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SSTR3 is a putative target for the medical treatment of gonadotroph adenomas of the pituitary

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Abstract

Gonadotroph pituitary adenomas (GPAs) often present as invasive macroadenomas not amenable to complete surgical resection. Radiotherapy is the only post-operative option for patients with large invasive or recurrent lesions. No medical treatment is available for these patients. The somatostatin analogues (SSAs) octreotide and lanreotide that preferentially target somatostatin receptor type 2 (SSTR2) have little effect on GPAs.

It is widely accepted that the expression of specific SSTR subtypes determines the response to SSAs. Given that previous studies on mRNA and protein expression of SSTRs in GPAs generated conflicting results, we investigated the expression of SSTR2, SSTR3 and SSTR5 (the main targets of available SSAs) in a clinically and pathologically well characterized cohort of 108 patients with GPAs. A total of 118 samples were examined by immunohistochemistry using validated and specific monoclonal antibodies. Matched primary and recurrent tissues were available for 10 patients. The results obtained were validated in an independent cohort of 27 GPAs.

We observed that SSTR3 was significantly more abundant than SSTR2 ($P<0.0001$) in GPAs, while full-length SSTR5 was only expressed in few tumors. SSTR3 expression was similar in primary and recurrent adenomas, was high in potentially aggressive lesions and did not significantly change in adenomas that recurred after irradiation.

In conclusion, low expression of SSTR2 may account for the limited response of GPAs to octreotide and lanreotide. Given the potent anti-proliferative, pro-apoptotic and anti-angiogenic activities of SSTR3, targeting this receptor with a multireceptor ligand SSA such as pasireotide may be indicated for potentially aggressive GPAs.

INTRODUCTION

Somatostatin receptors (SSTRs) are G-protein-coupled molecules encoded by five distinct genes (*SSTR1*-*SSTR5*) (Cuevas-Ramos & Fleseriu 2014, Theodoropoulou & Stalla 2013). SSTR1, SSTR2 and SSTR3 are constitutionally expressed in the normal human pituitary while SSTR4 and SSTR5 are expressed at low level. In pituitary adenomas, SSTRs expression varies among types and within each tumor type (Cuevas-Ramos & Fleseriu 2014, Theodoropoulou & Stalla 2013). When they bind to their natural ligands somatostatin-14 and -28, SSTRs exert both a regulatory function on pituitary hormone secretion by inhibiting their mRNA synthesis and release, and a potent anti-proliferative activity (Theodoropoulou & Stalla 2013). For these reasons, SSTRs are established targets for peptide receptor therapy with somatostatin analogs (SSAs).

The SSAs octreotide and lanreotide bind mainly to SSTR2 and to a lesser extent to SSTR3 and SSTR5, and represent the mainstay of medical therapy of secretory somatotroph and thyrotroph adenomas (Theodoropoulou & Stalla 2013, Grozinsky-Glasberg et al 2008, Ben-Shlomo & Melmed 2008). Treatment of acromegalic patients with these SSAs reduces or normalizes growth hormone (GH) and insulin-like growth factor 1 (IGF1) levels and induces tumor shrinkage (Theodoropoulou & Stalla 2013). Due to the restricted affinity of these two compounds for SSTRs, the multiligand SSA pasireotide, was developed. Pasireotide has a 158-, >30-, and 11-fold higher functional activity than octreotide on SSTR5, SSTR1 and SSTR3, respectively (Theodoropoulou & Stalla 2013, Schmid & Schoeffter 2004). Though not free of side effects, pasireotide has shown promising results in the treatment of acromegalic patients (Ben-Shlomo & Melmed 2008) and more recently of patients with Cushing's disease (Colao et al 2012, Webb et al 2014). A clinical trial led by Gadelha and colleagues is currently recruiting patients to evaluate the effect of pasireotide on re-growth of clinically non-functioning pituitary adenomas (NFPAs) (www.ClinicalTrial.gov; identifier NCT01620138).

Gonadotroph pituitary adenomas (GPAs) account for about 35% of all pituitary tumors. They are usually clinically nonfunctioning, and are often diagnosed at the occurrence of signs and symptoms of mass effects (Young et al 1996). Between 30-45% of GPAs extend to the cavernous sinus and fewer cases invade the sellar floor causing considerable morbidity to the patients (Brochier et al 2010). Large and invasive macroadenomas are not amenable to complete resection and can re-grow in up to almost half the cases (Brochier et al 2010, Berkmann et al 2014). Radiotherapy is the only post-operative option for residual and recurrent lesions as no effective medical treatment is available. Indeed, previous studies have documented little efficacy of octreotide and lanreotide in NFPAs (Colao et al 2011).

Since the response of pituitary adenomas to SSAs depends on the expression of specific SSTR subtypes (Gatto et al 2013), and their level of expression may vary among tumors, SSTR assessment on tumor tissue might allow a more accurate stratification of patients who may benefit from SSA therapy. Previous studies assessing SSTR expression in GPAs have produced conflicting results (Pawlikowski et al 2003, Pisarek et al 2009, Ramirez et al 2012). Therefore, we aimed to determine the expression profile of SSTR2, SSTR3, and SSTR5 in a large and homogeneous cohort of patients with primary or recurrent GPAs. As mRNA and protein levels of SSTRs do not always correlate in

pituitary adenomas (Nielsen et al 2001), we used immunohistochemistry with specific and validated antibodies. The results obtained in our cohort of 108 patients were validated in an independent series of 27 GPA patients collected at different Institutions.

Our results show that SSTR3 is the most abundant receptor subtype in GPAs. Atypical and recurrent GPAs show elevated expression of SSTR3, which is maintained after radiotherapy. SSTR3 could be the target of pharmacological treatment with pasireotide in patients with GPAs, particularly those with aggressive/recurrent disease.

PATIENTS AND METHODS

Patient selection and tumor samples (test cohort)

From the Brain & Pituitary Tumor Registry at Imperial College, London, UK, we retrieved all patients operated of trans-sphenoidal surgery (TSS) by the same neurosurgeon (NM) for GPA between January 2005 and December 2013. We identified 108 patients whose medical records and pre- and post-operative imaging were available for review. Patients with apoplexy were excluded. Seven patients were re-operated for recurrent disease within the study period and 11 of the 108 adenomas were recurrent lesions whereby the primary tumor had been removed prior to 2005. Overall, the tissue from the primary and recurrent adenoma was available for examination in 10 cases. Six patients showed measurable tumor re-growth at follow-up neuroimaging but were not re-operated on. The clinical characteristics of these patients are summarized in Table 1.

A total of 118 adenoma samples (108 consecutive cases and the corresponding primary tumors of 10 recurrences) were assessed for SSTR expression. All tumors except 2 were clinically nonfunctioning. Representative paraffin blocks were selected for SSTR immunohistochemistry using the original hematoxylin and eosin (H&E)-stained sections. The original H&E-stained sections and immunostains for anterior pituitary hormones, MIB-1 (Ki-67) and p53 were reviewed (Table 1). Steroidogenic factor 1 (SF1) was also performed in all cases to further confirm the diagnosis of GPA. Mitotic activity was evaluated in 100 fields at x40 (Nikon Plan Fluor x0.75, Nikon Ltd, Japan). Sixty-five adenomas did not show any mitoses. The remaining 43 adenomas showed a mitotic count ranging between 1x50 to 20x50 high power fields (HPFs). Immunostains for Ki67 were performed in all 118 tumor samples. The Ki67 labeling index was calculated as the mean percentage of stained nuclei of tumor cells irrespective of intensities for 1000 cells in three representative fields (total 3000 cells). It was equal or higher than 3% in 11/108 cases (10.2%). Six (5.6%) were atypical adenomas defined according the current WHO classification (Lloyd, et al. 2004). When classified following the grading criteria proposed by Trouillas et al. (Trouillas, et al. 2013), 63 GPAs were grade 1a, 9 were 1b, 39 were 2a and 7 (6.5%) were 2b. Pathological features of an example of grade 2b adenoma are shown in Supplementary Figure 1. Oncocytic changes were seen in 15/108 tumors (13.8%). Tumor characteristics are summarized in Table 1.

These studies were approved by the ethical committees of the Imperial College and patients signed an informed consent.

Independent validation cohort

In order to validate the results obtained in our 108 patients, we also examined SSTR expression in 27 GPAs operated between 2010 and 2014 at the University of Tübingen and at the Technical University of Munich, Germany. Five were females. The median age of the patients was 60 years (mean 59.5 years; range 25-81 years). All tumors were macroadenomas and clinically nonfunctioning. Medical records were available for 17 of the patients. Six of them showed uni- or bilateral extension to the cavernous sinus and none showed bone invasion. Ten were discovered incidentally at neuroimaging performed for other causes, six came to medical attention for visual defects and one for signs and symptoms secondary to hypopituitarism. One patient was operated for a recurrent adenoma (the primary tumor was operated elsewhere). None of the patients received any medical treatment or radiotherapy prior to initial surgery.

The original H&E-stained sections and immunostains for anterior pituitary hormones, MIB-1 (Ki-67) and p53 were available for review. No samples were classified either as atypical adenoma or as oncocytoma. Two expressed αGSU (alpha subunit), three FSH, 11 LH and 12 expressed combinations of the gonadotropin subunits. Ki-67 was equal or higher than 3% in 3 cases; the detection of p53 was positive in 2 cases.
These studies were approved by the ethical committees of the University of Tübingen and Technical University of Munich, and the patients signed an informed consent.

Immunohistochemistry (IHC)

Immunohistochemical stains for SSTRs were performed using an automated immunostainer (Ventana Medical Systems, Tucson, AZ, USA) as previously reported (Lee et al 2013). The SuperSentitive IHC detection system from BioGenex (Fremon, CA, USA) was used to visualize the antibody binding following the manufacturer's instructions. Sections were counterstained with Mayer's Haemalum, dehydrated and coverslipped. The primary antibodies directed against SSTR2 (clone UMB-1 reacting with the SSTR2a isoform, dilution: 1/500), SSTR3 (clone UMB-5, dilution: 1/750), SSTR5 (clone UMB-4, dilution: 1/75) were purchased from Abcam (Cambridge, MA, USA). Sections of normal pancreas were used as positive control and included in each run. Sections incubated without the primary antibody were included in each batch as a negative control.

Evaluation of immunostains for SSTRs

Immunostains were evaluated semi-quantitatively on acquired images. An immunoreactive score (IRS) was recorded for each section. The IRS was generated noting the intensity of the staining (no staining, 0; mild, 1; moderate, 2; strong, 3) and the percentage of cells showing membranous or cytoplasmic expression (no positive cells, 0; <10% of positive cells, 1; 10%–50% of positive cells, 2; 51%–80% of positive cells, 3; >80% of positive cells, 4). The overall IRS was calculated as [percentage of positive cells] x [intensity of staining]. We considered the staining as being negative for IRS 0 and 1, weakly positive for IRS 2 and 3, moderately positive for IRS 4-8, and strongly positive for IRS >8. The slides were scored semi-quantitatively by the three experienced neuropathologists coauthors of this study (RB, JS, FR). Scoring has been performed independently, with a double-blind method, according to

the criteria reported above with an inter-observer variability ranging from 1% to 3.7%. Discrepancies were discussed among the three pathologists.

Statistical analysis

A paired two-tailed Student's t test was used to detect significance between two series of data and P value (P) < 0.05 was considered significant. To compare the distribution of IRS scores between matched primary and recurrent samples we performed the Mann-Whitney paired test, and differences were taken to be statistically significant at $P < 0.05$.

RESULTS

Test patient cohort

The median age of the 108 GPA patients included in the study was 56 years (mean 56 years; range 24-84 years). Thirty-five patients were female. Eighty-two patients (75.9%) came to medical attention with visual field defects secondary to chiasm compression. Nine adenomas were discovered incidentally at neuroimaging performed for other causes; one occurred in a patient with MEN1. Two female patients had a functioning GPA and both presented with amenorrhea secondary to abnormal FSH secretion. Twenty-two patients had signs and symptoms of hypopituitarism and seven presented with headache; two patients had reduced libido, four had amenorrhea and one gynecomastia secondary to high prolactin.

All tumors were macroadenomas and all but two were clinically nonfunctioning. Twenty-seven cases showed uni- or bilateral extension to the cavernous sinus and 14 invaded the sellar floor (altogether 37.9%); of the bone-invasive lesions, four also extended to the cavernous sinus.

None of the patients received any medical treatment or radiotherapy prior to initial surgery. Eight were irradiated after the initial trans-sphenoidal surgery (TSS) to treat the residual adenoma and eleven had radiotherapy after subsequent radiological evidence of recurrent disease (altogether 19/108, 17.6%).

The clinico-pathological characteristics of the patients are summarized in Table 1.

SSTR expression in GPAs

SSTR2 and SSTR3 were expressed in 25.4% and 94.06% of the GPAs, respectively. SSTR2 showed membranous expression whereas SSTR3 showed both a membranous and cytoplasmic localization (Figure 1A), as previously reported (Lupp et al 2012). SSTR5 was expressed in two cases. Normal pancreas showed membranous expression of the three receptors. The mean immunoreactive score (IRS) for SSTR2 in GPAs was 1.4 ± 2.5, with 74.6% (88/118) of the samples showing IRS 0-1 (scored negative), and 16% (19/118) displaying moderate to strong immunoreactivity (IRS ≥4) (Figure 1B). The mean IRS for SSTR3 was 7 ± 3.45, with 83% (98/118) of the adenomas having moderate to high expression levels (IRS≥4) (Figure 1B). SSTR3 was significantly more expressed than SSTR2 (P=

7,29037E-45) in GPAs. We looked for possible associations between SSTR3 expression and invasion, recurrence or residual disease and found no significant correlation. SSTR3 expression in atypical and grade 2b adenomas was high (mean IRS 9 ± 2.35). The full-length SSTR5 was only weakly expressed (IRS 2) in two adenomas (Figure 1B).

SSTR expression in tumor recurrence

Eighteen of the 108 patients (16.7%) underwent a second operation for recurrent disease. The primary tumor was available for 10 patients; six of them underwent radiotherapy after the first surgery. Tumors that recurred showed a SSTR profile similar to the other GPAs. IRS for SSTR2 slightly increased in the recurrent samples (P=0.031). IRS for SSTR3 in 10 paired primary-recurrent lesions was similar in four cases, increased in the recurrence in four cases, and decreased in two recurrences (P=0.376) (Figure 2A and 2B). Following radiotherapy, IRS for SSTR3 did not change or increased in four recurrent samples, and it decreased in two cases, where it remained in any case ≥ 6.

SSTR expression in the independent validation series

To validate our results, we investigated SSTR2, SSTR3 and SSTR5 expression in series of 27 GPAs collected at two different Institutions. The mean immunoreactive score (IRS) for SSTR2 in the validation series was 1.04 ± 1.47, with 66.7% (18/27) of the samples showing IRS 0-1 (scored negative), and 7.4% (2/27) displaying moderate to strong immunoreactivity (IRS ≥4) (Figure 1C). The mean IRS for SSTR3 was 10 ± 2.25 and 96.3% (26/27) of the adenomas showed moderate to high expression levels (IRS≥4) (Figure 1C). SSTR3 was significantly more expressed than SSTR2 (P= 1.49947E-21). No samples in the validation cohort showed immunoreactivity for SSTR5.
Remarkably, GPA samples collected at different centers (validation cohort) show a SSTR expression profile virtually identical to our test cohort.

DISCUSSION

Using validated monoclonal antibodies (Gatto et al 2013, Lupp et al 2012, Korner et al 2012), we have assessed the expression of SSTR2, SSTR3 and SSTR5 in a cohort of 118 clinically and pathologically well-characterized primary and recurrent GPAs. Results were validated in an independent cohort of 27 GPAs operated in different Institutions. In our test cohort, we have demonstrated moderate to high expression of SSTR3 in 83% of cases while only 16% of them showed moderate to strong immunoreactivity for SSTR2. Importantly, highly similar results were obtained by analyzing our validation cohort of GPA samples. Indeed, in this series, moderate to high expression of SSTR3 was seen in 96.4% of cases, whereas only 7.4% of them displayed moderate to strong immunoreactivity for SSTR2.
Full-length SSTR5 was virtually absent in both sample series. Splice variants of the human *SSTR5* gene have been found in pituitary adenomas (Duran-Prado et al 2009), but the encoded truncated isoforms cannot be detected by the anti-SSTR5 antibody we used, which is directed against the receptor C-terminus. SSTR1 expression was investigated in 40 adenomas of the test cohort, including

247 the recurrences, and in 10 tumors of the validation cohort. As we observed weak, diffuse expression
248 (IRS 2-3) in 11/50 samples while the remaining cases were virtually negative (Supplementary Figure
249 2), SSTR1 was not investigated further.

250 Previous studies investigating the mRNA or protein level of SSTRs in NFPAs/GPAs produced
251 conflicting results. Using polyclonal anti-SSTRs antibodies, Pawlikowski et al. (Pawlikowski et al 2003)
252 reported high expression of SSTR1, SSTR2, and SSTR5, with only little or no expression of SSTR3 in
253 13 GPAs and five null cell adenomas. The same group later found SSTR3 to be the most commonly
254 expressed subtype in GPAs, followed by SSTR2, with no SSTR5 expression (Pisarek et al 2009).
255 More recently Ramirez et al. (Ramirez et al 2012) examined SSTRs expression on tissue microarrays
256 of 74 NFPAs using the same monoclonal antibodies of our study. They observed SSTR2 to be the
257 most prevalent receptor subtype (expressed in 60% of cases) while the immunopositivity for both
258 SSTR3 and SSTR5 was observed in about 45% of adenomas. Different scoring criteria (membranous
259 *versus* cytoplasmic staining of SSTR2), and/or the size of tissue samples (whole sections *versus*
260 cores) may explain the discrepancy between ours and Ramirez's et al. (2012) results. A study on 12
261 NFPAs, five of which recurred, confirms our observation of a tendency toward higher SSTRs levels in
262 the recurrent lesions compared to the corresponding primary adenoma (Pisarek et al 2011).

263 In tumor cells, upon ligand binding, SSTR3 is known to inhibit mitogenic pathways by activating
264 protein tyrosine phosphatases and ultimately inactivating Raf-1 and MAPK activities (Theodoropoulou
265 & Stalla 2013). SSTR3 also induces apoptosis by activating p53 and caspases, and represses
266 endothelial cell proliferation (Theodoropoulou & Stalla 2013, Florio et al 2003). Thus, targeting SSTR3
267 might induce cytostatic and cytotoxic effects, as well as inhibit angiogenesis. The evidence of
268 elevated SSTR3 expression in potentially aggressive adenomas (atypical or grade 2b, 6% in our test
269 cohort), and in all but 2 recurrent lesions (including those that recurred after irradiation) supports the
270 rationale of targeting SSTR3 in patients with aggressive/recurrent GPAs. Among the currently
271 available SSAs, pasireotide represents the most promising compound given its 11-fold higher
272 functional activity on SSTR3 than octreotide and lanreotide. *In vitro* evidence that pasireotide inhibits
273 the viability of NFPAs in primary cultures (Zatelli et al 2007) further supports the hypothesis that
274 SSTR3 is a suitable target for treatment. Indeed, the anti-proliferative effect of pasireotide on NFPAs
275 cultures, in addition to a suppressive action on VEGF secretion (Zatelli et al 2007), may also be
276 mediated by pathways downstream of SSTR3.

277 Similar to a previous series (Young et al 1996), about 80% of the patients in our test cohort presented
278 with visual impairment secondary to chiasm compression. TSS achieved chiasm decompression in all
279 but eight patients who required post-operative radiotherapy to treat residual tissue still encroaching on
280 the chiasm. Octreotide and lanreotide can cause shrinkage of a substantial number of somatotroph
281 and thyrotroph adenomas, while clinical data on pasireotide is limited to a few studies. Petersenn et al.
282 (Petersenn et al 2010) obtained a >20% reduction in tumor volume in 39% in acromegalic patients,
283 and Colao et al. (Colao et al 2012) observed a reduction of up to 43.8% in tumor volume in Cushing's
284 patients treated with pasireotide. To date, there are no published studies on the efficacy of pasireotide
285 in GPA patients, as these patients are just now being recruited in an ongoing clinical trial. The results
286 of this trial will help to determine whether pasireotide can induce tumor shrinkage in GPAs.

Two of the patients had functioning GPAs (FSH-secreting) that showed high SSTR3 expression (IRS 6 and 12). These tumors are uncommon. They usually occur in women in the reproductive age and present with menstrual irregularities, infertility or with ovarian hyperstimulation syndrome. Functioning GPAs are difficult to treat medically when surgery fails to completely remove the adenoma (Mor et al 2005; Ntali et al 2014). Our evidence of high SSTR3 in these tumors, and the fact that SSTR3 has anti-secretory action (Eigler et al 2014) similar to other SSTRs, may help guiding the medical therapy of such patients.

In conclusion, we show that SSTR3 is the predominant SSTR in GPAs. Our results may explain the limited efficacy of octreotide and lanreotide in patients with this type of adenoma and provide the rationale for investigating the effect of alternative SSAs with higher affinity for SSTR3 (such as pasireotide) in GPAs, especially in patients with large invasive or recurrent adenomas. Our data underpins the utility of profiling for SSTRs to stratify patients with pituitary adenomas for therapy with SSAs.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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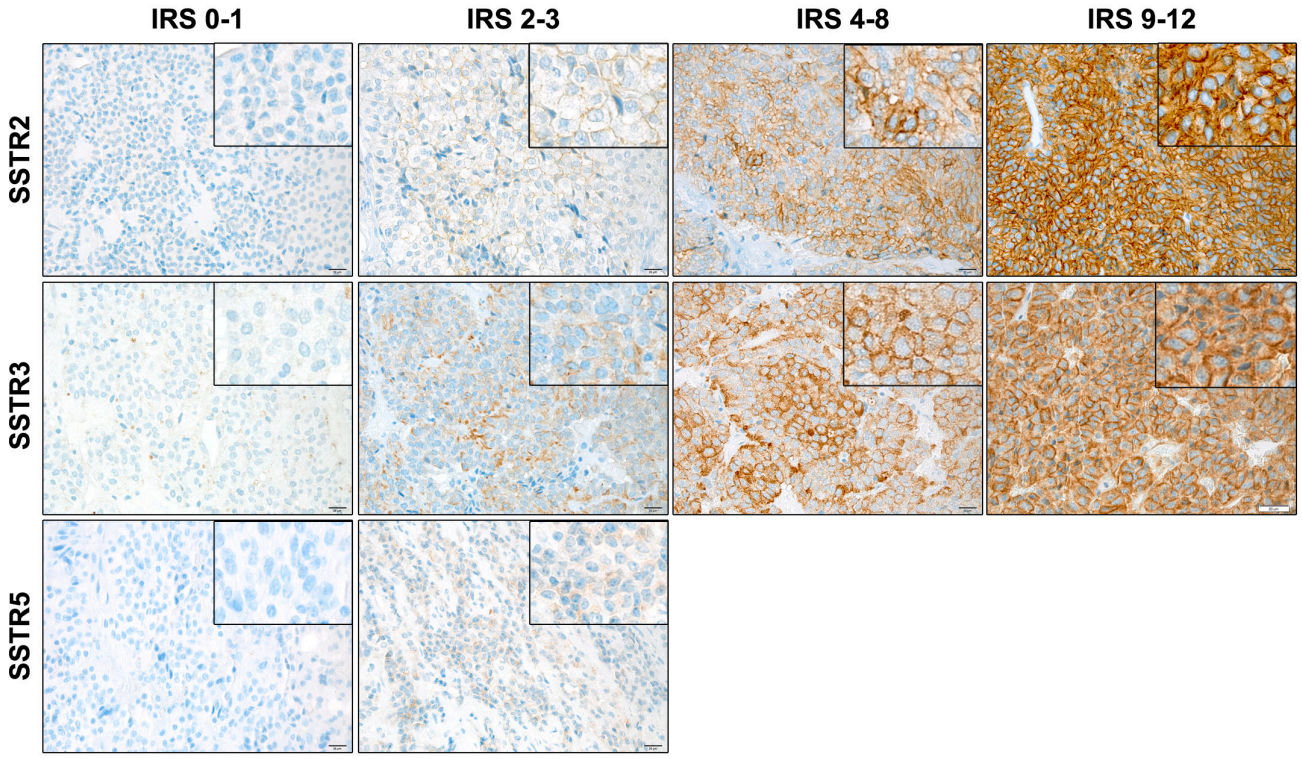
Figure legends

Figure 1. Expression of SSTR2, SSTR3 and SSTR5 in GPAs. (A) Immunohistochemical staining for SSTR2, SSTR3 and SSTR5 in representative GPA cases (immunoperoxidase, x200; insets x400) (scale bar = 20μm); (B) Immunoreactive scores (IRS) for SSTR2, SSTR3 and SSTR5 expression in our test cohort of 118 GPA samples; (C) Immunoreactive scores (IRS) for SSTR2, SSTR3 and SSTR5 expression in our validation cohort of 27 independent GPA samples.

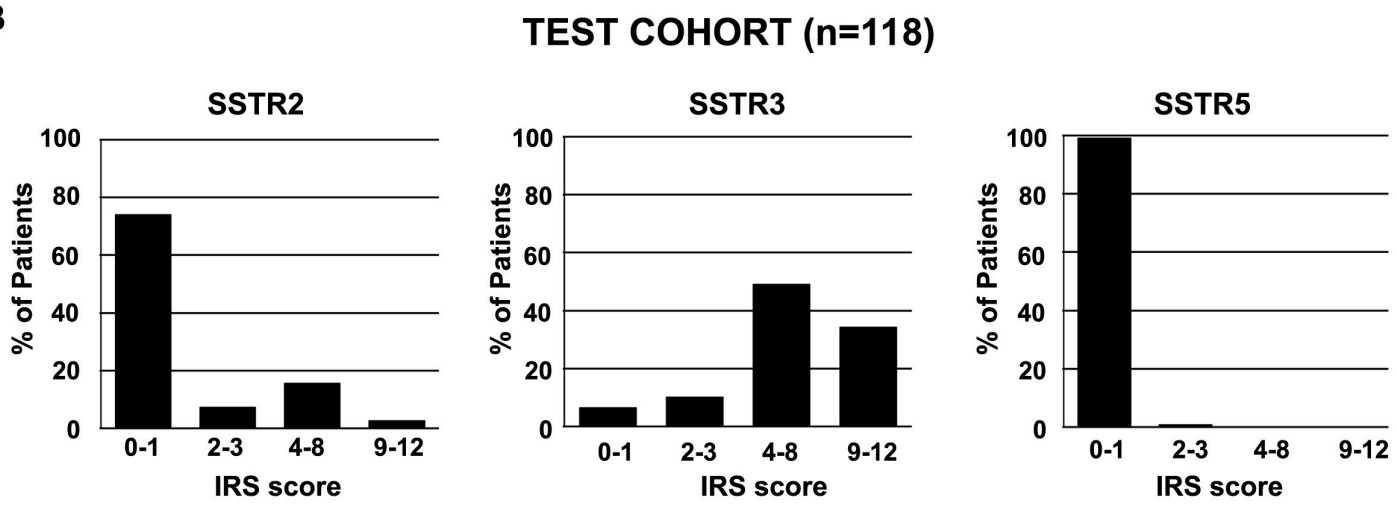
Figure 2. Expression of SSTRs in paired primary-recurrent tumor samples. (A) Immunohistochemical staining for SSTR3 in the matched primary-recurrent samples. Patient ID (P) is shown on the side. The boxed samples come from patients who received radiotherapy after the first surgery. (B) Table summarizing the IRS for SSTR2 and SSTR3, and Ki67 positivity of the paired primary-recurrent samples. The patients indicated in blue are those who received radiotherapy after the first surgery. These samples were negative for SSTR5.

Table 1: Essential clinical and pathological features of the GPAs in our test cohort.

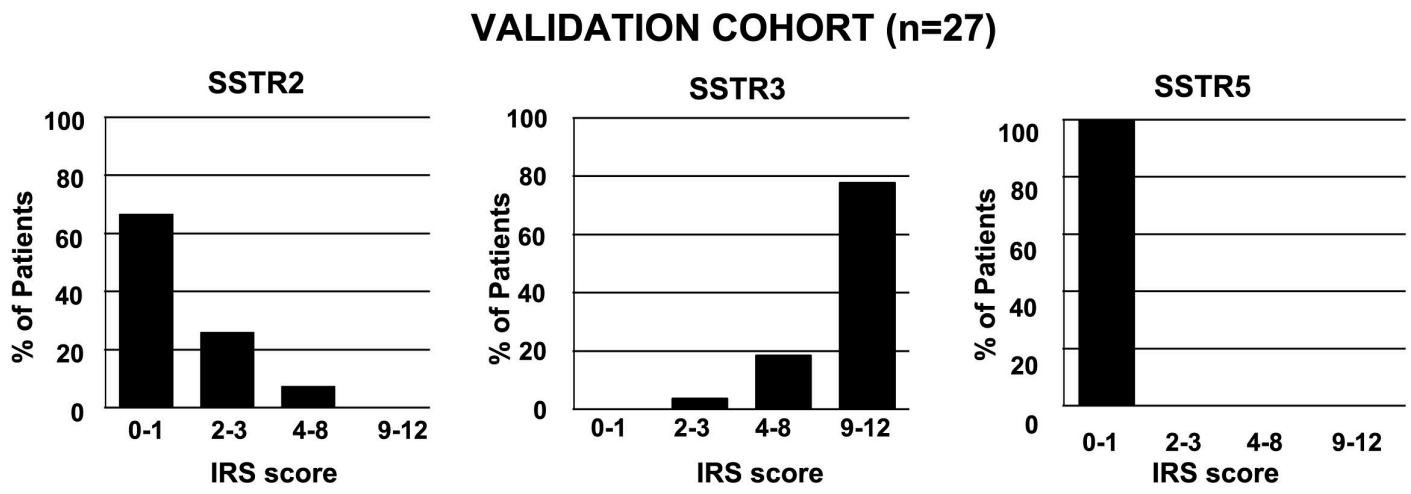
A


IRS 0-1
IRS 2-3
IRS 4-8
IRS 9-12
SSTR2
SSTR3
SSTR5

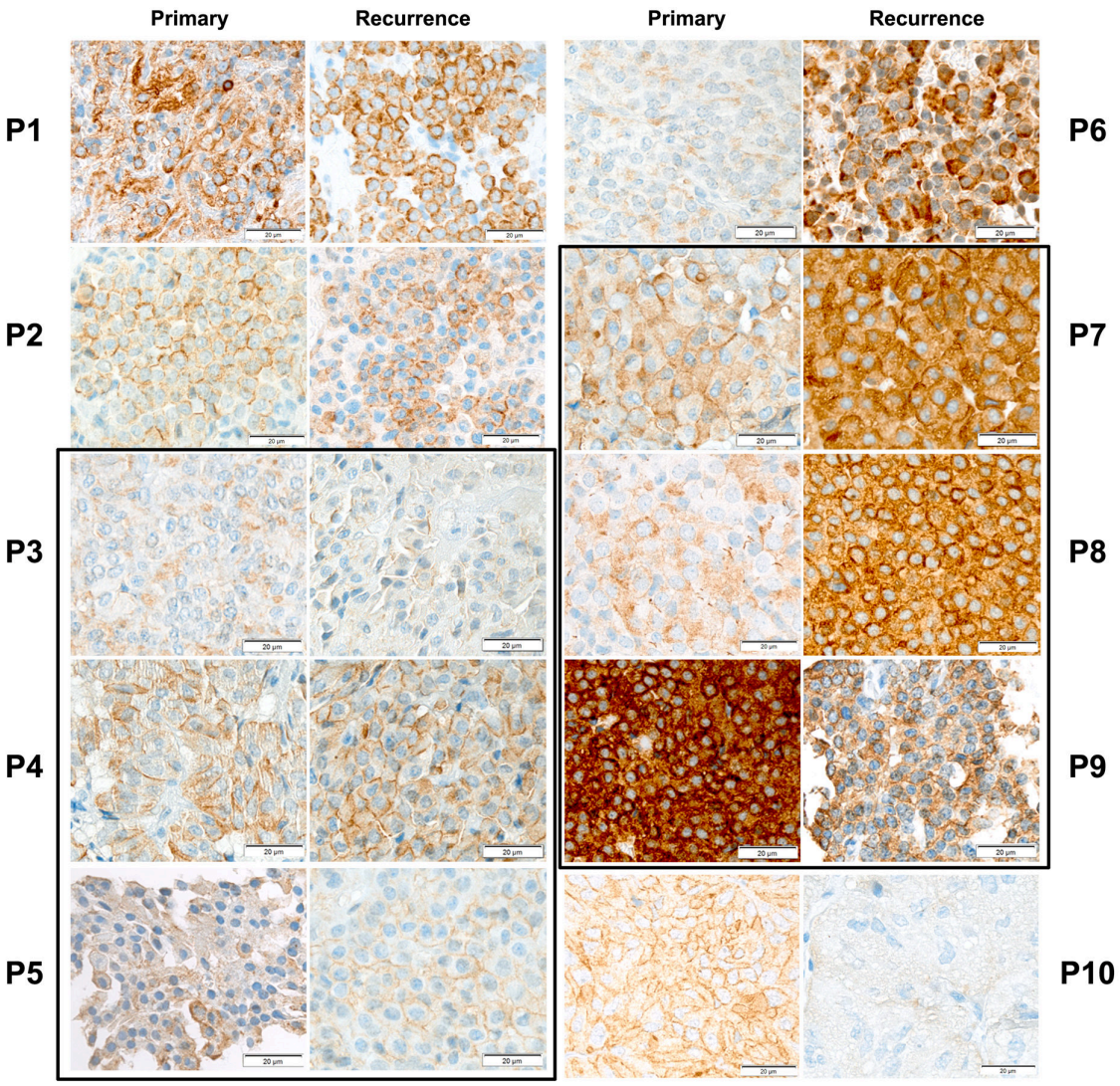
B


TEST COHORT (n=118)
SSTR2
SSTR3
SSTR5
% of Patients
100
80
60
40
20
0
0-1
2-3
4-8
9-12
IRS score

C


VALIDATION COHORT (n=27)
SSTR2
SSTR3
SSTR5
% of Patients
100
80
60
40
20
0
0-1
2-3
4-8
9-12
IRS score

A



B

Patient ID	IRS SSTR2		IRS SSTR3		Ki67 %	
	Primary	Recurrence	Primary	Recurrence	Primary	Recurrence
P1	0	1	6	6	<1	2
P2	0	0	4.5	6	2	7-8
P3	1	1	3	3	<1	2-3
P4	0	2	8	8	<1	<1
P5	3	4	10	10	<1	1
P6	0	0	2	10	<1	<1
P7	2	3	8	12	<1	1
P8	0	4	6	12	<1	3
P9	0	0	12	8	<1	1-2
P10	0	2	6	2	<1	<1

Number of patients (total)	Male/Female
108	73M/35F
Age	
Mean years (range)	56 (24-84)
Males	58
Female	53
Signs and symptoms at onset	**Percentage of patients**
Visual impairment	75.9% (82/108)
Hypopituitarism	20.4% (22/108)
Raised prolactin	11.1% (12/108)
Incidentalomas	8.3% (9/108)
Headache	6.5% (7/108)
Amenorrhea	5.6% (6/108)
Gynecomastia	0.9% (1/108)
Extension/Invasion	**Percentage of tumors**
Extension to cavernous sinus	25% (27/108)
Bone invasion	13.0% (14/108)
Follow-up	
Mean months (range)	45 (7-110)
Regrowth	5.5% (6/108)
Re-operation	6.5% (7/108)
Recurrence[a]	16.7% (18/108)
Post-operative radiotherapy	17.6% (19/108)
Pathological features	**Percentage of tumors**
Mitotic activity	
Absent	60.2% (65/108)
Present (range 1x50-20x50 HPF[b])	39.8% (43/108)
Immune profile	
FSHβ	88.9% (96/108)
LHβ	43.5%(47/108)
αGSU	75% (81/108)
Others	
Oncocytic changes	13.8% (15/108)
Ki67	
<3%	89.8% (97/108)
$\geq$3%	10.2% (11/108)
p53	
Negative	92.6% (100/108)
Positive	7.4% (8/108)
Atypical (WHO, ref. Lloyd et al. 2004)	5.6% (6/108)
Grade 2b (ref. Trouillas et al. 2013)	6.5% (7/108)

a, Seven patients recurred within the study period; the other 11 cases were recurrent lesions whose primary tumor was removed prior to 2005

b, HPF, high power field