Somatostatin Receptors in Human Neuroendocrine Tumors

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Peptide Receptors and NETs

Since the late twentieth century, somatostatin analogs (SSAs) have been used as a form of targeted therapies to control symptoms of hormonal hypersecretion by functional NETs. These agents are still an important treatment modality for NETs today. In recent years somatostatin receptors (SSTRs) have become a subject of active investigation as important targets for diagnosis and treatment of NETs.

Somatostatin and Somatostatin Receptors

Somatostatin (SST) is an endogenous cyclic peptide that regulates the secretion of various hormones by endocrine cells through its binding with a family of G protein-coupled transmembrane receptors, including five distinct subtypes (SSTRs 1–5) [1, 2]. SST also has a potent and broad antisecretory action, which makes it an invaluable drug target for the pharmacological management of NETs [3]. Somatostatin has a very short half-life (~3–4 min) [2], which limits its therapeutic efficacy in clinical setting. However, synthetic somatostatin analogs (SSAs) such as octreotide and lanreotide have high affinity for SSTR 2 and SSTR 5 and are without the undesirable effects of

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somatostatin [2, 4], supporting their efficacy in clinical setting. Furthermore, the ability of SSTRs to internalize and the development of radiolabeled somatostatin analogs have further contributed to improved diagnosis and treatment of NETs [3].

SSTR Signaling and Downstream Effects

SSTRs 1, 2, 3, and 5 mediate their antiproliferative actions and SSTR 4 its pro-proliferative action through unique signaling pathways [3]. The antiproliferative effect of SSTRs is mediated by either inhibiting mitogenesis or stimulating apoptosis [5]. In fact, SSTRs 1, 2, 4, and 5 can induce G1 cell cycle growth arrest, while SSTR 3 is pro-apoptotic via the induction of p53 and BAX [6, 7].

Activation of SSTRs and Effects on NET Cells

Activation of SSTRs has a number of direct and indirect effects on NET cells [3, 8]. Direct antiproliferative effects of SSTR activation include inhibition of cell cycle and growth factor effects and induction of apoptosis (Fig. 1), which may be mediated by the PI3K/mTOR, MAPK, and Ras/ERK signaling pathways [9, 10]. Indirect effects of SSTR activation include inhibition of the release of growth factors and trophic hormones, inhibition of angiogenesis, and modulation of the immune system (Fig. 1) [3, 8].

Investigating the function of each SSTR in various human tumor types has provided insightful information on the role of signaling pathways that can suppress tumor cell proliferation, survival, and angiogenesis [3]. This has also provided the rationale for developing multi-SSTR-targeted somatostatin analogs and combination therapies with various targeted agents like inhibitors of the mammalian target of rapamycin (mTOR) and dopamine receptors [3].

Expression of SSTRs in Human NET and Normal Tissues

Somatostatin receptors (SSTRs 1, 2A and 2B, 3, 4, and 5) belong to the G protein-coupled receptor family and have shown a wide expression pattern in both normal human tissues and solid tumors [3]. Specifically, these various SSTR subtypes (SSTRs 1, 2, 3, 4, 5) have been identified in human NETs and other tumors (Table 1) and their metastases, using a variety of techniques including autoradiography [11, 12], reverse transcriptase polymerase chain reaction (rt-PCR), and immunohistochemistry (IHC) [13–15].

Although somatostatin receptor incidence and density reported in various tumors depend on the methodology used, however, the majority of NETs express SSTRs in high density (Table 1) [16]. These tumors include pituitary adenomas (in particular GH- and TSH-producing adenomas), GEP and lung NETs, pheochromocytomas, and paragangliomas (Reubi 2003). Tumors of the nervous system, such

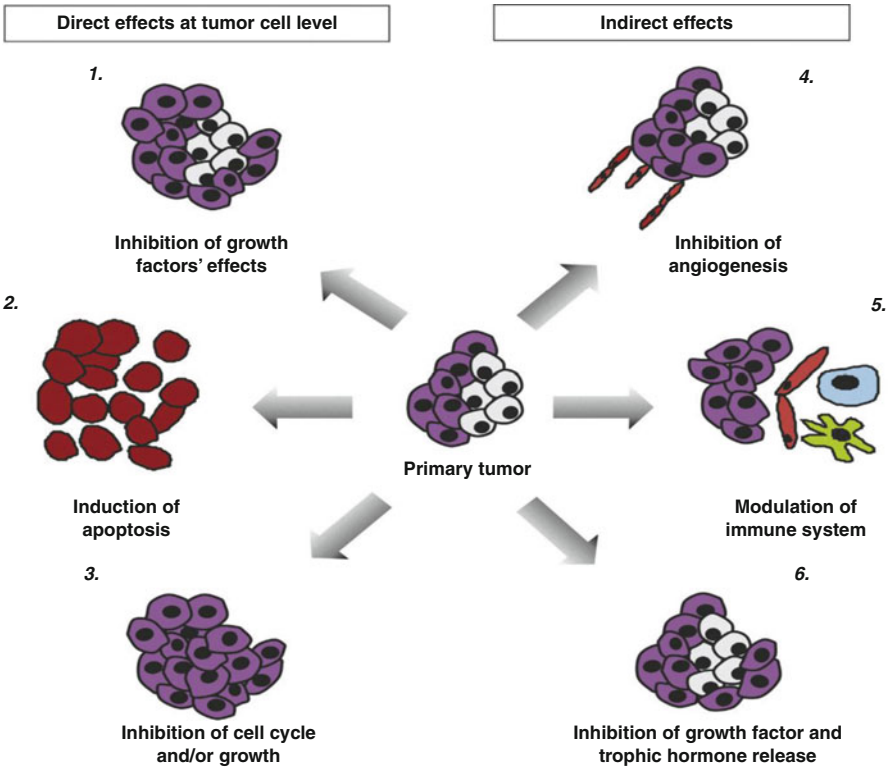

Fig. 1 Antiproliferative effect of somatostatin analogs on tumor cells. Somatostatin and its analogs may induce tumor shrinkage through direct action on the tumor cell [(*1*) inhibitory cross-talk of the SSTR signaling to the signaling induced by autocrine growth factors in the tumor cells, as well as inhibition of autocrine growth factor secretion from tumor cells; (*2*) cytostatic signaling mediated by the SSTR; (3) cytotoxic action of SSTR (e.g., by induction of apoptosis)] or indirectly by acting on components of the tumor microenvironment [(*4*) blocking of neovascularization; (*5*) inhibition of the secretion of tumor-promoting signals from immune cells; (*6*) blocking of the secretion of paracrine growth factor)]. Tumor cells are shown in fuchsia, vascular endothelial cells in red, immune cells in green and blue, and apoptotic cells in brown (Reproduced with permission from Theodoropoulou and Stalla [3])

Table 1 NETs expressing somatostatin receptors

Tumors with predominance of sst_2	Tumors with predominance of other ssts (with or without sst_2)
Pituitary adenomas (GH, TSH)	Selected GH Pituitary adenomas (sst_5; sst_2 + sst_5) ACTH pituitary adenomas (sst_5) Inactive pituitary adenomas (sst_3)
GEP NETs	Selected GEP NETs (insulinomas: sst_1, sst_5, with or without sst_2)
Lung NETs	Selected lung NETs
Pheochromocytomas	Medullary thyroid carcinomas
Paragangliomas	

as medulloblastomas, meningiomas, and neuroblastomas, also express somatostatin receptors at high density [16]. Furthermore, non-neural and non-NET tumors like breast, small cell lung, hepatic, renal, and gastric cancers and lymphoma also can express SSTRs with a lower incidence and/or density than NETs [16].

More recently, SSTR subtyping is being viewed as putative biomarkers of response of human NETs to somatostatin analog therapy [3]. This makes analytical and further clinical validation of SSTR subtyping methodologies an important goal for pathologists, who are engaged in supporting sub-specialty diagnostic and theranostic services in neuroendocrine pathology – at least at major referral centers.

Because of the pathobiological and clinical relevance of SSTRs, a large number of studies have investigated the expression and distribution of SSTRs in human NET tissues [13, 15, 17–19]. The distribution of SSTRs is widespread in GEP-NETs, with an overall prevalence of 50–100 % and frequent co-expression of multiple SSTRs in a given tumor [13, 15, 17–19]. Interestingly, the expression of SSTRs varies among various histological types of NETs and also among patients with the same tumor type. SSTR 2 and SSTR 5 are expressed in about 90 % and 80 % of pancreatic NET cells, respectively, making them potentially sensitive to hormone treatment [20].

In our experience, SSTR 2 was the most frequently expressed subtype in the hepatic metastases of NECs of the small intestine and pancreas, while SSTR 1 was the least commonly expressed SSTR (Fig. 2) [15]. In other studies, SSTR 2 has been shown to be absent or very low in insulinomas compared to nonfunctioning PNETs [16, 21]. Such differential expression of various SSTRs in primary and metastatic NETs/NECs further substantiates the relevance of implementation of reliable methodologies for SSTR subtyping as a potential biomarker approach to predict response to NETs/NECs to various somatostatin analog therapies.

SSTR Expression Correlates with the Pathology of Human NETs

In human NETs, the expression of SSTRs correlates with the degree of endocrine differentiation, lower histopathologic tumor grades [12], and clinical response to somatostatin analog (octreotide) therapy [22]. While the majority of well-differentiated NETs (Fig. 3) and islet cell carcinomas are SSTR-positive and respond favorably to somatostatin analog therapy, the poorly differentiated ETs are usually SSTR-negative (Figs. 4 and 5) [12] and rarely respond to somatostatin analog therapy.

Detection of SSTRs by Imaging

Overall, the most sensitive imaging modality for the detection of metastatic disease in NETs is SSTR scintigraphy (OctreoScan) [24]. This imaging technique also allows for noninvasive determination of the presence of SSTRs using radiolabeled octreotide (pentetreotide) [25]. Therefore, it is widely used for predicting the response of SSTR-positive NETs to somatostatin analogs. However, it does not identify expression of various SSTR subtypes in a given case of NET.

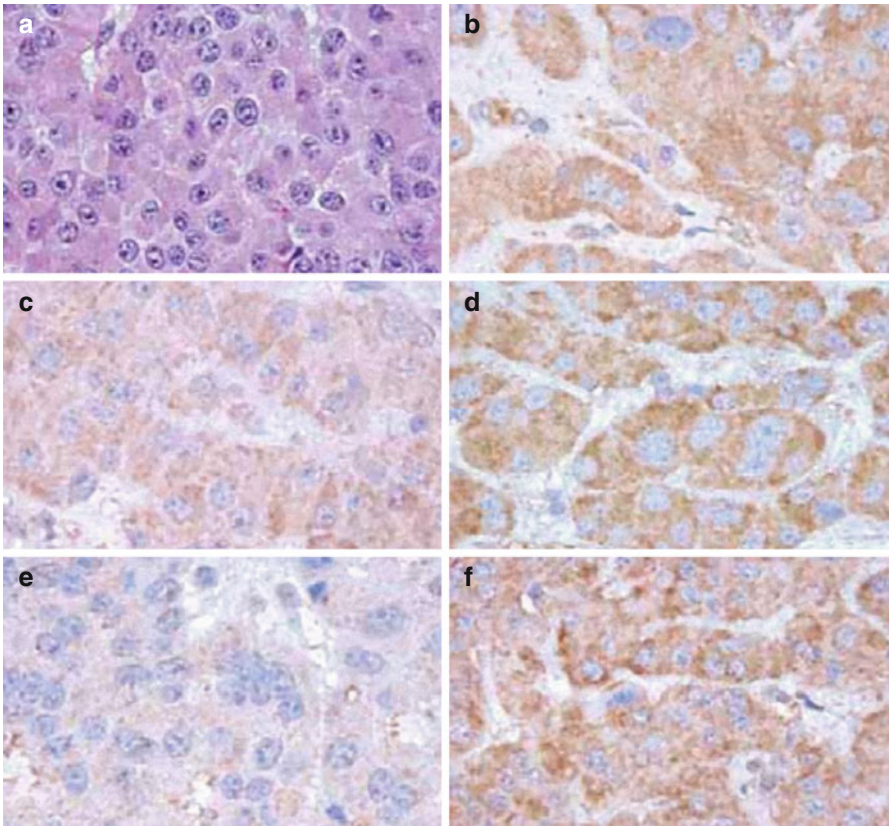

Fig. 2 (**a**) Moderately differentiated ECA of the pancreas; paraffin section from the primary pancreatic ECA (hematoxylin-eosin, ×630). (**b**–**f**) Paraffin sections from the primary pancreatic ECA featuring 2+, 1+, 2+, negative, and 2+ expression of SSTRs 1, 2, 3, 4, and 5, respectively (immunoperoxidase staining for SSTR subtypes 1–5, ×630) (Reproduced with permission from Nasir et al. [15])

SSTR Expression on NET Tissues and Response to Somatostatin Analogs

The expression of SSTRs on NET cells forms the basis for somatostatin analog treatment of patients with SSTR-positive NETs [26]. In malignant NETs, the presence of SSTRs has been shown to predict favorable clinical response to somatostatin analog (octreotide) therapy [22]. Therefore, SSTR subtyping is regarded as putative biomarkers of somatostatin analog response [3]. While SSTR subtyping may be useful in predicting favorable clinical response of many different types of NETs to SSA therapy, some clinical subsets of NETs may specifically benefit from SSTR subtyping on tumor tissues: These include [1] *SSTR-negative gastroenteropancreatic (GEP) NETs*, in which clinical response to somatostatin analog therapy is generally absent or suboptimal [2]; *nonfunctioning GEP-NETs*, in which role of octreotide therapy is controversial; and [3] *OctreoScan-positive GEP-NETs*, which

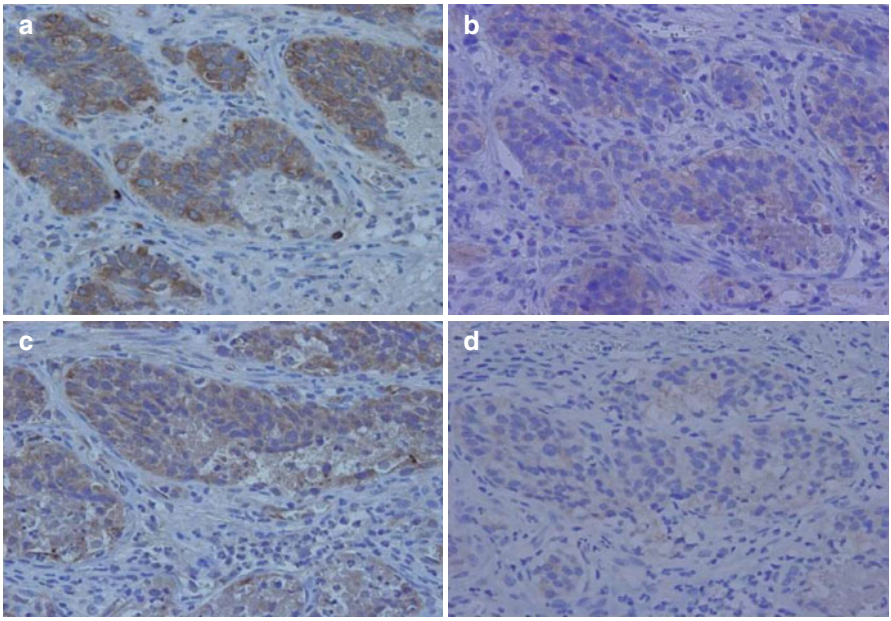

Fig. 3 Expression of SSTRs 1, 2, 3, and 5 in NET G1 (carcinoid tumor) of the appendix vermiformis. (**a**) SSTR 1, diffuse and intense positive staining identified mostly in cytoplasm of the tumor cells. (**b**) SSTR 2, weak but positive staining seen along the plasma membrane; (**c**) SSTR 3, diffuse cytoplasmic staining present; d) SSTR 5, very weak, but membranous staining seen along the plasma membrane. Original magnification ×400 (**a**–**d**) (Reproduced with permission from Mizutani et al. [23])

may show a variable clinical response to somatostatin analog treatment. For future studies, systematic analyses of SSTR status of NETs, based on imaging and tissue-based assays, will be an important consideration.

Clinical Usefulness of Somatostatin Analog Monotherapy and Combination

Treatment with radiolabeled somatostatin analogs is effective in the management of patients with inoperable or metastasized NETs [27]. Such therapy results in reduced hormonal overproduction and symptomatic relief in most of the NET patients, although it is seldom successful in reducing the tumor size [28]. Specifically, in patients with metastatic carcinoids, octreotide and lanreotide have shown biochemical response in 40–50 % cases, with temporary stabilization of tumor growth in more than 80 % and tumor regression in less than 10 % patients [29, 30]. More recently, co-targeting of SSTRs 1, 2, 3, and 5 (with SSA therapy) and dopamine receptor type 2 (D2DR) has been shown to yield potent therapeutic outcomes [3]. Furthermore, SSTR 2 targeting sensitizes NET cells to antitumor activity of mTOR inhibitors [3].

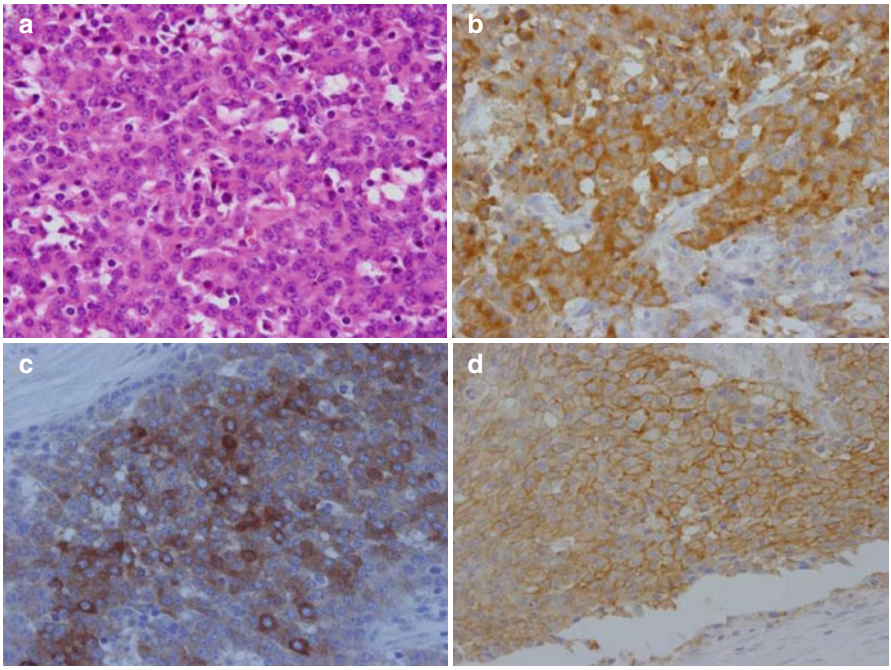

Fig. 4 Expression of SSTRs 1 and 2 in NEC G3 (neuroendocrine carcinoma) of the stomach. (**a**) H-E, rather solid growth of poorly differentiated tumor cells. (**b**) Synaptophysin: diffuse and intense cytoplasmic positive staining seen in tumor cells. (**c**) SSTR 1, diffuse cytoplasmic positivity seen in tumor cells. (**d**) SSTR 2, intense membranous staining along the plasma membrane. Original magnification ×400 (**a**–**d**) (Reproduced with permission from Mizutani et al. [23])

Other Peptide Receptors

With the clinical success of SSTRs in the management of NETs, patients have led to increased interest in other peptide receptors, including receptors for bombesin, cholecystokinin, and vasoactive intestinal peptide (VIP) in some types of pancreatic NETs [31–34].

Future Directions

In recent years, with the availability of newer subtype-specific ligands such as pasireotide (SOM-230) with selective affinity for various SSTR subtypes, compared to the older SSAs (like octreotide and lanreotide), it is becoming increasingly important to determine the relative expression of various SSTR subtypes in order to select the most relevant SSA(s) for optimal therapeutic effect in a given NET patient. Such a personalized approach will allow improved patient selection for SSA therapy, based on expression of various SSTRs in the patient's own GEP-NET tissues, and

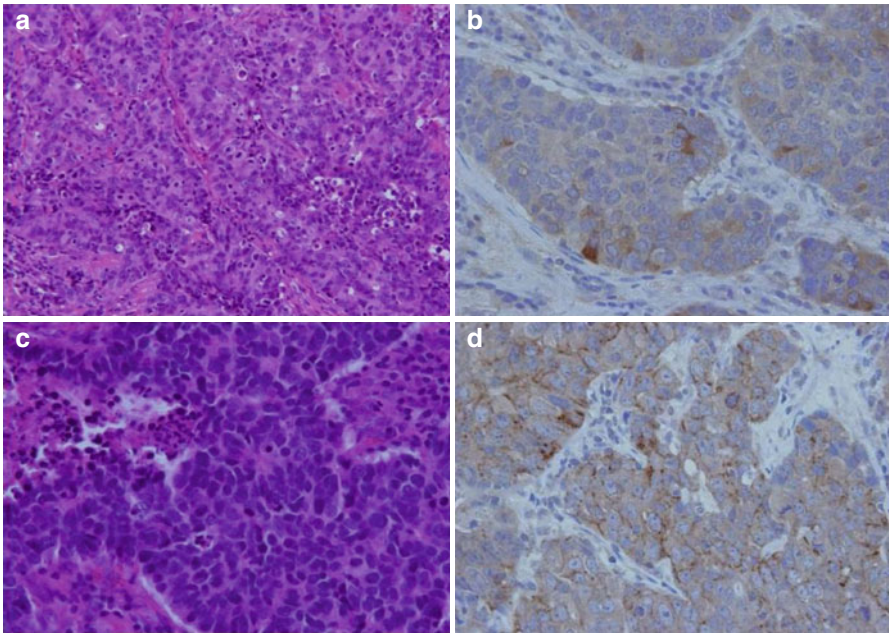

Fig. 5 Expression of SSTR 1 in two different NEC G3 (large cell neuroendocrine carcinoma; LCNEC) of lung. (**a**) H-E, rather solid growth of poorly differentiated tumor cells with incomplete peripheral nuclear palisading. (**b**) SSTR 1; in this case cytoplasmic positive staining seen. (**c**) H-E, another case of LCNEC. (**d**) Intense membranous and occasional cytoplasmic positivity identified in the tumor cells. Original magnification ×200 (**a**), ×400 (**b**–**d**) (Reproduced with permission from Mizutani et al. [23])

may contribute to higher clinical response rates for various SSA therapies in such patients.

Overall, lower grade NETs of the GI tract and pancreas have higher levels of various SSTRs, while PD-NECs tend to have infrequent and lower levels of SSTR expression in the tumor tissues. With the availability of newer somatostatin analogs such as pasireotide (SOM230), the determination of differential expression of various SSTR subtypes, and an assessment of heterogeneity of such expression in larger series of primary and metastatic GEP-NECs, is clinically relevant. Furthermore, SSTR-subtype expression should be correlated with the pattern of clinical response of the treated patients to somatostatin analog therapies. Based on our own experience with SSTR IHC, we believe that SSTR subtyping is feasible on formalin-fixed NET tissues. These findings merit additional SSTR-subtype analyses on larger series of patients with endocrine neoplasms. The predominance of cytoplasmic expression of various SSTR subtypes in our experience is best explained by prior Sandostatin therapy in our patients, a rational basis to explain internalization of the SSTRs.

Abbreviations

BAX	Gene promoter
ECA	Endocrine carcinoma
ERK	Extracellular regulated kinase
ET	Endocrine tumor
GEP-NET	Gastroenteropancreatic neuroendocrine tumor
G1	Growth phase 1
IHC	Immunohistochemistry
MAPK	Mitogen-activated protein kinase
mTOR	Mechanistic target of rapamycin (aka mammalian target of rapamycin)
NEC	Neuroendocrine carcinoma
NEC G3	Neuroendocrine carcinoma, grade 3
NET	Neuroendocrine tumor
NET G1	Neuroendocrine tumor, grade 1
PI3K	Phosphoinositide 3-kinase
p53	Gene promoter
rt-PCR	Reverse transcription polymerase chain reaction
SOM-230	Pasireotide (A newer somatostatin analog)
SSA	Somatostatin analog
SSTR	Somatostatin receptor
SSTR 2	Somatostatin receptor 2
SST	Somatostatin
VIP	Vasoactive intestinal peptide

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