Rationale for the use of somatostatin analogs as antitumor agents

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Background: There is a need for novel antitumor agents that demonstrate efficacy in currently refractory tumors without adding to the toxicity of therapy. The somatostatin analogs, which have demonstrated antineoplastic activities in experimental tumor models, and good tolerability and safety profiles are attractive candidates.

Materials and Methods: Data from preclinical studies provide evidence for direct and indirect mechanisms by which somatostatin analogs exert antitumor effects.

Results: Direct antitumor activities, mediated through somatostatin receptors (sst1–sst5) expressed in tumor cells, include blockade of autocrine/paracrine growth-promoting hormone and growth factor production, inhibition of growth factor-mediated mitogenic signals and induction of apoptosis. Indirect antitumor effects include inhibition of growth-promoting hormone and growth factor secretion, and antiangiogenic actions. Many human tumors express more than one somatostatin receptor subtype, with sst2 being predominant. Somatostatin analogs such as octreotide and lanreotide, which present a high affinity for sst2, are in current clinical use to alleviate symptoms in patients with endocrine tumors, and radiolabeled somatostatin analogs have been developed for diagnosis and radiotherapy.

Conclusions: While the rationale exists for the use of somatostatin analogs as antitumor agents, studies are ongoing to identify analogs with activity across the range of receptor subtypes to maximize the potential of such treatment.

Key words: angiogenesis, apoptosis, antitumor, receptor, refractory tumor, somatostatin analog

introduction

Without doubt, conventional chemotherapy is of benefit to many patients with cancer. However, many common neoplasms, such as those of the lung, breast, bowel, pancreas and kidney are refractory to current therapies, and late diagnosis typical of prostate and hepatocellular carcinomas, presents additional challenges. Treatment is often curtailed or reduced by the severe impact cytotoxic chemotherapy has on a patient’s quality of life.

Multimodal therapy, combining surgery, radio- and chemotherapy, is often used to treat refractory tumors. Nevertheless, recurrence rates are high and the value of adjuvant therapies remains to be established. In the search for innovative and less cytotoxic approaches to cancer treatment, the somatostatin analogs have been investigated. To the oncologist, somatostatin analogs will be most familiar as diagnostic and tumor-localization agents in somatostatin receptor (sst) scintigraphy, and as agents to control the symptoms associated with endocrine tumors. It was in this latter indication that accompanying tumor shrinkage was noted with octreotide use [1]. A detailed description for the use of somatostatin analogs in the management of neuroendocrine tumors of the gastroenteropancreatic (GEP) system is given in Oberg et al.’s consensus report [2]. Moreover, the antineoplastic activity of somatostatin analogs is not confined to endocrine tumors. Experimental studies have shown that some of these analogs also have antineoplastic activity in epithelial tumors [3–6].

Treatment with somatostatin analogs, however, has produced variable results in clinical practice especially when used as single agents. Poorer results were seen with rapidly progressive tumors, with high proliferation capacity despite the presence of somatostatin receptors [7, 8]. Conversely well-differentiated tumors such as mid-gut carcinoids respond well, with stabilization of tumor growth over many years [9–11].

Ideally, novel antineoplastic agents should be well tolerated in the clinical environment. Somatostatin analogs, therefore, represent attractive candidates having demonstrable good tolerability after two decades of use in conditions such as acromegaly and endocrine tumors. Over the past 15 years, studies have begun to reveal some of the molecular mechanisms underlying the observed antitumor activity of the somatostatin analogs. This review will discuss studies supporting the rationale for somatostatin analog use in clinical oncology practice.
somatostatin analogs: chemistry, biological activity and clinical experience

naturally occurring somatostatins

Naturally occurring somatostatins, which are also known as somatotropin release-inhibiting factors, have diverse biological effects in many cells and organs throughout the body. They are produced by normal endocrine, gastrointestinal, immune and neuronal cells, as well as by certain tumors [12, 13].

The effects of somatostatins are broadly inhibitory on the secretion of hormones, as well as on the proliferation and survival of cells. They inhibit both endocrine secretion (e.g. growth hormone, insulin, glucagons, gastrin, cholecystokinin, vasoactive intestinal peptide and secretin) and exocrine secretion (e.g. gastric acid, intestinal fluid and pancreatic enzymes) [12]. Somatostatins also inhibit proliferation of both normal and tumor cells [14].

Somatostatins are peptides, initially synthesized as a large precursor molecule that undergoes tissue-specific enzymatic degradation to yield either somatostatin-14 or somatostatin-28. In turn, further enzymatic degradation reduces their activity or renders them completely inactive. This occurs rapidly so naturally occurring somatostatins have only short half-lives (<3 min). The structure of somatostatins 14 and 28, and the enzymatic degradation sites of somatostatin-14 are shown in Figure 1.

The dual actions of natural somatostatins (inhibition of hormone release and cell growth) made them logical candidates as anticancer drugs, as well as for the treatment of neuroendocrine disorders. However, the short half-lives of the native somatostatin peptides presented a barrier to further therapeutic development.

synthetic derivatives

Synthetic derivatives of somatostatin have similar activity to native somatostatin but with a longer half-life. Of the many hundreds of somatostatin analogs synthesized, two are in common clinical use: octreotide and lanreotide. A third, RC-160 (vapreotide) has been well characterized in preclinical studies and applied in clinical trials (Figure 2) [15]. These analogs lack the key enzyme cleavage sites and are more stable than native somatostatin. As a result, they have relatively long half-lives, e.g. approximately 90 min for octreotide. The long-acting formulations of octreotide and lanreotide need to be administered only once every 4 weeks.

The actions of the somatostatin peptides are mediated through interaction with specific, cell-surface somatostatin receptors and five distinct receptor subtypes (sst1–5) have been characterized [12]. Somatostatin-14 and somatostatin-28 have approximately equivalent affinity for all the receptor subtypes (with only somatostatin-28 having slightly higher affinity for sst2). However, the synthetic analogs octreotide, lanreotide and RC-160 bind preferentially to sst2 and sst5, with moderate affinity for sst3 and low affinities for sst1 and sst4 (Table 1) [12, 16, 17].

Molecular engineering strategies have led to the discovery of peptide and non-peptide compounds with affinity either for one receptor subtype or combined affinities for two or more, or a universal binding profile. These analogs, such as the multiple sst receptor ligand SOM230, are currently in preclinical evaluation or in early clinical trials [17, 18].

Octreotide and lanreotide are approved for the control of hormonal symptoms of pituitary adenomas. They reduce and normalize the excessive growth hormone secretion and insulin-like growth factor (IGF-1) levels associated with acromegaly and reduce tumor size [19–25]. Pituitary tumors express all somatostatin receptors except sst4, but with variation in subtype expression between pituitary adenoma subtypes. The efficacy of treatment of acromegaly with somatostatin analogs is based on the expression of sst2 and sst5, which predominate in growth hormone-secreting adenomas [26, 27].

Both octreotide and lanreotide have potent activity against gastroenteropancreatic (GEP) endocrine tumors [28–30]. Each GEP tumor expresses more than one subtype, with sst2 the most

Figure 1. Primary structure of somatostatin-14 and somatostatin-28. Key sites of enzymatic degradation are marked with arrows for somatostatin-14.

Figure 2. Chemical structure of native somatostatin-14 and the synthetic analogs octreotide, lanreotide, and RC-160 (vapreotide).
frequently expressed subtype [31]. Immunohistochemistry and autoradiography reveals that sst proteins are highly expressed in gastrinomas, insulinomas, carcinoid tumors and their metastases, with a homogeneous receptor distribution. The frequency and pattern of expression of each subtype varies greatly in different tumor types but also in each patient [32–35]. Undifferentiated GEP endocrine tumors express sst in lower density than well-differentiated tumors. In studies of expression, the majority of the tumors expressed sst2, followed by sst1, sst5 and sst3 while sst4 is expressed in a minority of tumors [32, 36].

Table 1. Binding affinities of native somatostatin-14 and synthetic analogs for the five human sst subtypes, and properties of the receptors—adapted from [17, 18, 106]

<table>
<thead>
<tr>
<th></th>
<th>sst1</th>
<th>sst2</th>
<th>sst3</th>
<th>sst4</th>
<th>sst5</th>
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</thead>
<tbody>
<tr>
<td>Binding affinities (IC50 values; nmol/l)</td>
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<tr>
<td>Somatostatin-14</td>
<td>0.93</td>
<td>0.15</td>
<td>0.56</td>
<td>1.5</td>
<td>0.29</td>
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<tr>
<td>Synthetic analogs</td>
<td></td>
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<tr>
<td>Octreotide</td>
<td>280</td>
<td>0.38</td>
<td>7.1</td>
<td>&gt;1000</td>
<td>6.3</td>
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<tr>
<td>Lanreotide</td>
<td>180</td>
<td>0.54</td>
<td>14</td>
<td>230</td>
<td>17</td>
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<tr>
<td>SOM230</td>
<td>9.3</td>
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<td>1.5</td>
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<td>RC-160a</td>
<td>&gt;1000</td>
<td>5.4</td>
<td>31</td>
<td>45</td>
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<td>Phospholipase A2</td>
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<td>Tyrosine kinases</td>
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<td>PI3 kinase</td>
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<td>Nitric oxide synthases</td>
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<td>Expression of receptor</td>
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<td>Normal tissue distribution (sst1–5 are rather ubiquitous)</td>
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<tr>
<td>Brain, pituitary, pancreas, stomach, liver, kidneys</td>
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<tr>
<td>Brain, pituitary, pancreas, lymphocytes, vascular smooth muscle cell, stomach, kidneys</td>
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<td>Brain, pituitary, pancreas, T cells, stomach</td>
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<tr>
<td>Brain, pituitary, pancreas, lungs, placenta</td>
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<tr>
<td>Lymphoid cells, pituitary, pancreas, stomach</td>
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<td>Pancreatic, colon, prostatic, ovarian and renal cell cancers</td>
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</table>

Values represent K, values in nmol/l [16, 64].
The somatostatin analogs are usually considered to be well tolerated with a good safety profile [37]. Gastrointestinal complaints are the most common side-effects of long-acting analog therapy but are generally mild and improve in most patients with continued treatment. Somatostatin analogs are, therefore, unlikely to add significantly to the toxicity of cytotoxic chemotherapeutic regimens.

**antitumor actions**

Besides their recognized suppressive effects on secretory symptoms, somatostatin analogs appear also to induce an antiproliferative effect. In acromegaly patients treated with octreotide, 73% of patients showed >30% tumor shrinkage [38]. Tumor shrinkage has been rarely observed in patients with GEP endocrine tumors but tumor volume stabilization has been reported in 10%–45% of patients [10, 39, 40]. However, the exact mechanism responsible for this effect remains to be addressed.

Because it was thought that only tumors expressing receptors responded to somatostatin analogs, research has focused on receptor identification. However, Swarm chondrosarcomas, a tumor that is devoid of these receptors is inhibited by somatostatin analogs [3, 41]. This suggests a separate mechanism and two distinct effects have been proposed: direct actions, mediated by the somatostatin receptors, and indirect actions, independent of the receptors. Schematic representation of the mechanisms of antiproliferative effect induced by somatostatin analogs are outlined in Figure 3.

**direct antitumor actions**

Human prostatic tumors are useful models for determining the potential direct antitumor activities of somatostatin analogs. Prostatic tumors are regulated by growth hormone and prolactin, and binding of somatostatin inhibits the secretion of these hormones. In vitro, in vivo and clinical studies have demonstrated that somatostatin and its analogs exert a significant inhibitory effect on the proliferation of these cells (Table 2) [15, 42–44].

Somatostatin analogs may also inhibit the growth of other cancer cell lines, such as those of gastric, lung, colorectal or thyroid origin (Table 2) [6, 13]. In addition, experimental analogs, such as TT-232 and RC-160, also appear to have antiproliferative effects in some human tumor cells in vitro and in vivo [45–48]. These data suggest that somatostatin analogs have the potential to exert antitumor effects in clinical use.

### involvement of specific somatostatin receptor subtypes

The direct antiproliferative actions of somatostatin analogs may be the result of blocking cell division (by blockade of mitogenic growth factor signals) or result from the induction of apoptosis (programmed cell death) following interaction with somatostatin receptors. The mechanism by which a single somatostatin analog exerts direct antitumor actions is now known to depend on the subtype of somatostatin receptor to which it binds. Binding of an analog to a somatostatin receptor initiates specific signal transduction pathways. In this way, each receptor subtype is able to mediate different biological actions (see Table 1) [13, 18, 49].

Investigators report several mechanisms by which somatostatin and somatostatin analogs arrest cell growth (Tables 1 and 3). The receptor subtypes thought to mediate these mechanisms include sst1, sst2, sst4 and sst5 [50, 51].

An example of different sst subtype inducing differing activities occurs with the MAPK extracellular signal-regulated kinase (ERK) pathway, which is an important mediator of somatostatin-induced cell growth regulation. In neuroblastoma and small-cell lung cancer cells, sst1 and/or sst2 somatostatin analogs inhibit platelet-derived growth factor (PDGF)-stimulated ERK activity, and this effect is related to the antiproliferative action of these peptides [52]. Somatostatin analogs mediated by sst4 inhibit vascular endothelial growth factor, which in turn blocks ERK activity [53]. By contrast, human sst4, which is stably expressed in CHO-K1 cells, mediates proliferative activity of somatostatin by a mechanism involving a sustained protein kinase C-mediated activation of ERK1/2 pathway [54]. Other pathways affecting ERK activity are summarized in Table 3.

Recently, another mechanism for somatostatin-induced cell growth inhibition was identified: restoration of functional gap junctions. Gap junctions are composed of connexins and are critical for the maintenance of the differentiated state. In most cancer cells, connexin expression is impaired. We have found that the restoration of density-induced inhibition of the growth of human pancreatic cancer cells, which follows re-expression of sst2, is due to overexpression of endogenous connexin (Cx) 26 and Cx43, and consequent formation of functional gap junctions [55].

Somatostatin analogs are also thought to inhibit cell proliferation by inducing apoptosis, which may be mediated through the sst1 receptor subtype. When sst1 is transfected into previously sst-free cell lines, addition of octreotide causes the up-regulation of the tumor suppressor protein p53, which subsequently induces apoptosis. Other pathways mediated by somatostatin analogs leading to apoptosis are summarized in Table 3.

The sst2 receptor subtype, like sst3, also exerts anti-oncogenic properties. We initially demonstrated that human pancreatic carcinoma specifically lost the expression of sst2 receptor [56]. After correction of the sst2 gene defect, cell growth as well as...
**Table 2.** Evidence for direct antitumor actions of somatostatin and somatostatin analogs

<table>
<thead>
<tr>
<th>Cell line/tumor type</th>
<th>Somatostatin analog</th>
<th>Evidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prostate</td>
<td>Somatostatin, octreotide, RC-160</td>
<td>In both <em>in vitro</em> and <em>in vivo</em> studies somatostatin analogs exert a significantly inhibitory effect in prostate cell lines and models of prostate carcinoma. In clinical studies, some patients who are refractory to androgen ablation respond to somatostatin analog administration [42–44].</td>
</tr>
<tr>
<td>Gastric</td>
<td>Somatostatin analogs</td>
<td>Octreotide has a direct tumor-suppressive effect in both <em>in vitro</em> and <em>in vivo</em> studies of lung cancer cell lines and models expressing sst2 [6, 13].</td>
</tr>
<tr>
<td>Lung</td>
<td>Octreotide</td>
<td>Octreotide has a direct tumor-suppressive effect in both <em>in vitro</em> and <em>in vivo</em> studies of colorectal cancer cell lines and models expressing sst2 [6, 13].</td>
</tr>
<tr>
<td>Colorectal</td>
<td>Octreotide</td>
<td>Octreotide has a direct tumor-suppressive effect in both <em>in vitro</em> and <em>in vivo</em> studies of pancreatic cancer cell lines and models expressing sst2 [6].</td>
</tr>
<tr>
<td>Mammary</td>
<td>Octreotide</td>
<td>Octreotide has a direct tumor-suppressive effect in both <em>in vitro</em> and <em>in vivo</em> studies of mammary cell lines and models expressing sst2 Weckbecker et al. [6].</td>
</tr>
<tr>
<td>Thyroid</td>
<td>Somatostatin analogs</td>
<td>Somatostatins inhibit the growth of thyroid cancer cell lines [13].</td>
</tr>
<tr>
<td>Pancreatic</td>
<td>Octreotide</td>
<td>Octreotide has a direct tumor-suppressive effect in both <em>in vitro</em> and <em>in vivo</em> studies of mammary cell lines and models expressing sst2 Weckbecker et al. [6].</td>
</tr>
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</table>

**Table 3.** Mechanisms by which somatostatin and somatostatin analogs exert direct antitumor effects

<table>
<thead>
<tr>
<th>Receptor subtype(s) mediating pathway</th>
<th>Mechanism</th>
<th>Pathway(s) affected</th>
<th>Comment and evidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initiation factor</td>
<td>SHP-1</td>
<td>Cyclin-dependent kinase inhibitor p27Kip1</td>
<td>Activation of particular sst subtypes by somatostatin stimulates SHP activity, which induces p27Kip1 [107–109].</td>
</tr>
<tr>
<td>sst3</td>
<td>Vascular endothelial growth factor (VEGF)</td>
<td>MAPK extracellular signal-regulated kinase (ERK)</td>
<td>In endothelial cell lines, binding of somatostatin to sst3 inhibits VEGF-induced ERK1/2 activation and cell proliferation [53].</td>
</tr>
<tr>
<td>sst5</td>
<td>cGMP-dependent protein kinase G</td>
<td>ERK</td>
<td>In CHO and neuroblastoma cells, sst5-specific somatostatin analogs inhibit cGMP-dependent protein kinase G, which leads to inhibition of ERK and cell proliferation [52, 110].</td>
</tr>
<tr>
<td>sst1 and sst2</td>
<td>MAPK ERK1/2 which affects expression of p21Waf1 or p27Kip1 cyclin-dependent kinase inhibitors</td>
<td>MAPK ERK1/2 which affects expression of p21Waf1 or p27Kip1 cyclin-dependent kinase inhibitors</td>
<td>In nontumoral CHO cells, sst1 and sst2 mediate transient activation of ERK1/2 which exerts an antiproliferative effect [49].</td>
</tr>
<tr>
<td>sst1 and sst3</td>
<td>modulation of NO production</td>
<td>NO+ entry into the cell in exchange for H+</td>
<td>In sst2-mediated cell growth arrest, NO modulates inhibition of cell proliferation [49, 86].</td>
</tr>
<tr>
<td>sst1, sst3 and sst4</td>
<td>Na+–H+ exchanger NHE1</td>
<td>In endocrine and hepatic cells, somatostatin inhibits NHE1 activity, which affects intracellular pH homeostasis, cell volume regulation, and is associated with reduced cell proliferation [111].</td>
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<td>sst2</td>
<td>Overexpression of endogenous connexin (Cx) 26 and Cx43</td>
<td>Formation of functional gap junctions</td>
<td>Connexin overexpression results in the density-induced inhibition of the growth of human pancreatic cancer cells [55].</td>
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<tr>
<td>sst4</td>
<td>Tumor suppressor protein p53</td>
<td>Induction of apoptosis</td>
<td>Mediated by sst4, octreotide up-regulates p53, which subsequently induces apoptosis [30].</td>
</tr>
<tr>
<td>sst5</td>
<td>Bcl-2-associated protein (BAX) and acidic endonuclease</td>
<td>Induction of apoptosis</td>
<td>Octreotide induces BAX during apoptosis [50, 112].</td>
</tr>
<tr>
<td>sst6</td>
<td>Upregulation of the TRAIL and TNF receptors, DR4 and TNFRI and downregulation of the anti-apoptotic mitochondrial Bcl-2 protein</td>
<td>Induction of apoptosis</td>
<td>Mediated by sst6, octreotide upregulates TRAIL, DR4 and TNFRI, and downregulates Bcl-2, which results in apoptosis [113].</td>
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</table>
tumorigenicity were significantly reduced in sst2-expressing cells in the absence of exogenous ligand [57]. The synthesis and secretion of the natural ligand somatostatin-14 by sst2-transfected cells was responsible for an autocrine inhibitory loop (whereby somatostatin-14 affected the cells from which it was produced). Furthermore, in an experimental athymic mouse model, sst2 re-expression caused dramatic decrease in tumor growth, and both local and distant antitumoral bystander effects [58]. Further preclinical studies, in a hamster model demonstrated that re-expression of the sst2 gene caused inhibition of primary tumor growth and inhibited metastatic progression [59].

Specific somatostatin receptor subtypes may also be involved in direct antitumor actions by a less well understood mechanism in which two receptor subtypes act synergistically to enhance antiproliferative activity [60]. Somatostatin receptors can initiate their ligand-induced signaling cascades by receptor dimerization, although this has not yet been shown to occur in tumor cells. Dimerization of somatostatin receptors has been reported for sst2, sst3, and sst5 [61–63]. By contrast, sst3 does not form homodimers. sst4 can heterodimerize with other somatostatin receptor subtypes or other G protein-coupled receptor (GPCR), which may alter ligand binding affinity and/or signaling efficacy of GPCR. Furthermore, heterodimerization may be implicated in the enhanced efficacy of two receptor-specific compounds. First, the biselective sst2 and sst3 agonist BIM-23244 that suppresses growth hormone from octreotide-resistant human growth hormone-secreting adenomas [64] and, secondly, the chimeric somatostatin-dopamine molecule BIM-23A387, which selectively binds to sst2 and to dopamine D2 receptors, resulting in suppression of growth hormone and prolactin secretion from acromegalic tumors [65]. Whether these analogs inhibit cell proliferation remains to be established.

With respect to the direct effects on tumors, the therapeutic efficacy of somatostatin analogs in controlling pituitary adenomas tends to be long lasting and does not decrease with time. By contrast, desensitization of the effect of somatostatin analogs within weeks or months has been observed in the majority of islet cell or carcinoid tumor-bearing patients, and important differences occur among patients with respect to the induction of this tumor type-dependent tachyphylaxis [66].

Several potential mechanisms could be involved in somatostatin resistance among tumors that express different patterns of sst subtypes. One mechanism could be a change in sst expression at the cell surface of the tumors as a result of downregulation of sst subtypes for which octapeptide analogs have high affinity. Another could involve up-regulation of sst subtypes that do not bind currently available somatostatin analogs. Mechanisms inducing such changes in functional cell surface sst receptor number may include homologous and/or heterologous regulation of sst expression, and changes in sst trafficking, or perhaps more anaplastic tumors lose sst receptors from their surface [58, 66–69].

Appropriate receptor localization and stabilization at the cell surface and receptor turnover are due to dynamic interactions of the receptor with regulatory proteins, e.g. amphiphysin IIb, a multifunctional adaptor protein, critical for sst2 and sst5 targeting to the plasma membrane in the pituitary AtT-20 cells [70]. Changes in the expression of these regulatory protein(s), which are required for correct trafficking of specific sst subtypes, may account for the loss of response over time of tumors to the direct antitumor effects of somatostatin analogs. In addition, agonist-induced sst internalization, as well as their fate (recycling versus degradation) after endocytosis, differ according to the receptor subtype. After G protein-coupled receptor kinase-2 (GRK2)-dependent sst5 phosphorylation and internalization, sst5 is rapidly resensitized and recycled to the plasma membrane. By contrast, a large part of internalized sst2 is sequestered into intracellular vesicles and subjected to ubiquitin-dependent degradation after agonist activation. sst3 is unique in that it fails to internalize after agonist activation. Hence, the differential intracellular sorting of sst may provide important clues about the response of GEP tumors to somatostatin analog treatment [71].

The experimental evidence suggests that the direct antiproliferative activity of a somatostatin analog is dependent on its receptor selectivity (Table 1). The predominant expression of sst2 in human tumors results in the successful treatment with the somatostatin analogs octreotide and lanreotide of growth hormone-secreting pituitary adenomas, islet cell tumors, and carcinoid endocrine tumors. However, it seems that, for full antiproliferative action, a somatostatin analog would ideally have activity at more than just one receptor subtype. Therefore efforts have been concentrated into finding somatostatin analogs with multi-receptor selectivity, such as SOM230, which exhibits high affinity binding to sst2, sst3 and sst5 and moderate affinity for sst1 [17, 72]. SOM230 is a potent inhibitor of growth hormone- and prolactin-secreting pituitary adenomas and has the potency to increase the number of growth hormone-secreting adenomas responsive to treatment [73–75].

indirect antitumor mechanisms

Somatostatin and its synthetic analogs also exert a number of indirect antitumor actions, including: inhibition of the release of growth factors and hormones that drive tumor growth; antiangiogenic effects that reduce tumor blood flow; and immunomodulatory effects to stimulate the body’s natural antitumor mechanisms.

inhibition of the secretion of growth factors and growth-promoting hormones. Decrease in tumor growth may result from indirect effects of the peptide, through suppression of synthesis and secretion (and thereby diminution of the actions) of growth factors and hormones such as insulin, prolactin, IGF-1, EGF, transforming growth factor-β, gastrin, cholecystokinin and growth hormone. For example, IGF-1 is an important modulator of many neoplasms. The suppression IGF-1 secretion is significant because by suppressing growth hormone and dislocating growth hormone-driven tumor growth, somatostatin analogs may indirectly exert antiproliferative effects. Somatostatin analogs suppress the growth hormone-IGF-1 axis by both central and peripheral mechanisms. sst2 and sst5 are the primary subtypes mediating the inhibition of pituitary growth hormone release. The analogs also inhibit hepatic growth hormone-induced IGF-1 production via sst1- and/or sst5-mediated activation of a tyrosine phosphatase,
which leads to dephosphorylation of STAT5b and to a decrease in IGF-1 gene transcription [76].

**antiangiogenic effects.** Somatostatin analogs can also indirectly control tumor growth by inhibiting angiogenesis. Angiogenesis, the formation of new blood vessels from an existing capillary network, is necessary for tumor neovascularization, which is essential for tumor growth and metastasis. The value of antiangiogenesis as an approach to cancer treatment has previously been demonstrated with bevacizumab, a monoclonal antibody directed against one of the principal proangiogenic factors, VEGF [77].

Overexpression of peritumoral vascular somatostatin receptors with high-affinity for somatostatin and octreotide has been reported in human primary colorectal carcinomas, small-cell lung carcinoma, breast cancer, renal cell carcinoma and malignant lymphoma. Expression appears to be independent of receptor expression in the tumor and may be related to sst2 [78]. Furthermore, sst2 receptors have been detected by immunohistochemical staining and in vivo scintigraphy in proliferating angiogenic vessels of human vascular endothelium, while non-proliferative vessels lack sst2 [79]. Octreotide has been shown to inhibit angiogenesis in a number of experimental tumor models [80–82]. Octreotide and RC-160 showed significantly greater inhibition of neovascularization when compared to native somatostatin-14 [83]. In hypervascular tumors, such as hepatocellular carcinoma, the inhibition of angiogenesis may be the key pathway by which octreotide exerts its effects [84].

This inhibition may result from an up-regulation of sst2 during the angiogenic switch from resting to proliferating endothelium [78]. However, sst2 and sst5 proteins were preferentially expressed in proliferating human umbilical vein endothelial cells but not quiescent cells, which suggests that other sst subtypes, such as sst3, may also play a role [85]. In other cell systems, which only express sst3, such as EAHy926 cells, sst3 must be implicated with anti-angiogenic activity. At the molecular level, this effect results from somatostatin-mediated inhibition of MAP kinase activity and endothelial NO synthase (eNOS) activity, which in turn implicate sst1, sst2 and sst5 [53, 86].

There appear to be a myriad of mechanisms governing angiogenesis. One of the mechanisms by which somatostatin inhibits angiogenesis is through a decrease in VEGF synthesis [87, 88]. Somatostatin analogs also exert antiangiogenic actions through a broad inhibition of both the release and the effect of growth factors, some of which are angiogenic, including platelet-derived growth factor, IGF-1 and basic fibroblast growth factor [89]. These growth factors secreted by tumor cells and infiltrating inflammatory cells, stimulate endothelial and smooth muscle cell proliferation and migration, important processes in angiogenesis.

Somatostatin analogs, notably octreotide, appear to be promising candidates for targeted antiangiogenic therapies, either alone or in combination with cytostatic or cytotoxic agents.

**immunomodulatory effects**

Somatostatin analogs may also exert antiproliferative actions by modulating the immune system. Studies have shown that somatostatin receptors are present on human lymphocytes, monocytes [90] and lymphoma cells, and that somatostatin regulates immune cell secretion [91, 92]. According to experimental studies, activated somatostatin receptors can modulate inflammatory and immune mechanisms. For example, interferon-γ release by T cells [93] and tumor necrosis factor-α and interleukin-1β release by monocytes [94] are inhibited by somatostatin. Changes in natural killer cell activity have been reported during octreotide treatment of patients with metastatic carcinoid cancer that might contribute to the antiproliferative effects of this analog [95]. In addition, there is evidence that somatostatin exerts antiproliferative and apoptotic effects on human B and T cells [96, 97].

**potential use of somatostatin analogs in cancer treatment protocols**

Experimental studies of combinations of octreotide with antimiotic drugs, such as vincristine, methotrexate, fluorouracil and suramin, resulted in slightly additive actions [48, 98].

Currently, combinations of chemotherapy with somatostatin analogs are considered for use in patients with gastroenteropancreatic neuroendocrine tumors. Furthermore, somatostatin analogs have also been used as carriers to deliver cytotoxic agents to cancer cells. Schally and coworkers synthesized novel targeted cytotoxic somatostatin octapeptide conjugates, such as AN-238, which contains RC-121 coupled to doxorubicin derivative, 2-pyrrolino-DOX (AN-201). In human experimental cancer models, AN-238 was very effective [48, 59]. It suppressed the growth of H5746T and NCI-N87 human gastric cancers, which display a high concentration of sst1 and sst3 [99]. In addition, AN-238 appeared to target vascular st in a xenograft tumor model derived from sst-negative tumor cells [100].

The demonstrated ability of octreotide to reduce gastrointestinal toxicity associated with some cytotoxic agents [101] together with experimental evidence of antineoplastic activity, make somatostatin analogs attractive candidates for further trials in various cancers.

**other applications**

Radiolabeled somatostatin analogs are useful diagnostic tools (somatostatin receptor scintigraphy), to detect and localize small sst-expressing tumors, with particular utility in pinpointing primary and metastatic endocrine tumors. Because of the toxicities associated with targeted radiotherapy, a parallel field of development is focusing on the use of radiolabeled somatostatin analogs to treat patients with sst-expressing tumors. In the specific indication of endocrine tumors, new compounds bearing energetic isotopes, such as yttrium 90 (OctreoTher®, registration pending) or lutetium (prospectively tested), are expected to provide effective therapy of tumors due to the deep penetration of the radiation. The yttrium-labeled compound DOTATOC was found to stabilize disease in 60% of patients, with objective responses in 20% of patients [102, 103].

Finally, novel preclinical strategies based on sst2-receptor gene transfer, have resulted in a significant inhibition of tumor growth in vivo due to the anti-oncogenic properties of the sst2
gene in models for pancreatic cancer [104]. The antitumor effect of in vivo gene transfer may also be enhanced by systemic chemotherapy with targeted cytotoxic analogs or with the administration of radionuclide analogs [59, 105].

conclusion

Data from preclinical studies provide evidence for a number of direct and indirect mechanisms by which somatostatin analogs can exert antitumor effects. Direct antitumor effects require the tumor to express somatostatin receptors. Indirect antitumor activity does not require the presence of somatostatin receptors in tumor cells. Somatostatin analogs exert indirect antitumor activity by several mechanisms, such as inhibiting growth factor and hormone synthesis and angiogenesis. These findings, together with the good safety and tolerability profile that somatostatin analogs have demonstrated in clinical use, suggest their value in cancer treatment regimens. To date, octreotide is the best characterized, however, more preclinical and clinical studies are required to fully understand the antitumor activity of new somatostatin analogs. In addition, further research efforts are under way to develop somatostatin analogs, with activity across the range of SST subtypes, with the aim of maximizing the potential of this approach in antineoplastic therapy.

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