JNS

CLINICAL ARTICLE

Correlation between tumor invasion and somatostatin receptor subtypes in acromegaly

***Shaolin Zhang, MD, PhD,[1,2] Shun Yao, MD, PhD,[1] Jinping Chen, MD, PhD,[1] Farhana Akter, MD, PhD,[3,4] Jia Yang, MD, PhD,[1] Dimin Zhu, MD, PhD,[1] Ailiang Zeng, MD, PhD,[5] Wenli Chen, MD, PhD,[1] Zhigang Mao, MD, PhD,[1] Yonghong Zhu, PhD,[6] and Haijun Wang, MD, PhD[1]**

[1]Department of Neurosurgery, Center for Pituitary Tumor Surgery, The First Affiliated Hospital, Sun Yat-sen University, Guangzhou, Guangdong, China; [2]Department of Neurosurgery, The First Affiliated Hospital of Wannan Medical College, Wuhu, Anhui, China; [3]Faculty of Arts and Sciences, Harvard University, Cambridge, Massachusetts; [4]Department of Pharmacology, University of Oxford, United Kingdom; [5]Department of Cancer Biology, The University of Texas MD Anderson Cancer Center, Houston, Texas; and [6]Department of Histology and Embryology, Zhongshan School of Medicine, Sun Yat-sen University, Guangzhou, Guangdong, China

OBJECTIVE The low expression of somatostatin receptor (SSTR) subtypes in somatotropinoma is associated with a poor response to somatostatin analogs (SSAs). However, the correlation between SSTRs and tumor invasion has not yet been clarified. Therefore, the authors aimed to investigate the relationship between SSTRs and tumor invasion, as well as the correlation between tumor invasiveness and pharmacological response to SSAs.

METHODS A total of 102 patients with acromegaly who underwent surgery between December 2016 and December 2021 at the largest pituitary tumor surgery center in southern China were included in this retrospective study. Patients were divided into the noninvasive tumor group (Knosp grades 0–2 and Hardy-Wilson grade I or II) and invasive group (either Knosp grade 3 or 4 or Hardy-Wilson grade III or IV). The positive response to SSAs was defined by the following criteria after at least 3 months of SSA treatment: 1) ≥ 50% reduction or age- and sex-adjusted normal range of insulin-like growth factor–1 (IGF-1) level; 2) ≥ 80% reduction in or normal range of growth hormone (GH) level; or 3) > 20% reduction in tumor volume. The reference for the normal range of age- and sex-adjusted serum IGF-1 levels was derived from a survey of 2791 healthy adults (1339 males and 1452 females) in China. Demographics and clinical characteristics including tumor size, biochemical assessment, expression levels of SSTRs, and response to preoperative SSAs were compared between the invasive group and noninvasive group. Receiver operating characteristic (ROC) curve analysis was performed to assess the association between SSTR2 and tumor invasion.

RESULTS Compared with the noninvasive group, the invasive group presented with a larger tumor size (9.99 ± 10.41 cm^3 vs 3.50 ± 4.02 cm^3, $p < 0.001$), relatively lower SSTR2 expression ($p < 0.001$), and poorer response to SSAs (36.4% vs 91.7%, $p < 0.001$). In addition, there was a significant negative correlation between SSTR2 mRNA level and tumor size ($r = -0.214$, $p = 0.031$). However, there were no statistically significant differences in the expression of SSTR1, SSTR3, and SSTR5 between the groups. ROC analysis revealed that the low SSTR2 mRNA level was closely associated with tumor invasion (area under the curve 0.805, $p < 0.0001$).

CONCLUSIONS Tumor invasion is negatively correlated with SSTR2 level but is not associated with other SSTR subtypes. Patients with invasive tumors have a poorer response to SSA therapy, which may be due to the low level of SSTR2 expression. Therefore, SSTR2 could be considered as a routine investigative marker for aiding management of postoperative residual tumors.

https://thejns.org/doi/abs/10.3171/2023.7.JNS23858

KEYWORDS somatotropinoma; acromegaly; somatostatin receptor subtypes; response to somatostatin analogs; tumor invasion; pituitary surgery

ABBREVIATIONS AUC = area under the curve; BR = biochemical remission; CS = cavernous sinus; DAB = diaminobenzene; GH = growth hormone; GTR = gross-total resection; IGF-1 = insulin-like growth factor–1; IHC = immunohistochemical; OGTT = oral glucose tolerance test; qRT-PCR = quantitative real-time reverse transcription polymerase chain reaction; ROC = receiver operating characteristic; SSA = somatostatin analog; SSTR = somatostatin receptor.

SUBMITTED April 19, 2023. **ACCEPTED** July 12, 2023.
INCLUDE WHEN CITING Published online October 6, 2023; DOI: 10.3171/2023.7.JNS23858.
* S.Z., S.Y., and J.C. contributed equally to this work.

Approximately 10%–15% of resected neuroendocrine tumors of the pituitary are of somatotroph origin (somatotropinoma). These growth hormone (GH)–producing tumors cause acromegaly,[1–3] a condition that is often associated with several comorbidities such as hypertension, diabetes, colonic polyps, thyroid nodules, cardiac dysfunction, and sleep apnea. Histologically, somatotropinomas are heterogeneous and present with variable invasive properties and varied response to different pharmacological options.[4–6] Biochemically, they are characterized by elevated levels of circulating GH and insulin-like growth factor–1 (IGF-1).

The therapeutic goals for treatment of this tumor are a combination of maximal resection and normalization of GH and IGF-1 levels, reducing the morbidity and mortality of the acromegaly population.[7,8] Resection is the first line of treatment; unfortunately, normalized endocrinological function is achieved in only 50% of operated cases and is reduced to 10%–20% for invasive macroadenomas.[9,10] Preoperative and postoperative pharmacological agents such as somatostatin analogs (SSAs; e.g., octreotide and lanreotide) are therefore an important adjunct to surgery.[11–13] SSAs are ligands for somatostatin receptors (SSTRs), G-protein coupled receptors that are found in a variety of tissues and richly expressed in somatotropinomas. SSTRs are differentially expressed in somatotropinomas; the highest expressions are those of SSTR2 and SSTR5, followed by SSTR1 and SSTR3 and rarely, SSTR4.[14,15]

In our pituitary tumor surgery center, we observed that invasive somatotropinomas have a poor response to SSA treatment. To gain insight into this clinical phenomenon, we sought to investigate the differential expression of SSTRs between invasive and noninvasive tumors, as well as the correlation between SSTRs and tumor invasion.

Methods

Patient Characteristics

This prospective study enrolled 102 acromegaly patients (54.9% female) who underwent surgery between December 2016 and December 2021 at the Center for Pituitary Tumor Surgery, The First Affiliated Hospital of Sun Yat-sen University, Guangzhou, China. Inclusion criteria for the study were as follows: 1) disease diagnosed according to the 2010 consensus criteria of acromegaly,[8] 2) surgical treatment, 3) available tumor tissue, and 4) minimum follow-up of 3 months. Patients who received radiotherapy or had incomplete clinical data were excluded from the study. All patients provided informed consent for research use of their tumor tissue, and the study was approved by the ethics committee of The First Affiliated Hospital of Sun Yat-sen University.

Biochemical Measurement

Endogenous hormone levels, including serum GH, IGF-1, prolactin, cortisol, free T3, free T4, thyroid-stimulating hormone, follicle-stimulating hormone, luteinizing hormone, estrogen, adrenocorticotrophic hormone, and testosterone, were measured before surgery and 24 hours and 3 months after surgery in all patients by chemiluminescence immunoassay. An oral glucose tolerance test (OGTT) was conducted before surgery to measure the nadir GH value, and a further OGTT test was performed if the random GH level was over 1.0 ng/mL after surgery. The biochemical remission (BR) criteria consisted of random serum GH < 1.0 ng/mL or nadir GH < 0.4 ng/mL after the OGTT, as well as normal range of age- and sex-adjusted serum IGF-1.[7,8] The reference for the normal range of age- and sex-adjusted serum IGF-1 levels was derived from a survey of 2791 healthy adults (1339 males and 1452 females) in China.[16]

Fifty-seven (55.9%) patients were treated preoperatively with long-acting first-generation SSAs, either octreotide long-acting release (20 mg/4 weeks) or lanreotide (90 mg/4 weeks). Because of the lack of criteria for SSA resistance, the pharmacological response was defined according to previous studies.[5,12,17–20] A positive response to SSAs was defined by the following criteria after at least 3 months of SSA treatment: 1) ≥ 50% reduction or age- and sex-adjusted normal range of IGF-1 level (biochemical response),[5,17,18,20] 2) ≥ 80% reduction in or normal range of GH level (biochemical response),[5,12] or 3) > 20% reduction in tumor volume (tumor response).[5,12,19]

Imaging Assessments

MRI was performed before and after SSA administration and surgery using a 3.0-T MRI scanner (Magnetom Verio, Siemens Healthcare). T1-weighted, gadolinium-enhanced T1-weighted, and T2-weighted imaging data were collected from all patients. Tumor volume and maximum diameter before and after medication or before surgery were measured using 3D-Slicer software (version 5.0.3). Gross-total resection (GTR) was defined as no residual pituitary tumor on MRI at 3 months after surgery. Hardy-Wilson and Knosp grades were used to evaluate the tumor invasiveness on preoperative or premedication MRI.[21–23]

Definition of Pituitary Tumor Invasion

The Hardy-Wilson classification considers the degree of sellar destruction (grade) and extrasellar extension (stage), but it does not account for lateral extension (e.g., cavernous sinus [CS]), for which the Knosp classification is useful. We therefore used both classification systems, as this ensured that we considered all factors when considering invasion, with Knosp grade 3 or 4 or Hardy grade III or IV tumors classified as invasive and Knosp grade 0–2 and Hardy grade I or II tumors classified as noninvasive.

Quantitative Real-Time Reverse Transcription Polymerase Chain Reaction

To evaluate mRNA levels of the SSTRs (SSTR1, SSTR2, SSTR3, and SSTR5), all 102 samples were included in this study by quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR). According to the manufacturer's instructions, total RNA was extracted from tumor tissues using Trizol reagent (Invitrogen) and were reverse transcribed using commercially available reagents (TaKaRa), and qRT-PCR was performed using the StepOne SYBR Green Premix Pro Tag HS qPCR Kit (Accurate Biology).

GAPDH was used as a control for the mRNA expres-

sion of SSTR. The relative expression levels of SSTR mRNA were quantified by $2^{-\Delta Ct}$ methods. The sequences of primers were listed in Table 1, which were ordered from Tsingke Biotechnology Corporation.

Immunohistochemical Analysis

The differential expression of SSTR2 and SSTR5 between the invasive and noninvasive groups was evaluated at the protein level by immunohistochemical (IHC) analysis. Paraffin-embedded tumor specimens from 79 patients were cut into 5-μm slices and were stained using diaminobenzene (DAB) according to the manufacturer's instructions. In brief, the sections were treated with 3% H_2O_2 for 10 minutes at room temperature and then blocked with blocking serum for 20 minutes. Primary antibodies (SSTR2, ZSGB-BIO; and SSTR5, ABcom) were used at a dilution of 1:100, overnight at 4°C in a moist chamber. Then, the slides were incubated for 30 minutes at room temperature with a goat anti–rabbit immunoglobulin, and subsequently reacted with DAB (CWBio). Negative controls were incubated without primary antibodies.

The IHC results were scored semiquantitatively by the percentage of positive cells and staining intensity. The percentage of positive cells was evaluated using a four-tiered system: score 1 (negative or < 25% positive cells), score 2 (25%–49% positive cells), score 3 (50%–74% positive cells) and score 4 (≥ 75% of positive cells).[24,25] The intensity of staining was scored as score 1 (negative or light-yellow), score 2 (brown-yellow), and score 3 (brown).[24] The final score was quantified using the equation of the staining number score multiplied by the staining intensity score (range from 1 to 12). Scores of 1–6 were defined as low expression, and scores exceeding 6 were identified as high expression. The score assessments were performed independently by two researchers without knowledge of the clinical data.

Statistical Analysis

Data were analyzed using IBM SPSS version 22.0 (IBM Corp.). Numerical data were expressed as mean ± SD or mean ± SEM and were evaluated using the independent t-test or Mann-Whitney U-test. Categorical variables were expressed as percentages and were statistically analyzed using Pearson's chi-square or Fisher's exact test. Multivariate analysis was performed to determine independent variables related to tumor invasion. Receiver operating characteristic (ROC) curve analysis was used to assess the association between SSTR2 and tumor invasion using MedCalc version 20.023; $p < 0.05$ was considered statistically significant.

Results

Baseline Characteristics

The mean age was 41.42 ± 11.63 years. Seven tumors were categorized as Hardy-Wilson grade I, 72 tumors as grade II, 12 tumors as grade III, and 11 tumors as grade IV, while 17 tumors were classified as Knosp grade 0, 25 tumors as grade 1, 20 tumors as grade 2, 22 tumors as grade 3, and 18 tumors as grade 4. Only 4 tumors were microadenomas, and 98 tumors were macroadenomas. The mean tumor volume and maximum diameter were 6.55 ± 8.34 cm^3 (range 0.071–50.05 cm^3) and 24.57 ± 10.75 mm (range 6.46–56.64 mm), respectively. The minimum and maximum follow-up times were 3 months (16 patients, 15.69%) and 5 years (mean 18.12 ± 1.37 months), respectively. Other results are shown in Table 2.

TABLE 1. Primers designed for qRT-PCR

Primer Name	Sequence
SSTR1	Forward: 5′ CACATTTCTCATGGGCTTCCT 3′
	Reverse: 5′ ACAAACACCATCACCACCATC 3′
SSTR2	Forward: 5′ GGCATGTTTGACTTTGTGGTG 3′
	Reverse: 5′ GTCTCATTCAGCCGGGATTT 3′
SSTR3	Forward: 5′ TGCCTTCTTTGGGCTCTACTT 3′
	Reverse: 5′ ATCCTCCTCCTCAGTCTTCTCC 3′
SSTR5	Forward: 5′ CTGGTGTTTGCGGGATGTT 3′
	Reverse: 5′ GAAGCTCTGGCGGAAGTTGT 3′
GAPDH	Forward: 5′ CTGGGCTACACTGAGCACC 3′
	Reverse: 5′ AAGTGGTCGTTGAGGGCAATG 3′

Comparison of Characteristics Between the Invasive and Noninvasive Groups

Based on MRI findings, 48 patients were categorized in the invasive group and 54 patients in the noninvasive group. To investigate whether there were characteristic differences between the groups except for tumor grade (Knosp and Hardy grades), comparisons were performed and the results are listed in Table 2. GTR was achieved in 73 (71.6%) patients, including 50 (92.6%) in the noninvasive group and 23 (47.9%) in the invasive group ($p < 0.001$). Overall, 53 (52%) patients reached postoperative BR at the last follow-up (at least 3 months), namely, 40 patients in the noninvasive group and 13 patients in the invasive group (74.1% vs 27.1%, $p < 0.001$). Moreover, we found that patients with invasive tumor presented with both a larger tumor volume (9.99 ± 10.41 cm^3 vs 3.50 ± 4.02 cm^3, $p < 0.001$) and a wider maximum diameter (30.56 ± 11.2 mm vs 19.24 ± 6.88 mm, $p < 0.001$). Compared with patients with noninvasive tumor, patients with invasive tumor tended to have a lower pharmacological response rate to SSAs (36.4% vs 91.7%, $p < 0.001$). Baseline endocrinological evaluation revealed hypopituitarism in 26 (25.5%) patients: 17 (35.4%) patients in the invasive group and 9 (16.7%) in the noninvasive group ($p = 0.030$). However, there were no significant differences in age, BMI, disease duration, sex, empty sella, sella configuration, comorbidity, and GH, IGF-1, or normalized IGF-1 levels at diagnosis.

SSTR1/2/3/5 mRNA Expression

To investigate whether the differential SSTR1/2/3/5 expression occurred at a transcriptional level between the groups, we performed qRT-PCR analysis in all tumor tissue samples. As shown in Fig. 1A, the level of SSTR2 in the noninvasive group was significantly higher than in the invasive group ($p < 0.001$), which indicated that downregulation of SSTR2 might be associated with invasion of so-

TABLE 2. Group comparison between the invasive and noninvasive groups

Variable	Total (n = 102)	Noninvasive Group (n = 54)	Invasive Group (n = 48)	p Value
Age, yrs	41.42 ± 11.63	42.76 ±11.35	39.92 ± 11.88	0.169
BMI, kg/m^2	24.92 ± 3.05	24.67 ± 2.46	25.19 ± 3.61	0.400
Disease duration, mos	56.45 ± 70.53	60.19 ± 83.11	52.25 ± 53.55	0.694
Sex				0.291
Male	46 (45.1)	27 (50)	19 (39.6)	
Female	56 (54.9)	27 (50)	29 (60.4)	
Empty sella	15 (14.7)	10 (18.5)	5 (10.4)	0.249
Sella configuration				0.696
Sellar type	79 (77.5)	41 (75.9)	38 (79.2)	
Presellar	23 (22.5)	13 (24.1)	10 (20.8)	
Comorbidity*	48 (47.1)	30 (55.6)	18 (37.5)	0.068
GTR	73 (71.6)	50 (92.6)	23 (47.9)	<0.001
Remission	53 (52.0)	40 (74.1)	13 (27.1)	<0.001
Response to SSAs†	34 (59.6)	22 (91.7)	12 (36.4)	<0.001
GH at diagnosis, ng/mL	40.66 ± 46.72	34.8 ± 33.91	47.26 ± 57.53	0.367
IGF-1 at diagnosis, ng/mL	669.68 ± 258.54	649.06 ± 264.47	692.88 ± 252.44	0.311
Normalized IGF-1 at diagnosis	2.41 ± 0.95	2.38 ± 1.02	2.44 ± 0.86	0.488
Max tumor diameter, mm	24.57 ± 10.75	19.24 ± 6.88	30.56 ± 11.2	<0.001
Tumor vol, cm^3	6.55 ± 8.34	3.50 ± 4.02	9.99 ± 10.41	<0.001
Preop hypopituitarism‡	26 (25.5)	9 (16.7)	17 (35.4)	0.030

Values are presented as the number of patients (%) or mean ± SD unless stated otherwise.
* Diabetes, hypertension, colonic polyps, thyroid nodules, cardiac dysfunction, and sleep apnea.
† Fifty-seven patients received SSAs.
‡ Hypothyroidism, hypogonadism, hypoadrenocorticism, and panhypopituitarism.

matotroph tumors. Although the levels of SSTR1, SSTR3, and SSTR5 were also slightly higher in the noninvasive group than in the invasive group, the differences were not statistically significant (Fig. 1B–D). Thereafter, our further investigation showed that there was a significant negative correlation between SSTR2 mRNA level and tumor size, including maximum diameter (r = –0.218, p = 0.028) (Fig. 1E) and volume (r = –0.214, p = 0.031) (Fig. 1F).

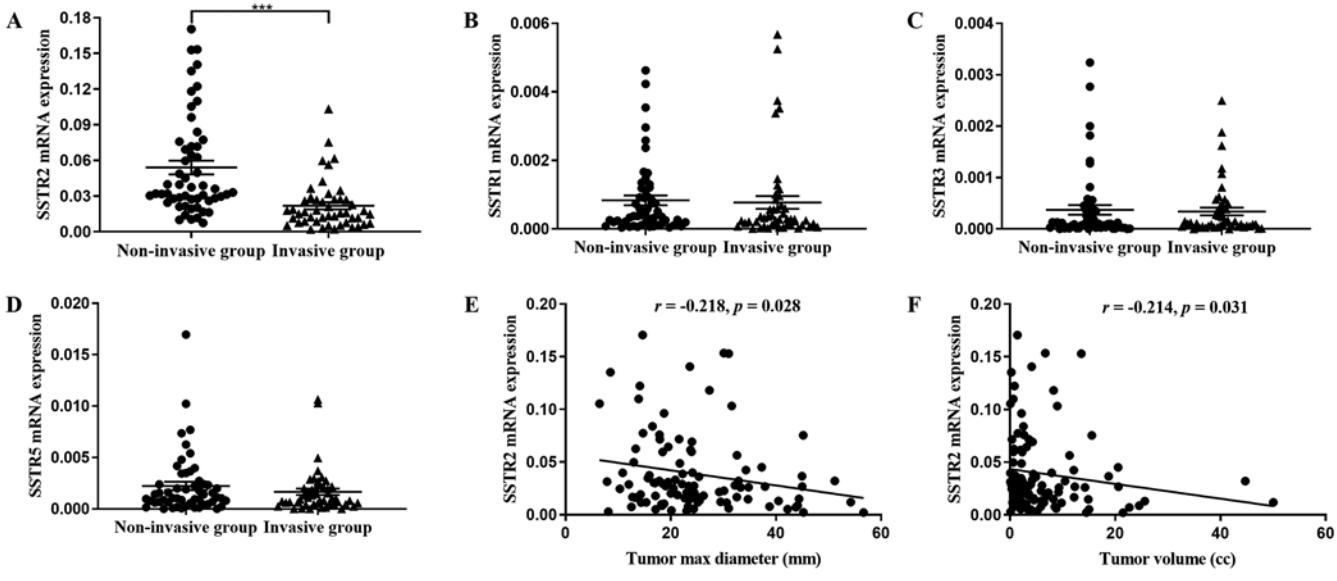

FIG. 1. SSTR mRNA levels between the two groups, and the correlation between SSTR2 mRNA expression and tumor size. **A–D:** Quantitative RT-PCR analysis of SSTR1/2/3/5 expression between the two groups. Mann-Whitney U-test. *Bars* represent mean ± SEM. ***p < 0.001. **E and F:** Spearman correlation analysis was used to test the correlation between SSTR2 mRNA level and maximum diameter (E) and tumor volume (F).

Factors Associated With Tumor Invasion

Multivariable analysis was performed for factors with $p < 0.1$ on univariate analysis that were possibly associated with tumor invasion, including comorbidity, maximum tumor diameter, tumor volume, preoperative hypopituitarism, and SSTR2 mRNA level. Tumor volume ($p = 0.021$), maximum tumor diameter ($p < 0.001$), and SSTR2 mRNA level ($p < 0.001$) were independent factors of tumor invasion. The results of univariate and multivariate analyses associated with tumor invasion are listed in Supplemental Table 1.

Using ROC curve analysis to evaluate the value of SSTR2, we found that the low mRNA level of SSTR2 in tumors was closely associated with tumor invasion, with an area under the curve (AUC) of 0.805 (95% CI 0.715–0.877, $p < 0.0001$) (Fig. 2).

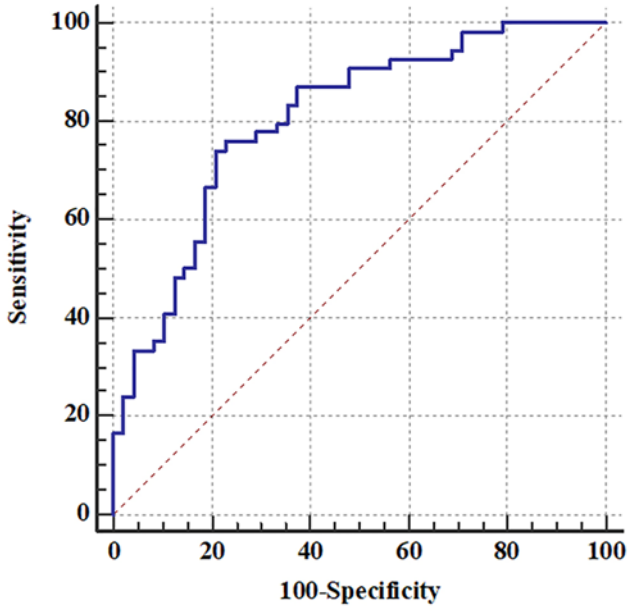

FIG. 2. ROC curve analysis for SSTR2 mRNA on evaluating tumor invasion. The low mRNA level of SSTR2 in tumors was closely associated with tumor invasion (AUC = 0.805, $p < 0.0001$). Figure is available in color online only.

IHC Results

Because of the relative higher mRNA levels, SSTR2 and SSTR5 underwent further investigation for differential protein expression by IHC staining. The results are demonstrated in Table 3. When compared with the noninvasive group, tumor samples from the invasive group had relatively lower SSTR2 expression. The final score for SSTR2 expression in the invasive group was 4.75 ± 3.27, lower than 7.91 ± 3.04 in the noninvasive group ($p < 0.001$); 11 (30.6%) patients had high expression in the invasive group and 30 (69.8%) had high expression in the noninvasive group ($p = 0.001$). However, there was no significant difference in SSTR5 expression between two groups regarding the percentage of positive cells or the intensity of positive staining. Representative SSTR2 and SSTR5 IHC images are shown in Fig. 3.

Evaluation of Whether Preoperative SSA Short-Term Therapy Could Influence the Expression of SSTR2

Given that the proportion of patients with preoperative SSA therapy in the invasive group was higher than that in the noninvasive group, to test whether SSA treatment can influence the expression level of SSTR2, we further investigated differential expressions at the levels of SSTR2 mRNA and protein in patients with and without SSA therapy between two groups and in patients with noninvasive and invasive tumors between the SSA therapy group and non-SSA therapy group. The results demonstrated that the SSTR2 mRNA and protein expressions in patients with SSA treatment were significantly higher in the noninvasive group than in the invasive group ($p < 0.001$ and $p = 0.002$, respectively) (Fig. 4A). The SSTR2 mRNA and protein levels in patients without preoperative SSA treatment were also higher in the noninvasive group compared with the invasive group but without difference in protein expression ($p < 0.001$ and $p = 0.491$, respectively) (Fig. 4B). However, there were no significant differences in the SSTR2 mRNA and protein levels between SSA therapy group and non-SSA therapy group, including patients in the invasive group ($p = 0.885$ and $p = 0.243$, respectively) (Fig. 4C) and the noninvasive group ($p = 0.224$ and $p = 0.925$, respectively) (Fig. 4D). These results indirectly indicated that short-term treatment with preoperative SSAs might not cause down- or upregulation of SSTR2 expression.

Correlation Between Response to Preoperative SSAs Therapy and Tumor Invasion

The above results revealed that the SSTR2 level was negatively correlated with tumor invasion. We therefore further investigated the correlation between pharmacological response to preoperative SSA therapy and tumor invasion. In our cohorts, 57 patients received SSA therapy before surgery, including 24 (44.4%) patients in the noninvasive group and 33 (68.8%) patients in the invasive group ($p = 0.014$). The results showed that 34 patients (59.6%) had a good response to SSAs, including tumor response in 30 (52.6%) patients and biochemical response in 29 (50.9%) patients. Interestingly, we also found that patients with noninvasive tumor tended to have a higher pharmacological response than patients with invasive tumor (91.7% vs 36.4%, $p < 0.001$) (Table 4). We therefore speculated that the mechanism of the poorer response to SSAs in invasive tumors was due to the lower level of SSTR2.

Moreover, we also compared the SSTR2 mRNA and protein levels, which were expressed significantly more in the SSA responder group than in the SSA nonresponder group. (qRT-PCR: $p < 0.01$ [Supplemental Fig. 1]; and IHC analysis: $p = 0.049$ [Supplemental Table 2]).

Discussion

In this study, we demonstrated that patients with invasive tumors had less favorable response rates to SSA therapy. We also showed that tumor invasion is inversely correlated with expression levels of SSTR2. It has been

TABLE 3. Immunohistochemical results of SSTR2 and SSTR5 in the noninvasive and invasive groups

Immunohistochemical Score	Total (n = 79)	Noninvasive Group (n = 43)	Invasive Group (n = 36)	p Value
SSTR2				<0.001
% positive cells				
Score 1	8 (10.1)	0 (0)	8 (22.2)	
Score 2	13 (16.5)	4 (9.3)	9 (25)	
Score 3	13 (16.5)	7 (16.3)	6 (16.7)	
Score 4	45 (56.9)	32 (74.4)	13 (36.1)	
Intensity of staining				0.005
Score 1	25 (31.6)	7 (16.3)	18 (50)	
Score 2	36 (45.6)	23 (53.5)	13 (36.1)	
Score 3	18 (22.8)	13 (30.2)	5 (13.9)	
Final score				0.001
Low expression (score ≤6)	38 (48.1)	13 (30.2)	25 (69.4)	
High expression (score >6)	41 (51.9)	30 (69.8)	11 (30.6)	
SSTR5				0.775
% positive cells				
Score 1	15 (19.0)	7 (16.3)	8 (22.2)	
Score 2	14 (17.7)	7 (16.3)	7 (19.4)	
Score 3	26 (32.9)	14 (32.6)	12 (33.3)	
Score 4	24 (30.4)	15 (34.9)	9 (25)	
Intensity of staining				0.555
Score 1	29 (36.7)	14 (32.6)	15 (41.7)	
Score 2	38 (48.1)	21 (48.8)	17 (47.2)	
Score 3	12 (15.2)	8 (18.6)	4 (11.1)	
Final score				0.245
Low expression (score ≤6)	54 (68.4)	27 (62.8)	27 (75)	
High expression (score >6)	25 (31.6)	16 (37.2)	9 (25)	

Values are presented as the number of patients (%).

observed that SSAs have a greater affinity for binding to SSTR2 as opposed to SSTR5.[26] Previous research has established a significant correlation between a positive response to SSAs and the level of SSTR2 expression.[15,27] Our study has demonstrated and highlighted the importance of SSTR2 in relation to tumor invasion.

SSTRs, considered important pharmacological targets, have been used to study the response to SSAs.[18,20] How-

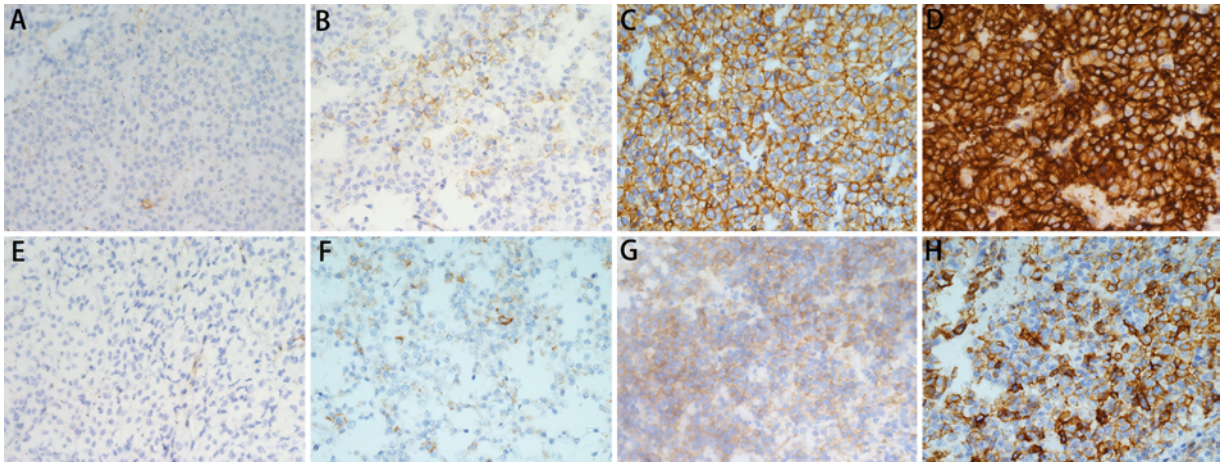

FIG. 3. Representative images of IHC staining (DAB stain) in tumors. **A–D:** SSTR2 stains were negative, light-yellow, brown-yellow, and brown, respectively. **E–H:** SSTR5 stains were negative, light-yellow, brown-yellow, and brown, respectively. Original magnification ×400. Figure is available in color online only.

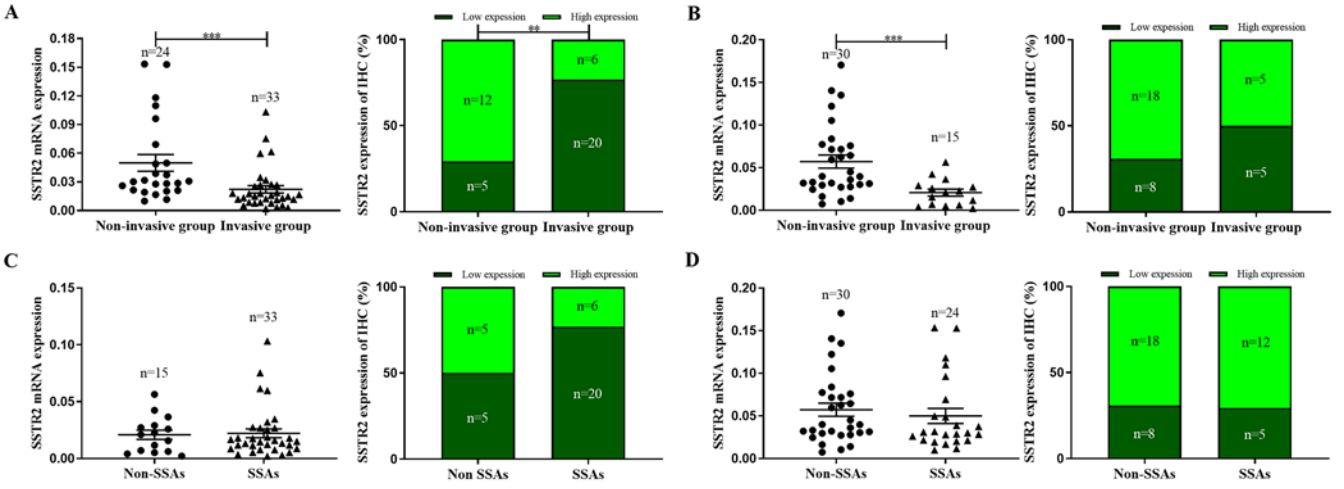

FIG. 4. Comparison results of the SSTR2 mRNA (*left*) and protein expression (*right*) in tumor samples. **A:** Comparisons in patients with SSA therapy between the noninvasive and invasive groups. **B:** Comparisons in patients without SSA therapy between the noninvasive and invasive groups. **C:** Comparisons in patients with invasive tumor between non-SSA therapy and SSA therapy. **D:** Comparisons in noninvasive group between non-SSA therapy and SSA therapy. Mann-Whitney U-test. *Bars* represent mean ± SEM. **p < 0.01; ***p < 0.001. Figure is available in color online only.

ever, the effect of SSTRs on tumor invasion has been seldom investigated. At our center, we found that patients with invasive tumors usually showed a poorer response to preoperative SSA therapy. This led us to hypothesize that differential expression of SSTRs might be related to tumor invasion. In this study, we first evaluated SSTR expression using a qRT-PCR technique in tumor samples obtained from 102 acromegaly patients with somatotropinomas and found that they expressed low mRNA levels of SSTR1 and SSTR3 in agreement with previous studies.[14,15,20] Meanwhile, our qRT-PCR results revealed no difference in SSTR1 and SSTR3 mRNA levels between the invasive and noninvasive groups. The protein expressions of SSTR1 and SSTR3 were not further investigated by IHC analysis in our study, as previous studies have shown that it is not a reliable method of detection of the proteins.[20] IHC analysis of SSTR2 and SSTR5 was also not performed in 23 cases because of the lack of sufficient tumor tissue. We observed that, in general, mRNA and protein levels of SSTR2 were significantly lower in invasive tumors than in noninvasive tumors. However, the other SSTRs (SSTR1, SSTR3, and SSTR5) were not associated with tumor invasion. Furthermore, multivariate and ROC analyses indicated that the low mRNA level of SSTR2 in tumors was an independent factor and a sensitive indicator of tumor invasion. Intriguingly, we also discovered that there was a significant negative correlation between SSTR2 mRNA expression level and tumor size.

Medical therapy is important for preoperative tumor shrinkage and reduction of GH levels; however, its importance and effectiveness are widely debated.[6,28] Multicenter studies have shown that the efficacy rates for SSAs range from 25% to 30%; however, many patients did not respond to SSA treatment.[29,30] Consequently, determining the correct SSA therapy for patients has been a challenge for clinicians. Our recent study revealed that preoperative SSA therapy does not improve BR rates in noninvasive tumors because the tumors are relatively easy to remove.[31] In our cohort, 59.6% patients had a good pharmacological response, with tumor volume response and biochemical

TABLE 4. Comparison of pharmacological response between the noninvasive and invasive groups

Response to SSAs	No. of Patients (%)			p Value
	Total (n = 57)	Noninvasive Group (n = 24)	Invasive Group (n = 33)	
Tumor response				<0.001
Positive response	30 (52.6)	21 (87.5)	9 (27.3)	
Negative response	27 (47.4)	3 (12.5)	24 (72.7)	
Biochemical response				<0.001
Positive response	29 (50.9)	20 (83.3)	9 (27.3)	
Negative response	28 (49.1)	4 (16.7)	24 (72.7)	
Total response				<0.001
Positive response	34 (59.6)	22 (91.7)	12 (36.4)	
Negative response	23 (40.4)	2 (8.3)	21 (63.6)	

response being 52.6% and 50.9%, respectively, showing a positive response rate that was consistent with a recent study.[28] Interestingly, the positive response to SSA therapy was only 36.4% in the invasive group. Therefore, we advise caution when prescribing preoperative SSA therapy for patients. As IHC analysis and qRT-PCR of SSTR2 are convenient and effective methods of evaluating the response to SSA therapy, we feel that qRT-PCR and IHC analyses should be used routinely as part of the postoperative investigative workup. For patients with postoperative residual tumor, SSA therapy use should not be universal but considered for patients with higher SSTR2 expression in tumor tissue, particularly invasive somatotroph tumors. In patients with lower SSTR2 expression, radiation therapy may be a superior option.

In this study, we also evaluated whether preoperative short-term SSA therapy could influence the expression level of SSTR2. Given that the number of patients who had preoperative SSA therapy in the invasive group was higher than the noninvasive group, patients were divided into different groups and compared to minimize the influence caused by sampling. Our results showed that there were no significant differences in mRNA and protein expression of SSTR2 between patients with SSA therapy and those without SSA therapy in both the invasive group (Fig. 4C) and the noninvasive group (Fig. 4D). Additionally, there were also differential expressions of SSTR2 between the invasive and noninvasive groups, except that the differential protein expression was not detected in patients without SSA therapy because of the small population size that underwent IHC analysis ($p = 0.491$, Fig. 4B). Results from this study suggest that short-term therapy using preoperative SSAs may not affect SSTR2 expression, which is inconsistent with what has been observed in other studies.[32,33] The impact of preoperative SSA treatment on SSTR2 expression over time has been less well studied, and determining baseline SSTR2 levels before surgery remains a challenge. Few patients met the positive response criteria of IGF-1 after a short course of SSA therapy, although IGF-1 reduction was also considered as an indicator.[20] Most positive responders had a significant reduction in GH and tumor size.

Despite advancements in surgical equipment and techniques, the rates of GTR and BR after surgery are still unsatisfactory due to the complexity of growth patterns, especially in acromegaly patients with invasive somatotroph tumors.[34–37] Tumors may invade surrounding structures including the sphenoid sinus, CS, and clivus bone.[38,39] The Knosp grade for pituitary neuroendocrine tumors is the most widely used to evaluate tumor invasion and has been shown to correlate with the possibility of achieving GRT and BR.[23,36,40,41] Knosp grade 3 or 4 tumors may invade the posterior and lateral areas of the CS and might be difficult to resect because of overlying and surrounding neurovascular structures and therefore result in residual tumor.[42] In addition, some Knosp grade 0–2 tumors may also be invasive and can infiltrate the sphenoid sinus or clivus bone, leading to postoperative nonremission despite achieving GTR.[31,36] Therefore, Hardy grade III or IV tumors were also classified as invasive in this study.

To the best of our knowledge, this is the first study to demonstrate a relationship between SSTR expression and invasion of somatotropinomas. Coincidentally, recent studies have indicated a significant association between a low expression of dopamine D2 receptor and tumor invasion.[43] The exact mechanism of SSTR2 affecting tumor invasion is still unclear and should be investigated in future studies.

Limitations

Several limitations should be considered. First, the data in this study were from a single medical center; a future multicenter study may generate different conclusions. Second, after transsphenoidal surgery, some acromegaly patients (approximately 20%) may experience delayed remission over a longer period of time;[31,44] however, our study only included a small number (16 patients) who were followed up for 3 months, which may have had a minor effect on the result of long-term endocrine remission. Third, the 3-month treatment time in the evaluation of the positive response to preoperative SSA treatment seems to be a little short; it would be interesting to know the response effect to SSAs over long-term treatment and follow-up. Further research could be conducted on surgical patients who have received medical treatment for more than 6 months to evaluate the positive response to SSAs. Finally, the Knosp grade has a limitation when evaluating tumor invasion; some lower-grade (0–2) tumors may also be invasive and can infiltrate the medial wall of the CS or clivus bone or intracranial invasion, while few grade 3 tumors may be noninvasive only because of their growth patterns.

Conclusions

Our study reveals that the invasiveness of somatotropinomas was negatively associated with SSTR2 expression, but not with SSTR1, SSTR3, and SSTR5. Patients with invasive tumors have a poorer response to SSA therapy than patients with noninvasive tumors, and this could be due to a low level of SSTR2 expression. Therefore, it is recommended that SSTR2 expression, especially for invasive somatotropinomas, should be routinely examined to select the most suitable therapeutic strategies (medication or radiation therapy) in case a postoperative residual tumor is present.

Acknowledgments

We appreciate the assistance of colleagues from the Department of Neurosurgery.

Funding support of this study was provided by National Natural Science Foundation of China (82203179) and Guangdong Basic and Applied Basic Research Foundation (2023A1515011644).

References

1. Asa SL, Ezzat S. The pathogenesis of pituitary tumors. *Annu Rev Pathol*. 2009;4:97-126.
2. Ezzat S, Asa SL, Couldwell WT, et al. The prevalence of pituitary adenomas: a systematic review. *Cancer*. 2004;101(3):613-619.
3. Mete O, Lopes MB. Overview of the 2017 WHO classification of pituitary tumors. *Endocr Pathol*. 2017;28(3):228-243.
4. Gil J, Marques-Pamies M, Valassi E, et al. Implications of heterogeneity of epithelial-mesenchymal states in acromegaly therapeutic pharmacologic response. *Biomedicines*. 2022;10(2):460.

5. Heck A, Emblem KE, Casar-Borota O, Bollerslev J, Ringstad G. Quantitative analyses of T2-weighted MRI as a potential marker for response to somatostatin analogs in newly diagnosed acromegaly. *Endocrine*. 2016;52(2):333-343.
6. Paragliola RM, Corsello SM, Salvatori R. Somatostatin receptor ligands in acromegaly: clinical response and factors predicting resistance. *Pituitary*. 2017;20(1):109-115.
7. Katznelson L, Laws ER Jr, Melmed S, et al. Acromegaly: an endocrine society clinical practice guideline. *J Clin Endocrinol Metab*. 2014;99(11):3933-3951.
8. Giustina A, Chanson P, Bronstein MD, et al. A consensus on criteria for cure of acromegaly. *J Clin Endocrinol Metab*. 2010;95(7):3141-3148.
9. Hazer DB, Işık S, Berker D, et al. Treatment of acromegaly by endoscopic transsphenoidal surgery: surgical experience in 214 cases and cure rates according to current consensus criteria. *J Neurosurg*. 2013;119(6):1467-1477.
10. Nomikos P, Buchfelder M, Fahlbusch R. The outcome of surgery in 668 patients with acromegaly using current criteria of biochemical 'cure'. *Eur J Endocrinol*. 2005;152(3):379-387.
11. Carlsen SM, Lund-Johansen M, Schreiner T, et al. Preoperative octreotide treatment in newly diagnosed acromegalic patients with macroadenomas increases cure short-term postoperative rates: a prospective, randomized trial. *J Clin Endocrinol Metab*. 2008;93(8):2984-2990.
12. Caron PJ, Bevan JS, Petersenn S, et al. Tumor shrinkage with lanreotide Autogel 120 mg as primary therapy in acromegaly: results of a prospective multicenter clinical trial. *J Clin Endocrinol Metab*. 2014;99(4):1282-1290.
13. Colao A, Auriemma RS, Pivonello R. The effects of somatostatin analogue therapy on pituitary tumor volume in patients with acromegaly. *Pituitary*. 2016;19(2):210-221.
14. Casarini APM, Jallad RS, Pinto EM, et al. Acromegaly: correlation between expression of somatostatin receptor subtypes and response to octreotide-lar treatment. *Pituitary*. 2009;12(4):297-303.
15. Taboada GF, Luque RM, Neto LV, et al. Quantitative analysis of somatostatin receptor subtypes (1-5) gene expression levels in somatotropinomas and correlation to in vivo hormonal and tumor volume responses to treatment with octreotide LAR. *Eur J Endocrinol*. 2008;158(3):295-303.
16. Zhu H, Xu Y, Gong F, et al. Reference ranges for serum insulin-like growth factor I (IGF-I) in healthy Chinese adults. *PLoS One*. 2017;12(10):e0185561.
17. Ferone D, de Herder WW, Pivonello R, et al. Correlation of in vitro and in vivo somatotropic adenoma responsiveness to somatostatin analogs and dopamine agonists with immunohistochemical evaluation of somatostatin and dopamine receptors and electron microscopy. *J Clin Endocrinol Metab*. 2008;93(4):1412-1417.
18. Soukup J, Hornychova H, Manethova M, et al. Predictive and prognostic significance of tumour subtype, SSTR1-5 and E-cadherin expression in a well-defined cohort of patients with acromegaly. *J Cell Mol Med*. 2021;25(5):2484-2492.
19. Tortora F, Negro A, Grasso LFS, et al. Pituitary magnetic resonance imaging predictive role in the therapeutic response of growth hormone-secreting pituitary adenomas. *Gland Surg*. 2019;8(suppl 3):S150-S158.
20. Venegas-Moreno E, Vazquez-Borrego MC, Dios E, et al. Association between dopamine and somatostatin receptor expression and pharmacological response to somatostatin analogues in acromegaly. *J Cell Mol Med*. 2018;22(3):1640-1649.
21. Hardy J, Vezina JL. Transsphenoidal neurosurgery of intracranial neoplasm. *Adv Neurol*. 1976;15:261-273.
22. Wilson CB. A decade of pituitary microsurgery. The Herbert Olivecrona lecture. *J Neurosurg*. 1984;61(5):814-833.
23. Knosp E, Steiner E, Kitz K, Matula C. Pituitary adenomas with invasion of the cavernous sinus space: a magnetic resonance imaging classification compared with surgical findings. *Neurosurgery*. 1993;33(4):610-618.
24. Guo Z, Zhang X, Zhu H, et al. TELO2 induced progression of colorectal cancer by binding with RICTOR through mTORC2. *Oncol Rep*. 2021;45(2):523-534.
25. Venegas-Moreno E, Flores-Martinez A, Dios E, et al. E-cadherin expression is associated with somatostatin analogue response in acromegaly. *J Cell Mol Med*. 2019;23(5):3088-3096.
26. Shimon I. Somatostatin receptors in pituitary and development of somatostatin receptor subtype-selective analogs. *Endocrine*. 2003;20(3):265-269.
27. Wildemberg LE, Neto LV, Costa DF, et al. Low somatostatin receptor subtype 2, but not dopamine receptor subtype 2 expression predicts the lack of biochemical response of somatotropinomas to treatment with somatostatin analogs. *J Endocrinol Invest*. 2013;36(1):38-43.
28. Lv L, Hu Y, Zhou P, et al. Presurgical treatment with somatostatin analogues in growth hormone-secreting pituitary adenomas: a long-term single-center experience. *Clin Neurol Neurosurg*. 2018;167:24-30.
29. Melmed S, Cook D, Schopohl J, Goth MI, Lam KSL, Marek J. Rapid and sustained reduction of serum growth hormone and insulin-like growth factor-1 in patients with acromegaly receiving lanreotide Autogel therapy: a randomized, placebo-controlled, multicenter study with a 52 week open extension. *Pituitary*. 2010;13(1):18-28.
30. Mercado M, Borges F, Bouterfa H, et al. A prospective, multicentre study to investigate the efficacy, safety and tolerability of octreotide LAR (long-acting repeatable octreotide) in the primary therapy of patients with acromegaly. *Clin Endocrinol (Oxf)*. 2007;66(6):859-868.
31. Zhang S, Chen J, Yao S, et al. Predictors of postoperative biochemical remission in lower Knosp grade growth hormone-secreting pituitary adenomas: a large single center study. *J Endocrinol Invest*. 2023;46(3):465-476.
32. Kontogeorgos G, Markussis V, Thodou E, et al. Association of pathology markers with somatostatin analogue responsiveness in acromegaly. *Int J Endocrinol*. 2022;2022:8660470.
33. Franck SE, Gatto F, van der Lely AJ, et al. Somatostatin receptor expression in GH-secreting pituitary adenomas treated with long-acting somatostatin analogues in combination with pegvisomant. *Neuroendocrinology*. 2017;105(1):44-53.
34. Asha MJ, Takami H, Velasquez C, et al. Long-term outcomes of transsphenoidal surgery for management of growth hormone-secreting adenomas: single-center results. *J Neurosurg*. 2019;133(5):1360-1370.
35. Park HH, Kim EH, Ku CR, Lee EJ, Kim SH. Outcomes of aggressive surgical resection in growth hormone-secreting pituitary adenomas with cavernous sinus invasion. *World Neurosurg*. 2018;117:e280-e289.
36. Wu X, Xie SH, Tang B, et al. Pituitary adenoma with posterior area invasion of cavernous sinus: surgical anatomy, approach, and outcomes. *Neurosurg Rev*. 2021;44(4):2229-2237.
37. Yao S, Chen WL, Tavakol S, et al. Predictors of postoperative biochemical remission in acromegaly. *J Neurooncol*. 2021;151(2):313-324.
38. Kasuki L, Raverot G. Definition and diagnosis of aggressive pituitary tumors. *Rev Endocr Metab Disord*. 2020;21(2):203-208.
39. Bai J, Li X, Ge A, Gu J. Correlation analysis of magnetic resonance imaging characteristics and prognosis of invasive pituitary adenomas in neurosurgery hospitals. *J Healthc Eng*. 2022;2022:8280540.
40. Micko A, Oberndorfer J, Weninger WJ, et al. Challenging Knosp high-grade pituitary adenomas. *J Neurosurg*. 2019;132(6):1739-1746.
41. Micko AS, Wöhrer A, Wolfsberger S, Knosp E. Invasion of the cavernous sinus space in pituitary adenomas: endoscopic

verification and its correlation with an MRI-based classification. *J Neurosurg*. 2015;122(4):803-811.
42. Fernandez-Miranda JC, Zwagerman NT, Abhinav K, et al. Cavernous sinus compartments from the endoscopic endonasal approach: anatomical considerations and surgical relevance to adenoma surgery. *J Neurosurg*. 2018;129(2):430-441.
43. Peverelli E, Giardino E, Treppiedi D, et al. Dopamine receptor type 2 (DRD2) inhibits migration and invasion of human tumorous pituitary cells through ROCK-mediated cofilin inactivation. *Cancer Lett*. 2016;381(2):279-286.
44. Guo X, Zhang R, Zhang D, et al. Determinants of immediate and long-term remission after initial transsphenoidal surgery for acromegaly and outcome patterns during follow-up: a longitudinal study on 659 patients. *J Neurosurg*. 2022;137(3):618-628.

Disclosures

The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

Author Contributions

Conception and design: Wang, Zhang, W Chen, Mao, Y Zhu. Acquisition of data: Wang, Zhang, Yao, J Chen, Yang, D Zhu, W Chen, Mao, Y Zhu. Analysis and interpretation of data: Zhang, Yao, J Chen, Zeng, W Chen. Drafting the article: Zhang, Yao, J Chen, Akter. Critically revising the article: Wang, Yao, Akter, Zeng, Mao, Y Zhu. Reviewed submitted version of manuscript: Wang, Zeng, Mao, Y Zhu. Approved the final version of the manuscript on behalf of all authors: Wang. Statistical analysis: Zhang, Yao, Yang. Administrative/technical/material support: D Zhu. Study supervision: Wang.

Supplemental Information

Online-Only Content

Supplemental material is available with the online version of the article.

Supplemental Figure and Tables. https://thejns.org/doi/suppl/10.3171/2023.7.JNS23858.

Correspondence

Haijun Wang: The First Affiliated Hospital, Sun Yat-sen University, Guangzhou, Guangdong, China. wanghaij@mail.sysu.edu.cn.