (1994)
96. K. Sharma, Y. C. Patel and C. B. Srikant: Subtype-selective induction of wild-type p53 and apoptosis, but not cell cycle arrest, by human somatostatin receptor 3. *Mol Endocrinol*, 10(12), 1688-1696 (1996)
97. D. B. Reardon, P. Dent, S. L. Wood, T. Kong and T. W. Sturgill: Activation in vitro of somatostatin receptor subtypes 2, 3, or 4 stimulates protein tyrosine phosphatase activity in membranes from transfected Ras-transformed NIH 3T3 cells: coexpression with catalytically inactive SHP-2 blocks responsiveness. *Mol Endocrinol*, 11(8), 1062-1069 (1997)
98. K. Sharma, Y. C. Patel and C. B. Srikant: C-terminal region of human somatostatin receptor 5 is required for induction of Rb and G1 cell cycle arrest. *Mol Endocrinol*, 13(1), 82-90 (1999)
99. C. B. Srikant and S. H. Shen: Octapeptide somatostatin analog SMS 201-995 induces translocation of intracellular PTP1C to membranes in MCF-7 human breast adenocarcinoma cells. *Endocrinology*, 137(8), 3461-3468 (1996)
100. C. Bousquet, N. Delesque, F. Lopez, N. Saint-Laurent, J. P. Esteve, K. Bedecs, L. Buscail, N. Vaysse and C. Susini: sst2 somatostatin receptor mediates negative regulation of insulin receptor signaling through the tyrosine phosphatase SHP-1. *J Biol Chem*, 273(12), 7099-7106 (1998)
101. T. Florio, S. Thellung, S. Arena, A. Corsaro, A. Bajetto, G. Schettini and P. J. Stork: Somatostatin receptor 1 (SSTR1)-mediated inhibition of cell proliferation correlates with the activation of the MAP kinase cascade: role of the phosphotyrosine phosphatase SHP-2. *J Physiol Paris*, 94(3-4), 239-250 (2000)
102. M. Zeggari, J. P. Esteve, I. Rauly, C. Cambillau, H. Mazarguil, M. Dufresne, L. Pradayrol, J. A. Chayvialle, N. Vaysse and C. Susini: Co-purification of a protein tyrosine phosphatase with activated somatostatin receptors from rat pancreatic acinar membranes. *Biochem J*, 303 (Pt 2), 441-448 (1994)
103. T. Florio, A. Scorizello, M. Fattore, V. D'Alto, S. Salzano, G. Rossi, M. T. Berlingieri, A. Fusco and G. Schettini: Somatostatin inhibits PC Cl3 thyroid cell proliferation through the modulation of phosphotyrosine activity. Impairment of the somatostatinergic effects by stable expression of E1A viral oncogene. *J Biol Chem*, 271(11), 6129-6136 (1996)
104. M. Thangaraju, K. Sharma, D. Liu, S. H. Shen and C. B. Srikant: Interdependent regulation of intracellular acidification and SHP-1 in apoptosis. *Cancer Res*, 59(7), 1649-1654 (1999)
105. N. Douziech, E. Calvo, Z. Coulombe, G. Muradia, J. Bastien, R. A. Aubin, A. Lajas and J. Morisset: Inhibitory and stimulatory effects of somatostatin on two human pancreatic cancer cell lines: a primary role for tyrosine phosphatase SHP-1. *Endocrinology*, 140(2), 765-777 (1999)
106. N. Benali, P. Cordelier, D. Calise, P. Pages, P. Rochaix, A. Nagy, J. P. Esteve, P. M. Pour, A. V. Schally, N. Vaysse, C. Susini and L. Buscail: Inhibition of growth and metastatic progression of pancreatic carcinoma in hamster after somatostatin receptor subtype 2 (sst2) gene expression and administration of cytotoxic somatostatin analog AN-238. *Proc Natl Acad Sci U S A*, 97(16), 9180-9185 (2000)
107. P. D. Zapata, R. M. Ropero, A. M. Valencia, L. Buscail, J. I. Lopez, R. M. Martin-Orozco, J. C. Prieto, J. Angulo, C. Susini, P. Lopez-Ruiz and B. Colas: Autocrine regulation of human prostate carcinoma cell proliferation by somatostatin through the modulation of the SH2 domain containing protein tyrosine phosphatase (SHP)-1. *J Clin Endocrinol Metab*, 87(2), 915-926 (2002)
108. M. C. Zatelli, D. Piccin, F. Tagliati, A. Bottoni, A. Luchin and E. C. degli Uberti: SRC homology-2-containing protein tyrosine phosphatase-1 restrains cell proliferation in human medullary thyroid carcinoma. *Endocrinology*, 146(6), 2692-2698 (2005)
109. M. Theodoropoulou, J. Zhang, S. Laupheimer, M. Paez-Pereda, C. Erneux, T. Florio, U. Pagotto and G. K. Stalla: Octreotide, a somatostatin analogue, mediates its antiproliferative action in pituitary tumor cells by altering phosphatidylinositol 3-kinase signaling and inducing Zac1 expression. *Cancer Res*, 66(3), 1576-1582 (2006)
110. F. Lopez, J. P. Esteve, L. Buscail, N. Delesque, N. Saint-Laurent, M. Theveniau, C. Nahmias, N. Vaysse and C. Susini: The tyrosine phosphatase SHP-1 associates with the sst2 somatostatin receptor and is an essential component of sst2-mediated inhibitory growth signaling. *J Biol Chem*, 272(39), 24448-24454 (1997)
111. P. Pages, N. Benali, N. Saint-Laurent, J. P. Esteve, A. V. Schally, J. Tkaczuk, N. Vaysse, C. Susini and L. Buscail: sst2 somatostatin receptor mediates cell cycle arrest and induction of p27(Kip1). Evidence for the role of SHP-1. *J Biol Chem*, 274(21), 15186-15193 (1999)
112. M. Hortala, G. Ferjoux, A. Estival, C. Bertrand, S. Schulz, L. Pradayrol, C. Susini and F. Clemente: Inhibitory

role of the somatostatin receptor SST2 on the intracrine-regulated cell proliferation induced by the 210-amino acid fibroblast growth factor-2 isoform: implication of JAK2. *J Biol Chem*, 278(23), 20574-20581 (2003)
113. G. Ferjoux, F. Lopez, J. P. Esteve, A. Ferrand, E. Vivier, F. Vely, N. Saint-Laurent, L. Pradayrol, L. Buscail and C. Susini: Critical role of Src and SHP-2 in sst2 somatostatin receptor-mediated activation of SHP-1 and inhibition of cell proliferation. *Mol Biol Cell*, 14(9), 3911-3928 (2003)
114. T. Florio, H. Yao, K. D. Carey, T. J. Dillon and P. J. Stork: Somatostatin activation of mitogen-activated protein kinase via somatostatin receptor 1 (SSTR1). *Mol Endocrinol*, 13(1), 24-37 (1999)
115. J. Held-Feindt, F. Forstreuter, T. Pufe and R. Mentlein: Influence of the somatostatin receptor sst2 on growth factor signal cascades in human glioma cells. *Brain Res Mol Brain Res*, 87(1), 12-21 (2001)
116. M. G. Cattaneo, G. Scita and L. M. Vicentini: Somatostatin inhibits PDGF-stimulated Ras activation in human neuroblastoma cells. *FEBS Lett*, 459(1), 64-68 (1999)
117. T. Florio, S. Arena, S. Thellung, R. Iuliano, A. Corsaro, A. Massa, A. Pattarozzi, A. Bajetto, F. Trapasso, A. Fusco and G. Schettini: The activation of the phosphotyrosine phosphatase eta (r-PTP eta) is responsible for the somatostatin inhibition of PC Cl3 thyroid cell proliferation. *Mol Endocrinol*, 15(10), 1838-1852 (2001)
118. M. G. Cattaneo, J. E. Taylor, M. D. Culler, E. Nisoli and L. M. Vicentini: Selective stimulation of somatostatin receptor subtypes: differential effects on Ras/MAP kinase pathway and cell proliferation in human neuroblastoma cells. *FEBS Lett*, 481(3), 271-276 (2000)
119. T. Florio, A. Scorziello, S. Thellung, S. Salzano, M. T. Berlingieri, A. Fusco and G. Schettini: Oncogene transformation of PC Cl3 clonal thyroid cell line induces an autonomous pattern of proliferation that correlates with a loss of basal and stimulated phosphotyrosine phosphatase activity. *Endocrinology*, 138(9), 3756-3763 (1997)
120. A. Massa, F. Barbieri, C. Aiello, R. Iuliano, S. Arena, A. Pattarozzi, A. Corsaro, V. Villa, A. Fusco, G. Zona, R. Spaziante, G. Schettini and T. Florio: The phosphotyrosine phosphatase eta mediates somatostatin inhibition of glioma proliferation via the dephosphorylation of ERK1/2. *Ann N Y Acad Sci*, 1030, 264-274 (2004)
121. S. Arena, A. Pattarozzi, A. Massa, J. P. Esteve, R. Iuliano, A. Fusco, C. Susini and T. Florio: An intracellular multi-effector complex mediates somatostatin receptor 1 activation of phospho-tyrosine phosphatase eta. *Mol Endocrinol*, 21(1), 229-246 (2007)
122. D. B. Reardon, S. L. Wood, D. L. Brautigan, G. I. Bell, P. Dent and T. W. Sturgill: Activation of a protein tyrosine phosphatase and inactivation of Raf-1 by somatostatin. *Biochem J*, 314 (Pt 2), 401-404 (1996)
123. T. Florio, M. Morini, V. Villa, S. Arena, A. Corsaro, S. Thellung, M. D. Culler, U. Pfeffer, D. M. Noonan, G. Schettini and A. Albini: Somatostatin inhibits tumor angiogenesis and growth via somatostatin receptor-3-mediated regulation of endothelial nitric oxide synthase and mitogen-activated protein kinase activities. *Endocrinology*, 144(4), 1574-1584 (2003)
124. P. Cordelier, J. P. Esteve, C. Bousquet, N. Delesque, A. M. O'Carroll, A. V. Schally, N. Vaysse, C. Susini and L. Buscail: Characterization of the antiproliferative signal mediated by the somatostatin receptor subtype sst5. *Proc Natl Acad Sci U S A*, 94(17), 9343-0348 (1997)
125. L. O. Murphy, S. Smith, R. H. Chen, D. C. Fingar and J. Blenis: Molecular interpretation of ERK signal duration by immediate early gene products. *Nat Cell Biol*, 4(8), 556-564 (2002)
126. D. Vaudry, P. J. Stork, P. Lazarovici and L. E. Eiden: Signaling pathways for PC12 cell differentiation: making the right connections. *Science*, 296(5573), 1648-1649 (2002)
127. H. Lahlou, N. Saint-Laurent, J. P. Esteve, A. Eychene, L. Pradayrol, S. Pyronnet and C. Susini: sst2 Somatostatin receptor inhibits cell proliferation through Ras-, Rap1-, and B-Raf-dependent ERK2 activation. *J Biol Chem*, 278, 39356-39371 (2003)
128. F. Alderton, P. P. Humphrey and L. A. Sellers: High-intensity p38 kinase activity is critical for p21(cip1) induction and the antiproliferative function of G(i) protein-coupled receptors. *Mol Pharmacol*, 59(5), 1119-1128 (2001)
129. K. Komatsuzaki, K. Terashita, T. B. Kinane and I. Nishimoto: Somatostatin type V receptor activates c-Jun N-terminal kinases via Galpha(12) family G proteins. *Biochem Biophys Res Commun*, 289(5), 1211-1217 (2001)
130. L. A. Sellers, W. Feniuk, P. P. Humphrey and H. Lauder: Activated G protein-coupled receptor induces tyrosine phosphorylation of STAT3 and agonist-selective serine phosphorylation via sustained stimulation of mitogen-activated protein kinase. Resultant effects on cell proliferation. *J Biol Chem*, 274(23), 16423-16430 (1999)
131. L. A. Sellers, F. Alderton, A. M. Carruthers, M. Schindler and P. P. Humphrey: Receptor isoforms mediate opposing proliferative effects through gbetagamma-activated p38 or Akt pathways. *Mol Cell Biol*, 20(16), 5974-5985 (2000)
132. C. Bousquet, J. Guillermet-Guibert, N. Saint-Laurent, E. Archer-Lahlou, F. Lopez, M. Fanjul, A. Ferrand, D. Fourmy, C. Pichereaux, B. Monsarrat, L. Pradayrol, J. P. Esteve and C. Susini: Direct binding of p85 to sst2 somatostatin receptor reveals a novel mechanism for inhibiting PI3K pathway. *Embo J*, 25(17), 3943-3954 (2006)
133. F. Lopez, G. Ferjoux, P. Cordelier, N. Saint-Laurent, J. P. Esteve, N. Vaysse, L. Buscail and C. Susini: Neuronal nitric oxide synthase: a substrate for SHP-1 involved in sst2 somatostatin receptor growth inhibitory signaling. *Faseb J*, 15(12), 2300-2302 (2001)
134. P. Cordelier, J. P. Esteve, S. Najib, L. Moroder, N. Vaysse, L. Pradayrol, C. Susini and L. Buscail: Regulation of neuronal nitric-oxide synthase activity by somatostatin analogs following SST5 somatostatin receptor activation. *J Biol Chem*, 281(28), 19156-19171 (2006)
135. H. Lahlou, M. Fanjul, L. Pradayrol, C. Susini and S. Pyronnet: Restoration of functional gap junctions through internal ribosome entry site-dependent synthesis of endogenous connexins in density-inhibited cancer cells. *Mol Cell Biol*, 25(10), 4034-4045 (2005)
136. K. Sharma and C. B. Srikant: Induction of wild-type p53, Bax, and acidic endonuclease during somatostatin-

signaled apoptosis in MCF-7 human breast cancer cells. *Int J Cancer*, 76(2), 259-266 (1998)
137. C. B. Srikant: Cell cycle dependent induction of apoptosis by somatostatin analog SMS 201-995 in AtT-20 mouse pituitary cells. *Biochem Biophys Res Commun*, 209(2), 400-406 (1995)
138. M. Thangaraju, K. Sharma, B. Leber, D. W. Andrews, S. H. Shen and C. B. Srikant: Regulation of acidification and apoptosis by SHP-1 and Bcl-2. *J Biol Chem*, 274(41), 29549-20557 (1999)
139. D. Liu, G. Martino, M. Thangaraju, M. Sharma, F. Halwani, S. H. Shen, Y. C. Patel and C. B. Srikant: Caspase-8-mediated intracellular acidification precedes mitochondrial dysfunction in somatostatin-induced apoptosis. *J Biol Chem*, 275(13), 9244-9250 (2000)
140. K. Sharma and C. B. Srikant: G protein coupled receptor signaled apoptosis is associated with activation of a cation insensitive acidic endonuclease and intracellular acidification. *Biochem Biophys Res Commun*, 242(1), 134-140 (1998)
141. R. Teijeiro, R. Rios, J. A. Costoya, R. Castro, J. L. Bello, J. Devesa and V. M. Arce: Activation of human somatostatin receptor 2 promotes apoptosis through a mechanism that is independent from induction of p53. *Cell Physiol Biochem*, 12(1), 31-38 (2002)
142. J. Guillermet, N. Saint-Laurent, P. Rochaix, O. Cuvillier, T. Levade, A. V. Schally, L. Pradayrol, L. Buscail, C. Susini and C. Bousquet: Somatostatin receptor subtype 2 sensitizes human pancreatic cancer cells to death ligand-induced apoptosis. *Proc Natl Acad Sci U S A*, 100(1), 155-160 (2003)
143. E. Ferrante, C. Pellegrini, S. Bondioni, E. Peverelli, M. Locatelli, P. Gelmini, P. Luciani, A. Peri, G. Mantovani, S. Bosari, P. Beck-Peccoz, A. Spada and A. Lania: Octreotide promotes apoptosis in human somatotroph tumor cells by activating somatostatin receptor type 2. *Endocr Relat Cancer*, 13(3), 955-962 (2006)
144. H. L. Liu, L. Huo and L. Wang: Octreotide inhibits proliferation and induces apoptosis of hepatocellular carcinoma cells. *Acta Pharmacol Sin*, 25(10), 1380-1386 (2004)
145. J. Guillermet-Guibert, N. Saint-Laurent, L. Davenne, P. Rochaix, O. Cuvillier, M. D. Culler, L. Pradayrol, L. Buscail, C. Susini and C. Bousquet: Novel synergistic mechanism for sst2 somatostatin and TNFalpha receptors to induce apoptosis: crosstalk between NF-kappaB and JNK pathways. *Cell Death Differ*, 14(2), 197-208 (2007)
146. C. Susini and L. Buscail: Rationale for the use of somatostatin analogs as antitumor agents. *Ann Oncol*, 17(12), 1733-1742 (2006)
147. V. M. Macaulay: Insulin-like growth factors and cancer. *Br J Cancer*, 65(3), 311-320 (1992)
148. G. R. Ambler, A. A. Butler, J. Padmanabhan, B. H. Breier and P. D. Gluckman: The effects of octreotide on GH receptor and IGF-I expression in the GH-deficient rat. *J Endocrinol*, 149(2), 223-231 (1996)
149. P. Hayry, A. Raisanen, J. Ustinov, A. Mennander and T. Paavonen: Somatostatin analog lanreotide inhibits myocyte replication and several growth factors in allograft arteriosclerosis. *Faseb J*, 7(11), 1055-1060 (1993)
150. B. D. Koch and A. Schonbrunn: The somatostatin receptor is directly coupled to adenylate cyclase in GH4C1 pituitary cell membranes. *Endocrinology*, 114(5), 1784-1790 (1984)
151. G. Schettini, T. Florio, O. Meucci, E. Landolfi, G. Lombardi and A. Marino: Somatostatin inhibition of anterior pituitary adenylate cyclase activity: different sensitivity between male and female rats. *Brain Res*, 439(1-2), 322-329 (1988)
152. W. Schlegel, F. Wuarin, C. B. Wollheim and G. R. Zahnd: Somatostatin lowers the cytosolic free Ca2+ concentration in clonal rat pituitary cells (GH3 cells). *Cell Calcium*, 5(3), 223-236 (1984)
153. B. D. Koch, J. B. Blalock and A. Schonbrunn: Characterization of the cyclic AMP-independent actions of somatostatin in GH cells. I. An increase in potassium conductance is responsible for both the hyperpolarization and the decrease in intracellular free calcium produced by somatostatin. *J Biol Chem*, 263(1), 216-225 (1988)
154. B. D. Koch and A. Schonbrunn: Characterization of the cyclic AMP-independent actions of somatostatin in GH cells. II. An increase in potassium conductance initiates somatostatin-induced inhibition of prolactin secretion. *J Biol Chem*, 263(1), 226-234 (1988)
155. M. Tallent, G. Liapakis, A. M. O'Carroll, S. J. Lolait, M. Dichter and T. Reisine: Somatostatin receptor subtypes SSTR2 and SSTR5 couple negatively to an L-type Ca2+ current in the pituitary cell line AtT-20. *Neuroscience*, 71(4), 1073-1081 (1996)
156. D. Roosterman, G. Glassmeier, H. Baumeister, H. Scherubl and W. Meyerhof: A somatostatin receptor 1 selective ligand inhibits Ca2+ currents in rat insulinoma 1046-38 cells. *FEBS Lett*, 425(1), 137-140 (1998)
157. H. Lahlou, J. Guillermet, M. Hortala, F. Vernejoul, S. Pyronnet, C. Bousquet and C. Susini: Molecular signaling of somatostatin receptors. *Ann N Y Acad Sci*, 1014, 121-131 (2004)
158. H. J. Kreienkamp, H. H. Honck and D. Richter: Coupling of rat somatostatin receptor subtypes to a G-protein gated inwardly rectifying potassium channel (GIRK1). *FEBS Lett*, 419(1), 92-94 (1997)
159. S. K. Yang, H. C. Parkington, A. D. Blake, D. J. Keating and C. Chen: Somatostatin increases voltage-gated K+ currents in GH3 cells through activation of multiple somatostatin receptors. *Endocrinology*, 146(11), 4975-4984 (2005)
160. M. Grimaldi, T. Florio and G. Schettini: Somatostatin inhibits interleukin 6 release from rat cortical type I astrocytes via the inhibition of adenylyl cyclase. *Biochem Biophys Res Commun*, 235(1), 242-248 (1997)
161. Y. Chowers, L. Cahalon, M. Lahav, H. Schor, R. Tal, S. Bar-Meir and M. Levite: Somatostatin through its specific receptor inhibits spontaneous and TNF-alpha- and bacteria-induced IL-8 and IL-1 beta secretion from intestinal epithelial cells. *J Immunol*, 165(6), 2955-2961 (2000)
162. R. D. Murray, K. Kim, S. G. Ren, M. Chelly, Y. Umehara and S. Melmed: Central and peripheral actions of somatostatin on the growth hormone-IGF-I axis. *J Clin Invest*, 114(3), 349-356 (2004)
163. J. Folkman: Fundamental concepts of the angiogenic process. *Curr Mol Med*, 3(7), 643-651 (2003)

164. P. Dasgupta: Somatostatin analogues: multiple roles in cellular proliferation, neoplasia, and angiogenesis. *Pharmacol Ther*, 102(1), 61-85 (2004)
165. E. A. Woltering, R. Barrie, T. M. O'Dorisio, D. Arce, T. Ure, A. Cramer, D. Holmes, J. Robertson and J. Fassler: Somatostatin analogues inhibit angiogenesis in the chick chorioallantoic membrane. *J Surg Res*, 50(3), 245-251 (1991)
166. J. C. Reubi, U. Horisberger and J. Laissue: High density of somatostatin receptors in veins surrounding human cancer tissue: role in tumor-host interaction? *Int J Cancer*, 56(5), 681-688 (1994)
167. R. Danesi and M. Del Tacca: The effects of the somatostatin analog octreotide on angiogenesis in vitro. *Metabolism*, 45(8 Suppl 1), 49-50 (1996)
168. R. Barrie, E. A. Woltering, H. Hajarizadeh, C. Mueller, T. Ure and W. S. Fletcher: Inhibition of angiogenesis by somatostatin and somatostatin-like compounds is structurally dependent. *J Surg Res*, 55(4), 446-450 (1993)
169. P. Dasgupta and R. Mukherjee: Lipophilization of somatostatin analog RC-160 with long chain fatty acid improves its antiproliferative and antiangiogenic activity in vitro. *Br J Pharmacol*, 129(1), 101-109 (2000)
170. R. Danesi, C. Agen, U. Benelli, A. D. Paolo, D. Nardini, G. Bocci, F. Basolo, A. Campagni and M. D. Tacca: Inhibition of experimental angiogenesis by the somatostatin analogue octreotide acetate (SMS 201-995). *Clin Cancer Res*, 3(2), 265-272 (1997)
171. A. Zalatnai and F. Timar: In vitro antiangiogenic effect of sandostatin (octreotide) on the proliferation of the placental vessels. *Anticancer Res*, 22(6C), 4225-4227 (2002)
172. N. Garcia de la Torre, J. A. Wass and H. E. Turner: Antiangiogenic effects of somatostatin analogues. *Clin Endocrinol (Oxf)*, 57(4), 425-441 (2002)
173. M. Koizumi, M. Onda, N. Tanaka, T. Seya, T. Yamada and Y. Takahashi: Antiangiogenic effect of octreotide inhibits the growth of human rectal neuroendocrine carcinoma. *Digestion*, 65(4), 200-206 (2002)
174. S. B. Curtis, J. Hewitt, S. Yakubovitz, A. Anzarut, Y. N. Hsiang and A. M. Buchan: Somatostatin receptor subtype expression and function in human vascular tissue. *Am J Physiol Heart Circ Physiol*, 278(6), H1815-H822 (2000)
175. W. D. Jia, G. L. Xu, R. N. Xu, H. C. Sun, L. Wang, J. H. Yu, J. Wang, J. S. Li, Z. M. Zhai and Q. Xue: Octreotide acts as an antitumor angiogenesis compound and suppresses tumor growth in nude mice bearing human hepatocellular carcinoma xenografts. *J Cancer Res Clin Oncol*, 129(6), 327-334 (2003)
176. E. A. Woltering: Development of targeted somatostatin-based antiangiogenic therapy: a review and future perspectives. *Cancer Biother Radiopharm*, 18(4), 601-609 (2003)
177. R. L. Adams, I. P. Adams, S. W. Lindow, W. Zhong and S. L. Atkin: Somatostatin receptors 2 and 5 are preferentially expressed in proliferating endothelium. *Br J Cancer*, 92(8), 1493-1498 (2005)
178. R. L. Adams, I. P. Adams, S. W. Lindow and S. L. Atkin: Inhibition of endothelial proliferation by the somatostatin analogue SOM230. *Clin Endocrinol (Oxf)*, 61(4), 431-436 (2004)
179. G. Garcia-Cardena and J. Folkman: Is there a role for nitric oxide in tumor angiogenesis? *J Natl Cancer Inst*, 90, 560-561 (1998)
180. S. Arena, A. Pattarozzi, A. Corsaro, G. Schettini and T. Florio: Somatostatin receptor subtype-dependent regulation of nitric oxide release: involvement of different intracellular pathways. *Mol Endocrinol*, 19(1), 255-267 (2005)
181. S. Arena, A. Pattarozzi, S. Thellung, V. Villa, A. Corsaro, A. Massa, F. Diana, G. Spoto, S. Forcella, G. Damonte, M. Filocamo, U. Benatti, G. Schettini and T. Florio: Nitric oxide production stimulated by the basic fibroblast growth factor requires the synthesis of ceramide. *Ann N Y Acad Sci*, 973, 94-104 (2002)
182. T. Florio, S. Arena, A. Pattarozzi, S. Thellung, A. Corsaro, V. Villa, A. Massa, F. Diana, G. Spoto, S. Forcella, G. Damonte, M. Filocamo, U. Benatti and G. Schettini: Basic fibroblast growth factor activates endothelial nitric-oxide synthase in CHO-K1 cells via the activation of ceramide synthesis. *Mol Pharmacol*, 63(2), 297-310 (2003)
183. S. Pola, M. G. Cattaneo and L. M. Vicentini: Anti-migratory and anti-invasive effect of somatostatin in human neuroblastoma cells: involvement of Rac and MAP kinase activity. *J Biol Chem*, 278(42), 40601-40606 (2003)
184. M. Kumar, Z. R. Liu, L. Thapa, Q. Chang, D. Y. Wang and R. Y. Qin: Antiangiogenic effect of somatostatin receptor subtype 2 on pancreatic cancer cell line: Inhibition of vascular endothelial growth factor and matrix metalloproteinase-2 expression in vitro. *World J Gastroenterol*, 10(3), 393-399 (2004)
185. M. N. Pollak and A. V. Schally: Mechanisms of antineoplastic action of somatostatin analogs. *Proc Soc Exp Biol Med*, 217(2), 143-152 (1998)
186. N. Ferrara: Molecular and biological properties of vascular endothelial growth factor. *J Mol Med*, 77(7), 527-543 (1999)
187. R. Mentlein, O. Eichler, F. Forstreuter and J. Held-Feindt: Somatostatin inhibits the production of vascular endothelial growth factor in human glioma cells. *Int J Cancer*, 92(4), 545-550 (2001)
188. S. Cascinu, E. Del Ferro, M. Ligi, M. P. Staccioli, P. Giordani, V. Catalano, R. Agostinelli, P. Muretto and G. Catalano: Inhibition of vascular endothelial growth factor by octreotide in colorectal cancer patients. *Cancer Invest*, 19, 8-12 (2001)
189. J. W. Sall, D. D. Klisovic, M. S. O'Dorisio and S. E. Katz: Somatostatin inhibits IGF-1 mediated induction of VEGF in human retinal pigment epithelial cells. *Exp Eye Res*, 79(4), 465-476 (2004)
190. J. W. Miller: Vascular endothelial growth factor and ocular neovascularization. *Am J Pathol*, 151(1), 13-23 (1997)
191. H. H. Li, X. C. Wang, J. R. Lu, K. J. He and Z. Yang: Effects of short-term treatment of somatostatin on angiogenesis of gastric carcinoma. *Ai Zheng*, 22(9), 990-993 (2003)
192. N. Ferrara, H. P. Gerber and J. LeCouter: The biology of VEGF and its receptors. *Nat Med*, 9(6), 669-676 (2003)

193. C. J. Wiedermann, N. Reinisch and H. Braunsteiner: Stimulation of monocyte chemotaxis by human growth hormone and its deactivation by somatostatin. *Blood*, 82(3), 954-960 (1993)

Abbreviations: basic fibroblast growth factor (bFGF), bovine artery endothelial cells (BAEC), corioallanthoic membrane model (CAM), cyclin dependent kinase inhibitors (CDKI), death receptor 4 (DR4), dopamine D2 receptor (D2R), epidermal growth factor (EGF), G protein coupled receptors (GPCR), gastrointestinal stromal tumors (GIST), glycogen synthase kinase 3β (GSK3β), human umbilical vein endothelial cells (HUVEC), insulin-like growth factor-1 (IGF-1), μ opioid receptor 1 (MOR1), Na^+-H^+ exchanger (NHE1), nitric oxide (NO), nitric oxide synthase (NOS), phosphatidyl inositol 3 kinase (PI3K), phosphotyrosine phosphatases (PTP), platelet derived growth factor (PDGF), somatostatin (SST), somatostatin receptors (SSTRs). tumor necrosis factor alpha (TNFalpha), TNFalpha receptor 1 (TNFR1), vascular endothelial growth factor (VEGF).

Key Words: somatostatin, somatostatin receptors, cell proliferation, apoptosis, angiogenesis, phosphotyrosine phosphatase, MAP kinase, calcium channel, potassium channel, cyclic AMP, Review

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