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Association Between Somatostatin Receptor Expression and Clinical Outcomes in Neuroendocrine Tumors

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Use of standardized official symbols: We use HUGO (Human Genome Organisation)-approved official symbols for genes and gene products, including MKI67, SST, SSTR1, SSTR2, SSTR3, SSTR4 and SSTR5; all of which are described at www.genenames.org. Gene names are italicized, and gene product names are non-italicized.

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Abstract

Objective—Somatostatin receptors (SSTRs), products of gene superfamily *SSTR1-5,* are commonly expressed in neuroendocrine tumors (NETs). Somatostatin analogs (SSAs) bind to SSTRs and are used as therapeutic agents in patients with advanced NETs. We hypothesized that tumor SSTR expression status would be associated with clinical outcomes in NET.

Methods—Expression of the five SSTRs was evaluated by immunohistochemistry, using tissue microarrays comprising 173 primary NETs, 24 matched metastases, and 22 metastatic NETs from 195 patients. Cox proportional hazards regression analysis was used to assess the association of SSTR expression status (high vs. low) with clinical outcomes, adjusting for potential confounders.

Results—High expression of SSTR2 was associated with longer overall survival in the cohort overall (multivariate hazard ratio 0.42, 95% confidence interval 0.21–0.84; $P = 0.013$). In a subgroup of patients with metastatic small intestine NET treated with SSAs and evaluable for progression, SSTR2 expression was associated with both longer progression-free and overall survival. No associations with progression-free or overall survival were observed with expression of other SSTRs.

Conclusions—Our study demonstrated that expression of SSTR2, but not other SSTRs, is associated with longer overall survival. In patients treated with SSAs, expression of SSTR2 is associated with longer progression-free survival.

Keywords

somatostatin receptor; somatostatin analog; prognosis; progression-free survival and overall survival; carcinoid; neuroendocrine tumor

INTRODUCTION

Somatostatin (SST) was first described in 1968 as an inhibitor of hormone secretion.[1] The effects of somatostatin are mediated through interaction with five somatostatin receptor family gene products (*SSTR1* to *SSTR5*).[2,3] Somatostatin signaling pathways inhibit both

cell secretion and cell growth through a variety of signaling pathways, including modulation of mitogen-activated protein kinase (MAPK) signaling and induction of apoptosis.[2–4] SSTRs are commonly expressed on neuroendocrine tumors (NETs), and somatostatin analogs (SSAs) have long been used to control secretory symptoms in such patients.[3–8] Recent studies have demonstrated that SSAs also inhibit tumor growth, both in vitro and in the clinical setting.[3,9–20]

Octreotide and lanreotide, two synthetic SSAs, bind primarily to SSTR2 and SSTR5.[5,9,10,21] Several prior studies have examined associations between expression of somatostatin receptors and prognosis, though most studies have focused primarily on SSTR2.[6–8,22] In a study evaluating 79 pancreatic NETs for expression of SSTR2, high expression of SSTR2 was associated with a favorable prognosis.[22] Other studies including a range of gastroenteropancreatic NET have similarly found that SSTR2 expression is associated with favorable outcomes.[7,8]

To optimize and expand the clinical applications of SSAs, newer multi-targeted somatostatin analogs have been developed. These analogs bind to several SSTR subtypes, and include SOM230 (pasireotide), with high affinity for SSTR2, SSTR3 and SSTR5 (but lower affinity for SSTR1), and KE108, with high affinity for all five SSTRs.[2,3] Because of the development of new SSAs, as well as radiopeptides that target multiple SSTR subtypes,[2,3] assessment of SSTR subtypes has become increasingly relevant. Comprehensive studies evaluating expression patterns of SSTR1 to SSTR5 in neuroendocrine tumors are limited; and the SSTR subtypes most critical in mediating a cytostatic effect remains unclear.[2,3]

To help address these questions, we used immunohistochemical techniques to comprehensively evaluate the expression of SSTR1 to SSTR5 in a large cohort of NETs, comprising small intestinal NET, pancreatic NET, and other NET subtypes. We further evaluated co-expression of SSTR subtypes. Finally, we evaluated whether SSTR subtype expression was associated with survival, both in the cohort overall and in a highly characterized cohort of patients with small intestinal NET that had been treated with SSAs and followed for both progression-free survival and overall survival.

MATERIALS AND METHODS

Study Population

Tissue blocks were obtained from patients with a confirmed diagnosis of NET, from 1991 to 2009, recruited to an IRB-approved study at Dana-Farber Cancer Institute. Additional IRB approval was obtained for the molecular analysis of tumor blocks and correlation with clinical variables performed in this study. Demographic and clinical information was obtained from medical records; the American Joint Committee on Cancer TNM classification system was used for staging. If not available in the medical record, survival data was obtained from the Social Security Death Index.

Tissue Microarray (TMA) Construction

Tissue microarrays were constructed from formalin-fixed, paraffin-embedded tissue comprising 216 resection specimens and 4 liver wedge biopsies using a tissue-array

instrument (Beecher Instruments, Silver Spring, MD). Three representative 0.6-mm-diameter tissue cores were taken from each specimen. Two TMA blocks were designed containing 145 and 75 samples, respectively, from a total of 195 patients. Multiple 4-μm sections were cut and transferred to adhesive-coated slides for immunohistochemical staining.

Immunohistochemistry

Anti-SSTR1, anti-SSTR2, anti-SSTR3, and anti-SSTR5 antibodies were obtained from Epitomics Inc, Burlingame. (Cambridge, MA, USA). Anti-SSTR4 was obtained from Cell Signaling Technology (Millipore, MA, USA). Anti-MKI67 mouse monoclonal antibody was obtained from DakoCytomatin (Glostrup, Denmark).

Immunohistochemistry was based on the labeled streptavidin-biotin method, and was performed on TMA sections for SSTRs and full sections for MKI67. After deparaffinization and antigen retrieval using an autoclave oven technique, sections were incubated overnight at 4°C with antibodies : anti-SSTR1 (1:200, rabbit monoclonal, clone UMB-7, ab137083), anti- SSTR2 (1:400, rabbit monoclonal, clone UMB-1, ab134152), anti-SSTR3 (1:100, rabbit monoclonal, clone UMB-5, ab137026), anti-SSTR4 (1:400, rabbit polyclonal, AB9487)[23,24], anti-SSTR5 (1:100, rabbit monoclonal, clone UMB-4, ab109495) and anti-MKI67 antibody (1:100, mouse monoclonal, clone JC70A). Antigen–antibody complexes were detected using the cobalt-3, 3′-diaminobenzidine (Co-DAB) reaction. The antibodies used for SSTRs 1, 2, 3, and 5 in this study have been previously used and validated.[25,26] In our studies, normal pancreatic islet cells, which are known to be positive for SSTRs 1-5, were used as positive controls.[27,28] We used omission of the primary antibody as one method of negative control for SSTRs 1-5. Additionally SSTR2 peptide (ab171899, Abcam, Cambridge, Mass.) and SSTR5 peptide (ab178801 Abcam) were available to absorb the respective antibodies and were used as negative controls. Antibodies for SSTR4 have been less well studied. To further validate anti-SSTR4, we used a CHO-SSTR4 cell line that overexpressed SSTR4 as a positive control, using previously described techniques,[29,30] and used normal CHO cells as negative controls.

The MKI67 (Ki-67) labeling index was determined by counting the number of positive cells in a total of up to 2000 tumor cells observed within areas of highest immunostaining by high-power fields (× 400). Membranous expression of SSTR2, and the assessed-combined expression of cytoplasmic and membrane staining of the receptor proteins SSTR1, SSTR3, SSTR4, and SSTR5 were scored by applying a semi-quantitative immunohistochemistry scoring (IHCS) system, as previously described.[26,31–33] In brief, staining intensity was scored as 0 (no immunostaining), 1 (weak), 2 (moderate) or 3 (strong). The percentage of immunoreactive cells was scored as 0 (none), 1 (< 10%), 2 (10–50%), 3 (51–80%) or 4 (> 80%). Multiplication of the staining intensity score and the percent immunoreactivity score resulted in an IHCS score, which ranged from 0 to 12 for each tissue core. The overall IHCS score for each case was calculated by averaging the IHCS scores in three tissue cores. The median IHCS score for each marker, based on analysis of all cases in the cohort, was used as the cutoff to define high vs. low expression. High IHCS scores were as follows: ≥4 for SSTR1, ≥6 for SSTR2, ≥4 for SSTR3, ≥6 for SSTR4, and ≥8 for SSTR5. To

independently confirm the scores, a random selection of 70 cases was examined for each marker by a second blinded observer (YM). Concordance scores (all $p < 0.0001$) were: $\kappa = 0.74$ (SSTR1), $\kappa = 0.73$ (SSTR2), $\kappa = 0.72$ (SSTR3), $\kappa = 0.84$ (SSTR4), and $\kappa = 0.78$ (SSTR5).

Statistical analysis

Overall survival was calculated from the date of initial diagnosis to the date of death. Overall survival from time of metastatic disease was calculated from the date of diagnosis distant metastasis until death. Progression-free survival (PFS) was defined as period from when the patient initiated treatment to radiologic or clinical progression of disease, next treatment, last follow-up or death. SAS software (version 9.2; SAS Institute Inc, Cary, North Carolina) was used. All *P* values were two sided at alpha = 0.05. We categorized protein expression level as low *vs.* high. The Chi-Square test was used to assess associations between the categorized expression levels, the tumor subgroups, and MKI67 (Ki-67) labeling index status. The Spearman rank order correlation was used for the pairwise correlation analyses of expression between proteins. To compare mean age, an ANOVA, assuming equal variances, was performed. The Kaplan-Meier method was used to compute survival probabilities and comparisons were assessed with the log-rank test. Cox proportional hazards regression models were used to compute mortality hazard ratios (HR) and 95% confidence intervals (CI). Multivariate Cox proportional hazards regression models included sex, age at diagnosis, TNM stage, and MKI67 labeling index. A backward elimination with threshold of $P = 0.05$ was used to select variables in the final models.

RESULTS

Clinicopathologic characteristics

We evaluated primary neuroendocrine tumor (NET) samples from 173 patients, together with 24 matched metastases. Patient demographics are described in Table 1, and included 112 patients with small intestinal NETs, 19 patients with pancreatic NETs, and 42 patients with other NETs derived from other sites. Approximately half (50%) of the cohort had localized disease (Stages I–III); the remainder had advanced metastatic disease (Table 1). All tumors were well differentiated; 55% had MKI67 (Ki-67) labeling index ≤2; and 45% had a MKI67 (Ki-67) labeling index > 2%.

Expression of SSTRs in NET according to tumor subtype

Expression (any level) of SSTR1, SSTR2, SSTR3, SSTR4 and SSTR5 was detected in 65%, 76%, 90%, 86%, and 93% of 173 primary NETs, respectively (Figure 1A–C). The frequency of expression according to tumor primary location is shown in Figure 2A. We further categorized expression of SSTRs as either high or low based on the scoring system described in the methods section. High expression of SSTR1, SSTR2, SSTR3, SSTR4 and SSTR5 was detected in 47%, 51%, 23%, 36%, and 43% NETs, respectively. Expression levels of SSTRs varied according to both receptor subtype and tumor subtype. High expression of both SSTR1 and SSTR2 were frequently observed in pancreatic NET (Figure 2B). High SSTR2 expression was observed in >50% of both pancreatic NET and small intestine NET (Figure 2B), whereas high expression of SSTR3, SSTR4, and SSTR5 was

uncommon and observed in less than half of NETs, regardless of tumor subtype. We found no major differences in patterns of SSTR expression between primary tumors and metastases, nor did we find differences in SSTR expression patterns based on clinical characteristics such as sex, age and TNM stage.

Association between expression of SSTRs and MKI67 (Ki-67) labeling index

All NETs in our cohort were classified as well differentiated [MKI67 (Ki-67) labeling index <20%]. To examine whether SSTR expression was associated with differences in MKI67 (Ki-67) index within this group, we categorized tumors according to whether the proliferative index was less than or greater than 2%. We found that high expression of SSTR2 and SSTR3 was more common in tumors with low MKI67 (Ki-67) labeling index [59% and 29% in 95 MKI67 (Ki-67) labeling index ≤2% vs. 42% and 15% in 78 MKI67 (Ki-67) labeling index > 2%] (Chi-square: $P = 0.029$ and $P = 0.028$; respectively).

Correlations between expression of SSTR subtypes

We further identified correlations between expressions of SSTR subtypes. Co-expression of SSTR1, SSTR2, SSTR3, and SSTR5 was common; whereas expression of SSTR4 appeared to be independent of expression of the other receptor subtypes (Table 2).

Association between expression of SSTRs and somatostatin receptor scintigraphy

Eighty-one patients in our study had tumors that were evaluated with somatostatin scintigraphy (Octreoscan). Of these patients, 74 (91%) had tumors that demonstrated uptake, and 7 (9%) had no evidence of uptake. We did not observe any clear correlations between high vs. low expression of SSTRs 1-5 and uptake on somatostatin scintigraphy, nor did we observe any correlations when we classified expression of SSTRs as either present or absent.

Association between expression of SSTRs and clinical outcomes

To evaluate associations between SSTR expression and clinical outcomes, we first assessed whether expression of SSTRs was associated with overall survival in our overall cohort. Among 173 cases with primary tumors available for evaluation, there were 40 deaths with median follow up time of 5.5 years. High expression of SSTR2 was associated with improved overall survival [multivariate HR, 0.42; 95% confidence interval (CI), 0.21 to 0.84; $P = 0.013$] (Table 3). We additionally assessed associations between SSTR expression and overall survival from time of metastatic disease. Among 103 patients who had metastatic disease either at diagnosis or during follow up, there were 38 deaths with median follow up time of 5.6 years. In a multivariate analysis, expression of SSTR2 was also associated with favorable overall survival from time of metastatic disease (HR, 0.46; 95% CI 0.23 to 0.92; $P = 0.027$). Expression of other four SSTRs was not significantly associated with overall survival or overall survival from time of metastatic disease.

We evaluated associations between SSTR2 expression and survival in each subgroup of patients, those with small intestinal NETs, pancreatic NETs and other NETs, respectively, adjusting for age, gender, tumor stage and MKI67 (Ki-67) labeling index. We found that SSTR2 was most strongly associated with survival in patients with small intestine NETs [multivariate HR, 0.45; 95% CI, 0.20 to 0.97; $P = 0.042$; Log-rank $P = 0.02$] (Table 4, Figure

3A); although a trend toward improved survival in patients with pancreatic NETs was also evident (Table 4). No clear association between survival and SSTR2 expression was observed in patients with other NET subtypes (Table 4).

SSTR Expression, PFS, and OS in small intestine NET treated with SSAs

The reason for the association between SSTR2 expression and outcome in our patient population is uncertain; however, it is known that currently available SSAs target primarily SSTR2. We explored whether expression of SSTR2 or other SSTRs was associated with PFS in NET patients treated with SSA in a highly characterized cohort of patients who had been treated with single-agent SSAs and were evaluable for progression-free survival. To minimize patient heterogeneity, we focused on the group of 54 patients with small intestinal NETs who had received treatment with SSAs. We found that high expression of SSTR2 was associated with longer PFS (multivariate HR 0.40, $P = 0.012$; Log-rank $P = 0.023$; median PFS: 2.6 years for SSTR2-high *v* 1.3 years for SSTR2-low) (Table 5, Figure 3B). We confirmed that that high expression of SSTR2 was significantly associated with longer overall survival in this cohort as well (Table 5). There were no significant associations between PFS, overall survival, or expression of the other four SSTRs (Table 5).

DISCUSSION

Studies comprehensively investigating expression of all five SSTRs and correlating expression with clinical outcomes in NET are limited.[34,35] We found that expression, at some level, of all receptor subtypes is relatively common in NETs.[3,36,37] We further evaluated expression of SSTRs using an IHCS system that differentiates between high and low expression.[26,31–33] SSTR2 was highly expressed in 51% of cases, followed by SSTR1 (47%) and SSTR5 (43%); while the remaining SSTR4 (36%) and SSTR3 (23%) generally had low expression levels. High expression of SSTR1 and SSTR2 was more frequently observed in pancreatic NETs and small intestinal NETs than in other NETs. Our observations are consistent with previous, more limited studies demonstrating that expression of receptor subtypes tends to be similar between small intestine and pancreatic NET.[34,35,38]

We further observed evidence of co-expression of SSTR1, SSTR2, SSTR3 and SSTR5. Our observation of a high concordance between expression SSTR2, SSTR3 and SSTR5 is consistent with prior studies.[39,40] Interestingly, we found no correlation between expression of SSTR4 and other SSTRs, suggesting that SSTR4 may play different biologic role. Previous studies have reported that high expression of SSTR2 is correlated with lower MKI67 (Ki-67) labeling index in NETs, though many of these studies included both low and high grade tumors,[22,33,38,41–44] Our study was limited to well differentiated NETs. We found that, even within this group, expression of SSTR2 is inversely associated with MKI67 (Ki-67) labeling index. We did not observe any clear correlation between expression of SSTRs 1-5 and uptake on somatostatin scintigraphy (Octreoscan). While this observation is perhaps surprising, particularly with regard to SSTR2 which is known to strongly bind octreotide, the low number (9%) of negative scans in our patient cohort may have precluded our ability to detect this association.

Among the SSTR1 to SSTR5, we found that only SSTR2 expression was independently associated with overall survival. Several studies have evaluated the potential prognostic value of SSTR expression profiles in NET.[6–8,22] However, most have included a relatively small number of samples or examined a limited number of SSTR subtypes. Recently, one comparatively comprehensive study evaluated expression of SSTR1 to SSTR5, in 31 midgut and 55 pancreatic NETs. This study similarly found that expression of SSTR2, but not other SSTRs is associated with a favorable prognosis.[8] Our study confirms a key role for SSTR2 as a prognostic marker, and suggests that SSTR2 may play a significant role in mediating tumor growth.

First-generation SSAs (such as octreotide and lanreotide) exhibit high affinity for SSTR2 and lower affinity to SSTR3 and SSTR5.[21] Few studies have examined the potential predictive role of SSTR expression in SSA treatment response. Toboada et al. reported that elevated tumor expression of SSTR1, SSTR2, and dopamine receptor 2 may help improve responsiveness to SSA in somatotropinomas.[45] In other study of 21 advanced well-differentiated pancreatic neuroendocrine carcinoma treated with SSA, there was no significant association between expression of SSTR2 or SSTR5, and patient survival.[46]

Our study is unique in its ability to correlate SSTR expression with clinical outcomes in a large, highly characterized population of patients treated with somatostatin analogs, and our observation that high expression of SSTR2 was associated with both improved PFS and overall survival in patients treated with SSAs targeting primarily SSTR2, suggests that such an association may exist. Limitations of our study include our use of paraffin-embedded tissue and consequent reliance on immunohistochemistry rather than other, potentially more specific, techniques to assess expression of SSTRs 1-5. Additionally, due to the prevalence of SSA treatment in patients with NETs, we were unable to assess either PFS or overall survival in a non-treatment control group, limiting our ability to make firm conclusions regarding the association of SSTR expressions and treatment response to SSAs.

In conclusion, SSTR2 is commonly expressed in a range of neuroendocrine tumors. SSTRs co-expressed with SSTR2 include SSTR3, and SSTR5. Among the five SSTR subtypes, only expression of SSTR2 was associated with PFS in patients treated with SSAs, and with overall survival in patients overall. Further assessment of the mechanisms by which SSTR2 modulates tumor growth is warranted.

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References

1. Krulich L, Dhariwal AP, McCann SM. Stimulatory and inhibitory effects of purified hypothalamic extracts on growth hormone release from rat pituitary in vitro. Endocrinology. 1968; 83:783–790. [PubMed: 4879544]
2. Theodoropoulou M, Stalla GK. Somatostatin receptors: from signaling to clinical practice. Front Neuroendocrinol. 2013; 34:228–252. [PubMed: 23872332]
3. Reubi JC, Schonbrunn A. Illuminating somatostatin analog action at neuroendocrine tumor receptors. Trends Pharmacol Sci. 2013; 34:676–688. [PubMed: 24183675]
4. Florio T. Molecular mechanisms of the antiproliferative activity of somatostatin receptors (SSTRs) in neuroendocrine tumors. Front Biosci. 2008; 13:822–840.
5. Gatto F, Hofland LJ. The role of somatostatin and dopamine D2 receptors in endocrine tumors. Endocr Relat Cancer. 2011; 18:R233–251. [PubMed: 22135243]
6. Abdel-Rahman O, Lamarca A, Valle JW, et al. Somatostatin receptor expression in hepatocellular carcinoma: prognostic and therapeutic considerations. Endocr Relat Cancer. 2014; 21:R485–R493. [PubMed: 25336571]
7. Kim HS, Lee HS, Kim WH. Clinical significance of protein expression of cyclooxygenase-2 and somatostatin receptors in gastroenteropancreatic neuroendocrine tumors. Cancer Res Treat. 2011; 43:181–188. [PubMed: 22022296]
8. Pinato DJ, Tan TM, Toussi ST, et al. An expression signature of the angiogenic response in gastrointestinal neuroendocrine tumours: correlation with tumour phenotype and survival outcomes. Br J Cancer. 2014; 110:115–122. [PubMed: 24231952]
9. Rinke A, Muller HH, Schade-Brittinger C, et al. Placebo-controlled, double-blind, prospective, randomized study on the effect of octreotide LAR in the control of tumor growth in patients with metastatic neuroendocrine midgut tumors: a report from the PROMID Study Group. J Clin Oncol. 2009; 27:4656–4663. [PubMed: 19704057]
10. Caplin ME, Pavel M, Cwikla JB, et al. Lanreotide in metastatic enteropancreatic neuroendocrine tumors. N Engl J Med. 2014; 371:224–233. [PubMed: 25014687]
11. Arnold R, Rinke A, Klose KJ, et al. Octreotide versus octreotide plus interferon-alpha in endocrine gastroenteropancreatic tumors: a randomized trial. Clin Gastroenterol Hepatol. 2005; 3:761–771. [PubMed: 16234004]
12. Faiss S, Pape UF, Bohmig M, et al. 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. 2003; 21:2689–2696. [PubMed: 12860945]
13. Aparicio T, Ducreux M, Baudin E, et al. Antitumour activity of somatostatin analogues in progressive metastatic neuroendocrine tumours. Eur J Cancer. 2001; 37:1014–1019. [PubMed: 11334727]
14. Arnold R, Trautmann ME, Creutzfeldt W, et al. Somatostatin analogue octreotide and inhibition of tumour growth in metastatic endocrine gastroenteropancreatic tumours. Gut. 1996; 38:430–438. [PubMed: 8675099]
15. Ducreux M, Ruszniewski P, Chayvialle JA, et al. The antitumoral effect of the long-acting somatostatin analog lanreotide in neuroendocrine tumors. Am J Gastroenterol. 2000; 95:3276–3281. [PubMed: 11095353]
16. Palazzo M, Lombard-Bohas C, Cadiot G, et al. Ki67 proliferation index, hepatic tumor load, and pretreatment tumor growth predict the antitumoral efficacy of lanreotide in patients with malignant digestive neuroendocrine tumors. Eur J Gastroenterol Hepatol. 2013; 25:232–238. [PubMed: 23108416]
17. Toumpanakis C, Caplin ME. Update on the role of somatostatin analogs for the treatment of patients with gastroenteropancreatic neuroendocrine tumors. Semin Oncol. 2013; 40:56–68. [PubMed: 23391113]
18. Sideris L, Dube P, Rinke A. Antitumor effects of somatostatin analogs in neuroendocrine tumors. Oncologist. 2012; 17:747–755. [PubMed: 22628056]

19. Susini C, Buscail L. Rationale for the use of somatostatin analogs as antitumor agents. Ann Oncol. 2006; 17:1733–1742. [PubMed: 16801334]
20. Grozinsky-Glasberg S, Shimon I, Korbonits M, et al. Somatostatin analogues in the control of neuroendocrine tumours: efficacy and mechanisms. Endocr Relat Cancer. 2008; 15:701–720. [PubMed: 18524947]
21. Veenstra MJ, de Herder WW, Feelders RA, et al. Targeting the somatostatin receptor in pituitary and neuroendocrine tumors. Expert Opin Ther Targets. 2013; 17:1329–1343. [PubMed: 23991721]
22. Okuwaki K, Kida M, Mikami T, et al. Clinicopathologic characteristics of pancreatic neuroendocrine tumors and relation of somatostatin receptor type 2A to outcomes. Cancer. 2013; 119:4094–4102. [PubMed: 24022344]
23. Montironi R, Cheng L, Mazzucchelli R, et al. Immunohistochemical detection and localization of somatostatin receptor subtypes in prostate tissue from patients with bladder outlet obstruction. Cell Oncol. 2008; 30:473–482. [PubMed: 18936524]
24. Mazzucchelli R, Morichetti D, Santinelli A, et al. Immunohistochemical expression and localization of somatostatin receptor subtypes in androgen ablated prostate cancer. Cell Oncol (Dordr). 2011; 34:235–243. [PubMed: 21533650]
25. Casar-Borota O, Heck A, Schulz S, et al. Expression of SSTR2a, but not of SSTRs 1, 3, or 5 in somatotroph adenomas assessed by monoclonal antibodies was reduced by octreotide and correlated with the acute and long-term effects of octreotide. J Clin Endocrinol Metab. 2013; 98:E1730–1739. [PubMed: 24092823]
26. Specht E, Kaemmerer D, Sanger J, et al. Comparison of immunoreactive score, HER2/neu score and H score for the immunohistochemical evaluation of somatostatin receptors in bronchopulmonary neuroendocrine neoplasms. Histopathology. 2015
27. Kumar U, Sasi R, Suresh S, et al. Subtype-selective expression of the five somatostatin receptors (hSSTR1-5) in human pancreatic islet cells: a quantitative double-label immunohistochemical analysis. Diabetes. 1999; 48:77–85. [PubMed: 9892225]
28. Portela-Gomes GM, Stridsberg M, Grimelius L, et al. Expression of the five different somatostatin receptor subtypes in endocrine cells of the pancreas. Appl Immunohistochem Mol Morphol. 2000; 8:126–132. [PubMed: 10937060]
29. Xu Y, Song J, Berelowitz M, et al. Ligand Binding and Functional Properties of the Rat Somatostatin Receptor SSTR4 Stably Expressed in Chinese Hamster Ovary Cells. Mol Cell Neurosci. 1993; 4:245–249. [PubMed: 19912929]
30. Arena S, Pattarozzi A, Corsaro A, et al. Somatostatin receptor subtype-dependent regulation of nitric oxide release: involvement of different intracellular pathways. Mol Endocrinol. 2005; 19:255–267. [PubMed: 15388796]
31. Daniel K, Maria A, Amelie L, et al. Somatostatin receptor immunohistochemistry in neuroendocrine tumors: comparison between manual and automated evaluation. Int J Clin Exp Pathol. 2014; 7:4971–4980. [PubMed: 25197368]
32. Qian ZR, Ter-Minassian M, Chan JA, et al. Prognostic significance of MTOR pathway component expression in neuroendocrine tumors. J Clin Oncol. 2013; 31:3418–3425. [PubMed: 23980085]
33. Kaemmerer D, Specht E, Sanger J, et al. Somatostatin receptors in bronchopulmonary neuroendocrine neoplasms: new diagnostic, prognostic, and therapeutic markers. J Clin Endocrinol Metab. 2015; 100:831–840. [PubMed: 25494861]
34. Papotti M, Bongiovanni M, Volante M, et al. Expression of somatostatin receptor types 1-5 in 81 cases of gastrointestinal and pancreatic endocrine tumors. A correlative immunohistochemical and reverse-transcriptase polymerase chain reaction analysis. Virchows Arch. 2002; 440:461–475. [PubMed: 12021920]
35. Zamora V, Cabanne A, Salanova R, et al. Immunohistochemical expression of somatostatin receptors in digestive endocrine tumours. Dig Liver Dis. 2010; 42:220–225. [PubMed: 19819769]
36. Kulaksiz H, Eissele R, Rossler D, et al. Identification of somatostatin receptor subtypes 1, 2A, 3, and 5 in neuroendocrine tumours with subtype specific antibodies. Gut. 2002; 50:52–60. [PubMed: 11772967]

37. Oberg KE, Reubi JC, Kwekkeboom DJ, et al. Role of somatostatins in gastroenteropancreatic neuroendocrine tumor development and therapy. Gastroenterology. 2010; 139:742–753. 753 e741. [PubMed: 20637207]

38. Schmid HA, Lambertini C, van Vugt HH, et al. Monoclonal antibodies against the human somatostatin receptor subtypes 1–5: development and immunohistochemical application in neuroendocrine tumors. Neuroendocrinology. 2012; 95:232–247. [PubMed: 22156600]

39. Diakatou E, Alexandraki KI, Tsolakis AV, et al. Somatostatin and Dopamine receptor expression in Neuroendocrine Neoplasms: correlation of immunohistochemical findings with Somatostatin Receptor Scintigraphy visual scores. Clin Endocrinol (Oxf). 2015

40. Righi L, Volante M, Tavaglione V, et al. Somatostatin receptor tissue distribution in lung neuroendocrine tumours: a clinicopathologic and immunohistochemical study of 218 'clinically aggressive' cases. Ann Oncol. 2010; 21:548–555. [PubMed: 19759190]

41. O'Toole D, Saveanu A, Couvelard A, et al. The analysis of quantitative expression of somatostatin and dopamine receptors in gastro-entero-pancreatic tumours opens new therapeutic strategies. Eur J Endocrinol. 2006; 155:849–857. [PubMed: 17132755]

42. Nakayama Y, Wada R, Yajima N, et al. Profiling of somatostatin receptor subtype expression by quantitative PCR and correlation with clinicopathological features in pancreatic endocrine tumors. Pancreas. 2010; 39:1147–1154. [PubMed: 20717067]

43. Korner M, Waser B, Reubi JC. Does somatostatin or gastric inhibitory Peptide receptor expression correlate with tumor grade and stage in gut neuroendocrine tumors? Neuroendocrinology. 2015; 101:45–57. [PubMed: 25591947]

44. Srirajaskanthan R, Watkins J, Marelli L, et al. Expression of somatostatin and dopamine 2 receptors in neuroendocrine tumours and the potential role for new biotherapies. Neuroendocrinology. 2009; 89:308–314. [PubMed: 19307732]

45. Taboada GF, Neto LV, Luque RM, et al. Impact of gsp oncogene on the mRNA content for somatostatin and dopamine receptors in human somatotropinomas. Neuroendocrinology. 2011; 93:40–47. [PubMed: 21079388]

46. Butturini G, Bettini R, Missiaglia E, et al. Predictive factors of efficacy of the somatostatin analogue octreotide as first line therapy for advanced pancreatic endocrine carcinoma. Endocr Relat Cancer. 2006; 13:1213–1221. [PubMed: 17158766]

FIGURE 1 A

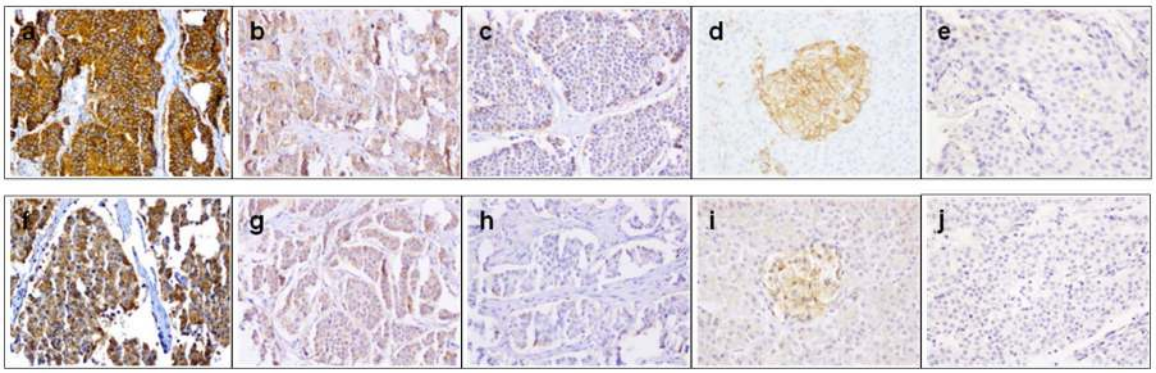

FIGURE 1 B

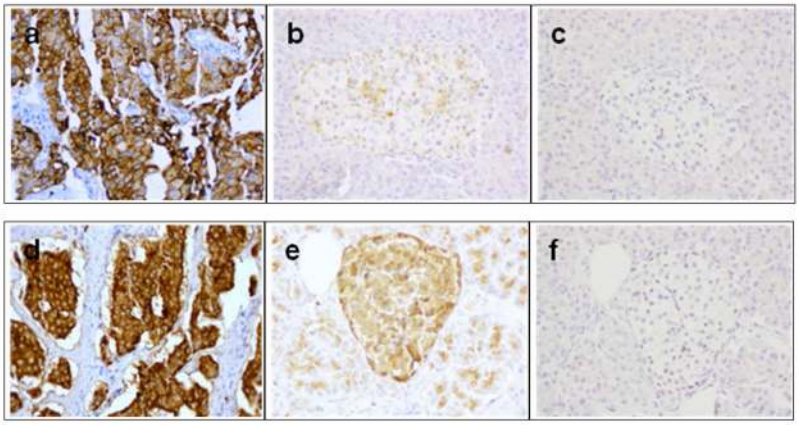

FIGURE 1 C

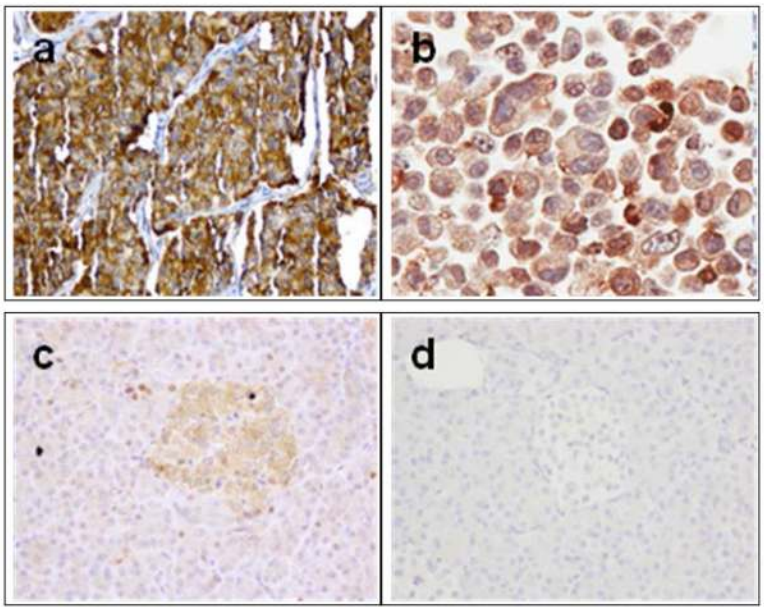

Figure 1. Expression of SSTRs in small intestine NET

A. Representative expression of SSTR2 and SSTR5 in small intestinal NET. Strong staining (**a** and **f**), weak staining (**b** and **g**), negative staining in (**c** and **h**) are shown, respectively. Positive staining in normal pancreatic islet cells in (**d** and **i**), and negative staining after using blocking peptides are shown in (**e** and **j**), respectively.

B. Representative expression of SSTR1 and SSTR3 in small intestinal NET. Strong staining is shown in **a** and **d,** respectively. Positive staining in normal pancreatic islet cells is shown in **b** and **e**, with negative staining after omitting the primary antibodies shown in **c** and **f**.

C. Representative expression of SSTR4 in small intestinal NET. Strong staining is shown in (**a**). Positive staining is additionally shown in an SSTR4-expressing CHO cell line (**b**), and in normal pancreatic islet cells (**c**). Negative staining is shown after omitting the primary antibody (**d**).

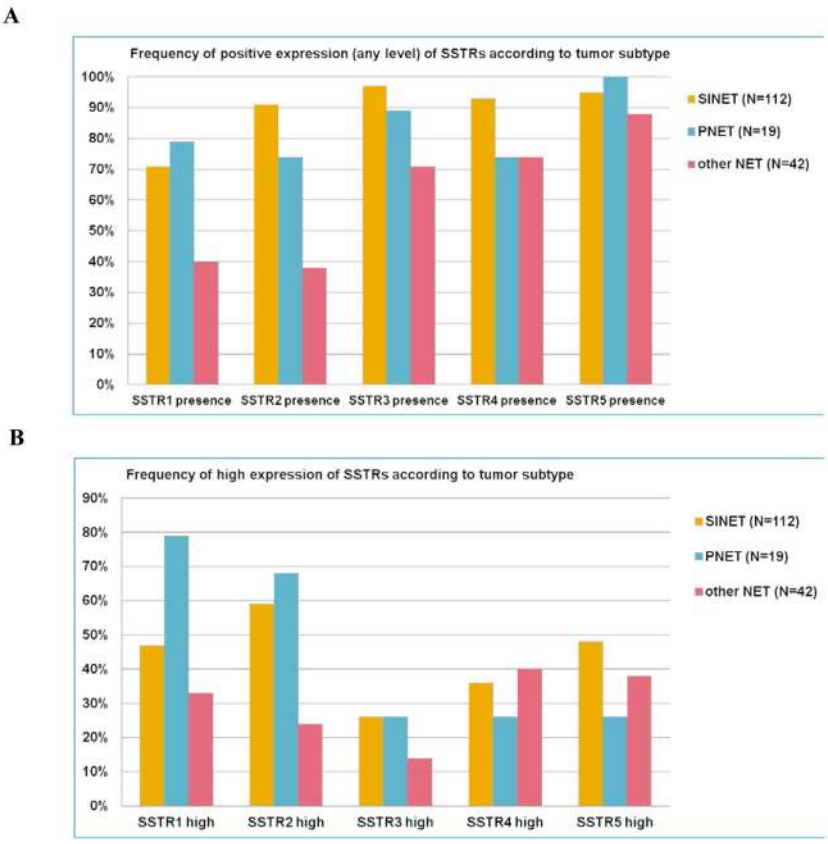

Figure 2.

A. Frequency of expression (at any level) of SSTR1-5 is shown according to tumor primary sites (small intestinal NETs, pancreatic NETs and other NETs). **B.** Frequency of high expression of SSTR1-5 is shown according to tumor primary site.

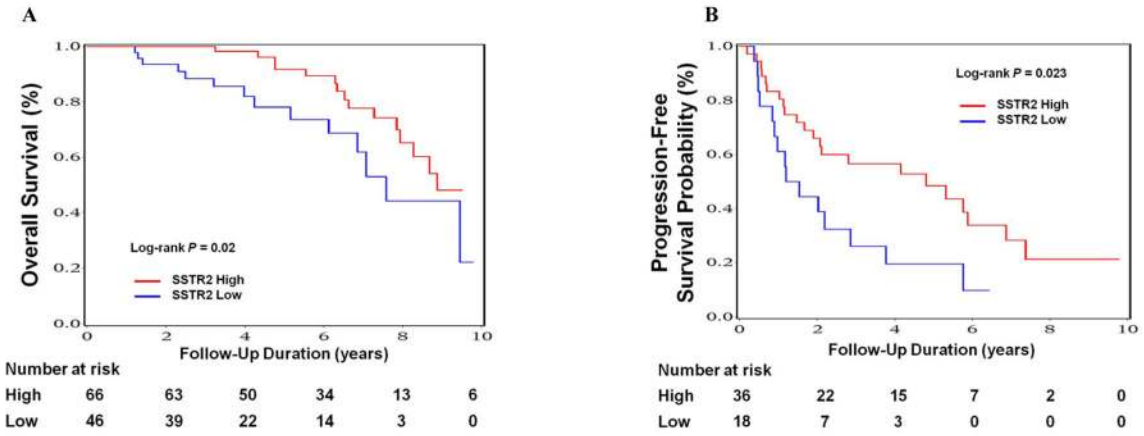

Figure 3.

Progression-free survival (**A**) and overall survival (**B**) according to expression of SSTR2 in patients with small intestine NET receiving treatment with an SSA.

TABLE 1

Characteristics of the Patient Population

	No. (%) 173
Sex	
Female	88 (51)
Male	85 (49)
Mean age (yrs) ± SD	54.7 ± 13
TNM stage	
I	15 (9)
II	32 (19)
III	46 (27)
IV	78 (46)
Tumor primary site	
Small intestine	112 (65)
Pancreas	19 (11)
Other [a]	42 (24)
MKI67 Libeling Index	
≤2%	95 (55)
> 2%	78 (45)

[a]Others: appendix, colon, lung, ovary, rectum, stomach, thyroid, and unknown.

TABLE 2

Co-Expression of SSTRs (N=173)

	SSTR1	SSTR2	SSTR3	SSTR4
SSTR2	R: 0.28 P = 0.0002			
SSTR3	R: 0.34 P < 0.0001	R: 0.55 P < 0.0001		
SSTR4	R: 0.094 P = 0.22	R: 0.14 P = 0.058	R: 0.21 P = 0.0057	
SSTR5	R: 0.33 P < 0.0001	R: 0.39 P < 0.0001	R: 0.46 P < 0.0001	R: 0.10 P = 0.17

TABLE 3

Expression of SSTRs and Overall Survival in 173 Primary NETs

SSTRs expression		No./events	Multivariate HR [a](95% CI)	P
SSTR1	–/Low	91 / 19	1 (reference)	0.67
	High	82 / 21	1.15 (0.61 to 2.17)	
SSTR2	–/Low	84 / 20	1 (reference)	0.013
	High	89 / 20	0.42 (0.21 to 0.84)	
SSTR3	–/Low	133 / 33	1 (reference)	0.31
	High	40 / 7	0.64 (0.27 to 1.51)	
SSTR4	–/Low	111 / 25	1 (reference)	0.56
	High	62 / 15	1.22 (0.64 to 2.32)	
SSTR5	–/Low	98 / 22	1 (reference)	0.87
	High	75 / 18	0.95 (0.51 to 1.78)	

Abbreviation: CI, confidence interval; HR, hazard ratio.

[a]Hazard Ratios adjusted for tumor primary site, age at diagnosis (continuous), sex, tumor stage and MKI67 LI (continuous).

TABLE 4

SSTR2 Expression and Overall Survival in Patients with NETs according to Primary Site

Primary sites	SSTR2 expression	No./events	Multivariate HR [a](95% CI)	P
Small intestinal NETs (N=112)	–/Low	46 / 14	1 (reference)	0.027
	High	66 / 16	0.44 (0.21 to 0.91)	
Pancreatic NETs (N=19)	–/Low	6 / 2	1 (reference)	0.69
	High	13 / 3	0.69 (0.11 to 4.32)	
Other NETs (N=42)	–/Low	32 / 4	1 (reference)	0.9
	High	10 / 1	1.18 (0.08–16.7)	

Abbreviation: CI, confidence interval; HR, hazard ratio.

[a]Hazard Ratios adjusted for age at diagnosis (continuous), sex, tumor stage and MKI67 LI (continuous).

TABLE 5

Progression-Free Survival and Overall Survival in Patients with Advanced Small Intestine NET Treated with SSAs, according to SSTR Expression

SSTRs expression		Overall survival		Progression-Free Survival on SSA	
		No./events	Multivariate [a] HR (95% CI)	No./events	Multivariate [a] HR (95% CI)
SSTR1	–/Low	29/9	1 (reference)	29/20	1 (reference)
	High	25/12	1.69 (0.70–4.09)	25/17	1.52 (0.79–2.92)
			P = 0.24		*P* = 0.21
SSTR2	–/Low	18/8	1 (reference)	18/15	1 (reference)
	High	36/13	0.37 (0.15–0.93)	36/22	0.40 (0.19–0.82)
			P = 0.035		*P* = 0.012
SSTR3	–/Low	41/17	1 (reference)	41/29	1 (reference)
	High	13/4	0.99 (0.32–3.09)	13/8	0.97 (0.44–2.16)
			P = 0.99		*P* = 0.95
SSTR4	–/Low	38/15	1 (reference)	38/27	1 (reference)
	High	16/6	1.28 (0.48–3.39)	16/10	1.07 (0.50–2.30)
			P = 0.63		*P* = 0.87
SSTR5	–/Low	27/10	1 (reference)	27/18	1 (reference)
	High	27/11	1.17 (0.49–2.80)	27/19	1.06 (0.55–2.04)
			P = 0.73		*P* = 0.85

Abbreviation: CI, confidence interval; HR, hazard ratio.

[a]Hazard Ratios adjusted for age at diagnosis (continuous), sex, tumor stage and MKI67 LI (continuous).