REFRACTORY PITUITARY ADENOMA—CURRENT CHALLENGES AND EMERGING TREATMENTS

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PUBLISHED IN: Frontiers in Endocrinology

frontiers Research Topics

ISSN 1664-8714
ISBN 978-2-88974-799-3
DOI 10.3389/978-2-88974-799-3

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REFRACTORY PITUITARY ADENOMA—CURRENT CHALLENGES AND EMERGING TREATMENTS

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Citation: Wang, R., Zhou, C., McCormack, A., Mamelak, A., eds. (2022). Refractory Pituitary Adenoma—Current Challenges and Emerging Treatments. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-88974-799-3

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frontiers in Endocrinology

EDITORIAL
published: 09 March 2022
doi: 10.3389/fendo.2022.868174

Editorial: Refractory Pituitary Adenoma—Current Challenges and Emerging Treatments

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Keywords: refractory pituitary adenomas, diagnosis, surgical treatment, medical therapy, temozolomide, targeted and immunotherapy, drug resistance, pathogenesis

Editorial on the Research Topic

Refractory Pituitary Adenoma—Current Challenges and Emerging Treatments

OPEN ACCESS

Edited and reviewed by:
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Specialty section:
This article was submitted to Pituitary Endocrinology, a section of the journal Frontiers in Endocrinology

Received: *02 February 2022*
Accepted: *11 February 2022*
Published: *09 March 2022*

Citation:
Wang R, Zhou C, McCormack AI and Mamelak AN (2022) Editorial: Refractory Pituitary Adenoma—Current Challenges and Emerging Treatments. Front. Endocrinol. 13:868174. doi: 10.3389/fendo.2022.868174

Refractory pituitary adenomas (PAs) are defined as aggressive-invasive PAs characterized by rapid growth, frequent recurrence, a high Ki-67 index, and resistance to standard therapeutic approaches (1, Dai et al.). It is notoriously difficult to manage these refractory PAs due to limited efficacy of current therapeutic options. After initial treatment, refractory PAs frequently re-grow or recur and require continued therapies including medical therapy, radiotherapy, and re-operation. However, these patients with refractory PAs often do not respond to these treatments and have a very poor prognosis (2). Recently, temozolomide (TMZ) has shown moderate efficacy for refractory PAs and pituitary carcinomas (PCs) (3). In 2018, the European Society of Endocrinology Clinical Practice Guidelines recommended TMZ as the first-line treatment for refractory PAs and PCs when conventional treatments have failed to control tumor growth (4). However, many refractory PAs demonstrate only transient response to TMZ therapy, and subset of them develop drug resistance during treatment (5, 6). Although targeted therapies such as anti-epidermal growth factor receptor (EGFR), anti-vascular endothelial growth factor (VEGF), and inhibitors of mammalian target of rapamycin (mTOR) signal pathway have also been used to treat refractory PAs and PCs, effectiveness of these targeted therapies is still largely unknown due to a lack of data from preclinical research and clinical trials (7, 8). Cancer immunotherapy is also a promising treatment option for refractory PAs and PCs, but more basic research and clinical trials are needed to further verify its efficacy (Dai et al., 9).

In the present Research Topic in Frontiers in Endocrinology, we collected 16 studies and reviews on recent advancements of preclinical research and clinical cases in diagnosis and treatment of refractory PAs.

Eight articles of clinical findings and case reports addressed imaging, immunotherapy, fluconazole and TMZ treatment of refractory pituitary tumors, and raised attention on silent corticotroph adenoma (SCA). Yan et al. showed that increased distance between the bilateral internal carotid arteries (ICAs) is related to disease duration, however, is not associated with preoperative levels of GH and IGF-1 in acromegalic patients. Both longer disease duration and refractory clinical presentation are risk factors of increased ICAs distance in acromegalic patients.

Furthermore, Zheng's and Zhang's groups addressed SCA, a high-risk aggressive subtype of refractory PA in 2 separate articles. Zheng et al. retrospectively investigated the characteristics of SCA that transformed to typical Cushing's syndrome at a single medical center and reviewed relevant literatures. All cases that converted from SCA to adrenocorticotropic hormone (ACTH)-secreting tumors were macroadenomas and the median time of conversion was 30 (13.0, 68.3) months. Once transformed, the ACTH-secreting tumors exhibited higher recurrence frequency, increased invasiveness and more serious hypercortisolism. Zhang et al. explored differences of clinical features and surgical outcomes between ACTH-negative and ACTH-positive SCA patients with positive Tpit immunostaining but no evidence for Cushing's Syndrome. These findings indicated that the prevalence of SCA is substantially underestimated, thus more attention and long-term follow-up is important for SCAs. Moreover, Lamb et al. reported a case of PC responding to immune checkpoint inhibitor (ICI) therapy and subsequent anti-VEGF therapy. The PC presented satisfied initial response to ICI therapy combining anti-PD-1 and anti-CTLA4. Survival was lengthened and morbidity reduced in the patient. This case raised the potential of sequential or combining ICI and anti-VEGF therapy as a possible strategy treating aggressive pituitary tumors. Xiang et al. described a case of autoimmune hypophysitis (AH) coexisting with systemic lupus erythematosus (SLE) and summarized all case reports of AH with SLE. After treatments with immunosuppressant and glucocorticoids, symptoms of hyponatraemia and hypopituitarism gradually disappeared, and long-term remission was achieved. This case report implied a potential relationship between SLE and AH, and AH should be concerned when evaluating patients with SLE. Zhao et al. reported a case of a patient with Cushing's disease and pulmonary cryptococcus neoformans treating with fluconazole, both cryptococcus infection and hypercortisolism were relieved. Therefore, as a possible alternative treatment, fluconazole may be effective for patients with Cushing's disease and cryptococcal pneumonia. Tang et al. presented a case of aggressive prolactinoma that was resistant to dopamine agonists treatment but responded to TMZ. After a total of six-cycle TMZ treatment, tumor was completely disappeared and PRL level normalized. Authors further performed genome sequencing to investigate the underlying mechanism of the tumor aggressiveness and high sensitivity to TMZ. Additionally, Cooper et al. summarized 9 cases of aggressive PAs to demonstrate the importance and significance of multidisciplinary, individualized treatment. The choice of treatment timing and rationality for different therapeutic approaches in each case were reviewed, as well as strategies of individualized treatment were discussed.

Three preclinical and basic studies investigated mechanisms underlying refractory/aggressive PAs. Wang et al. studied the mechanism underlying miR-134 effect on alleviating growth of nonfunctioning pituitary adenoma cells. miR-134 significantly inhibited αT3-1 cell proliferation, VEGFA expression, and G1/S cell cycle transition. This inhibition effect can be reversed by stromal cell-derived factor-1alpha and overexpressed VEGFA. In primary nonfunctioning PAs, miR-134 expression level negatively correlated with invasiveness of tumor. These findings implied a novel mechanism of pituitary tumor pathogenesis and provided potential therapeutic target. Moreover, Shen et al. discovered the role of heat-shock protein 90 (Hsp90) in ACTH-secreting adenomas cells and the possible clinical application of Hsp90 inhibitor 17-N-allylamino-17-demethoxygeldanamycin (17-AAG) in Cushing's disease. 17-AAG attenuated cell viability and decreased secretory function of human ACTH-secreting adenomas cells. Inhibitory effect of 17-AAG on USP8 mutant tumor cells were increased than the effect on USP8 wild-type cells, indicating 17-AAG as a potential treatment option for Cushing's disease. Furthermore, Xi et al. explored significant differences of immunologic profiles in normal pituitaries vs PAs, and in benign vs aggressive PAs. Authors quantitatively analyzed mRNA expression levels of CD86, CD80, CLTA-4, PD-1, PD-L1 and PD-L2. Aggressive PAs showed increased CD80, CD86 and PD-L2 than normal pituitaries, as well as elevated CD80 and CD86 than non-aggressive PAs. These results suggested scientific rationale of ICI therapy for PAs.

Several review articles discussed current progress of refractory PA treatment and addressed new hypotheses for PC. Dai et al. addressed recent progress and current controversies of the classification and definition of pituitary tumors. Terms and classifications of PitNET, aggressive PAs and refractory PAs were discussed. Authors suggested that the term of refractory PAs accurately represents characteristics of these tumors, and the diagnostic criteria are more strict, objective, and accurate. This diagnostic criterion may help to early identify patients with refractory PAs and adopt aggressive therapeutic strategies, therefore achieve better clinical outcomes. Dai et al. also summarized the progress of immunotherapy in refractory PAs and PCs. The expression of immune factors including PD-1, PD-L1 macrophages, and lymphocytes were significantly related to clinicopathological characteristics of PAs. Clinical studies suggested the benefit of immunotherapy for refractory PA or PC patients, while more preclinical study and clinical trials are required to further elucidate the efficacy. Furthermore, Nakano-Tateno et al. provided comprehensive review on the efficacies and protocols of medical treatments for aggressive PAs. Aggressive PAs can be resistant to conventional therapies. Some novel therapeutic strategies, such as TMZ, TMZ-Based combination therapies (CAPTEM), inhibitors of mTOR signal pathway and CDK2, anti-EGFR, immunotherapy, peptide receptor radionuclide therapy (PRRT), and retinoic acid were discussed in this review. In addition, Yamamoto et al. introduced several aggressive types of ACTHomas including Crooke's cell adenoma (CCA), Nelson's syndrome and SCAs, summarized current knowledge of the definition, pathophysiology, and treatment of refractory ACTHomas, and provided directions for future research. Moreover, Dai et al. proposed the hypothesis of metastatic spread of PCs. Authors reviewed previously reported cases of PCs and surmised that different secretory types of PCs prefer their own favorable metastatic organs through different patterns of metastatic spread. Therefore, the

"seed and soil" theory could be adopted to explain the metastatic pattern of PCs.

In summary, studies on diagnosis and treatment of refractory PAs have achieved significant advances. However, this Research Topic only contains a limited fraction of many important aspects of refractory PAs. We expect future issues will collect more studies on basic and translational research and large-scale clinical trials, bringing a better understanding of characteristics and underlying mechanisms of refractory PAs. Moreover, new therapeutic concepts and strategies such as personalized and precision medicine may also play a role in future treatment. However, a significantly more preclinical and clinical research is needed to further understand the nature of the mechanism underlying refractory PA growth, and to improve the clinical outcomes for patients suffering with these tumors.

AUTHOR CONTRIBUTIONS

RW and CZ drafted this manuscript. AIM and ANM polished and revised the text. All authors contributed to the article and approved the submitted version.

REFERENCES

1. Dai C, Feng M, Liu X, Ma S, Sun B, Bao X, et al. Refractory Pituitary Adenoma: A Novel Classification for Pituitary Tumors. *Oncotarget* (2016) 7(50):83657–68. doi: 10.18632/oncotarget.13274
2. Liu X, Dai C, Feng M, Li M, Chen G, Wang R. Diagnosis and Treatment of Refractory Pituitary Adenomas: A Narrative Review. *Gland Surg* (2021) 10 (4):1499–507. doi: 10.21037/gs-20-873
3. Elbelt U, Schlaffer SM, Buchfelder M, Knappe UJ, Vila G, Micko A, et al. Efficacy of Temozolomide Therapy in Patients With Aggressive Pituitary Adenomas and Carcinomas-A German Survey. *J Clin Endocrinol Metab* (2020) 105(3):e660–e675. doi: 10.1210/clinem/dgz211
4. Raverot G, Burman P, McCormack A, Heaney A, Petersenn S, Popovic V, et al. European Society of Endocrinology Clinical Practice Guidelines for the Management of Aggressive Pituitary Tumours and Carcinomas. *Eur J Endocrinol* (2018) 178(1):G1–G24. doi: 10.1530/EJE-17-0796
5. Santos-Pinheiro F, Penas-Prado M, Kamiya-Matsuoka C, Waguespack SG, Mahajan A, Brown PD, et al. Treatment and Long-Term Outcomes in Pituitary Carcinoma: A Cohort Study. *Eur J Endocrinol* (2019) 181(4):397–407.
6. Lizzul L, Lombardi G, Barbot M, Ceccato F, Gardiman MP, Regazzo D, et al. Long-Course Temozolomide in Aggressive Pituitary Adenoma: Real-Life Experience in Two Tertiary Care Centers and Review of the Literature. *Pituitary* (2020) 23(4):359–66.
7. Araki T, Cooper O, Fukuoka H. Editorial: Targeted Therapy for Pituitary Adenomas. *Front Endocrinol (Lausanne)* (2019) 10:358.
8. Dai C, Liang S, Sun B, Li Y, Kang J. Anti-VEGF Therapy in Refractory Pituitary Adenomas and Pituitary Carcinomas: A Review. *Front Oncol* (2021) 11:773905.
9. Nie D, Fang Q, Li B, Cheng J, Li C, Gui S, et al. Research Advances on the Immune Research and Prospect of Immunotherapy in Pituitary Adenomas. *World J Surg Oncol* (2021) 19(1):162.

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The handling editor declared a past collaboration with several of the authors (AIM, ANM).

frontiers in Endocrinology

ORIGINAL RESEARCH
published: 07 July 2020
doi: 10.3389/fendo.2020.00429

The Change in Distance Between Bilateral Internal Carotid Arteries in Acromegaly and Its Risk Factors

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OPEN ACCESS

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Specialty section:
This article was submitted to Pituitary Endocrinology, a section of the journal Frontiers in Endocrinology

Received: *08 March 2020*
Accepted: *01 June 2020*
Published: *07 July 2020*

Citation:
Yan X, Chen X, Ge H, Zhu S, Lin Y, Kang D, Lin Z, Jiang C and Ding C (2020) The Change in Distance Between Bilateral Internal Carotid Arteries in Acromegaly and Its Risk Factors. Front. Endocrinol. 11:429. doi: 10.3389/fendo.2020.00429

Background: Studies investigating the change in distance between the bilateral internal carotid arteries (ICAs) in acromegalic patients have provided ambiguous results. The influencing factors of these changes have not been well-identified.

Objective: To further investigate the change in distance between bilateral ICAs in acromegaly patients and identify the influencing factors of the change.

Method: Patients diagnosed as acromegaly from Jan 2016 to Sep 2019 in the Department of Neurosurgery of the First Affiliated Hospital of Fujian Medical University, were included in this study. Computed tomography angiography (CTA) or magnetic resonance angiography (MRA) data were obtained for all patients for three-dimensional reconstruction of the ICAs. Distance between bilateral ICAs was measured and recorded for assessment.

Result: 172 patients including 86 cases with acromegaly in the study group and 86 cases with non-functional pituitary adenoma in the control group were enrolled in this study. The difference of adenoma sizes between two groups was not statistically significant. Patients in acromegaly group had significantly larger maximum distances between bilateral siphon carotid ectasias (25.5 ± 4.1 vs. 23.4 ± 3.5 mm, $P = 0.001$) and between bilateral lacerum segments (26.2 ± 3.2 vs. 24.1 ± 4.3 mm, $P < 0.001$) compared with those of patients with non-functional pituitary adenomas. Multivariate analysis showed that the increased bilateral ICAs distance was associated with disease duration (odds ratio = 1.01, 95% confidence interval = 1.01–1.02, $P = 0.005$) and refractory pituitary adenoma (odds ratio = 9.8, 95% confidence interval = 1.1–88.7, $P = 0.043$) but not with level of growth hormone (GH), insulin-like growth factor-1 (IGF-1) and adenoma size in acromegaly.

Conclusion: Our study showed significant change in distance between the bilateral ICAs in acromegalic patients, comparing to patients with non-functional pituitary adenomas. The increased intercarotid artery distance is associated with disease duration but not with preoperative level of GH and IGF-1. Refractory pituitary adenoma and longer disease duration are the both risk factors of the increased ICAs distance in patient with acromegly.

Keywords: refractory pituitary adenoma, internal carotid artery, acromegaly, disease duration, computed tomography angiography, magnetic resonance angiography

INTRODUCTION

Acromegaly is mainly caused by the growth hormone (GH)-secreting pituitary adenoma, and characterized by excessive GH secretion. The excessive GH stimulates the growth of various tissues and impairs the structures of the heart and great vessels, which further affects their functions. Acromegalic patients also showed a higher risk of co-morbidities such as hypertension, diabetes and hypopituitarism than general population. Furthermore, there is a higher chance for these patients to have intracranial aneurysms (1, 2), which is known as a main cause of cerebrovascular accidents (3, 4). In acromegalic patients, excessive GH has been proven a primary factor contributing to intracranial aneurysms (1), while co-morbidities, previous surgery and other clinical findings seems not related.

Several studies showed that tortuosity and ectasia of intracranial vessels were increased in acromegalic patients (5), which was associated with increased vascular mortality. Therefore, the distance between bilateral internal carotid arteries (ICAs) also changed. As we know, transsphenoidal surgery is a first-line treatment for GH-secreting pituitary adenoma, therefore, these changes were regarded as the possible risk factors for intraoperative vascular injury. Knowing detailed information of the bilateral ICAs preoperatively could impact intraoperative tumor exposure and reduce intraoperative adverse events.

However, studies investigating the changes of distance between ICAs in acromegalic patients have provided ambiguous results (5–7). Furthermore, the influencing factors of these changes have not been well-identified. With the aid of computed tomography angiography (CTA) and magnetic resonance angiography (MRA), intracranial vascular and bony structure could be modeled. Our study aimed at determining the changes of the distance between the bilateral ICAs in acromegalic patients and investigating the possible risk factors for those changes.

MATERIALS AND METHODS

Subjects

Patients diagnosed as acromegaly from Jan 2016 to Sep 2019 in the Department of Neurosurgery of the First Affiliated Hospital of Fujian Medical University were included in this study as research subjects. Eighty six patients with acromegaly were included in this study. And 86 patients with non-functional pituitary adenoma, matched for age, gender and adenoma size were recruited in the control group.

The inclusion criteria of acromegaly group were as follows: [1] clinical diagnosis of acromegaly; [2] image diagnosis of pituitary adenoma according to contrast-enhanced magnetic resonance imaging, as well as fasting GH level higher than that of normal; [3] pathologically diagnosed as GH-secreting pituitary adenoma. Non-functional pituitary adenoma patients diagnosed by preoperative image, laboratory examination and postoperative pathology were recruited as the control group. The exclusion criteria were as follows: [1] age <18 years; [2] history of surgery, radiotherapy of other brain tumor; [3] medication of somatostatin analogs before admission to our hospital; [4] clinical, laboratory, imaging, and pathologic data set was incomplete.

The following criteria were used to diagnose acromegaly: [1] typical symptoms of acromegaly such as enlarged hand/foot and thickened soft tissue; [2] not able to lower GH to < 1.0 ng/mL after oral administration of 75 g glucose; [3] a high level of fasting GH (>2.5 ng/mL); and [4] a high level of serum insulin-like growth factor-1 (IGF-1) controlled for age and gender. After documentation of their disease information, all patients were admitted, had hormone tests and CTA evaluation. Our study analyzed data of those patients. For comparison purpose, patients with non-functional adenoma, admitted during the same period in our institution were 1:1 matched for age, gender and adenoma size, and recruited as the control group. The study protocol was approved by the Ethics Committee of the hospital. The consent of all subjects was obtained in accordance with the Declaration of Helsinki.

Data Collection and Imaging Examination

Clinical information, such as medical history (prior and current illness), admission status, image files, treatment received and other relevant hospitalization information was collected for each patient. Invasiveness of pituitary adenoma was evaluated using the Knosp classification on the enhanced MRI. Duration of disease of acromegaly was counted from the onset of typical symptoms such as enlarged hand/foot and thickening of the soft tissue. The time for headache, decreased visual acuity or identification of tumor by physical examination was recorded as duration of non-functional pituitary adenoma by medical history. The defintination of atypical adnoma and refractory adenoma was according to the 2004 WHO classification (8) and the published classification for pituitary tumors (9) as follow: Atypical adnoma: [1] elevated mitotic index (>2 mitoses per high-power field [HPF]); [2] positive p53 staining; [3] an ki-67 index >3%. Refractory adenoma: [1] tumor infltrates adjacent structures according to radiological results or intraoperative fndings; [2] tumor Ki-67 index is >3% and grow fast; [3] current treatments fail to control tumor growth and/or hormonal hypersecretion; [4] tumor recurrence occurs within 6 months after surgery.

All included patients received CTA (AquilionTM ONE systems, Toshiba, Japan) or MRA (Magneto Verio MRI system with Tim technology or Skyra MRI system, Siemens, Germany). The image data were exported as DICOM format file and further imported to three-dimensional printing software (PolyJet Studio^{TM}, Aojie, China). After reconstructing the ICAs, distance between the bilateral ICAs was measured. Based on the Bouthillier classification, parameters collected were as follows: (a). the distance between the inner walls of the bilateral carotids at the level of distal dural ring (ophthalmic segment); (b). the distance between ICAs at the level of the most concave point of the C4-C5 bend (siphon carotid ectasias segment); (c). the distance between ICAs at the level of posterior ascending portion of the C4 segment (cavernous segment); (d). the maximum distance between ICAs at the level of the C3 segment (lacerum segment). Illustration was showed in **Figure 1**.

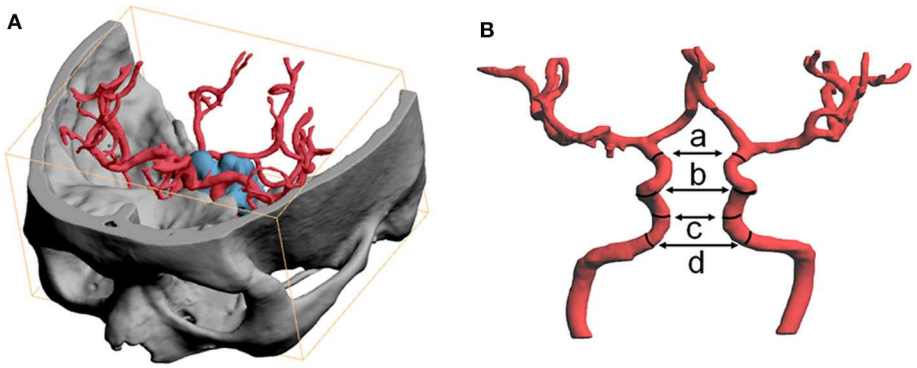

FIGURE 1 | Three-dimensional image software was used to reconstruct the ICAs and to measure intercarotid distance. **(A)** The image data were exported as DICOM format file and further imported to three-dimensional image process software to reconstruct skull, pituitary adenoma and the ICAs. Fused image of skull and ICA was used to determine segments of ICA. **(B)** Measurement of intercarotid distance, parameters including (a) the distance between the inner walls of the bilateral carotids at the level of distal dural ring (ophthalmic segment); (b) the istance between ICAs at the level of the most concave point of the C4-C5 bend (siphon carotid ectasias segment); (c) the distance between ICAs at the level of posterior ascending portion of C4 segment (cavernous segment); (d) the maximum distance between ICAs at the level of C3 segment (lacerum segment).

TABLE 1 | Comparison of demographic and clinical data between patients with acromegaly and non-functional pituitary adenomas.

Characteristics	Acromegaly group (n = 86)	Control group (n = 86)	P-value
DEMOGRAPHICS			
Age, year	40.1 ± 10.6	41.1 ± 10.3	0.531
Gender, female	40 (46.5%)	40 (46.5%)	1.000
Height, cm	167.3 ± 6.3	165.6 ± 6.6	0.114
BMI, kg/m^2	24.8 ± 4.1	24.2 ± 3.5	0.309
MRI FOUNDING			
Pituitary adenoma volume, cm^3	2.6 (0.7, 5.4)	3.1 (0.6, 6.4)	0.418
Pituitary adenoma diameter, cm	1.7 ± 0.8	1.9 ± 1.0	0.455
Knosp grade	2 (1–3)	2 (1–3)	0.512
LABORATORY			
GH, μg/L	40.6 (11.4, 60.2)	0.9 (0.5, 1.4)	<0.001
IGF-1, μg/L	526.2 ± 204.4	-	-
MEDICAL HISTORY			
Hypertension	23 (26.7%)	18 (20.9%)	0.374
Diabetes mellitus	19 (22.1%)	16 (18.6%)	0.570
Hyperlipidemia	14 (16.3%)	13 (15.1%)	0.834
Somking history	18 (20.9%)	21 (24.4%)	0.585
Duration of disease, months	90 (48, 132)	12 (5–20)	<0.001
BILATERAL ICA DISTANCES			
Ophthalmic segments	16.0 ± 5.2	16.2 ± 3.4	0.730
Siphon carotid ectasias segments	25.5 ± 4.1	23.4 ± 3.5	0.001
Cavernous segments	21.9 ± 3.2	22.5 ± 2.7	0.205
Lacerum segments	26.2 ± 3.2	24.1 ± 4.3	<0.001

Acromegaly group: patients with GH-secreting pituitary adenoma. Control group: patients diagnosed with non-functional pituitary adenomas. Values are n (%), mean (SD), median (25%−75%).

Laboratory Examination

All patients had a nine-hour fasting period before surgery for collection of serum samples. Both pituitary hormones and IGF-1 were measured according to a chemiluminescence assay (ADVIA Centaur XP, Siemens, Germany).

Statistical Analysis

The results were expressed as mean ± standard deviation or [M(QL,QU)]. Comparisons of categorical variables were analyzed using the Chi-squared test. The 2-sample t-test or Wilcoxon rank-sum test was used to compare numerical variables.

The bilateral ICAs distances (a, b, c, and d, **Figure 1**) were compared between the acromegaly group and control group. After comparing the distances, the acromegaly group was divided into two subgroups by indicators with significant difference (distance between bilateral siphon carotid ectasias segments and distance between bilateral lacerum segments). After adding the two numbers together, patients were equally distributed into two subgroups with larger number and smaller one.

Clinical characteristics were compared to find factors related to change of ICAs distance in acromegalic patients, based on the difference of two subgroups. Multivariate logistic regression model analysis was used to assess the change of ICAs distance in patient with acromegaly. To find variables to be used in the multivariate evaluation, univariate assessment was first performed. Briefly, candidate variables for univariate logistic regression analysis included all available variables that had univariate associations $P < 0.15$ between the two acromegaly subgroups as divided by the sum of ICAs distances. All variables having $P < 0.05$ from univariate logistic regression analyses were included in multivariate analysis. Then, the final model was created using the backward stepwise multivariate regression, in which the least non-significant variables were removed from the model one at a time, until all remaining variables had $P < 0.05$. Statistical analysis was performed with SPSS version 17.0 (SPSS Inc., Chicago, Illinois). $P < 0.05$ was considered as statistically significant.

RESULTS

Demographic and Clinical Data

After selection with the inclusion and the exclusion criteria, 86 cases of acromegalic patients, 46 males and 40 females, were included as study group. The mean age of patients in the acromegaly group was 40.1 ± 10.6 years. Eighty six patients with non-functional pituitary adenoma, 1:1 matched for age, gender and adenoma size, were recruited as the control group. In this series of patients, the incidences of atypical GH adenomas and refractory GH adenomas were 16.3% (14/86) and 10.5% (9/86), respectively.

The demographic information, adenoma size, laboratory results, medical history and ICAs distances of the patients are shown in **Table 1**. Between the two groups, there were no significant differences in gender, age, body mass index (BMI), adenoma size, smoking habit, hypertension, diabetes, and dyslipidemia.

Comparison of Distance Between the Bilateral ICAs

Compared with patients in the control group, the maximum distance between bilateral siphon carotid ectasias (25.5 ± 4.1 vs. 23.4 ± 3.5 mm, $P = 0.001$) and distance between bilateral lacerum segments (26.2 ± 3.2 vs. 24.1 ± 4.3 mm, $P < 0.001$) were significantly higher for patients with acromegaly. On the other hand, the distance between bilateral ophthalmic segments—(16.0 ± 5.2 vs. 16.2 ± 3.4, $P = 0.730$) and the distance between bilateral cavernous segments (21.9 ± 3.2 vs. 22.5 ± 2.7, $P = 0.205$) were not significantly different between the two groups (**Table 1**). A typical illustration was showed in **Figure 2**.

Determination of Related Factors of Change in ICAs Distance for Acromegalic Patients

The acromegaly group was divided into two subgroups by indicators with significant difference (distance between bilateral siphon carotid ectasias segments and distance between bilateral lacerum segments). After adding the two numbers together, patients were equally divided into large size group ($n = 43$) and small size group ($n = 43$). Six variables, including the age, volume of pituitary adenoma, diameter of pituitary adenoma, duration of the disease, atypical pituitary adenoma, and refractory pituitary adenoma had potential univariate associations ($p < 0.15$) with the large ICAs distance (**Table 2**) and were put into logistic regression model analysis. This analysis revealed that the refractory pituitary adenomas [odds ratio [OR] = 9.8, 95% confidence interval [CI] = 1.1–88.7, $P = 0.043$] and longer disease duration [OR = 1.01, 95%CI = 1.01–1.02, $P = 0.005$] were both the significant factors associated with the larger bilateral ICAs distance, while age, volume of pituitary adenoma, diameter of pituitary adenoma and did not show statistically significant difference (**Table 3**).

DISCUSSION

Patients with acromegaly often undergo several treatments, which include transsphenoid or transcranial surgery, radiotherapy and several medical approaches. Transsphenoidal surgery is a first-line treatment for GH-secreting pituitary adenoma. Rupture of intracranial carotid artery during the surgery and formation of pseudoaneurysm after surgery were the common operative complications. Usually, these complications were caused by injury of the intracranial carotid artery during the surgery. Comparing to non-functional pituitary adenoma, functional pituitary adenoma has a higher risk of bleeding during the surgery (7). Furthermore, of pituitary adenomas, GH secreting tumors are considered at higher risk for ICA tear during surgery due to the higher occurrence of macroadenoma invading the neighboring cavernous sinus (10, 11). The impact of changed intercarotid distance on transsphenoidal surgery has been proven in several studies (12, 13). Banu et al. stated that the difference of intercarotid distance could affect the transsphenoidal angle and have further impact on the extent of tumor dissection (12). In their report, the volume of the

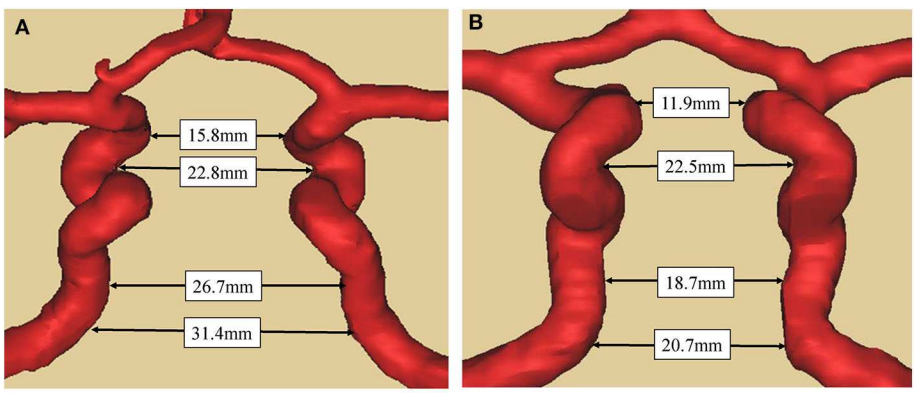

FIGURE 2 | Comparison of bilateral ICAs distance between acromegaly and non-functional pituitary adenoma patient **(A)**. Case 1, a 59-year-old man with acromegaly. The tumor diameter is 1.8cm with knosp gradell. The distance between the bilateral ICAs at a-d level (as in **Figure 1**) were respectively 15.8, 22.8, 26.7, and 31.4 mm. **(B)**. Case 2, a 61-year-old male with no-functional pituitary adenoma. The tumor diameter is 2.0cm with knosp gradell. The distance between the bilateral ICAs at a-d level (as in **Figure 1**) were respectively 11.9, 22.5, 18.7, and 20.7 mm. The two patients have the same gender and similar age, and similar diameter of pituitary adenoma and same knosp grade.

TABLE 2 | Comparison of demographic and clinical data in acromegalic patients according to bilateral ICAs distance.

Characteristics	Patients with larger bilateral ICA distance ($n = 43$)	Patients with smaller bilateral ICA distance ($n = 43$)	*P*-value
DEMOGRAPHICS			
Age, year	41.8 ± 10.8	38.4 ± 10.2	0.140
Gender, female	22 (51.2%)	18 (41.9%)	0.387
Height, cm	166.2 ± 5.6	168.3 ± 6.8	0.162
BMI, kg/m^2	24.5 ± 4.6	25.1 ± 3.6	0.506
MRI FOUNDING			
Pituitary adenoma volume, cm^3	3.7 (0.9, 6.8)	1.6 (0.6, 5.2)	0.107
Pituitary adenoma diameter, cm	1.9 ± 0.8	1.7 ± 0.7	0.146
Knosp grade	2 (1–3)	1 (1–3)	0.153
Grade 0	4	10	
Grade II	10	13	
Grade III	15	7	
Grade VI	11	9	
Grade V	3	4	
LABORATORY			
GH, μg/L	45.1 ± 36.0	49.9 ± 42.5	0.585
IGF-1, μg/L	511.2 ± 211.9	541.2 ± 198.2	0.520
MEDICAL HISTORY			
Hypertension	14 (32.6%)	9 (20.9%)	0.223
Diabetes mellitus	12 (27.9%)	7 (16.3%)	0.194
Hyperlipidemia	8 (18.6%)	6 (14.0%)	0.559
Somking history	8 (18.6%)	10 (23.3%)	0.596
Duration of disease, months	108 (72, 156)	72 (36, 96)	<0.001
Atypical pituitary adenoma	11 (25.6%)	3 (7.0%)	0.019
Refractory pituitary adenomas	8 (18.6%)	1 (2.3%)	0.030

The acromegaly group was divided into two subgroups by indicators with significant difference (distance between bilateral siphon carotid ectasias segments and distance between bilateral lacerum segments). After adding the two numbers together, patients were equally distributed into two subgroups with larger numbers and smaller ones. Values are n (%), mean (SD), median (25%−75%).

sphenoid sinus and nare-sellar distance were proved to be correlated with intercarotid distance. That means change of intercarotid distance could affect neurosurgeon on the overview of basic cranial and surgical approaches. In addition, change of intercarotid distance has been demonstrated to be a predictive factor for surgical outcome and postoperative complications (13). Therefore, distances of the bilateral ICAs could not be ignored when planning for surgical strategy. For functional pituitary adenoma, those changes and characteristics may affect tumor resection and surgical safety to a great extent (14).

Because of excessive GH for a long time, morphology of ICAs in acromegalic patients has been changed. In addition, changes of vascular structure and function such as aortosclerosis, endothelial dysfunction, reconstruction of vessel walls and increased tortuosity of vessels exist in some patients (15, 16). According to comparison to patients with non-functional pituitary adenomas, our study aimed at determining changes of the distance between bilateral ICAs in acromegalic patients. We found that for the acromegalic patient group, the maximum distance between bilateral siphon carotid ectasias and distance between bilateral lacerum segments were significantly higher than those of the control group, respectively; and the distance between bilateral cavernous segments tended to be smaller for acromegalic patients, but the difference was not statistically significant. However, studies investigating the changes of ICAs distance in acromegalic patients have provided ambiguous results (5–7). In a study, Manara et al. reported different results comparing to ours. Based on image data of 177 acromegalic patients, they found a reduced intercarotid distance in C3 segment and increased intercarotid distance in C4 segment. Besides, Manara et al. selected subjects with headache or transient neurological deficits as their control group while we chose patients with non-functional pituitary adenomas. Mass effect of non-functional pituitary adenomas could affect the distance between bilateral ICAs because of compression. However, in contrast to acromegalic patients, there is no excess of GH secretion to stimulate the growth of various tissues that could impair the structures of ICAs. Thus, indicate that the GH exposure might impact the morphology of ICAs.

In the comparison between larger and smaller bilateral ICAs distance groups, the level of GH and IGF-1 were proved not to be the significant factor in the univariate analysis, whereas the duration of disease show significant different between these two groups in both univariate and multiple analysis. This means that the longer exposure duration of GH might be one of the key factors in changing the ICA morphology in patient with acromegaly. Meanwhile, in the univariate analysis, our study also revealed that atypical pituitary adenoma, refractory pituitary adenoma were significant factors associated with the occurrence of larger intercarotid artery distance, nevertheless, only refractory pituitary adenoma and duration of disease show statistically significant difference in the multivariate model. Therefore, refractory pituitary adenoma and longer disease duration with excessive GH might be the determining factors for the distance change of bilateral ICAs in acromegalic patients.

With the increasing application and importance of transsphenoidal surgery, anatomical structure of ICAs has drawn more attention in the past decades. Nowadays, transsphenoidal surgery has become a routine method to manage sellar tumors. The space-occupying lesion of hypophysial fossa, middle skull base and anterior skull base could affect anatomical structure of ICAs. Be aware of the morphology and anatomical variation of ICAs is the key to ensure surgical safety. Our study reported changes in the structure of ICAs, which was valuable to achieve greater security during the transsphenoidal surgery in acromegalic patients. For a long period of time in the past, bilateral ICAs and optic nerve have been considered as exclusion zone in the transsphenoidal surgery. With the obvious improvement of surgical techniques, surgeons could extend the

TABLE 3 | Logistic regression model analysis of larger bilateral ICA distance in acromegaly with possible factors*.

Predictors	Univariate analysis				Multivariate analysis#	
	Patients with larger bilateral ICA distance ($n = 43$)	Patients with smaller bilateral ICA distance ($n = 43$)	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
Age, yr	41.8 ± 10.8	38.4 ± 10.2	1.03 (0.99–1.08)	0.142	-	-
Pituitary adenoma volume, cm^3	3.7 (0.9, 6.8)	1.6 (0.6, 5.2)	1.10 (0.98–1.24)	0.112	-	-
Pituitary adenoma diameter, cm	1.9 ± 0.8	1.7 ± 0.7	1.55 (0.86–2.79)	0.146	-	-
Duration of disease, months	108 (72, 156)	72 (36, 96)	1.01 (1.01–1.02)	0.002	1.01 (1.01–1.02)	0.005
Atypical pituitary adenoma	11 (25.6%)	3 (7.0%)	4.6 (1.2–18.0)	0.028	-	-
Refractory pituitary adenomas	8 (18.6%)	1 (2.3%)	9.6 (1.1–81.0)	0.037	9.8 (1.1–88.7)	0.043

*Values are n (%), mean (SD), median (25%−75%), OR = odds ratio. *Among all the items belong to demographics, MRI finding, laboratory, medical history and duration of disease in **Table 2**, factors which had univariate associations P < 0.15 were included for further analysis. #Multivariate analysis: factors with P < 0.05 in univariate analysis were included in multivariate analysis. Backward stepwise regression methods were used to produce the final model.*

approach to expose cavernous sinus, pterygopalatine fossa and other intracranial structure. ICAs was exposed to the operative field of vision and considered as an anatomical landmark to avoid injuries to other structure. Therefore, it is particularly important to determine the location and anatomical structure of ICAs before surgery.

Compared to patients with non-functional pituitary adenomas, acromegalic patients had significantly larger distances between bilateral siphon carotid ectasias, and between bilateral lacerum segments. These differences may be caused by local factors in combination with systematic factors. Mass effect is the main local factor for macroadenoma which is usually found in pituitary adenomas (17). The invasion of cavernous sinus and compression of ICAs in GH-secreting pituitary adenoma could expand the distance between bilateral cavernous segments of ICAs. However, some studies reported that invasion of cavernous sinus did not affect ICA morphology and found increased ICA tortuosity in microadenoma (5). Therefore, it is highly possible that ICA ectasia and tortuosity are not determined by vascular infiltration, mechanical effects or increased blood flow demand. In our study, there was no difference in diameter and Knosp grade of pituitary adenomas between experimental and control groups. However, the maximum distance between bilateral siphon carotid ectasias and distance between bilateral lacerum segments were significantly different between the two groups. The results further explained that local factors did not make a huge impact on the distances between bilateral ICAs in acromegalic patients.

Excessive growth hormone secretion and its related complications may be the systematic factors for the change in distance between bilateral ICAs in acromegalic patients. Growth hormone has shown a mitogenic effect on vascular endothelial cells, fibroblasts, macrophages and smooth muscle cells. It also plays a key role on the process of cell growth and metabolism (18, 19), which may affect the distance between bilateral ICAs. Moreover, collagen I to collagen III ratio could be altered by excessive GH, which has been suggested to be related to GH-dependent vascular alterations and aneurysm formation (20). That may be the reason for the changes of distances between bilateral ICAs in our study.

There were some limitations in our study. The current study is a single center retrospective study and has the known inherent limitations. We only compared bilateral ICAs distance according to CTA or MRA. The outcome and follow-up of subjects have not been linked to the change of distance. Further analysis should be conducted to confirm whether the change in distance is related to surgical outcome and postoperative complications. Further perspective study with large sample should be conducted to confirm whether the change in distance is related to surgical outcome and postoperative complications and investigate the clinical valuation of ICA distance as a tool in decision in acromegaly treatment.

CONCLUSION

Our study showed significant change of the distance between the bilateral ICAs in patients with acromegaly. Compared to patients with non-functional pituitary adenomas, acromegalic patients had significantly larger distance between bilateral siphon carotid ectasias and distance between bilateral lacerum segments. The increased bilateral ICAs distance is associated with disease duration but not with the level of GH, IGF-1 or adenoma size. Refractory pituitary adenoma and longer duration of physiological stimulation by the obviously increased GH and IGF-1 might lead to the larger bilateral ICAs distance. Further large-scale studies are still needed to confirm this finding.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/supplementary material.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of the First Affiliated Hospital of Fujian Medical University. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s)

for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

ZL, XY, and CD revised the manuscript for important intellectual content. ZL, CJ, and CD take final responsibility for this article. All authors provided contributions to the study conception and design, acquisition of data or analysis and interpretation of data, drafting of the article, or revising it critically for important intellectual content, final approval of the version to be published, and analyzed and interpreted the data. All authors contributed to the article and approved the submitted version.

FUNDING

This study was supported by grants from the Science and Technology Innovation Joint Foundation of Fujian Province (No. 2017Y9085) and the National Natural Science Foundation of China (No. 81902547).

REFERENCES

1. Oshino S, Nishino A, Suzuki T, Arita H, Tateishi A, Matsumoto K, et al. Prevalence of cerebral aneurysm in patients with acromegaly. *Pituitary.* (2013) 16:195–201. doi: 10.1007/s11102-012-0404-x
2. Satyarthee GD, Raheja A. Unruptured internal carotid artery aneurysm associated with functional pituitary adenoma: a true association. *Asian J Neurosurg.* (2017) 12:701–4. doi: 10.4103/1793-5482.215760
3. Schofl C, Petroff D, Tonjes A, Grussendorf M, Droste M, Stalla G, et al. Incidence of myocardial infarction and stroke in acromegaly patients: results from the German acromegaly registry. *Pituitary.* (2017) 20:635–42. doi: 10.1007/s11102-017-0827-5
4. Powlson AS, Gurnell M. Cardiovascular disease and sleep-disordered breathing in acromegaly. *Neuroendocrinology.* (2016) 103:75–85. doi: 10.1159/000438903
5. Manara R, Gabrieli J, Citton V, Ceccato F, Rizzati S, Bommarito G, et al. Intracranial internal carotid artery changes in acromegaly: a quantitative magnetic resonance angiography study. *Pituitary.* (2014) 17:414–22. doi: 10.1007/s11102-013-0516-y
6. Ebner FH, Kuerschner V, Dietz K, Bueltmann E, Naegele T, Honegger J. Reduced intercarotid artery distance in acromegaly: pathophysiologic considerations and implications for transsphenoidal surgery. *Surg Neurol.* (2009) 72:456–60; discussion 60. doi: 10.1016/j.surneu.2009.07.006
7. Sasagawa Y, Tachibana O, Doai M, Hayashi Y, Tonami H, Iizuka H, et al. Carotid artery protrusion and dehiscence in patients with acromegaly. *Pituitary.* (2016) 19:482–7. doi: 10.1007/s11102-016-0728-z
8. Delellis RA, Lloyd RV, Heitz PU. Pathology and genetics of tumours of endocrine organs. *Pathol Genet.* (2004) :147–66.
9. Dai C, Feng M, Liu X, MA S, Sun B, Bao X, et al. Refractory pituitary adenoma: a novel classification for pituitary tumors. *Oncotarget.* (2016) 7:83657–68. doi: 10.18632/oncotarget.13274
10. Shigemori M, Shirahama M, Hara K, Tokutomi T, Kawaba T. [Traumatic aneurysms of intracranial internal carotid arteries. Case reports (author's transl)]. *Neurol Med Chir (Tokyo).* (1982) 22:241–7. doi: 10.2176/nmc.22.241
11. Vieira JO Jr, Cukiert A, Liberman B. Evaluation of magnetic resonance imaging criteria for cavernous sinus invasion in patients with pituitary adenomas: logistic regression analysis and correlation with surgical findings. *Surg Neurol.* (2006) 65:130–5. doi: 10.1016/j.surneu.2005.05.021
12. Banu MA, Guerrero-Maldonado A, McCrea HJ, Garcia-Navarro V, Souweidane MM, Anand VK, et al. Impact of skull base development on endonasal endoscopic surgical corridors. *J Neurosurg Pediatr.* (2014) 13:155–69. doi: 10.3171/2013.10.PEDS13303
13. Banu MA, Rathman A, Patel KS, Souweidane MM, Anand VK, Greenfield JP, et al. Corridor-based endonasal endoscopic surgery for pediatric skull base pathology with detailed radioanatomic measurements. *Neurosurgery.* (2014) 10 (Suppl. 2):273–93. doi: 10.1227/NEU.0000000000000252
14. Nishioka H, Fukuhara N, Horiguchi K, Yamada S. Aggressive transsphenoidal resection of tumors invading the cavernous sinus in patients with acromegaly: predictive factors, strategies, and outcomes. *J Neurosurg.* (2014) 121:505–10. doi: 10.3171/2014.3.JNS132214
15. Guo X, Gao L, Zhang S, Li Y, Wu Y, Fang L, et al. Cardiovascular system changes and related risk factors in acromegaly patients: a case-control study. *Int J Endocrinol.* (2015) 2015:573643. doi: 10.1155/2015/573643
16. Yaron M, Izkhakov E, Sack J, Azzam I, Osher E, Tordjman K, et al. Arterial properties in acromegaly: relation to disease activity and associated cardiovascular risk factors. *Pituitary.* (2016) 19:322–31. doi: 10.1007/s11102-016-0710-9
17. Lenzi J, Lapadula G, D'amico T, Delfinis CP, Iuorio R, Caporlingua F, et al. Evaluation of trans-sphenoidal surgery in pituitary GH-secreting micro- and macroadenomas: a comparison between microsurgical and endoscopic approach. *J Neurosurg Sci.* (2015) 59:11–8.
18. Messias de Lima CF, Dos Santos Reis MD, da Silva Ramos FW, Ayres-Martins S, Smaniotto S. Growth hormone modulates *in vitro* endothelial cell migration and formation of capillary-like structures. *Cell Biol Int.* (2017) 41:577–84. doi: 10.1002/cbin.10747
19. Shen J, Zhang N, Lin YN, Xiang P, Liu XB, Shan PF, et al. Regulation of vascular calcification by growth hormone-releasing hormone and its agonists. *Circ Res.* (2018) 122:1395–408. doi: 10.1161/CIRCRESAHA.117.312418
20. Acqui M, Ferrante L, Matronardi L, d'Addetta R. Alteration of the collagen type III/type I ratio and intracranial saccular aneurysms in GH-secreting hypophyseal adenomas. *Ital J Neurol Sci.* (1988) 9:365–8. doi: 10.1007/BF02334000

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Endocrinology

ORIGINAL RESEARCH
published: 23 September 2020
doi: 10.3389/fendo.2020.558593

Clinical, Laboratory, and Treatment Profiles of Silent Corticotroph Adenomas That Have Transformed to the Functional Type: A Case Series With a Literature Review

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OPEN ACCESS

Edited by:
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Specialty section:
This article was submitted to Pituitary Endocrinology, a section of the journal Frontiers in Endocrinology

Received: 03 May 2020
Accepted: 13 August 2020
Published: 23 September 2020

Citation:
Zheng G, Lu L, Zhu H, You H, Feng M, Liu X, Dai C, Yao Y, Wang R, Zhang H, Sun X and Lu Z (2020) Clinical, Laboratory, and Treatment Profiles of Silent Corticotroph Adenomas That Have Transformed to the Functional Type: A Case Series With a Literature Review. Front. Endocrinol. 11:558593. doi: 10.3389/fendo.2020.558593

Purpose: Silent corticotroph adenoma (SCA) is clinically non-functional pituitary adenoma with expression of corticotropin or Tpit. To further understand the characteristics of this rare type of SCA transforming to a functional SCA, we retrospectively reviewed SCAs that converted to typical Cushing's syndrome at a tertiary medical center and the relevant literature.

Methods: Patients were identified based on the diagnosis of pituitary adenoma without symptoms of hypercortisolism at the initial visit with positive Immunohistochemical (IHC) staining for corticotropin or Tpit after surgery and subsequent transformation to functional SCAs during the follow-up period from March 1990 to January 2020 at Peking Union Medical College Hospital and in the literature. The characteristics of the clinical manifestations, biochemical results, imaging findings, pathology findings and outcome were analyzed.

Results: Altogether, 16 patients were included in the study with an average age of 42.0 ± 12.48 (18–65) years at the first visit. Females were slightly predominant (F:M = 1.3:1). The median time of conversion from the nonfunctional to the functional type was 30 (13.0, 68.3) months. Once a functional SCA developed, the adrenocorticotropic hormone (ACTH) level and 24-h urine free cortisol were increased 3.8- (2.6, 12.9) and 5.3- (2.6, 19.3) fold, respectively, above the normal range. Approximately 50% of the patients had macrocystic changes on pituitary MRI. All 16 patients experienced 1–5 surgeries with a median of 2.5 (2.0, 4.0) surgeries. The proportion of patients with Ki-67 ≥ 3% increased from 22.2% (2/9) at the beginning to 50% (7/14) at the time of functional SCA diagnosis. Thirteen patients received radiotherapy, and 4 patients (30.8%) achieved remission. Four patients with refractory functional SCAs received temozolomide treatment with the normalization of cortisol in 4 cases and reduced tumor volume in 3 cases.

Conclusion: In this study, all cases that transformed to functional SCAs were macroadenomas. Hypercortisolism was more severe in functional SCA patients. The tumors tended to have frequent recurrence and were highly invasive. Temozolomide could be a promising treatment for refractory functional SCA cases. Long-term follow-up is needed for nonfunctional SCAs since some cases have the potential to transform to clinical Cushing's syndrome.

Keywords: treatment, diagnosis, cushing syndrome, silent corticotroph adenoma, nonfunctional pituitary adenoma, temozolomide

INTRODUCTION

Pituitary adenomas originate from the pituitary gland and are slow-growing and benign in most cases. An autopsy series reported that the prevalence could be as high as 10–15%, accounting for ∼15–20% of intracranial tumors (1–5). A study showed that the prevalence of pituitary adenomas in 2012 was 115.57/100, 000 in a surgical series (6). Pituitary adenomas can be classified into non-functional adenomas and functional adenomas according to the presence or absence of hormone overproduction. The most common type of pituitary adenoma is a non-functional pituitary adenoma (NFPA), 20% of which might not be diagnosed due to the absence of typical symptoms (7, 8). In epidemiological data from Iceland between 1955 and 2012, the relative prevalence of NFPAs was 42.32 per 100,000 in a surgical series. NFPAs were the most common (43.0%) pituitary adenomas, followed by prolactinomas (39.9%), acromegaly (11.3%), and Cushing's disease (5.7%) (6). However, there were some special cases of "clinically silent but pathological active" pituitary adenomas among the NFPAs; they were called "silent pituitary adenomas," which indicated that these pituitary adenomas had immunohistochemical (IHC) expression of one or more anterior pituitary hormones but no relevant clinical symptoms. Recently, it was recommended that, in addition to the expression of hormone levels, transcription factors should be measured to define the actual lineage of the tumor according to the 2017 WHO classification of pituitary adenomas. Nishioka et al. (9) investigated the complementary role of transcription factors in consecutive NFPAs ($n = 516$). Depending on the IHC findings for hormones, these NFPAs could be classified as follows: gonadotroph adenomas (58.1%), hormone-negative adenomas ($n = 119$, 23.1%), silent corticotroph adenomas (SCAs) (9.9%), and 46 other silent adenomas. Further evaluation of hormone-negative adenomas showed that 95% ($n = 116$) of those tumors expressed transcription factors as follows: gonadotroph adenomas (SF-1 positive, 66.4%), SCAs (Tpit positive, 26.9%), etc. Thus, the SCAs, distinguished by integrating IHC staining for hormones with transcription factors, were the second most common (16.1%, 83/516) of the above mentioned clinically silent NFPAs.

The first SCA case in the literature was reported by Kovacs et al. (10). A 43-year-old man diagnosed with NFPA presented with positive adrenocorticotropic hormone (ACTH) expression in the tumor tissue without symptoms of hypercortisolism, increased cortisol, or ACTH levels. It is commonly known that SCAs have no clinical manifestations of Cushing's syndrome, but they are highly aggressive and often invade the cavernous and sphenoid sinuses. Histologically, SCAs can be divided into three subtypes: densely granulated corticotroph adenomas, sparsely granulated corticotroph adenomas, and Crooke cell adenoma (11). It is worth noting that the first case of NFPA that transformed into a functional corticotroph adenoma, after 7 years of follow-up, was reported by Cooper et al. (12), causing some concern among clinicians. Subsequently, a few cases of SCAs that transformed from silent to clinically functional corticotroph adenomas have been reported (13–18). However, the underlying mechanism of the transformation of function has not yet been elucidated.

To further explore the characteristics of transformation from non-functional to functional SCA types, which may have clinical significance for the management of these patients, patients with SCAs who underwent transformation were identified from the Peking Union Medical College Hospital records and the published literature. The clinical manifestations, biochemical tests, imaging features, and IHC features of these cases were further analyzed.

METHODS

Subjects

The clinical data of patients who were initially diagnosed with NFPAs and presented later with typical Cushing's syndrome and abnormal hormone tests during long-term follow-up were collected at Peking Union Medical College Hospital from March 1990 to December 2019. In addition, we reviewed the literature (from PubMed) about SCAs with the transformation from non-functional to functional types.

Silent Corticotroph Adenoma Cases That Transformed Into Functional Corticotroph Adenomas at Peking Union Medical College Hospital

The patients' data were obtained through a search of the hospital information system at Peking Union Medical College Hospital from 1990 to January 2020. The Ethics Committee of Peking Union Medical College Hospital approved this retrospective study.

The inclusion criteria included the following: (1) patients with pituitary adenomas who underwent surgery, (2) pituitary

adenoma's that were totally silent without typical manifestations of Cushing's syndrome at the first visit, (3) no tests indicating hypercortisolism at the first visit (normal ACTH, 24-h urinary free cortisol [UFC], and midnight cortisol or suppressed low-dose dexamethasone suppression test [DST] if ACTH or cortisol was elevated), (4) positive IHC staining for ACTH and/or Tpit on the first or subsequent pathological results after surgery, (5) gradual appearance of the typical clinical manifestations of Cushing's syndrome and the laboratory examination of ACTH-dependent Cushing's syndrome (normal or increased ACTH, increased midnight cortisol or 24-h UFC, no suppression on the low-dose DST) during the postoperative follow-up period, and (6) detailed medical information that could be collected. The exclusion criteria included the following: (1) abnormality on at least one of the mentioned tests for hypercortisolism at the first endocrine evaluation (despite no clinical manifestations of hypercortisolism), (2) diagnosis of ectopic ACTH syndrome, (3) incomplete medical information, or (4) no findings of either ACTH or Tpit expression in tumor tissues on IHC. Finally, seven SCA patients initially diagnosed with NFPAs met the enumerated inclusion/exclusion criteria and had transformation to typical Cushing's syndrome during the follow-up period. We collected information about these cases, including (1) demographic features such as age and sex; (2) the time of appearance of Cushing's syndrome; (3) clinical symptoms; in particular, clinical manifestations relevant to Cushing's syndrome including central obesity, hypertension, purple striae, buffalo hump, weight gain, skin thinning, moon face, osteoporosis, hypokalemia, and hyperglycemia; (4) biochemical tests including blood glucose, serum potassium, and blood lipids; (5) hormonal tests including ACTH, serum cortisol, 24-h UFC, and DST; (6) results of enhanced pituitary magnetic resonance imaging (MRI); (7) pathological results and IHC staining for pituitary hormones, and transcription factors; and (7) details of treatment.

The diagnostic procedure for Cushing's syndrome depends on the typical symptoms and signs, including increased midnight cortisol (>1.8 μg/dl), 24-h UFC, and no suppression on the low-dose DST (including overnight DST or 2 mg/day 48-h low-dose DST). The 48-h, 8-mg/day high-dose DST was also carried out for the patients admitted to our center. Suppression rates above 50% after the high-dose DST indicated suppression. Because all the patients had macroadenomas, International Prostate Symptom Score was not necessary.

Literature Review of Silent Corticotroph Adenoma Cases That Transformed Into Functional Corticotroph Adenomas

We conducted a literature search of the PubMed online database through February 1, 2020; we searched for keywords including [Cushing's syndrome] AND [SCA] OR [silent ACTH adenomas] OR [silent corticotroph adenomas] OR [non-functional pituitary adenomas] OR [silent pituitary adenomas]. The inclusion criteria were the same as those mentioned, and the literature exclusion criteria were as follows: (1) there was no transformation to Cushing's syndrome in the description; (2) cases could not be diagnosed as SCA according to the pathological IHC staining; (3) cases were not reported in English; (4) the literature had already been reviewed, and there were no new cases; or (5) there was a lack of detailed data on the clinical manifestations and biochemical tests related to this study. Finally, 65 studies were identified, 13 of which described the transformation from "silent" SCA to functional corticotroph adenoma, and 6 of them had detailed data. Altogether, nine cases from six studies met the inclusion/exclusion criteria, and relevant medical information was collected.

Statistical Methods

Qualitative data are presented as frequencies and percentages. Quantitative data are presented as the means ± standard deviations or medians (ranges). If the data did not conform to the normal distribution, the median (25th percentile, 75th percentile) was used. Independent two-sample t-tests were used to evaluate the statistical significance. The significant differences in the data were analyzed using the Wilcoxon rank-sum test and the Kruskal–Wallis test. Statistical analysis was performed using SPSS version 19 (IBM, Armonk, New York). $P < 0.05$ indicated significance. A statistical chart was made with GraphPad Prism 7 (GraphPad Software, La Jolla, California, USA).

RESULTS

A total of 16 SCA patients who had a diagnosis of progressive change from non-functional to functional SCA were identified in our hospital ($n = 7$) and the literature ($n = 9$) (14, 16–18).

Demographics

Among the 16 patients, there were seven males (43.8%) and nine females (56.2%) with a sex ratio (M:F) of 1:1.3. The average age at their first visit was 42.0 ± 12.5 years (18–65 years), 46.4 ± 14.3 years (18–65 years), and 38.9 ± 9.6 years (18–49 years) in all patients, male patients, and female patients, respectively. The average age at diagnosis of a clinically functional corticotroph adenoma was 45.7 ± 11.8 years (21–67 years), 48.6 ± 13.88 years (21–67 years), and 43.4 ± 9.25 years (24–54 years) in all patients, male patients, and female patients, respectively. The average age at the first visit and the diagnosis of typical Cushing's syndrome in males tended to be older than that in females, but there was no significant difference. The most common age group at the initial visit was 40–49 years (nine cases, 56.3%). The interval between the diagnosis of SCA at the initial visit to the diagnosis of overt Cushing's syndrome was 12–120 months, with a median of 30 (13.0, 68.3) months. The most common interval of transformation was 12–24 months in eight cases (50%).

Clinical Manifestations

Among the 16 patients with SCA, none showed obvious relevant symptoms of Cushing's syndrome initially according to their medical charts and descriptions in the literature. At the first visit, the symptoms of the patients manifested as visual impairment (62.5%, 10/16), headache (12.5%, 2/16), and hypopituitarism (12.5%, 2/16) and incidentally discovered after traffic accident without symptoms (2/16, 12.5%). However, during the follow-up period, they developed several symptoms, including weight

gain, purple striae, skin thinning, central obesity, buffalo hump, and hypertension. No information about hypertension or hyperlipidemia was reported in the literature. Therefore, it was only from the seven patients from our medical center that we could obtain the frequency of hypertension (57.1%, 4/7) and hyperlipidemia (42.9%, 3/7). In addition to symptoms of the Cushing's syndrome, there were other symptoms, such as blurred vision (43.8%, 7/16), defect of the visual field (25%, 4/16), ptosis of the eyelid (6.3%, 1/16), dizziness (6.3%, 1/16), headache (6.3%, 1/16), and lactation (6.3%, 1/16). These symptoms, which are unusual in common Cushing's disease, may have been caused by compression due to the large size of the tumors in these patients.

Hormonal Tests

The main results of the hormonal tests after the transformation to functional corticotroph adenoma are shown in **Table 1**. Serum potassium was available in the seven patients from our medical center. They all had hypopotassemia, and the average potassium level was 2.6 ± 0.2 (2.3–3.0) mmol/L. The morning ACTH, cortisol, and 24-h UFC levels ranged between 21.5 and 1,500 pg/ml, 20.2–75 μg/dl, and 210–2,454.6 μg/24 h, respectively, in the 16 patients. Considering that the 16 cases were reported from different medical centers, the normal ranges for hormone tests were different. The fold increase above the upper limit of the normal range (ULN) was used to show the degree of increased ACTH, cortisol, and 24-h UFC. These patients showed variable elevation of 24-h UFC (3–23.7-fold increase) with a median 5.3-fold (2.6, 19.3) increase above the ULN. The morning serum cortisol was 1.7-fold (0.9- to 3.4-fold) above the ULN. The morning plasma ACTH levels were 1.7- to 14.5-fold above the ULN with a median 3.8-fold increase (2.6, 12.9). In all patients, the low-dose DST could not be suppressed. The high-dose DST was carried out in five patients, with four patients showing no suppression. The suppression rates of the high-dose DST were 41.6, −43.4, −72, and −33.7% in these four patients. It was unexpected that three patients had a paradoxical increase in 24-h UFC after the high-dose DST, which is more commonly observed in some cases of primary pigmented nodular adrenal dysplasia.

Imaging Features

The MRI results were available in 15 patients, and CT results were available in one patient who had an implanted pacemaker at the time of functional SCA diagnosis; these imaging results were used to identify tumor invasion. All the tumors were macroadenomas. The invasion was detected as follows: cavernous sinus invasion in seven cases (43.8%) (17, 18), sphenoid sinus invasion in two cases (12.5%) (13), simultaneous sphenoid sinus and cavernous sinus invasion in two cases (12.5%) (14), and no sphenoid sinus or cavernous sinus invasion in five cases (31.3%) (15, 16). The MRI images from six cases and CT images from one case in our center are shown in **Figure 1**. Contrast MRI or CT images were obtained in 14 cases, and in 7/14 cases (50%), they demonstrated cystic changes (most of them were macrocystic changes). However, the size of the tumor could be obtained only for the seven cases from our medical center. The range of tumor volume was 1.7–29.2 cm^3 with a median of 11.4 (2.3, 23.0) cm^3 and maximal diameter of 2.1–4.7 cm at the first visit ($n = 6$), and increased to 3–35.5 cm^3 with a median of 10.6 (3.2, 30.8) cm^3 and maximal diameter of 2–5.6 cm at the time of diagnosis of functional SCAs ($n = 7$).

Immunohistochemical Features on Pathological Examination

For the IHC staining of ACTH, although only eight patients (8/15) showed positive expression of ACTH and one patient did not have available information about ACTH staining after the first surgery, positive IHC staining for ACTH was found in all 16 patients at the time of diagnosis of Cushing's syndrome, which supported the diagnosis of SCA. For the IHC staining of transcription factors, Tpit staining was performed in only one case and showed positive results (case 2). Although the SCA tumors tended to be invasive and recurred easily, the range of IHC staining for the Ki-67 index was 1–8%, with a median of 2% (1%, 3.5%) after the first surgery ($n = 9$ patients with available data). However, the Ki-67 index had a wide range of 1–20% with a median of 2.5% (1%, 9%) after surgery for functional corticotroph adenomas ($n = 14$ patients with available data). However, the proportion of patients with Ki-67 $\geq$ 3% increased from 22.2% (2/9) at the first surgery to 50% (7/14) at the time of diagnosis of a functional SCA.

In addition, Crooke's cell adenomas are more aggressive and invasive in clinical practice, with a high recurrence rate and the possibility of developing into pituitary cancer (11). Crooke's cell adenoma was occasionally found either in functional corticotroph adenomas or SCAs depending on the hyaline changes and the expression of circular low molecular weight keratin under pathological examinations. There was no Crooke's cell adenoma in this group of SCA patients after reviewing by pathology experts.

Treatment

Because the SCAs recurred frequently, surgery could only temporarily reduce the tumor size and relieve the compression symptoms. Even after several surgeries and adjuvant radiotherapy, the tumors often relapsed in a short time, which indicated that these cases were refractory pituitary adenomas. All 16 patients experienced one to five surgeries (median of 2.5 [2.0, 4.0] surgeries), which was much more than the surgeries needed for patients with common pituitary adenomas. Because most of the tumors were invasive macroadenomas, the tumor could not be completely removed by surgery. Thirteen patients received radiotherapy with remission in four cases (30.8%) and uncontrolled in nine cases (69.2%). In the patients who achieved remission, one patient underwent proton knife (case 3), and three underwent fractionated radiotherapy (cases 1, 6, and 7). In all four cases, the tumor volume shrank, and ACTH and cortisol were controlled within the normal range after radiotherapy with a follow-up time of 3–44 months. The other nine patients showed progression or recurrence after radiotherapy (five cases with fractionated radiotherapy and four cases with gamma knife). Because the treatment was so challenging, with quick recurrence and lost opportunity for surgery, five patients (cases 2, 5, 6, 13, and 15) were treated with temozolomide (TMZ). Case 6 developed extensive skin rashes after taking TMZ for 2 days; the rashes recovered after drug withdrawal and were aggravated

TABLE 1 | Clinical features, imaging features, IHC features, and treatment of 16 patients with a transformation to functional SCA.

	Cases from our center							Cases from the literature								
	Case 1	**Case 2**	**Case 3**	**Case 4**	**Case 5**	**Case 6**	**Case 7**	**Case 8**	**Case 9**	**Case 10**	**Case 11**	**Case 12**	**Case 13**	**Case 14**	**Case 15**	**Case 16**
Sex	F	M	F	M	M	M	F	F	F	F	M	F	M	M	F	F
Age at first visit	42	43	31	42	62	46	33	47	18	37	49	49	65	18	45	48
Time between onset and functional SCA (months)	120	56	72	12	12	12	57	84	72	24	24	12	24	36	46	16
Laboratory examination at the diagnosis of CS																
Serum potassium (mmol/L)	2.8	2.7	2.5	2.3	2.6	3.0	2.6	NA	NA	NA	NA	NA	NA	NA	NA	NA
ACTH at 8 am (pg/ml) (NR: 0–46) (fold increase above the ULN)	119 (2.6)	361 (7.8)	201 (4.4)	76.5 (1.7)	669 (14.5)	376 (8.2)	173 (3.8)	1008† (5–60) (16.8)	21.5 (0.5)	1,500† (9–52) (28.8)	127.9† (5–52) (2.5)	140† (7.5–51.9) (2.7)	151.8 (3.3)	173 (3.8)	677 (14.7)	140 (3)
Cortisol at 8 am (μg/dl) (NR: 4–22.3) (fold increase above the ULN)	20.2 (0.9)	75 (3.4)	39.7 (1.8)	47 (2.1)	54.8 (2.5)	55.9 (2.5)	47.3 (2.1)	34.1‡ (7–21) (1.6)	41.2‡ (5.6–23) (1.8)	44.5‡ (6.2–19.4) (2.3)	30.6‡ (7–25) (1.2)	26.8‡ (5–20) (1.3)	24.5 (1.1)	32.5‡ (5–25) (1.3)	23.2 (1)	21.6 (1)
24-h UFC (μg/24 h) (NR: 12.3–103.5) (fold increase above the ULN)	312.3 (3)	2,367.4 (22.9)	1,261.7 (12.2)	2,454.6 (23.7)	623.6 (6)	1,981.3 (19.1)	2,072.8 (20)	240* (35–137) (1.8)	210* (20–90) (2.3)	630* (36–137) (4.6)	1,307.5* (50–190) (3.3)	NA	1,132.8 (10.9)	267 (1.4)	279 (2.7)	NA
Imaging Features																
Tumor size at the first visit (cm) (volume cm^3)	4 × 4 × 2.5 (20.9)	1.5 × 1.5 × 2.1 (2.5)	4.1 × 4.7 × 2.9 (29.2)	2.1 × 1.6 × 2.3 (4)	2.3 × 1.3 × 1.1 (1.7)	3.5 × 3.3 × 3.1 (18.7)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Tumor size at the time of diagnosis of a functional SCA (cm) (volume cm^3)	5.4 × 4 × 3.2 (35.5)	2 × 1.5 × 2 (3.2)	4.4 × 3.3 × 4 (30.4)	2.5 × 2.7 × 3 (10.6)	2.3 × 2 × 1.5 (3.6)	5.6 × 3.5 × 3 (30.8)	1.5 × 1. 9 × 2 (3)	NA	NA	NA	NA	NA	NA	NA	NA	NA
Tumor invasion	Cavernous+ sphenoid	Cavernous	Cavernous	None	Sphenoid	Cavernous	None	Cavernous	Cavernous	Cavernous	None	Cavernous	sphenoid	None	Cavernous + sphenoid	None
Cystic changes	Cystic degeneration	None	Macro cystic and microcystic	Macro cystic and microcystic	None	None	None	Macrocystic	Macrocystic	Macrocystic	Macrocystic	None	None	Macrocystic	NA	NA
Ki-67 index in pathological IHC examination after surgery																
At the first surgery	NA	NA	1%	1%	5%	NA	NA	2%	1%	2%	2%	NA	1%	NA	8%	NA
At the time of diagnosis of a functional SCA	1%	8%	1%	15%	5%	5%	20%	2%	2%	3%	2%	NA	1%	1%	12%	NA
Specific Treatments																
Surgery (number)	5	5	2	4	4	2	3	4	4	3	1	2	1	2	2	2
Radiotherapy (courses)	2	3	1	1	1	1	1	2	0	0	0	1	1	1	2	1

(Continued)

TABLE 1 | Continued

	Cases from our center								Cases from the literature							
	Case 1	**Case 2**	**Case 3**	**Case 4**	**Case 5**	**Case 6**	**Case 7**	**Case 8**	**Case 9**	**Case 10**	**Case 11**	**Case 12**	**Case 13**	**Case 14**	**Case 15**	**Case 16**
TMZ therapy	No	Yes	No	No	Yes	Yes	No	No	No	No	No	No	Yes	No	Yes	No
Follow-up																
Duration (months)	357	141	112	107	85	81	115	57	24	24	72	120	168	NA	48	228
Hormone remission[#]	Yes	Yes	Yes	No	Yes	Yes	Yes	No	No	No	Yes	No	Yes	No	Yes	No
Tumor reduction	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes

NR, normal range.

ULN, upper limit of the normal range.

Tumor volume: calculated as (1/6πABC), A, B, and C are the length, height, and width of the tumor, respectively.

[†]*ACTH: Reference ranges for cases 8, 9, 10, 11, and 14 were different from the others, so they are listed separately.*

[‡]*Serum cortisol: Reference ranges for cases 8, 9, 10, 11, 12, and 14 were different from the others, so they are listed separately.*

[*]*Urinary free cortisol: Reference ranges for cases 8, 9, 10, and 11 were different from others, so they are listed separately.*

after reuse; therefore, TMZ was discontinued. In addition, case 2 started TMZ (150 mg/m^2 for 5 days every 28 days) in September 2018 after five surgeries and three courses of radiotherapy. The serum cortisol and plasma ACTH levels returned to the normal ranges, the tumor volume was significantly reduced after 12 cycles of TMZ, and adrenal insufficiency occurred after 13 cycles of TMZ. As of January 2020, this patient had completed 17 cycles of TMZ treatment, and the treatment was ongoing with a rather good therapeutic effect (**Figure 2**). Cases 13 and 15 were also treated with TMZ at 150–200 mg/m^2 for 5 days every 28 days for 15 cycles and 11 cycles, respectively. These two cases also obtained both tumor volume reduction and normalization of ACTH and cortisol levels. However, ACTH and cortisol levels decreased significantly in case 5 after six cycles of TMZ treatment, whereas the tumor continued to progress. Finally, the patient in case 5 died of tumor progression that compressed the brain stem. Among the four patients with long-term therapy with TMZ, there were no significant adverse reactions, such as rash, gastrointestinal reaction, leukopenia, or abnormal liver function tests. Therefore, TMZ is a promising drug for the treatment of SCA that has transformed into clinically functional Cushing's syndrome.

Follow-Up

The median follow-up time for these patients was 110.9 ± 85.3 months (24–357 months). Although surgery and radiotherapy could reduce the tumor tissue and relieve the compression symptoms caused by the tumor, recurrence and progression of the tumor occurred in a short time after the earlier mentioned treatment. After comprehensive treatment, 10 patients were in remission at the last follow-up. Eight patients remained in remission with both tumor and hormone control: one patient (case 11) underwent only one surgery with a follow-up time of 72 months; four patients (cases 1, 2, 3, and 7) underwent surgeries and radiotherapy with follow-up times of 357, 141, 112, and 115 months, respectively; and three patients (cases 6, 13, and 15) underwent surgeries, radiotherapy, and TMZ treatment with follow-up times of 81, 168, and 48 months, respectively. A reduction in the tumor without effective hormone control was observed in case 8 (after four surgeries and two courses of radiotherapy with a follow-up time of 57 months) and case 9 (after four surgeries with a follow-up time of 24 months). Two patients died. The patient of case 4 died of severe hydrocephalus due to rapid tumor progression, although he received three surgeries, one hydrocephalus shunt operation, one course of radiotherapy, and 3 months of 20-mg long-acting octreotide every month intramuscularly. The patient of case 5 died of progressive tumor compression of the brainstem, although he received radiotherapy, surgery, and six cycles of TMZ treatment.

DISCUSSION

SCAs account for 3 to 6% of all pituitary adenomas, 10 to 20% of silent pituitary adenomas, and ∼40% of all corticotroph cell tumors (19–23). SCA was also the second most common tumor (16%) in NFPAs differentiated by combined IHC staining

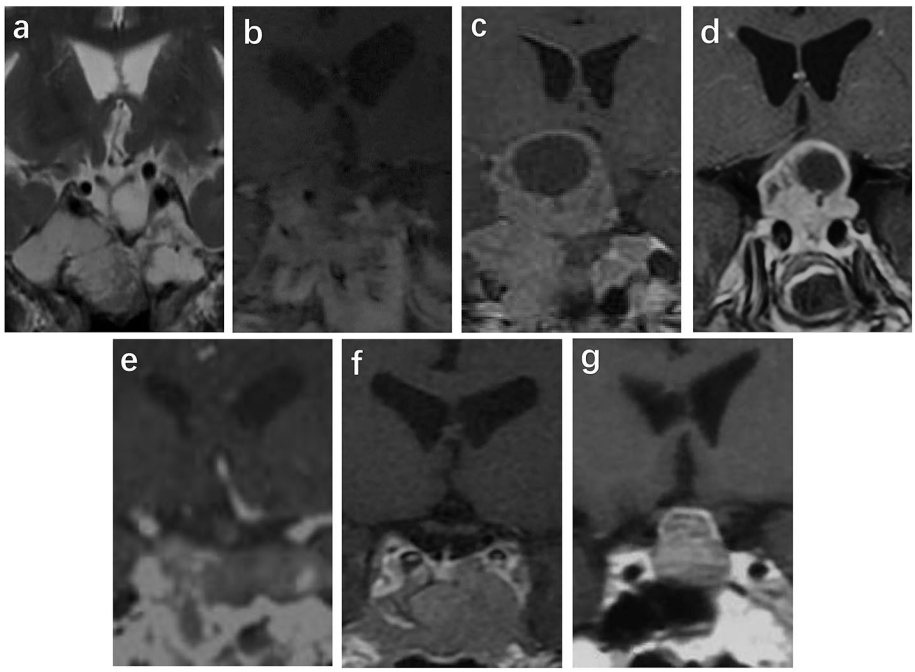


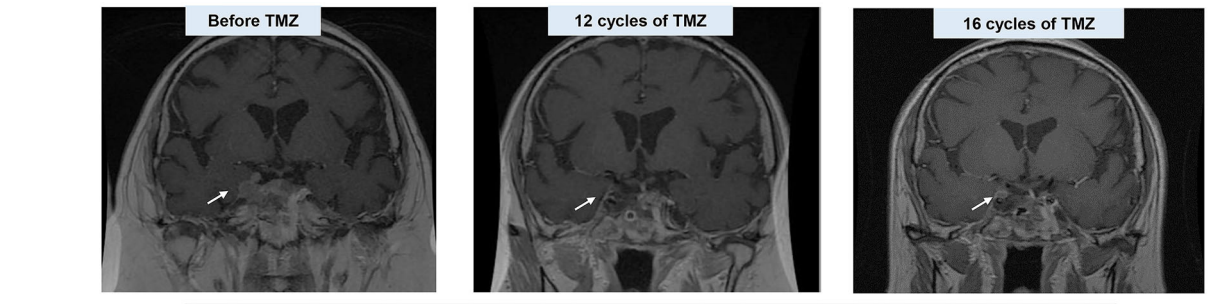
FIGURE 1 | Images at the time of diagnosis of a functional SCA in cases 1–7 **(a–g)**. Sella MRI was performed in cases 1 **(a)**, 2 **(b)**, 3 **(c)**, 4 **(d)**, 6 **(f)**, and 7 **(g)**. Sella CT was performed in case 5 **(e)** because a pacemaker was installed. There is cavernous invasion in **(b)**, sphenoid sinus invasion in **(d)**, and both types of invasion in **(a)**. There are macrocystic changes and surrounding microcystic changes in **(b,c)**. According to T2-phase MRI of case 1 (a), the tumor tended to have cystic degeneration.

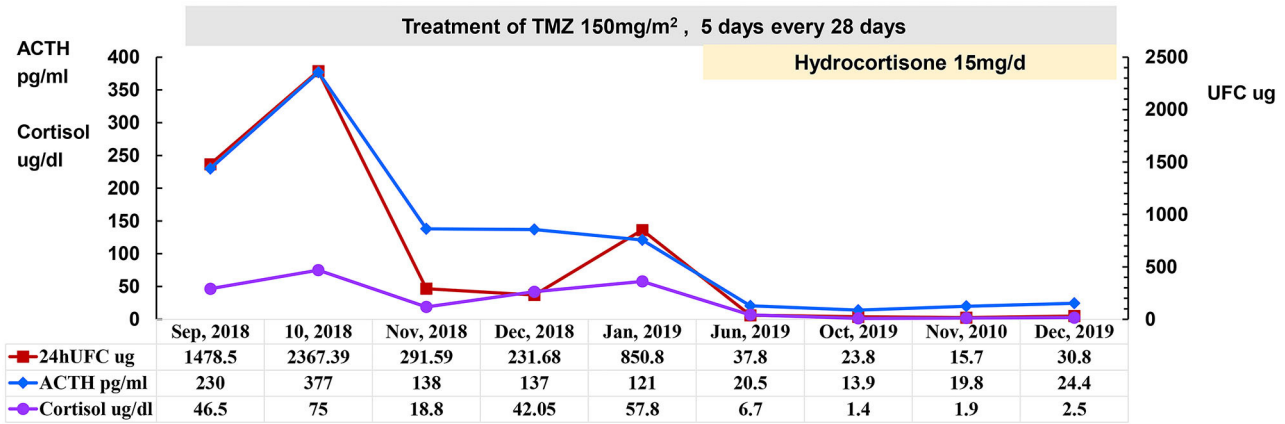
for ACTH and Tpit. Although the pathological results of SCAs showed positive expression of ACTH in most cases, the SCAs were usually diagnosed as NFPAs before the first surgery, as their clinical manifestations, laboratory examinations, and imaging were very similar to NFPAs. Most SCA cases remained "silent" for a long time. Very rare cases of SCAs have the potential to transform into clinically functional corticotroph adenomas (13–18). To investigate the clinical features of SCAs with transformation, a total of 16 SCAs with detailed data were collected from our medical center and the literature.

The sex ratio of males to females was 1:1.3 in this group of converted SCAs, which was much lower than that of common Cushing's disease (1:3–8) (24). The most common age group of patients with SCAs at the time of diagnosis of Cushing's syndrome was 30–49 years (11 cases, 68.8%), which coincided with the observed age range for other patients with Cushing's disease (mostly diagnosed at the age of 25–45 years). The typical interval from the initial visit to the diagnosis of a functional SCAs was 12–36 months (9/16, 56.3%), and the longest interval was 120 months. One study showed that the recurrence rate of SCAs was 31%, which is similar to that of NFAs (25), whereas, in our study, the recurrence rate of functional SCAs was 100%. As a result, SCA patients with repeated recurrence must be carefully monitored for the potential of conversion to a functional type. The long-term follow-up of evaluation of the HPA axis was required for recurrent SCAs.

It was suspected that even SCA microadenomas might have the chance to convert to clinical Cushing syndrome. However, in this group of converted SCAs, the tumors were all macroadenomas, and none were microadenomas. The reason might be that the diagnosis of SCA can only be initially confirmed by the pathological results with positive ACTH or Tpit immunostaining after surgery. SCA microadenomas were usually too small to be detected without the relevant symptoms of tumor-mass effects; thus, these patients may not have the indications for surgery or pathological examination. As a result, it was difficult to investigate the incidence of microadenomas among SCAs.

The pathogenesis of transformation of corticotroph adenomas from the silent type to the functional type is still unclear, but it was speculated that the biological activity of ACTH decreased in SCAs, which led to the fact that, although ACTH was IHC positive, the efficacy was not enough to cause biochemical and clinical symptoms of Cushing's syndrome (26). The other cause for the silent nature of ACTH among most SCAs has been demonstrated by lysosome dysfunction and the inability to destroy ACTH before it is secreted (20). Other theories include Golgi complex dysplasia leading to the mispackaging of ACTH (27, 28). In addition, PC1/3 is an enzyme that can cleave the precursor of ACTH, proopiomelanocortin (POMC), into ACTH. Whereas, overexpression of POMC has been found in SCA tumor tissues, the expression of PC1/3 was decreased; thus, POMC could not be broken down into enough ACTH to cause typical hypercortisolism (16, 29). Therefore,





	Sep, 2018	10, 2018	Nov, 2018	Dec, 2018	Jan, 2019	Jun, 2019	Oct, 2019	Nov, 2010	Dec, 2019
24hUFC ug	1478.5	2367.39	291.59	231.68	850.8	37.8	23.8	15.7	30.8
ACTH pg/ml	230	377	138	137	121	20.5	13.9	19.8	24.4
Cortisol ug/dl	46.5	75	18.8	42.05	57.8	6.7	1.4	1.9	2.5

FIGURE 2 | Changes in 24-h UFC, morning ACTH, and morning cortisol levels after treatment with TMZ after transformation to a functional SCA in case 2 (September 2018–December 2019). Patient in case 2 started TMZ in September 2018 after five surgeries and three courses of radiotherapy. Serum cortisol and plasma ACTH levels returned to within the normal ranges after treatment with TMZ for 12 cycles. Hydrocortisone was temporarily suspended on the day of the 24-h UFC collection in October, November, and December 2019. Prior to TMZ treatment, enhanced MRI showed irregular boundaries and an uneven texture of the tumor tissue that invaded the right cavernous sinus; the tumor tissue was significantly reduced after TMZ treatment.

it might take a long time for ACTH to rise gradually to a higher level to cause clinical Cushing's syndrome. The time interval for transformation from the silent type to the functional type of SCA took a median of 30 months with the longest interval of 120 months, which possibly accounts for this speculation.

Patients with SCAs usually do not have symptoms of hypercortisolism before conversion; thus, their Cushing's syndrome remains silent (30). Patients often come for the first clinical visit because of intracranial compression symptoms, including visual disturbances, headache, and hypopituitarism, etc. Compared with a study reported by Adriana et al., the incidence rates of visual impairment were 39.4% (13/33) in SCAs and 46.8% (59/126) in NFAs (31), and visual impairment was more common in the functional SCAs in our study (10/16, 62.5%). After the appearance of functional corticotroph adenoma, the biochemical changes and hormonal changes seemed more serious. Frequent hypokalemia was observed in seven patients (100%), and it was often significantly higher than that in patients with common Cushing's disease (10–25.64%) (24, 32). In addition, the median increase values in morning ACTH, serum cortisol, and 24-h UFC in SCA patients were 3.8-, 1.7-, and 5.3-fold, respectively, above the ULN, respectively. In a study including 197 patients with common Cushing's disease, the average morning ACTH (72.90 pg/ml), morning cortisol (28.13 μg/dl), and 24-h UFC (474.90 μg) were increased 1.6-, 1.5-, and 3.7-fold, respectively (32). As a result, the decrease in potassium and increase in ACTH and 24-h UFC in functional SCAs were more significant than those in common Cushing's disease. As we know, microadenomas accounted for most cases of Cushing's disease, whereas all SCAs that transformed to functional types were macroadenomas initially, and the tumor sizes became larger after conversion. In general, it was thought that the larger tumors caused the stronger ability of hormone production. However, before conversion to Cushing's syndrome, the SCAs had already been macroadenomas without overproduction of hormones; therefore, the mechanism that triggered the tumor conversion to produce more hormones still needs to be investigated. In addition, five functional SCA patients underwent high-dose DST with a paradoxical increase in three cases, which is quite different from the suppression in ∼80% of cases that were typically observed in Cushing's disease (24). The high-dose DST was not mentioned in the other nine cases from the relevant literature, so it was impossible to know whether they had the same characteristics. This result suggested that the function of SCA tumors was more autonomic and that it was not easily suppressed by dexamethasone. Raverot et al. observed that following intravenous dexamethasone,

the 24-h UFC and nadir ACTH levels were significantly higher in the corticotroph macroadenoma group than those in the microadenoma group. A subsequent molecular study revealed that there were no differences among the SCA, macroadenoma, and microadenoma groups in terms of the level of glucocorticoid receptor α or messenger RNA expression. Therefore, resistance to glucocorticoid suppression could not be explained by changes in glucocorticoid receptor α expression in SCAs (33).

In the 16 patients in this study, the tumors not only were macroadenomas but also had obvious invasiveness. Altogether, 68.8% of cases in this study had cavernous invasion, which led to the difficulty of complete removal of the tumor and possible residual tumor progression or recurrence after surgery. In addition, in five cases with available tumor sizes both initially and at the time of functional SCA diagnosis, the tumor volume increased from 1.7–29.2 to 3.2–30.8 cm^3. The tumor volume increased 1.71 ± 0.6-fold (1.0- to 2.65-fold) from the initial non-functional SCA to the transformation to a functional SCA for each patient. This suggested that the tumor had more progression than previously when it converted to a functional SCA. Although the value of Ki-67 in predicting tumor aggressiveness remains controversial (34–36), the proportion of patients with Ki-67 ≥ 3% reached 50% and was consistent with the larger tumor volume at the time of functional SCA diagnosis (11, 34). The other fact worth noting is that the cystic changes were found on pituitary MRI in 50% (7/14) of the functional SCA patients. The MRI images suggested that the cyst changes were macrocystic, whereas the lack of a description of T2-phase images in most patients made it impossible to assess further microcystic changes. Langlois et al. reported a case series of SCAs ($n = 39$), and 33% of cases demonstrated >50% fluid content on MRI T2-phase sequences (37). Cazabat et al. reported that cystic changes could be found in all SCAs ($n = 17$), half of corticotroph macroadenomas ($n = 14$), and only 17% of silent gonadotroph macroadenomas ($n = 60$) on T2-weighted pituitary MRI sequences in their case series (38). Multiple microcysts were present in most SCAs (13/17, 76.5%), and macrocysts were observed in the remaining four SCAs (4/17, 23.6%). In contrast, the specific finding of multiple microcysts was present in only 5% of silent gonadotroph macroadenomas. The presence of multiple microcysts had a sensitivity of 76% and a specificity of 95% for predicting an SCA (38, 39). Kasuki also found that microcystic changes were more common in SCAs (7/12, 58.3%) than in other pituitary tumors (6.9%) and had a sensitivity of 58%, a specificity of 93%, and an accuracy of 90% for defining an SCA (40). In this study, cystic changes, mostly macrocystic changes, were found in 50% of the functional SCAs (7/14) on pituitary MRI. Therefore, we should pay attention to SCA patients with macrocystic changes on MRI who may have the possibility of a transformation of functional type.

SCA, which can sometimes transform into functional Cushing's syndrome, is not only a rare disease but also a kind of refractory pituitary adenoma. During the disease, patients often underwent several surgeries and radiotherapy, but the tumor recurred ∼1–3 years after treatment, and some patients did not have the possibility of reoperation. Therefore, the choice of radiotherapy and medical treatment became more important for adjuvant therapy. In total, 13 patients received radiotherapy, and only four patients (30.8%) achieved a remission. However, the remission rate of radiotherapy in common Cushing's disease was 66.7–83% (41, 42).

The European Society of Endocrinology survey from 2016 reported that for patients with failed surgeries or radiotherapy, TMZ was still effective in some aggressive pituitary adenomas with a remission rate of 37% ($n = 156$) (43). In addition, clinically functioning pituitary tumors were more likely to demonstrate regression on TMZ treatment compared with non-functioning tumors (45 vs. 17%, $P = 0.01$). Furthermore, there was no clear difference in efficacy among pituitary tumors with different subtypes of function. According to the subtype of pathology, the most effective response to TMZ was lactotroph adenomas (50%), followed by somatotroph adenomas (43%), corticotroph adenomas (38%), thyrotroph adenomas (25%), and gonadotroph adenomas (17%) (44). Four patients in our study were treated with TMZ for a long time, and three patients achieved long-term hormone control and tumor control with a treatment duration of 6–17 cycles. The additional one patient with TMZ treatment had been under hormone control, but the tumor continued to progress and caused death. The relatively preferable effective result of TMZ treatment was achieved in three patients in our group, probably because the tumors had become functional when receiving TMZ treatment. To date, TMZ has been a very helpful and well-tolerated treatment for patients with functional SCA.

In general, SCAs that have completely transformed into functional SCAs are quite rare. Limited cases have been collected to investigate their clinical features. SCAs that can transform into functional SCAs tend to be macroadenomas with a more frequent invasion of the cavernous sinus and higher recurrence, even after multiple steps of treatment. The tumor volume became larger at the time of diagnosis of a functional SCA, combined with an increase in the Ki-67 index. After the diagnosis of Cushing's syndrome, the ACTH and 24-h UFC increased, and the potassium decreased more significantly than in common Cushing's disease. In addition, macrocystic changes in pituitary MRI were observed in half of this group of SCA patients. During treatment, surgery was able to remove the resectable tumor tissues and decompress the oppression symptoms. Radiotherapy was helpful to nearly 1/3 of patients but led to a lower remission rate than in patients with common Cushing's disease. TMZ is a well-tolerated and promising adjuvant medical treatment with good efficacy in patients with functional SCAs. Above all, although it is rare, long-term follow-up is required to monitor the occurrence of hypercortisolemia once a clinically non-functional SCA is diagnosed.

DATA AVAILABILITY STATEMENT

All datasets presented in this study are included in the article.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of Peking Union Medical College Hospital Peking Union Medical College Hospital, Chinese Academy of Medical Science and Peking Union Medical College. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

GZ wrote the main manuscript text and prepared the figures and tables. LL, HZhu, HY, MF, XL, CD, YY, RW, HZha, XS, and ZL collected and analyzed the data. LL and ZL designed the study and critically revised it for important intellectual content. All authors reviewed the manuscript and approved it for publication.

REFERENCES

1. Day PF, Loto MG, Glerean M, Picasso MF, Lovazzano S, Giunta DH. Incidence and prevalence of clinically relevant pituitary adenomas: retrospective cohort study in a health management organization in buenos Aires, Argentina. *Arch Endocrinol Metab.* (2016) 60:554–61. doi: 10.1590/2359-3997000000195
2. Gittleman H, Ostrom QT, Farah PD, Ondracek A, Chen Y, Wolinsky Y, et al. Descriptive epidemiology of pituitary tumors in the United States, 2004-2009. *J Neurosurg.* (2014) 121:527–35. doi: 10.3171/2014.5.JNS131819
3. Gruppetta M, Mercieca C, Vassallo J. Prevalence and incidence of pituitary adenomas: a population based study in Malta. *Pituitary.* (2013) 16:545–53. doi: 10.1007/s11102-012-0454-0
4. McDowell BD, Wallace RB, Carnahan RM, Chrischilles EA, Lynch CF, Schlechte JA. Demographic differences in incidence for pituitary adenoma. *Pituitary.* (2011) 14:23–30. doi: 10.1007/s11102-010-0253-4
5. Raappana A, Koivukangas J, Ebeling T, Pirila T. Incidence of pituitary adenomas in Northern Finland in 1992-2007. *J Clin Endocrinol Metab.* (2010) 95:4268–75. doi: 10.1210/jc.2010-0537
6. Agustsson TT, Baldvinsdottir T, Jonasson JG, Olafsdottir E, Steinthorsdottir V, Sigurdsson G, et al. The epidemiology of pituitary adenomas in Iceland, 1955-2012: a nationwide population-based study. *Eur J Endocrinol.* (2015) 173:655–64. doi: 10.1530/EJE-15-0189
7. Buurman H, Saeger W. Subclinical adenomas in postmortem pituitaries: classification and correlations to clinical data. *Eur J Endocrinol.* (2006) 154:753–8. doi: 10.1530/eje.1.02107
8. Tomita T, Gates E. Pituitary adenomas and granular cell tumors. Incidence, cell type, and location of tumor in 100 pituitary glands at autopsy. *Am J Clin Pathol.* (1999) 111:817–25. doi: 10.1093/ajcp/111.6.817
9. Nishioka H, Inoshita N, Mete O, Asa SL, Hayashi K, Takeshita A, et al. The complementary role of transcription factors in the accurate diagnosis of clinically nonfunctioning pituitary adenomas. *Endocr Pathol.* (2015) 26:349–55. doi: 10.1007/s12022-015-9398-z
10. Kovacs K, Horvath E, Bayley TA, Hassaram ST, Ezrin C. Silent corticotroph cell adenoma with lysosomal accumulation and crinophagy. A distinct clinicopathologic entity. *Am J Med.* (1978) 64:492–9. doi: 10.1016/0002-9343(78)90236-X
11. Mete O, Lopes MB. Overview of the 2017 WHO classification of pituitary tumors. *Endocr Pathol.* (2017) 28:228–43. doi: 10.1007/s12022-017-9498-z
12. Cooper ME, Murray RM, Kalnins R, Woodward J, Jerums G. The development of cushing's syndrome from a previously silent pituitary tumour. *Aust N Z J Med.* (1987) 17:249–51. doi: 10.1111/j.1445-5994.1987.tb00055.x
13. Annamalai AK, Dean AF, Kandasamy N, Kovacs K, Burton H, Halsall DJ, et al. Temozolomide responsiveness in aggressive corticotroph tumours: a case report and review of the literature. *Pituitary.* (2012) 15:276–87. doi: 10.1007/s11102-011-0363-7
14. Flores L, Sleightholm R, Neilsen B, Baine M, Drincic A, Thorell W, et al. Highly aggressive and radiation-resistant, "Atypical" and silent pituitary corticotrophic carcinoma: a case report and review of the literature. *Case Rep Oncol.* (2019) 12:139–46. doi: 10.1159/000496019
15. Jahan S, Hasanat MA, Mahmood T, Morshed S, Haq R, Fariduddin M. Postoperative expression of cushing disease in a young male: metamorphosis of silent corticotroph adenoma? *Endocrinol Diabetes Metab Case Rep.* (2019) 2019:19-0046. doi: 10.1530/EDM-19-0046
16. Sano T, Kovacs K, Asa SL, Yamada S, Sanno N, Yokoyama S, et al. Pituitary adenoma with "honeycomb Golgi" appearance showing a phenotypic change at recurrence from clinically nonfunctioning to typical cushing disease. *Endocr Pathol.* (2002) 13:125–30. doi: 10.1385/EP:13:2:125
17. Yokoyama S, Kawahara Y, Sano T, Nakayama M, Kitajima S, Kuratsu J. A case of non-functioning pituitary adenoma with cushing's syndrome upon recurrence. *Neuropathology.* (2001) 21:288–93. doi: 10.1046/j.1440-1789.2001.00409.x
18. Zoli M, Faustini-Fustini M, Mazzatenta D, Marucci G, De Carlo E, Bacci A, et al. ACTH adenomas transforming their clinical expression: report of 5 cases. *Neurosurg Focus.* (2015) 38:E15. doi: 10.3171/2014.11.FOCUS14679
19. Cooper O. Silent corticotroph adenomas. *Pituitary.* (2015) 18:225–31. doi: 10.1007/s11102-014-0624-3
20. Saeger W, Ludecke DK, Buchfelder M, Fahlbusch R, Quabbe HJ, Petersenn S. Pathohistological classification of pituitary tumors: 10 years of experience with the German pituitary tumor registry. *Eur J Endocrinol.* (2007) 156:203–16. doi: 10.1530/eje.1.02326
21. Yamada S, Ohyama K, Taguchi M, Takeshita A, Morita K, Takano K, et al. A study of the correlation between morphological findings and biological activities in clinically nonfunctioning pituitary adenomas. *Neurosurgery.* (2007) 61:580–4. doi: 10.1227/01.NEU.0000290906.53685.79
22. Cheres AF, ElAsmar N, Rajpal A, Selman WR, Arafah BM. Perioperative hypothalamic pituitary adrenal function in patients with silent corticotroph adenomas. *Pituitary.* (2017) 20:471–6. doi: 10.1007/s11102-017-0809-7
23. Cho HY, Cho SW, Kim SW, Shin CS, Park KS, Kim SY. Silent corticotroph adenomas have unique recurrence characteristics compared with other nonfunctioning pituitary adenomas. *Clin Endocrinol.* (2010) 72:648–53. doi: 10.1111/j.1365-2265.2009.03673.x
24. Kronenberg H, Williams RH. *Williams Textbook of Endocrinology*. 11th ed. Philadelphia, PA: Saunders/Elsevier (2008).
25. Fountas A, Lavrentaki A, Subramanian A, Toulis KA, Nirantharakumar K, Karavitaki N. Recurrence in silent corticotroph adenomas after primary treatment: a systematic review and meta-analysis. *J Clin Endocrinol Metab.* (2018) 104:1039–48. doi: 10.1210/jc.2018-01956
26. Matsuno A, Okazaki R, Oki Y, Nagashima T. Secretion of high-molecular-weight adrenocorticotropic hormone from a pituitary adenoma in a patient without cushing stigmata. Case report. *J Neurosurg.* (2004) 101:874–7. doi: 10.3171/jns.2004.101.5.0874
27. Jahangiri A, Wagner JR, Pekmezci M, Hiniker A, Chang EF, Kunwar S, et al. A comprehensive long-term retrospective analysis of silent corticotrophic adenomas vs hormone-negative adenomas. *Neurosurgery.* (2013) 73:8–17. doi: 10.1227/01.neu.0000429858.96652.1e
28. Scheithauer BW, Jaap AJ, Horvath E, Kovacs K, Lloyd RV, Meyer FB, et al. Clinically silent corticotroph tumors of the pituitary gland. *Neurosurgery.* (2000) 47:723–9. doi: 10.1227/00006123-200009000-00039
29. Tateno T, Izumiyama H, Doi M, Akashi T, Ohno K, Hirata Y. Defective expression of prohormone convertase 1/3 in silent corticotroph adenoma. *Endocr J.* (2007) 54:777–82. doi: 10.1507/endocrj.K07-059
30. Ben-Shlomo A, Cooper O. Silent corticotroph adenomas. *Pituitary.* (2018) 21:183–93. doi: 10.1007/s11102-018-0864-8
31. Ioachimescu AG, Eiland L, Chhabra VS, Mastrogianakis GM, Schniederjan MJ, Brat D, et al. Silent corticotroph adenomas: emory University cohort and comparison with ACTH-negative nonfunctioning pituitary adenomas. *Neurosurgery.* (2012) 71:296–303. doi: 10.1227/NEU.0b013e318257c1f0

32. Fan L, Zhuang Y, Wang Y, Liu X, Liu D, Xiang B, et al. Association of hypokalemia with cortisol and ACTH levels in cushing's disease. *Ann N Y Acad Sci.* (2019) 1463:60–66. doi: 10.1111/nyas.14205
33. Raverot G, Wierinckx A, Jouanneau E, Auger C, Borson-Chazot F, Lachuer J, et al. Clinical, hormonal and molecular characterization of pituitary ACTH adenomas without (silent corticotroph adenomas) and with cushing's disease. *Eur J Endocrinol.* (2010) 163:35–43. doi: 10.1530/EJE-10-0076
34. Sen A, Das C, Mukhopadhyay M, Mukhopadhyay S, Deb S, Mukhopadhyay B. Cytohistological correlation in pituitary tumor and immunological assessment with the help of Ki-67. *J Postgrad Med.* (2017) 63:96–9. doi: 10.4103/0022-3859.192797
35. Nishioka H, Inoshita N. New WHO classification of pituitary adenomas (4th edition): assessment of pituitary transcription factors and the prognostic histological factors. *Brain Tumor Pathol.* (2018) 35:57–61. doi: 10.1007/s10014-017-0307-7
36. Liu X, Feng M, Zhang Y, Dai C, Sun B, Bao X, et al. Expression of matrix metalloproteinase-9, pituitary tumor transforming gene, high mobility group A 2, and Ki-67 in adrenocorticotropic hormone-secreting pituitary tumors and their association with tumor recurrence. *World Neurosurg.* (2018) 113:e213–21. doi: 10.1016/j.wneu.2018.01.214
37. Langlois F, Lim DST, Yedinak CG, Cetas I, McCartney S, Cetas J, et al. Predictors of silent corticotroph adenoma recurrence; a large retrospective single center study and systematic literature review. *Pituitary.* (2018) 21:32–40. doi: 10.1007/s11102-017-0844-4
38. Cazabat L, Dupuy M, Boulin A, Bernier M, Baussart B, Foubert L, et al. Silent, but not unseen: multimicrocystic aspect on T2-weighted MRI in silent corticotroph adenomas. *Clin Endocrinol.* (2014) 81:566–72. doi: 10.1111/cen.12443
39. Drummond J, Roncaroli F, Grossman AB, Korbonits M. Clinical and pathological aspects of silent pituitary adenomas. *J Clin Endocrinol Metab.* (2019) 104:2473–89. doi: 10.1210/jc.2018-00688
40. Kasuki L, Antunes X, Coelho MCA, Lamback EB, Galvao S, Silva Camacho AH, et al. Accuracy of microcystic aspect on T2-weighted MRI for the diagnosis of silent corticotroph adenomas. *Clin Endocrinol.* (2020) 92:145–9. doi: 10.1111/cen.14133
41. Estrada J, Boronat M, Mielgo M, Magallon R, Millan I, Diez S, et al. The long-term outcome of pituitary irradiation after unsuccessful transsphenoidal surgery in cushing's disease. *N Engl J Med.* (1997) 336:172–7. doi: 10.1056/NEJM199701163360303
42. Castinetti F, Brue T, Ragnarsson O. Radiotherapy as a tool for the treatment of cushing's disease. *Eur J Endocrinol.* (2019) 180:D9–18. doi: 10.1530/EJE-19-0092
43. Raverot G, Burman P, McCormack A, Heaney A, Petersenn S, Popovic V, et al. European society of endocrinology clinical practice guidelines for the management of aggressive pituitary tumours and carcinomas. *Eur J Endocrinol.* (2018) 178:G1–24. doi: 10.1530/EJE-17-0796
44. McCormack A, Dekkers OM, Petersenn S, Popovic V, Trouillas J, Raverot G, et al. Treatment of aggressive pituitary tumours and carcinomas: results of a European society of endocrinology (ESE) survey 2016. *Eur J Endocrinol.* (2018) 178:265–76. doi: 10.1530/EJE-17-0933

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Endocrinology

CASE REPORT
published: 07 October 2020
doi: 10.3389/fendo.2020.579436

Autoimmune Hypophysitis With Systemic Lupus Erythematosus: A Case Report and Literature Review

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Background: Autoimmune hypophysitis (AH) is a primary autoimmune inflammatory disorder of the pituitary gland, which usually presents as a mass in the sella turcica. Systemic lupus erythematosus (SLE) is another inflammatory disorder in which the immune system attacks healthy cells and tissues throughout the body. Although both diseases are autoimmune disorders, they rarely coexist, and the relationship between them is unclear.

Case Report: A 66-year-old man was evaluated at the endocrinology clinic because of worsening fatigue, anorexia, drowsiness, and leg oedema. Examination revealed alertness impairment and lower limb oedema. Laboratory tests showed anterior pituitary hypofunction. The treatment approach, with glucocorticoids and immunosuppressive agents, resulted in long-term remission of symptoms of hypopituitarism and hyponatraemia.

Conclusions: Our case demonstrates a potential association between AH and SLE. AH may need to be considered in the evaluation of SLE patients with headache, hyperprolactinemia, a pituitary mass, and hypopituitarism.

Keywords: autoimmune hypophysitis, systemic lupus erythematosus, anterior pituitary hypofunction, autoimmune disease, sella turcica

OPEN ACCESS

Edited by:
Renzhi Wang,
Peking Union Medical College Hospital (CAMS), China

Reviewed by:
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Specialty section:
This article was submitted to Pituitary Endocrinology, a section of the journal Frontiers in Endocrinology

Received: *02 July 2020*
Accepted: *14 September 2020*
Published: *07 October 2020*

Citation:
Xiang P, Wu Q, Zhang H, Luo C and Zou H (2020) Autoimmune Hypophysitis With Systemic Lupus Erythematosus: A Case Report and Literature Review. Front. Endocrinol. 11:579436. doi: 10.3389/fendo.2020.579436

INTRODUCTION

Autoimmune hypophysitis (AH), also known as lymphocytic hypophysitis, is a rare inflammatory disorder that can affect the anterior, posterior or both pituitary lobes (1). Most cases have been diagnosed in women during late pregnancy or the postpartum period (2). Systemic lupus erythematosus (SLE) is a condition where the immune system attacks healthy cells as well as tissues around the body (3). AH is rarely associated with rheumatic diseases, and only 1.3% of AH cases have previously been described to be associated with SLE (4). Here, we describe a case of AH and SLE in a 66-year-old man presenting with fatigue, anorexia, drowsiness, and leg oedema, and we summarize all case reports of AH with SLE to provide some evidence of the pathogenesis, diagnosis and treatments.

CASE PRESENTATION

A 66-year-old man with a history of diabetes and percutaneous transluminal coronary intervention (PCI) was evaluated at the endocrinology clinic because of worsening fatigue, anorexia, drowsiness, and leg oedema.

The patient had been followed up by one of us (P X) for 5 years because of non-insulin-dependent diabetes mellitus (NIDDM). Three years ago, the patient was diagnosed with cardiovascular disease (CVD) and treated with percutaneous coronary intervention (PCI) in the Department of Cardiology. Five months before presentation, the patient was readmitted to the Department of Cardiology because of fatigue, anorexia, drowsiness, and leg oedema. His temperature was 36.5°C, heart rate was 72 beats/min, and blood pressure was 135/85 mmHg. At the Department of Cardiology, the white-cell and differential counts and blood levels of erythrocyte sedimentation rate (ESR), high-sensitivity C-reactive protein, thyroid stimulating hormone (TSH), and free T3 (FT3) were normal; the level of free T4 (FT4) was decreased; and levels of glycated haemoglobin A1c (HbA1c), triglycerides, and brain natriuretic peptide (BNP) were elevated. The blood sodium level was 131.7 mmol/L [normal range (NR), 137 to 147]. During hospitalization, he received treatment with laxatives due to constipation, and his blood sodium level decreased to 110.3 mmol/L. Therefore, treatment with 3% NaCl was initiated, with obvious relief in symptoms. The patient was discharged when the sodium level reached 130.1 mmol/L.

At the last visit in the endocrinology clinic, the patient complained that these symptoms had worsened during the past week. He reported no headache, dizziness, polydipsia, polyuria, or vomiting. Examination revealed alertness impairment and lower limb oedema. No trochlear nerve palsy, oculomotor nerve paralysis, or visual field defects were detected during examination. As shown in **Table 1**, laboratory tests showed anterior pituitary hypofunction. The FT3 (1.93 pg/ml; NR, 2.27 to 4.22) and FT4 (0.63 ng/dl; NR, 0.9 to 1.76) decreased without a corresponding increase in TSH (3.00 uIU/ml; NR, 0.55 to 4.78), suggesting central hypothyroidism. Follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone levels were all below the sensitivity of the assay, consistent with hypogonadotropic hypogonadism. Laboratory examination also showed low sodium (124 mmol/L; NR, 137 to 147) and chloride (89 mmol/L; NR, 99 to 110) and low morning cortisol (0.49 μg/dl; NR, 4.26 to 24.85) without a corresponding increase in corticotrophin (ACTH 8.14 pg/ml, NR 7.2–63.4), suggesting central hypercortisolism. The insulin-like growth factor 1 (IGF-1) level was decreased (53 ng/ml; NR, 69-211), and the serum growth hormone (GH) level was normal. Interestingly, prolactin (PRL) was slightly elevated (26.6 ng/ml; NR, 3.6 to 16.3), which occurs in approximately 18% of AH patients (5) and can decrease LH and FSH levels. Test results for rheumatoid factor (RF), antinuclear antibody (ANA), anti-Sm antibody, anti-Sjögren's syndrome B (anti-SSB), anti-double-stranded DNA antibodies (anti-dsDNA), anti-nRNP/Sm, and anticardiolipin antibody-IgG (ACL-IgG) were positive. Test results for other relevant antibodies were negative (**Table 1**). The levels of all

TABLE 1 | Laboratory findings.

Variable	Results	Reference values
Blood routine		
Hemoglobin (g/L)	115	130–175
Hematocrit (%)	0.320	0.4–0.5
White-cell count (*10^9/L)	4.18	3.5–9.5
Platelet count (*10^9/L)	253	125–350
Electrolyte		
Sodium (mmol/L)	124	137–147
Potassium (mmol/L)	3.4	3.5–5.3
Chloride (mmol/L)	89.0	99–100
Calcium (mmol/L)	2.22	2.1–2.9
Blood biochemical		
Urea nitrogen (mmol/L)	2.51	3.6–9.5
Creatinine (μmol/L)	54.7	57–111
Glucose (mmol/L)	6.32	4.11–5.89
HbA1c (%)	6.80	4.27–6.07
Total protein (g/L)	67.1	65–85
Albumin (g/L)	40.9	40–55
cTnI (ng/ml)	<0.02	0–0.06
Endocrine hormones		
FT4 (ng/dl)	0.63	0.9–1.76
FT3 (pg/ml)	1.93	2.27–4.22
TSH (uIU/ml)	3.00	0.55–4.78
GH (μg/L)	0.04	≤2.47
IGF-1 (ng/ml)	53	69–211
ACTH (μg/ml)	8.14	7.2–63.4
Cortisol peak (ng/ml)	0.49	4.26–24.85
PRL (ng/ml)	26.6	3.6–16.3
FSH (mIU/ml)	<1.00	2.1–18.6
LH (mIU/ml)	<0.20	1.7–11.2
Estradiol (pg/ml)	<25.00	<75
Progesterone (ng/ml)	<0.10	<0.46
Testosterone (ng/dl)	3.12	262–870
Antibodies		
RF (KIU/L)	31.8	<14.0
AKA	Negative	Negative
CCP (RU/ml)	3.3	≤5.0
RA33 (AU/ml)	6.42	<25
ANA	Positive (1:1,000)	Negative
Anti-Sm	Positive (++)	Negative
Anti-SSA	Negative	Negative
Anti-SSB	Positive	Negative
Anti-dsDNA	Positive (+)	Negative
ACA	Negative	Negative
AHA	Negative	Negative
Anti-nucleosome	Negative	Negative
ARPA	Negative	Negative
Anti-Jo-1	Negative	Negative
Anti-nRNP	Positive (++)	Negative
Anti-Ro-52	Negative	Negative
Anti-Scl-70	Negative	Negative
ACL-IgG	Positive	Negative
LA1 (s)	27.10	31–44
LA2 (s)	30.5	30–38
LA1-LA2 (ratio)	0.89	1.0–1.2
Image		
Pituitary MRI	Empty sella	Normal
Adrenal MRI	Normal	Normal

HbA1c, glycated hemoglobin A1c; cTnI, cardiac troponin I; FT4, free T4; FT3, free T3; TSH, thyroid stimulating hormone; GH, growth hormone; IGF-1, insulin-like growth factor 1; ACTH, corticotrophin; PRL, prolactin; FSH, follicle-stimulating hormone; LH, luteinizing hormone; RF, rheumatoid factors; AKA, anti-keratin antibody; CCP, cyclic citrullinated peptide antibody; ANA, antinuclear antibodies; anti-SSA, anti- Sjögren's syndrome A; anti-SSB, anti- Sjögren's; syndrome B; anti-dsDNA, anti-double-stranded DNA antibodies; ACA, anti-centromere autoantibody; AHA, anti-histone antibody; ARPA, anti-ribosomal P protein antibodies; ACL-IgG, anticardiolipin antibody-IgG; LA1, lupus anticoagulant 1; LA2, lupus anticoagulant 2; MRI, magnetic resonance imaging.

tumour markers were normal. The magnetic resonance imaging (MRI) scan of the pituitary gland revealed an empty sella (**Figure 1**). No abnormality was observed in the adrenal MRI.

Based on the above, the patient was diagnosed with AH, anterior pituitary hypofunction, SLE, NIDDM, and CVD. According to the clinical guidelines (6, 7), the patient was treated with 30 mg/d prednisone, 80 mg/d testosterone undecanoate, and 50 μg/d levothyroxine for the treatment of anterior pituitary hypofunction; 0.4 g/d hydroxychloroquine for the treatment of SLE; 22 U/d premixed human insulin for the treatment of NIDDM; and 75 mg/d clopidogrel, 10 mg/d atorvastatin calcium tablets, 50 mg/d captopril and 100 mg/d metoprolol for the treatment of CVD. Three days after the treatment, the sodium level had risen to 134 mmol/L, and the patient's symptoms resolved gradually. Then, the patient was discharged with prescriptions for oral administration on the 11th hospital day. The dosage of prednisone was decreased after one month, with a monthly decrease of 5 mg until it was maintained at 10 mg/d. Two months after discharge, the patient reported no fatigue, anorexia, drowsiness, or leg oedema, and the patient's sodium level was normal at the follow-up visit.

REVIEW OF THE LITERATURE

We conducted a literature search with the terms "autoimmune hypophysitis" or "lymphocytic hypophysitis" and "systemic lupus erythematosus" in the PubMed, Embase, and CNKI databases for case reports of AH with SLE from inception to August 10, 2020. Then, manual searching was conducted according to the references of relevant acquired articles. Five cases of AH with SLE were reported (2, 8–11). Unfortunately, we did not obtain the complete publication of the first case reported by Hasegawa et al. (8). We summarized the remaining 4 cases in **Table 2** (2, 9–11).

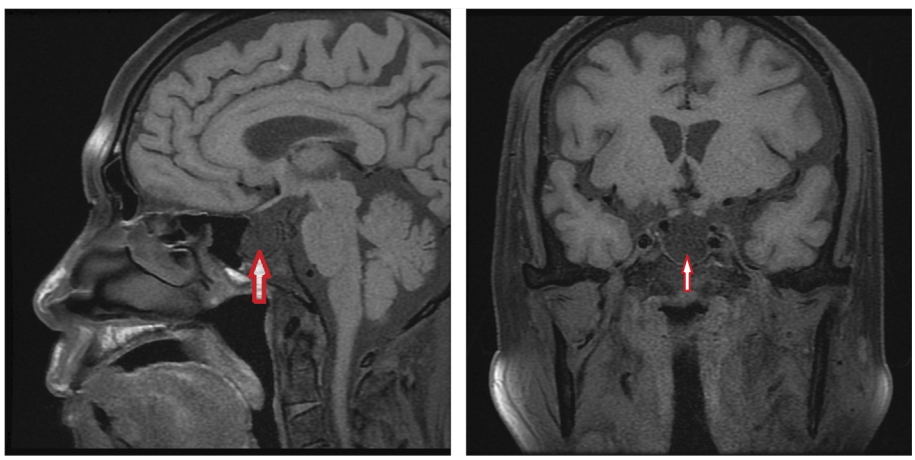

FIGURE 1 | Brain MRI.

TABLE 2 | Reported cases of AH associated with SLE.

No.	Source	Age (years)/ Gender	Diagnosis	Clinical presentation	Pituitary defects	Treatment	Glucocorticoid dose	Prognosis
1	Katano et al., (9)	26/Female	AH with SLE	Visual disturbances	Panhypopituitarism	Surgery	Not provide	Not provide
2	Ji et al. (2)	20/Female	AH with SLE	Headache and nausea	Anterior pituitary hypofunction	Trans-sphenoidal surgery and medication with prednisolone and hydroxychloroquine	Prednisolone 15 mg/d	Remission
3	Hashimoto et al. (10)	27/Male	AH with SLE	Polyuria and polydipsia, facial erythema and general malaise.	Panhypopituitarism	Medication with hydrocortisone and prednisolone.	Prednisolone 30 mg/d	Remission
4	Huang et al. (11)	19/Female	AH with SLE	Amenorrhea, polyuria and polydipsia	Normal	Medication with methylprednisolone, desmopressin, azathioprine, and hydroxychloroquine	Methylprednisolone: 200 mg/d*3d→160 mg/d*3d→ 100 mg/d*3d→80 mg/d*3d Prednisone: 40mg/d→30mg/d	Died

AH, autoimmune hypophysitis; SLE, systemic lupus erythematosus.

In the four cases of AH with SLE, only one patient was male, and the remaining patients were female. The onset age ranged from 19 to 27 years. Three of them were SLE patients who then developed visual impairment, headache, nausea, polyuria, or polydipsia. One patient was previously diagnosed with diabetes insipidus and then presented with facial erythema and general malaise. Most of the patients were diagnosed based on clinical and imaging features. Two patients underwent surgery, and postoperative pathological examination confirmed the diagnosis. Three patients were treated with glucocorticoid medication. One patient received hydroxychloroquine and azathioprine for the treatment of SLE. Three patients achieved remission, but one died of severe hyponatraemia.

DISCUSSION

AH is related to a disruption of tissue organization and frequently leads to glandular malfunction, which generally manifests as a mass within the sella turcica, with headache and/or visual disturbances (5). Pituitary enlargement is often secondary to infiltration and oedema. The progression of AH includes remission, spontaneous or pharmacological resolution of the inflammation, or progressive diffuse destruction with glandular atrophy for fibrotic replacement, thus resulting in varying degrees of pituitary dysfunction (1) and symptoms of hypopituitarism, including low ACTH, low TSH, low gonadotropins, or low prolactin (5). Currently, a definitive diagnosis of AH can be based only on pathological examination of a pituitary biopsy sample after invasive surgical intervention. For patients with glandular atrophy, as in the present case, clinicians should make a non-invasive diagnosis through clinical and imaging features and then treat with conservative management with glucocorticoids and other anti-inflammatory and immunosuppressive agents (methotrexate, azathioprine) (12), which aims to reduce inflammation and restore pituitary function.

SLE is a chronic autoimmune disease in which the immune system attacks healthy cells and tissues throughout the body, and the most frequently targeted organs are the kidneys, skin, lungs, brain, and heart (13). AH and SLE are both autoimmune diseases, but we are not aware of any histologically proven description or mechanism that links AH with SLE. It is still unclear whether the pituitary is a target of SLE. Our case demonstrates a potential association between AH and SLE. AH may need to be considered in the evaluation of SLE patients with headache, hyperprolactinemia, a pituitary mass, and hypopituitarism.

CONCLUSION

In conclusion, we report the case of a 66-year-old man with AH and SLE who presented with hypopituitarism and hyponatraemia. The diagnosis was made mainly based on clinical manifestations, laboratory assessment, and imaging studies. The treatment approach, with glucocorticoids and immunosuppressive agents, resulted in long-term remission of symptoms of hypopituitarism and hyponatraemia.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material; further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

HJZ and PX collected data and wrote the manuscript. QW, HZ, and CL provided suggestion during the diagnosis and treatment in this case. All authors contributed to the article and approved the submitted version.

REFERENCES

1. Falorni A, Minarelli V, Bartoloni E, Alunno A, Gerli R. Diagnosis and classification of autoimmune hypophysitis. *Autoimmun Rev* (2014) 13:412–6. doi: 10.1016/j.autrev.2014.01.021
2. Ji JD, Lee SY, Choi SJ, Lee YH, Song GG. Lymphocytic hypophysitis in a patient with systemic lupus erythematosus. *Clin Exp Rheumato* (2000) 78–80. doi: 10.1186/ar132
3. Kiriakidou M, Cotton D, Taichman D, Williams S. Systemic lupus erythematosus. *Ann Intern Med* (2013) 159:ITC4–1. doi: 10.7326/0003-4819-159-7-201310010-01004
4. da Silva BSP, Bonin C, Bueno CBF, Glezer A, Bronstein MD, Carvalho JF. Primary antiphospholipid syndrome and panhypopituitarism: a unique presentation. *Acta Reumatol Port* (2012) 37:272–5. doi: 10.1186/1471-2474-13-117
5. Caturegli P, Lupi I, Landek-Salgado M, Kimura H, Rose NR. Pituitary autoimmunity: 30 years later. *Autoimmun Rev* (2008) 7:631–7. doi: 10.1016/j.autrev.2008.04.016
6. Fanouriakis A, Kostopoulou M, Alunno A, Aringer M, Bajema I, Boletis JN, et al. Update of the EULAR recommendations for the management of systemic lupus erythematosus. *Ann Rheum Dis* (2019) 78:736–45. doi: 10.1136/annrheumdis-2019-215089
7. Takagi H, Iwama S, Sugimura Y, Takahashi Y, Oki Y, Akamizu T, et al. Diagnosis and treatment of autoimmune and IgG4-related hypophysitis: Clinical guidelines of the Japan endocrine society. *Endocr J* (2020) 67:373–8. doi: 10.1507/endocrj.EJ19-0569
8. Hasegawa Y, Matsumoto M, Kamimura AYM. A case of systematic lupus erythematosus with autoimmune hypophysitis. *Nihon Naika Gakkai Zasshi* (1993) 82:582–3. doi: 10.2169/naika.82.582
9. Katano H, Umemura A, Kamiya K, Kanal H, Yamada K. Visual disturbance by lymphocytic hypophysitis in a non-pregnant woman with systemic lupus erythematosus. *Lupus* (1998) 7:554–6. doi: 10.1191/096120398678920514
10. Hashimoto K, Asaba K, Tamura K, Takao T, Nakamura T. A case of lymphocytic infundibuloneurohypophysitis associated with systemic lupus erythematosus. *Endocr J* (2002) 49:605–10. doi: 10.1507/endocrj.49.605

11. Huang A, Liu Y, Zhang N, Zhang L, Wang Z, Su X, et al. A case of systemic lupus erythematosus with hypophysitis as the first manifestation. *Chin J Rheumatol* (2013) 17:285–6. doi: 10.3760/cma.j.issn.1007-7480.2013.04.018
12. Xu C, Ricciuti A, Caturegli P, Keene CD, Kargi AY. Autoimmune lymphocytic hypophysitis in association with autoimmune eye disease and sequential treatment with infliximab and rituximab. *Pituitary* (2015) 18:441–7. doi: 10.1007/s11102-014-0592-7
13. Tsokos GC. Systemic Lupus Erythematosus. *N Engl J Med* (2011) 365:2110–21. doi: 10.1056/NEJMra1100359

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Endocrinology

CASE REPORT
published: 18 November 2020
doi: 10.3389/fendo.2020.576027

Case Report: A Case of Pituitary Carcinoma Treated With Sequential Dual Immunotherapy and Vascular Endothelial Growth Factor Inhibition Therapy

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Aggressive pituitary tumors (APTs) are associated with significant morbidity and mortality, and effective treatment options are limited. Immune checkpoint inhibitors (ICIs) have revolutionized clinical cancer care; however, there is little experience with these agents in the management of APTs. Vascular endothelial growth factor (VEGF) targeted therapy has reported success in a small number of APT case reports. Here we describe a case of pituitary carcinoma responding to ICI therapy and subsequently VEGF inhibition. We discuss the possible mechanisms and experience with ICI therapy and VEGF inhibitors in the management of APTs, biomarkers that may predict response, and the potential role of combination therapies including ICIs and temozolomide.

Keywords: aggressive pituitary tumors, pituitary carcinoma, immunotherapy, immune checkpoint inhibitor, vascular endothelial growth factor inhibitor, biomarker, combination therapy

OPEN ACCESS

Edited by:
Mônica Gadelha, Federal University of Rio de Janeiro, Brazil

Reviewed by:
Gerald Raverot, Hospices Civils de Lyon, France
Leandro Kasuki, Instituto Estadual do Cérebro Paulo Niemeyer (IECPN), Brazil

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Specialty section:
This article was submitted to Pituitary Endocrinology, a section of the journal Frontiers in Endocrinology

Received: *25 June 2020*
Accepted: *19 October 2020*
Published: *18 November 2020*

Citation:
Lamb LS, Sim H-W and McCormack AI (2020) Case Report: A Case of Pituitary Carcinoma Treated With Sequential Dual Immunotherapy and Vascular Endothelial Growth Factor Inhibition Therapy. Front. Endocrinol. 11:576027. doi: 10.3389/fendo.2020.576027

INTRODUCTION

Aggressive pituitary tumors (APTs) including pituitary carcinomas (PCs) are a subset of pituitary tumors with a more aggressive course defined clinically by significant invasion, rapid tumor growth rate, and resistance to optimal standard therapies (1). Morbidity and mortality associated with these tumors are high with overall mortality rates for APT and PC reported at 28 and 42.5% respectively at a median duration of 11 years following diagnosis (2). Treatment options include a combination of surgical resection, radiotherapy and medical therapy for functioning tumors. Temozolomide (TMZ) is the only chemotherapeutic agent demonstrated to have a significant effect on APTs and is recommended as first line chemotherapy for APTs in the European Society of Endocrinology (ESE) Clinical Practice Guidelines (3). TMZ is associated with an improved 5 year overall survival in APTs of up to 90% in responders; however, only 37% of patients have a response to TMZ. One third of patients continue to progress despite treatment, and 35% initially responding to TMZ progress following cessation of treatment (2–4). There is typically a poor response to a second course of TMZ with just 11% of patients reported to have partial regression following a second course in the ESE survey (2). Alternative treatment options remain experimental and include use of targeted therapies such as VEGF, mTOR, or EGFR inhibitors and peptide receptor radionucleotide therapy (5).

Immune checkpoint inhibitors (ICIs) have revolutionized clinical cancer care and are now approved for use in many malignancies including melanoma, lung cancer, renal cell carcinoma, squamous cell, head and neck cancer, lymphoma, urothelial carcinoma and gastro-esophageal carcinoma (6). ICIs are monoclonal antibodies targeting the immunosuppressive CTLA4 and PD-1 receptors on the surface of T lymphocytes and PD-L1 on the surface of tumor cells, inhibiting the downregulation of T lymphocytes and resulting in improved anti-tumor immune response.

Thirteen cases of APTs treated with VEGF inhibition therapy have been reported, with the majority responding to treatment (2, 7–12). Published experience with ICI therapy in APTs is limited to two cases with markedly different outcomes (13, 14). No cases to our knowledge have previously been reported of treatment with ICI and VEGF inhibition therapy. Here we describe a case of PC responding to ICI treatment and subsequently VEGF inhibition. We discuss mechanisms supporting the use of ICIs in APTs and explore the potential for sequential and combination therapy of novel agents in the management of APTs.

CASE DESCRIPTION

A 72 year old female was diagnosed with a silent lactotroph pituitary carcinoma in August 2018 (**Figure 1**). She underwent an initial transsphenoidal surgery (TSS) in 2014 following presentation with headaches and demonstration of a macroadenoma on MRI. Pituitary hormone profile was normal, and specifically she has never had hyperprolactinemia. Histopathology (2014) revealed a pituitary tumor with diffusely strong prolactin immunoexpression, mitoses 5 per 10 high power field and elevated Ki67 10%. Gross total resection was achieved at the initial surgery, however; she had subsequent rapid tumor recurrences necessitating repeat TSS in 2015 and 2016 followed by sellar radiotherapy in 2017. In 2018 the patient represented with headache, anorexia, and weight loss and MRI demonstrated extra-sellar disease progression with evidence of dural metastases. She underwent debulking surgery of the spinal metastatic disease in August 2018. Histopathology was consistent with a lactotroph (Pit-1 positive) pituitary carcinoma with high MGMT expression and Ki67 20%. On the recommendation of recent guidelines, albeit noting the high MGMT expression, she commenced a trial of TMZ, but progression was expected and demonstrated following 3 months of therapy (3). She also received concomitant radiotherapy delivered to the sites of spinal metastatic disease. In the meantime, tumor genomic profiling was undertaken. The tumor was mismatch repair-proficient, harbored no actionable variants, and the tumor mutation burden was low at 6.8 Mut/Mb. Tumor PD-L1 expression by immunohistochemistry was <1%. Beyond the use of temozolomide as first line chemotherapy for APT and PC, treatment options remain experimental and were presented to the patient. Extrapolating from the isolated case report of successful response to dual immunotherapy in an ACTH-secreting hypermutated pituitary carcinoma (13), as well as increasing use of immunotherapy in the cancer field, our patient elected to receive self-funded dual immunotherapy with ipilimumab 3 mg/kg IV and nivolumab 1 mg/kg IV thrice weekly. Following the second cycle, treatment was complicated by autoimmune nephritis with acute kidney injury requiring hospital admission. Creatinine peaked at 468 umol/L, eGFR 8 ml/min/1.73 m^2. This responded to high dose glucocorticoid therapy with recovery over 10 days. Ipilimumab was discontinued and maintenance nivolumab 3 mg/kg IV twice weekly was continued

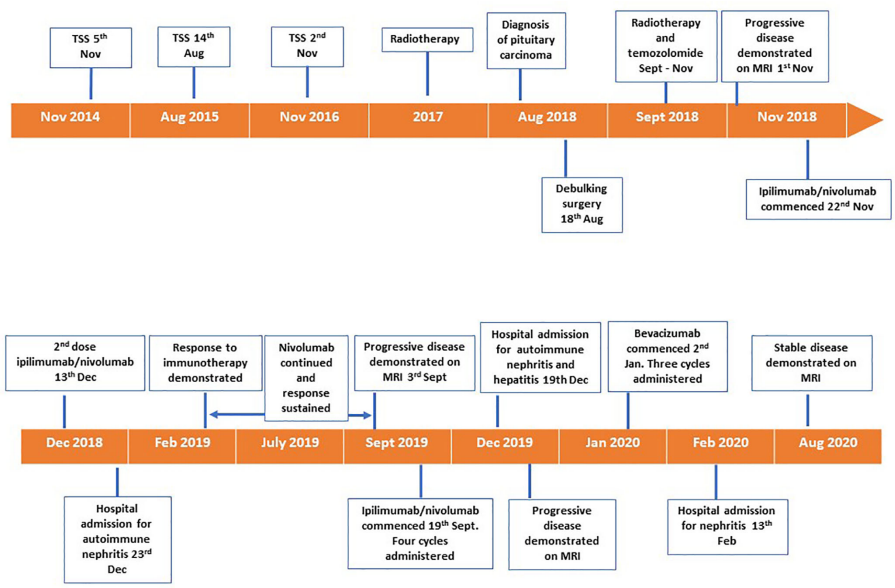


FIGURE 1 | Timeline of disease progression and treatment.

for 17 cycles over 8 months. There was a marked clinical and radiological response of the primary pituitary carcinoma and all metastatic lesions that was sustained for eight months (**Figures 2** and **3**). In September 2019 tumor progression was again demonstrated despite continued use of nivolumab, and a decision was made to rechallenge with addition of ipilimumab to nivolumab. Following four cycles of combination ipilimumab and nivolumab, treatment was once again complicated by ICI-related nephritis and hepatitis which responded to high dose glucocorticoid therapy. Unfortunately, on this occasion MRI demonstrated tumor progression, and ipilimumab and nivolumab were ceased. Based on a small selection of successful case reports (2, 7–10, 12), further treatment was trialed with the VEGF monoclonal antibody bevacizumab 10 mg/kg IV twice weekly, and three cycles were administered. Treatment was interrupted by nephritis, and she was managed for both potential autoimmune and VEGF inhibitor induced nephritis with good response to high dose glucocorticoid therapy and antihypertensives. Six months after ceasing bevacizumab, she has stable disease on MRI.

DISCUSSION

To our knowledge, this is the first case reported of an APT treated with sequential ICI and VEGF targeted therapy. ICIs are now well established as effective treatments in a number of cancers; however, only two cases of use in APTs have previously been described. The case we have described highlights a number of interesting and relevant considerations for ICI and VEGF inhibitor use in the management of APTs.

Immune Checkpoint Inhibitors and Normal Pituitary

The well-documented side effect of hypophysitis resulting from ICI treatment of other cancers provides a rationale for the use of these agents in the management of pituitary tumors. A recent meta-analysis reported the incidence of hypophysitis following ICI therapy was greatest with combination therapy (6.4%) and was significantly greater in those receiving CTLA-4 inhibitors (3.2%) compared with PD-1 inhibitors (0.4%) (OR, 0.29; 95% CI, 0.18–0.49; P <.001) (15). The mechanism of action of CTLA4 inhibitors on the pituitary seems to be multifactorial. Pituitary antibodies have been demonstrated in CTLA-4 inhibitor related hypophysitis (16). Pituitary expression of CTLA-4 has been demonstrated in normal pituitary and pituitary adenomas and may provide a direct target for CTLA4 antibodies (16, 17). Pituitary specific complement deposition in mice administered CTLA-4 antibody suggests that CTLA-4 monoclonal antibodies induce a type 2 hypersensitivity reaction which targets CTLA-4 expressed on pituitary cells and initiates tissue destruction (16). Direct binding of CTLA-4 antibody to anterior pituitary cells may also activate antibody dependent cell-mediated cytotoxicity (ADCC). By contrast, the IgG4-based PD-1 and PD-L1 antibodies do not activate the classical complement pathway and are less effective in activating ADCC (17). Despite this, treatments targeting PD-1 or PD-L1 remain intriguing as a potential treatment option for pituitary tumors based on the findings that pituitary tumors express variable levels of PD-L1 with increased expression in functioning tumors and an association with higher tumor Ki67 index (18–20).

Clinical Experience With Immune Checkpoint Inhibitors in Aggressive Pituitary Tumors

Clinical experience with ICIs to treat APTs is limited with just two other cases reported in the literature to date. Lin et al. described the case of a 35 year old female with an aggressive ACTH secreting pituitary tumor that initially responded to combination TMZ and capecitabine prior to metastasizing to the liver. She subsequently received dual ICI therapy with ipilimumab and nivolumab resulting in a 92% reduction in volume of the liver metastasis, 59% reduction in intracranial tumor volume, and normalization of ACTH levels. The liver metastasis had low (<1%) PD-L1 expression by immunohistochemistry (13). Interestingly, genetic analysis of the hepatic metastasis demonstrated development of a hypermutated phenotype (5,275 mutations or 93 mutations/Mb) classic for TMZ exposure including an MSH6 mutation. TMZ is known to induce inactivating mutations in MSH6 in malignant gliomas which confers resistance to TMZ, and there is at least one other case of a PC with development of MSH6 deficiency and progression following TMZ (21–24). Caccese et al. reported the case of a 47 year old male with a silent corticotroph adenoma which transformed to an aggressive ACTH secreting tumor with progression despite surgery, radiotherapy, and TMZ. Immunohistochemical analysis following TMZ demonstrated complete loss of MSH2 and MSH6, and PD-L1 expression was 0%. The patient received four cycles of pembrolizumab; however, he continued to have radiological and biochemical disease progression (14).

Combination Immune Checkpoint Inhibitor Therapy

It is possible that the excellent response to therapy observed in our case and that of Lin et al. was due to the use of combination CTLA4 and PD-L1 inhibition, compared with the use of pembrolizumab monotherapy as reported by Caccese et al. Pre-clinical studies suggest improved response to ICI combination therapy when compared to monotherapy by acting synergistically, increasing the number of tumor infiltrating lymphocytes, reducing T regulatory (T reg) lymphocytes, and retarding tumor growth (25, 26). Improved overall response and survival have been demonstrated with combination therapy compared with monotherapy for the treatment of melanoma and other cancers, albeit with an increased rate of autoimmune adverse effects (27–31).

Biomarkers Predicting Response to Immune Checkpoint Inhibitor Therapy

Several potential biomarkers for response to ICI therapy have been proposed in the management of other cancers which can be considered in the context of the cases described. High PD-L1 expression on tumor cells has been associated with improved response rate and survival in a number of cancers treated with PD-L1/PD1 inhibition including melanoma, non-small cell lung

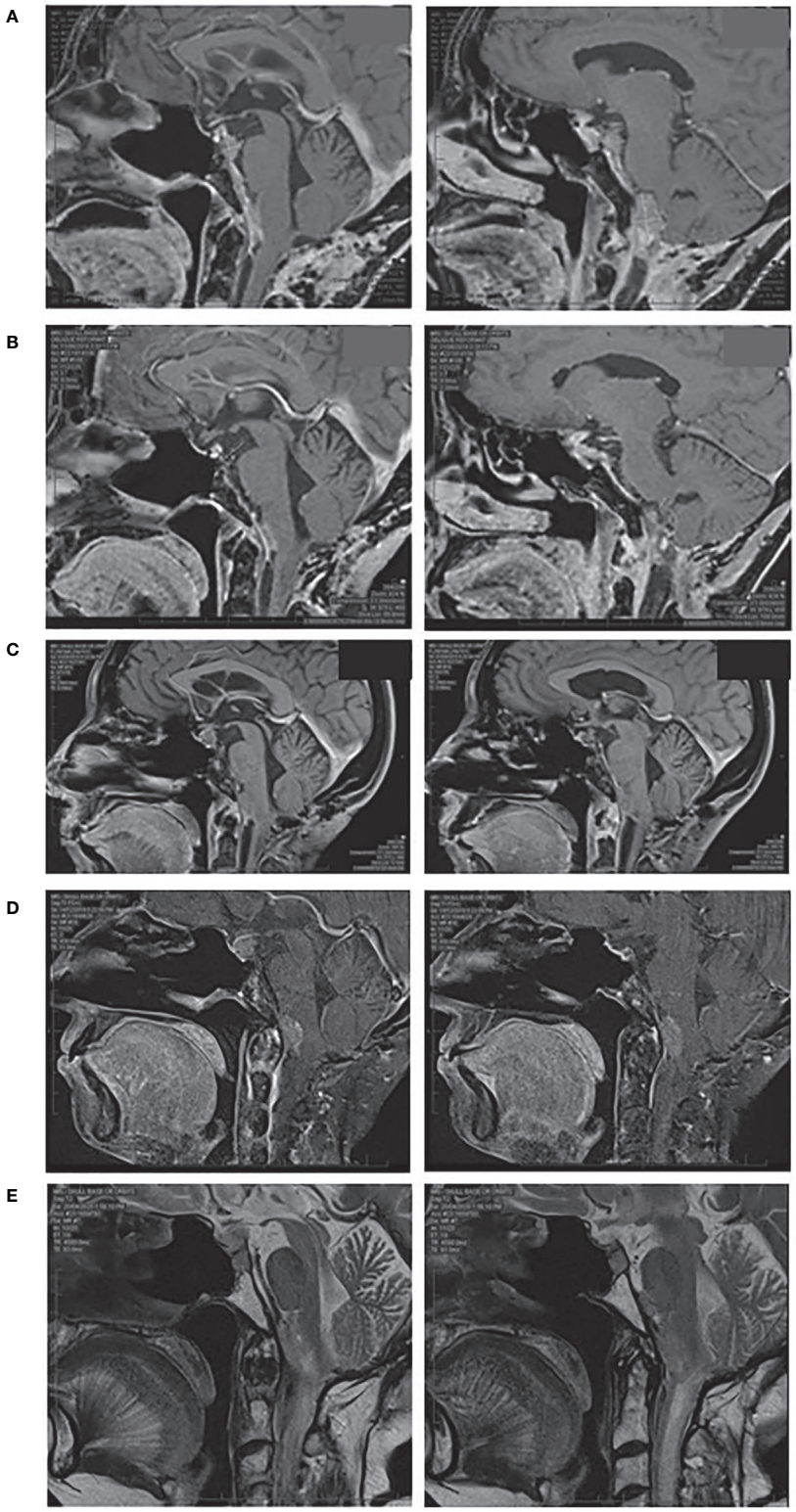
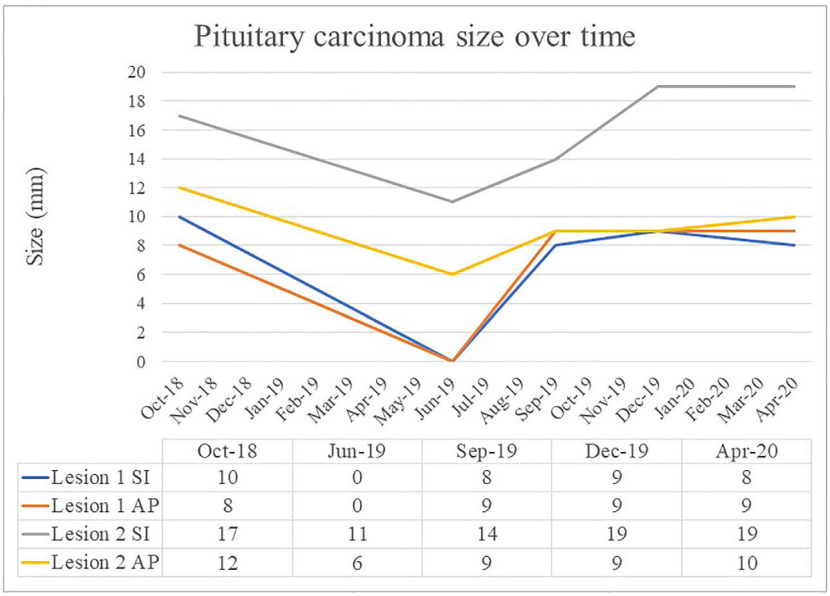


FIGURE 2 | MRI pituitary, sagittal views. **(A)** October 2018 prior to dual ICI therapy, **(B)** June 2019 demonstrating response to dual ICI therapy, **(C)** September 2019 demonstrating progressive disease, **(D)** December 2019 demonstrating progressive disease following repeat dual ICI therapy, **(E)** April 2020 demonstrating stable disease following bevacizumab. **(A–D)** T1 weighted images post gadolinium, **(E)** T2 weighted images without gadolinium due to renal impairment.



	Oct-18	Jun-19	Sep-19	Dec-19	Apr-20
Lesion 1 SI	10	0	8	9	8
Lesion 1 AP	8	0	9	9	9
Lesion 2 SI	17	11	14	19	19
Lesion 2 AP	12	6	9	9	10

FIGURE 3 | Pituitary carcinoma size (mm) over time. Superior to inferior measurement (SI), Anterior to posterior measurement (AP).

cancer, urothelial cancer, and renal cell carcinoma (32–38). However, other studies have demonstrated no association between PD-L1 expression and response to treatment, and patients with PD-L1 negative disease can still achieve clinical benefit from PD-L1/PD1 inhibition (27, 39–41). Several reasons for the conflicting findings have been proposed. There is significant variability in the scoring systems for PD-L1 expression as well as the immunohistochemistry antibodies and platforms used in clinical trials (32, 33). Other factors that may account for PD-L1 variation include T cell derived cytokines, intracellular signaling pathways and transcription factors which upregulate PD-L1 expression (42). Furthermore, PD-L1 expression can be transient and variable depending on factors relating to treatment and cancer progression, and intratumoral heterogeneity may exist (43–45). These factors all contribute to the poor reliability of PD-L1 as a biomarker for response to ICI therapy. Our case as well as the other two published pituitary cases had low PD-L1 expression, yet two of the three cases demonstrated response to ICI therapy.

Elevated tumor mutational burden (TMB) has emerged as a predictor of improved survival and response to ICI in tumors such as melanoma and non-small cell lung cancer. It is thought that tumors with a high mutation burden express higher numbers of neoantigens that can be recognized by the immune system in response to immune checkpoint inhibitor therapy (46–48). A greater benefit with anti-PD-L1 and PD-1 therapy has been reported in tumors with both high TMB and high PD-L1 expression (46). However, response to combination ICI therapy with both PD-L1/PD-1 and CTLA4 blockade seems to be predicted by TMB but not PD-L1 expression (46).

In addition to TMB, improved survival and response to ICI is associated with mismatch repair deficiency (dMMR) which is defined by defects in one of four key genes (*MLH1*, *PMS2*, *MSH2*, and *MSH6*) that encode the MMR complex (48, 49). dMMR induces microsatellite instability (MSI) and results in a deficiency of DNA repair mechanisms, increased TMB, and associated neoantigens (48–50). dMMR/MSI high tumors selectively demonstrate upregulated expression of multiple immune checkpoints to offset the activated immune response and PD-L1 expression, as a regulatory mechanism has been shown to be correlated with dMMR/MSI high in multiple cancer types (49). The correlation between high TMB and MSI-high is, however, variable between tumor types with concordance high in gastrointestinal cancers but low in lung cancer and melanoma (51). The majority of dMMR or MSI-high tumors have a high TMB; however, only a small number (16%) of tumors with high TMB are MSI-high across multiple different cancer types (51).

The pituitary cases reported thus far do not support the use of established biomarkers utilized in other cancer types. The patient we have described responded to dual ICI therapy despite being mismatch repair proficient, having a low mutation burden and undetectable PD-L1. Lin et al. reported significant response to dual ICI therapy in a patient with increased TMB and MSH6 mutation and proposed that TMZ-induced hypermutation may increase tumor response to ICIs. In contrast, Caccese et al. reported no response to single agent anti-PD-1 therapy in a patient with MSH2 and MSH6 mutation who was expected to respond given the association with dMMR, TMB, and response to ICI therapy in other cancers. These cases highlight the need for further research to identify biomarkers for response in pituitary disease and suggest that patients with aggressive disease who have failed to respond to standard therapy should not be excluded based on a lack of currently established biomarkers on tumor analysis.

Timing of Immunotherapy in Relation to Temozolomide

TMZ is well established as first line chemotherapy for APTs (52). As described above, high TMB may predict response to ICI. It is on this basis, Lin et al. proposed the potential that a TMZ induced hypermutation may increase pituitary tumor response to ICI. However, TMZ may have other effects on the tumor immune environment that may potentially decrease the response to ICIs. Furthermore, a number of studies in different cancers have reported improved response to systemic chemotherapy *following* immunotherapy (53–58). Although there is no similar clinical data for pituitary tumors, pre-clinical studies in murine glioblastoma models suggest that TMZ may impair response to immune checkpoint blockade. TMZ causes systemic immunosuppression, depletion of tumor infiltrating lymphocytes and inhibits JAK/STAT pathway signaling which decreases PD-L1 expression and may limit the effect of PD-1/PD-L1 checkpoint inhibitors in the treatment of these tumors (59–61). In murine glioblastoma models, systemic TMZ was inferior to locally administered TMZ in combination with anti-PD-1 due to the immunosuppressive effects of systemic TMZ (60). Some effects of TMZ on the immune microenvironment seem to be dose related. Standard compared with protracted low dose TMZ dosing causes an upregulation of gene signatures of T cell exhaustion and inhibitory checkpoint markers (62). PD-1 monotherapy for murine glioma models is associated with increased survival which is negated by the addition of standard dose TMZ therapy while being preserved with addition of the lower dose regimen (62). The effects of TMZ on the immune microenvironment in pituitary tumors, the interaction with ICI treatment, and consideration of timing of ICI and TMZ require further investigation.

Vascular Endothelial Growth Factor Inhibition Therapy and Pituitary Tumors

The VEGF signaling pathway has been implicated in the tumorigenesis of many cancer types. It has a physiological and pathological role in angiogenesis and vascular permeability as well as modulating the immune microenvironment *via* several mechanisms which promote a pro-tumor immunosuppressive microenvironment (12, 63, 64). VEGF targeted therapies including antibody mediated inhibition of VEGF and VEGF receptor tyrosine kinase inhibitors are now used successfully in the treatment of many cancers (64). In pituitary tumors, markers of angiogenesis such as VEGF expression and vascular density are increased in APTs compared with non-APTs; however, the significance of this with respect to anti-VEGF treatment response is uncertain (65–69). Several potential biomarkers such as VEGF expression have been investigated in other cancers with inconclusive findings, and there are currently no validated biomarkers for response to VEGF inhibition (VEGFi) therapy (70–75).

Clinical experience with VEGFi therapy for the treatment of APTs has been limited but promising. Thirteen cases of APT or PC treated with VEGFi therapy have been described, ten of which responded to treatment (2, 7–10). Nine of these were treated with bevacizumab, four in combination with TMZ, and five following unsuccessful treatment with TMZ (2, 7–9, 11). One case has been reported of response to the VEGF-2 inhibitor apatinib in combination with TMZ (10). Of the cases which progressed, two were treated with bevacizumab and one with the VEGF receptor inhibitor sunitinib (2).

Combination Immune Checkpoint Inhibitor and Vascular Endothelial Growth Factor Inhibition Therapy

There are no cases reported previously of the use of ICI and VEGFi therapy in the same patient for the management of an APT. In our case, a good response to ICI therapy with subsequent progression was followed by a stable response to VEGFi therapy. Whether an improved response to VEGF inhibitor therapy could have been seen if used prior to or concomitant with ICI therapy is not clear. However, a rationale for combination therapy has been established in other cancer types. Tumor angiogenesis contributes to an immunosuppressive microenvironment by decreasing the abundance and function of tumor infiltrating lymphocytes, increasing markers of T cell exhaustion and increasing the abundance of pro-tumor Treg lymphocytes (76). Targeting angiogenesis with anti-VEGF therapies converts the immunosuppressive tumor microenvironment to an immunosupportive one which in turn promotes the effect of ICIs (76, 77). A number of clinical trials have examined the efficacy of combination ICI and VEGFi therapy in melanoma, renal cell carcinoma, and non-small cell lung cancer with favorable results, demonstrating improved response and survival for combination therapy when compared directly and indirectly with treatment regimens consisting of single agent ICI or anti-VEGF therapy (78–80). Currently, in the management of APT, the effectiveness of ICI and VEGF inhibition therapy as monotherapies still needs to be established; however, consideration of timing of ICI and VEGF therapy may be important and should be investigated further.

Adverse Effects of Novel Therapies for Aggressive Pituitary Tumors

The use of ICIs may be limited by the occurrence of immune related adverse events (irAEs) which can occur in up to 60% of patients treated with anti-CTLA4 antibodies and up to 20% of patients treated with anti-PD-1 and anti-PD-L1 antibodies (81). Fatal irAEs occur in 0.3–1.3% of treated patients and tend to occur early in the course of treatment (82). The most common irAEs are skin rash and colitis and less commonly include hepatitis, nephritis, pneumonitis, pancreatitis, myocarditis, episcleritis, uveitis, and a number of endocrinopathies and neuropathies. The time to onset and severity of irAEs depend on the type of irAE as well as the dose and class of ICI administered. Anti-CTLA antibodies have a higher incidence of irAEs than anti-PD-1 and anti-PD-L1 antibodies, and combination ICI therapy is associated with the highest incidence of irAEs. The mainstay of treatment of irAEs is immunosuppression with corticosteroids or other immunosuppressive agents (81, 83–85). ICIs should be withheld and reintroduced following resolution of the irAE considered on an individual basis although in the case of certain

severe irAEs, rechallenge is not recommended (81). The risk of developing irAEs is difficult to predict in patients receiving ICI therapy, and there are no formal recommended prevention strategies. Patients receiving ICI therapy should undergo regular surveillance for irAEs (81).

Adverse effects of VEGFi occur as a result of endothelial dysfunction and include hypertension in up to 32% of patients and proteinuria in 23% of patients, as well as venous and arterial thromboembolic events, cardiotoxicity, impaired wound healing, gastrointestinal perforation, and increased risk of hemorrhagic events (86, 87).

There is emerging experience using the combination of ICI and VEGFi therapy. Seminal phase III trials of atezolizumab plus bevacizumab in non-small cell lung cancer and hepatocellular carcinoma did not reveal any new safety signals (80, 88). Reassuringly, the safety profile of the combination appeared to be consistent with the safety profile of the individual medications (80).

When considering the use of ICIs or VEGFi in the management of APTs, judicious risk assessment is paramount, taking into account the limited clinical experience thus far in APTs, the potential but unproven efficacy of these drugs, and the risk of adverse effects.

CONCLUSION

The case we have reported demonstrates excellent initial response of a pituitary carcinoma to combination anti-CTLA4 and anti-PD-1 ICI therapy despite exhibiting an absence of biomarkers considered predictive of response. In this case, ICI therapy undoubtedly prolonged survival and reduced morbidity in this patient. Our experience and that of the previously published pituitary cases suggest that the combination of CTLA4 and PD-1 blockade but not PD-1 blockade alone may be more effective in the treatment of APT and PC and a rationale for this has been described. In addition, the case raises the possibility of sequential or combination ICI and VEGFi therapy as novel therapy for APTs. These new approaches to treatment of APTs represent promising new avenues for research.

DATA AVAILABILITY STATEMENT

All datasets presented in this study are included in the article/ supplementary material.

ETHICS STATEMENT

Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

FUNDING

LL is supported by a University Postgraduate Award from the University of New South Wales.

ACKNOWLEDGMENTS

The authors would like to acknowledge Dr Sebastian Fung who assisted with the preparation of the images in **Figures 2** and **3**.

REFERENCES

1. Lloyd R, Osamura R, Kloppel G, Rosai J. *WHO classification of tumours of endocrine organs. 4th ed* Vol. 10. Lyon: IARC (2017).
2. McCormack A, Dekkers OM, Petersenn S, Popovic V, Trouillas J, Raverot G, et al. Treatment of aggressive pituitary tumours and carcinomas: results of a European Society of Endocrinology (ESE) survey 2016. *Eur J Endocrinol* (2018) 178(3):265–76. doi: 10.1530/EJE-17-0933
3. Raverot G, Burman P, McCormack A, Heaney A, Petersenn S, Trouillas J, et al. European Society of Endocrinology Clinical Practice Guidelines for the management of aggressive pituitary tumours and carcinomas. *Eur J Endocrinol* (2018) 178:G1–24. doi: 10.1530/EJE-17-0796
4. Ji Y, Vogel RI, Lou E. Temozolomide treatment of pituitary carcinomas and atypical adenomas: systematic review of case reports. *Neurooncol Pract* (2016) 3(3):188–95. doi: 10.1093/nop/npv059
5. Dworakowska D, Grossman AB. Aggressive and malignant pituitary tumours: state-of-the-art. *Endocr Rel Cancer* (2018) 25(11):R559–75. doi: 10.1530/ERC-18-0228
6. Havel JJ, Chowell D, Chan TA. The evolving landscape of biomarkers for checkpoint inhibitor immunotherapy. *Nat Rev Cancer* (2019) 19(3):133–50. doi: 10.1038/s41568-019-0116-x
7. Rotman LE, Vaughan TB, Hackney JR, Riley KO. Long-Term Survival After Transformation of an Adrenocorticotropic Hormone-Secreting Pituitary Macroadenoma to a Silent Corticotroph Pituitary Carcinoma. *World Neurosurg* (2019) 122:417–23. doi: 10.1016/j.wneu.2018.11.011
8. Touma W, Hoostal S, Peterson R, Wiernik A, SantaCruz K, Lou E. Successful treatment of pituitary carcinoma with concurrent radiation, temozolomide, and bevacizumab after resection. *J Clin Neurosci* (2017) 41:75–7. doi: 10.1016/j.jocn.2017.02.052
9. O'Riordan LM, Greally M, Coleman N, Breathnach OS, Hennessy B, Thompson CJ, et al. Metastatic ACTH-producing pituitary carcinoma managed with combination pasireotide and bevacizumab following failure of temozolamide therapy: A case report. *JCO* (2013) 31(15_suppl):e13022–2. doi: 10.1200/jco.2013.31.15_suppl.e13022
10. Wang Y, He Q, Meng X, Zhou S, Zhu Y, Xu J, et al. Apatinib (YN968D1) and Temozolomide in Recurrent Invasive Pituitary Adenoma: Case Report and Literature Review. *World Neurosurg* (2019) 124:319–22. doi: 10.1016/j.wneu.2018.12.174
11. Ortiz LD, Syro LV, Scheithauer BW, Ersen A, Uribe H, Fadul CE, et al. Anti-VEGF therapy in pituitary carcinoma. *Pituitary* (2012) 15(3):445–9. doi: 10.1007/s11102-011-0346-8
12. Lamb LS, Sim H-W, McCormack AI. Exploring the Role of Novel Medical Therapies for Aggressive Pituitary Tumors: A Review of the Literature—"Are We There Yet?" *Cancers* (2020) 12(2):308. doi: 10.3390/cancers12020308
13. Lin AL, Jonsson P, Tabar V, Yang TJ, Cuaron J, Beal K, et al. Marked Response of a Hypermutated ACTH-Secreting Pituitary Carcinoma to Ipilimumab and

Nivolumab. *J Clin Endocrinol Metab* (2018) 103(10):3925–30. doi: 10.1210/jc.2018-01347
14. Caccese M, Barbot M, Ceccato F, Padovan M, Gardiman MP, Fassan M, et al. Rapid disease progression in patient with mismatch-repair deficiency pituitary ACTH-secreting adenoma treated with checkpoint inhibitor pembrolizumab. *Anti Cancer Drugs* (2020) 31(2):199–204. doi: 10.1097/CAD.0000000000000856
15. Barroso-Sousa R, Barry WT, Garrido-Castro AC, Hodi FS, Min L, Krop IE, et al. Incidence of Endocrine Dysfunction Following the Use of Different Immune Checkpoint Inhibitor Regimens. *JAMA Oncol* (2018) 4(2):173–82. doi: 10.1001/jamaoncol.2017.3064
16. Iwama S, Remigis AD, Callahan MK, Slovin SF, Wolchok JD, Caturegli P. Pituitary Expression of CTLA-4 Mediates Hypophysitis Secondary to Administration of CTLA-4 Blocking Antibody. *Sci Trans Med* (2014) 6 (230):230ra45–230ra45. doi: 10.1126/scitranslmed.3008002
17. Faje A. Immunotherapy and hypophysitis: clinical presentation, treatment, and biologic insights. *Pituitary* (2016) 19(1):82–92. doi: 10.1007/s11102-015-0671-4
18. Wang P-F, Wang T-J, Yang Y-K, Yao K, Li Z, Li YM, et al. The expression profile of PD-L1 and CD8+ lymphocyte in pituitary adenomas indicating for immunotherapy. *J Neurooncol* (2018) 139(1):89–95. doi: 10.1007/s11060-018-2844-2
19. Mei Y, Bi WL, Greenwald NF, Du Z, Agar NYR, Kaiser UB, et al. Increased expression of programmed death ligand 1 (PD-L1) in human pituitary tumors. *Oncotarget* (2016) 7(47):76565–76. doi: 10.18632/oncotarget.12088
20. Wang P, Wang T, Yang Y, Yu C, Liu N, Yan C. Detection of programmed death ligand 1 protein and CD8+ lymphocyte infiltration in plurihormonal pituitary adenomas: A case report and review of the literatures. *Medicine* (2017) 96(49). doi: 10.1097/MD.0000000000009056
21. Murakami M, Mizutani A, Asano S, Katakami H, Ozawa Y, Yamazaki K, et al. A Mechanism of Acquiring Temozolomide Resistance During Transformation of Atypical Prolactinoma Into Prolactin-Producing Pituitary Carcinoma: Case Report. *Neurosurgery* (2011) 68:E1761–7; discussion E1767. doi: 10.1227/NEU.0b013e318217161a
22. Hunter C, Smith R, Cahill DP, Stephens P, Stevens C, Teague J, et al. A Hypermutation Phenotype and Somatic MSH6 Mutations in Recurrent Human Malignant Gliomas after Alkylator Chemotherapy. *Cancer Res* (2006) 66(8):3987–91. doi: 10.1158/0008-5472.CAN-06-0127
23. Cahill DP, Levine KK, Betensky RA, Codd PJ, Romany CA, Reavie LB, et al. Loss of the mismatch repair protein MSH6 in human glioblastomas is associated with tumor progression during temozolomide treatment. *Clin Cancer Res* (2007) 13(7):2038–45. doi: 10.1158/1078-0432.CCR-06-2149
24. Yip S, Miao J, Cahill DP, Iafrate AJ, Aldape K, Nutt CL, et al. MSH6 Mutations Arise in Glioblastomas during Temozolomide Therapy and Mediate Temozolomide Resistance. *Clin Cancer Res* (2009) 15(14):4622–9. doi: 10.1158/1078-0432.CCR-08-3012
25. Curran MA, Montalvo W, Yagita H, Allison JP. PD-1 and CTLA-4 combination blockade expands infiltrating T cells and reduces regulatory T and myeloid cells within B16 melanoma tumors. *PNAS* (2010) 107(9):4275–80. doi: 10.1073/pnas.0915174107
26. Spranger S, Koblish HK, Horton B, Scherle PA, Newton R, Gajewski TF. Mechanism of tumor rejection with doublets of CTLA-4, PD-1/PD-L1, or IDO blockade involves restored IL-2 production and proliferation of CD8(+) T cells directly within the tumor microenvironment. *J Immunother Cancer* (2014) 2:3. doi: 10.1186/2051-1426-2-3
27. Wolchok JD, Chiarion-Sileni V, Gonzalez R, Rutkowski P, Grob J-J, Cowey CL, et al. Overall Survival with Combined Nivolumab and Ipilimumab in Advanced Melanoma. *New Engl J Med* (2017) 377(14):1345–56. doi: 10.1056/NEJMoa1709684
28. Postow MA, Chesney J, Pavlick AC, Robert C, Grossmann K, McDermott D, et al. Nivolumab and Ipilimumab versus Ipilimumab in Untreated Melanoma. *N Engl J Med* (2015) 372(21):2006–17. doi: 10.1056/NEJMoa1414428
29. Wu K, Yi M, Qin S, Chu Q, Zheng X, Wu K. The efficacy and safety of combination of PD-1 and CTLA-4 inhibitors: a meta-analysis. *Exp Hematol Oncol* (2019) 8(1):26. doi: 10.1186/s40164-019-0150-0
30. Wolchok JD, Kluger H, Callahan MK, Postow MA, Rizvi NA, Lesokhin AM, et al. Nivolumab plus ipilimumab in advanced melanoma. *N Engl J Med* (2013) 369(2):122–33. doi: 10.1056/NEJMoa1302369
31. Larkin J, Chiarion-Sileni V, Gonzalez R, Grob JJ, Cowey CL, Lao CD, et al. Combined Nivolumab and Ipilimumab or Monotherapy in Untreated Melanoma. *New Engl J Med* (2015) 373(1):23–34.
32. Gibney GT, Weiner LM, Atkins MB. Predictive biomarkers for checkpoint inhibitor-based immunotherapy. *Lancet Oncol* (2016) 17(12):e542–51. doi: 10.1016/S1470-2045(16)30406-5
33. Yi M, Jiao D, Xu H, Liu Q, Zhao W, Han X, et al. Biomarkers for predicting efficacy of PD-1/PD-L1 inhibitors. *Mol Cancer* (2018) 17(129). doi: 10.1186/s12943-018-0864-3
34. Rosenberg JE, Hoffman-Censits J, Powles T, van der Heijden MS, Balar AV, Necchi A, et al. Atezolizumab in patients with locally advanced and metastatic urothelial carcinoma who have progressed following treatment with platinum-based chemotherapy: a single-arm, multicentre, phase 2 trial. *Lancet* (2016) 387(10031):1909–20.
35. Garon EB, Rizvi NA, Hui R, Leighl N, Balmanoukian AS, Eder JP, et al. Pembrolizumab for the Treatment of Non–Small-Cell Lung Cancer. *New Engl J Med* (2015) 372(21):2018–28. doi: 10.1056/NEJMoa1501824
36. Reck M, Rodríguez-Abreu D, Robinson AG, Hui R, Csőszi T, Fülöp A, et al. Pembrolizumab versus Chemotherapy for PD-L1–Positive Non–Small-Cell Lung Cancer. *New Engl J Med* (2016) 375(19):1823–33. doi: 10.1056/NEJMoa1606774
37. Motzer RJ, Rini BI, McDermott DF, Redman BG, Kuzel TM, Harrison MR, et al. Nivolumab for Metastatic Renal Cell Carcinoma: Results of a Randomized Phase II Trial. *JCO* (2014) 33(13):1430–7. doi: 10.1200/JCO.2014.59.0703
38. Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. *N Engl J Med* (2012) 366(26):2443–54.
39. Sharma P, Callahan MK, Bono P, Kim J, Spiliopoulou P, Calvo E, et al. Nivolumab monotherapy in recurrent metastatic urothelial carcinoma (CheckMate 032): a multicentre, open-label, two-stage, multi-arm, phase 1/2 trial. *Lancet Oncol* (2016) 17(11):1590–8. doi: 10.1016/S1470-2045(16)30496-X
40. Brahmer J, Reckamp KL, Baas P, Crinò L, Eberhardt WEE, Poddubskaya E, et al. Nivolumab versus Docetaxel in Advanced Squamous-Cell Non–Small-Cell Lung Cancer. *New Engl J Med* (2015) 373(2):123–35. doi: 10.1056/NEJMoa1504627
41. Motzer RJ, Escudier B, McDermott DF, George S, Hammers HJ, Srinivas S, et al. Nivolumab versus Everolimus in Advanced Renal-Cell Carcinoma. *New Engl J Med* (2015) 373(19):1803–13. doi: 10.1056/NEJMoa1510665
42. Chen S, Crabill GA, Pritchard TS, McMiller TL, Wei P, Pardoll DM, et al. Mechanisms regulating PD-L1 expression on tumor and immune cells. *J Immunother Cancer* (2019) 7(1):305. doi: 10.1186/s40425-019-0770-2
43. Li D, Chen R, Wang Y-WJr, Fornace AJ, Li H-H. Prior irradiation results in elevated programmed cell death protein 1 (PD-1) in T cells. *Int J Radiat Biol* (2018) Apr 294(5):488–94. doi: 10.1080/09553002.2017.1400192
44. Vilain RE, Menzies AM, Wilmott JS, Kakavand H, Madore J, Guminski A, et al. Dynamic Changes in PD-L1 Expression and Immune Infiltrates Early During Treatment Predict Response to PD-1 Blockade in Melanoma. *Clin Cancer Res* (2017) 23(17):5024–33. doi: 10.1158/1078-0432.CCR-16-0698
45. Munari E, Zamboni G, Lunardi G, Marchionni L, Marconi M, Sommaggio M, et al. PD-L1 Expression Heterogeneity in Non–Small Cell Lung Cancer: Defining Criteria for Harmonization between Biopsy Specimens and Whole Sections. *J Thoracic Oncol* (2018) 13(8):1113–20. doi: 10.1016/j.jtho.2018.04.017
46. Chan TA, Yarchoan M, Jaffee E, Swanton C, Quezada SA, Stenzinger A, et al. Development of tumor mutation burden as an immunotherapy biomarker: utility for the oncology clinic. *Ann Oncol* (2019) 30(1):44–56. doi: 10.1093/annonc/mdy495
47. Steuer CE, Ramalingam SS. Tumor Mutation Burden: Leading Immunotherapy to the Era of Precision Medicine? *JCO* (2018) 36(7):631–2. doi: 10.1200/JCO.2017.76.8770
48. Li X, Song W, Shao C, Shi Y, Han W. Emerging predictors of the response to the blockade of immune checkpoints in cancer therapy. *Cell Mol Immunol* (2019) 16(1):28–39. doi: 10.1038/s41423-018-0086-z
49. Zhao P, Li L, Jiang X, Li Q. Mismatch repair deficiency/microsatellite instability-high as a predictor for anti-PD-1/PD-L1 immunotherapy efficacy. *J Hematol Oncol* (2019) 12(1):54. doi: 10.1186/s13045-019-0738-1
50. Mandal R, Chan TA. Personalized Oncology Meets Immunology: The Path toward Precision Immunotherapy. *Cancer Discovery* (2016) 6(7):703–13. doi: 10.1158/2159-8290.CD-16-0146

51. Chalmers ZR, Connelly CF, Fabrizio D, Gay L, Ali SM, Ennis R, et al. Analysis of 100,000 human cancer genomes reveals the landscape of tumor mutational burden. *Genome Med* (2017) 9(1):34. doi: 10.1186/s13073-017-0424-2
52. Burman P, Lamb L, McCormack A. Temozolomide therapy for aggressive pituitary tumours – current understanding and future perspectives. *Rev Endocr Metab Disord* (2020) 21:263–76. doi: 10.1007/s11154-020-09551-y
53. Schvartsman G, Peng SA, Bis G, Lee JJ, Benveniste MFK, Zhang J, et al. Response rates to single-agent chemotherapy after exposure to immune checkpoint inhibitors in advanced non-small cell lung cancer. *Lung Cancer* (2017) 112:90–5. doi: 10.1016/j.lungcan.2017.07.034
54. Park SE, Lee SH, Ahn JS, Ahn M-J, Park K, Sun J-M. Increased Response Rates to Salvage Chemotherapy Administered after PD-1/PD-L1 Inhibitors in Patients with Non–Small Cell Lung Cancer. *J Thoracic Oncol* (2018) 13 (1):106–11. doi: 10.1016/j.jtho.2017.10.011
55. Brahmer JR, Rodriguez-Abreu D, Robinson AG, Hui R, Csőszi T, Fülöp A, et al. Progression after the next line of therapy (PFS2) and updated OS among patients (pts) with advanced NSCLC and PD-L1 tumor proportion score (TPS) ≥50% enrolled in KEYNOTE-024. *JCO* (2017) 35(15_suppl):9000–0. doi: 10.1200/JCO.2017.35.15_suppl.9000
56. Dwary AD, Master S, Patel A, Cole C, Mansour R, Mills G, et al. Excellent response to chemotherapy post immunotherapy. *Oncotarget* (2017) 8 (53):91795–802. doi: 10.18632/oncotarget.20030
57. Alsuwaigh R, Lee J, Chan G, Chee CE, Choo SP. Response to targeted therapy or chemotherapy following immunotherapy in patients with gastrointestinal cancers - a case series. *J Immunother Cancer* (2019) 7(1):162. doi: 10.1186/s40425-019-0637-6
58. Simon A, Kourie HR, Kerger J. Is there still a role for cytotoxic chemotherapy after targeted therapy and immunotherapy in metastatic melanoma? A case report and literature review. *Chin J Cancer* (2017) 36(1):10. doi: 10.1186/s40880-017-0179-6
59. Heynckes S, Daka K, Franco P, Gaebelein A, Frenking JH, Doria-Medina R, et al. Crosslink between Temozolomide and PD-L1 immune-checkpoint inhibition in glioblastoma multiforme. *BMC Cancer* (2019) 19(1):117. doi: 10.1186/s12885-019-5308-y
60. Mathios D, Kim JE, Mangraviti A, Phallen J, Park C-K, Jackson CM, et al. Anti–PD-1 antitumor immunity is enhanced by local and abrogated by systemic chemotherapy in GBM. *Sci Trans Med* (2016) 8(370):370ra180–370ra180. doi: 10.1126/scitranslmed.aag2942
61. Park J, Kim CG, Shim J-K, Kim JH, Lee H, Lee JE, et al. Effect of combined anti-PD-1 and temozolomide therapy in glioblastoma. *Oncoimmunology* (2019) 8(1):e1525243. doi: 10.1080/2162402X.2018.1525243
62. Karachi A, Yang C, Dastmalchi F, Sayour EJ, Huang J, Azari H, et al. Modulation of temozolomide dose differentially affects T-cell response to immune checkpoint inhibition. *Neuro-Oncology* (2019) 21(6):730–41. doi: 10.1093/neuonc/noz015
63. Ferrara N, Gerber H-P, LeCouter J. The biology of VEGF and its receptors. *Nat Med* (2003) 9(6):669–76. doi: 10.1038/nm0603-669
64. Goel HL, Mercurio AM. VEGF targets the tumour cell. *Nat Rev Cancer* (2013) 13(12):871–82. doi: 10.1038/nrc3627
65. Lloyd RV, Scheithauer BW, Kuroki T, Vidal S, Kovacs K, Stefaneanu L. Vascular endothelial growth factor (VEGF) expression in human pituitary adenomas and carcinomas. *Endocr Pathol* (1999) 10(3):229–35. doi: 10.1007/BF02738884
66. Wang Y, Li J, Tohti M, Hu Y, Wang S, Li W, et al. The expression profile of Dopamine D2 receptor, MGMT and VEGF in different histological subtypes of pituitary adenomas: a study of 197 cases and indications for the medical therapy. *J Exp Clin Cancer Res* (2014) 33(1):56. doi: 10.1186/s13046-014-0056-y
67. Sánchez-Ortiga R, Sánchez-Tejada L, Moreno-Perez O, Riesgo P, Niveiro M, Picó Alfonso AM. Over-expression of vascular endothelial growth factor in pituitary adenomas is associated with extrasellar growth and recurrence. *Pituitary* (2013) 16(3):370–7. doi: 10.1007/s11102-012-0434-4
68. Cristina C, Perez-Millan MI, Luque G, Dulce RA, Sevlever G, Berner SI, et al. VEGF and CD31 Association in Pituitary Adenomas. *Endocr Pathol* (2010) 21 (3):154–60. doi: 10.1007/s12022-010-9119-6
69. Vidal S, Kovacs K, Horvath E, Scheithauer BW, Kuroki T, Lloyd RV. Microvessel density in pituitary adenomas and carcinomas. *Virchows Arch* (2001) 438(6):595–602. doi: 10.1007/s004280000373
70. Miles D, Cameron D, Bondarenko I, Manzyuk L, Alcedo JC, Lopez RI, et al. Bevacizumab plus paclitaxel versus placebo plus paclitaxel as first-line therapy for HER2-negative metastatic breast cancer (MERiDiAN): A double-blind placebo-controlled randomised phase III trial with prospective biomarker evaluation. *Eur J Cancer* (2017) 70:146–55. doi: 10.1016/j.ejca.2016.09.024
71. Jain RK. Normalizing Tumor Microenvironment to Treat Cancer: Bench to Bedside to Biomarkers. *J Clin Oncol* (2013) 31(17):2205–18. doi: 10.1200/JCO.2012.46.3653
72. Hegde PS, Jubb AM, Chen D, Li NF, Meng YG, Bernaards C, et al. Predictive Impact of Circulating Vascular Endothelial Growth Factor in Four Phase III Trials Evaluating Bevacizumab. *Clin Cancer Res* (2013) 19(4):929–37. doi: 10.1158/1078-0432.CCR-12-2535
73. Jain RK, Duda DG, Willett CG, Sahani DV, Zhu AX, Loeffler JS, et al. Biomarkers of response and resistance to antiangiogenic therapy. *Nat Rev Clin Oncol* (2009) 6(6):327–38. doi: 10.1038/nrclinonc.2009.63
74. Gianni L, Romieu GH, Lichinitser M, Serrano SV, Mansutti M, Pivot X, et al. AVEREL: A Randomized Phase III Trial Evaluating Bevacizumab in Combination With Docetaxel and Trastuzumab As First-Line Therapy for HER2-Positive Locally Recurrent/Metastatic Breast Cancer. *JCO* (2013) 31 (14):1719–25. doi: 10.1200/JCO.2012.44.7912
75. Cutsem EV, de Haas S, Kang Y-K, Ohtsu A, Tebbutt NC, Xu JM, et al. Bevacizumab in Combination With Chemotherapy As First-Line Therapy in Advanced Gastric Cancer: A Biomarker Evaluation From the AVAGAST Randomized Phase III Trial. *J Clin Oncol* (2012) 30(17):2119–27. doi: 10.1200/JCO.2011.39.9824
76. Yi M, Jiao D, Qin S, Chu Q, Wu K, Li A. Synergistic effect of immune checkpoint blockade and anti-angiogenesis in cancer treatment. *Mol Cancer* (2019) 18. doi: 10.1186/s12943-019-0974-6
77. Fukumura D, Kloepper J, Amoozgar Z, Duda DG, Jain RK. Enhancing cancer immunotherapy using antiangiogenics: opportunities and challenges. *Nat Rev Clin Oncol* (2018) 15(5):325–40. doi: 10.1038/nrclinonc.2018.29
78. Hodi FS, Lawrence D, Lezcano C, Wu X, Zhou J, Sasada T, et al. Bevacizumab plus ipilimumab in patients with metastatic melanoma. *Cancer Immunol Res* (2014) 2(7):632–42. doi: 10.1158/2326-6066.CIR-14-0053
79. Wallin JJ, Bendell JC, Funke R, Sznol M, Korski K, Jones S, et al. Atezolizumab in combination with bevacizumab enhances antigen-specific T-cell migration in metastatic renal cell carcinoma. *Nat Commun* (2016) 7(1):1–8. doi: 10.1038/ncomms12624
80. Socinski MA, Jotte RM, Cappuzzo F, Orlandi F, Stroyakovskiy D, Nogami N, et al. Atezolizumab for First-Line Treatment of Metastatic Nonsquamous NSCLC. *New Engl J Med* (2018) 378(24):2288–301. doi: 10.1056/NEJMoa1716948
81. Martins F, Sofiya L, Sykiotis GP, Lamine F, Maillard M, Fraga M, et al. Adverse effects of immune-checkpoint inhibitors: epidemiology, management and surveillance. *Nat Rev Clin Oncol* (2019) 16(9):563–80. doi: 10.1038/s41571-019-0218-0
82. Wang DY, Salem J-E, Cohen JV, Chandra S, Menzer C, Ye F, et al. Fatal Toxic Effects Associated With Immune Checkpoint Inhibitors: A Systematic Review and Meta-analysis. *JAMA Oncol* (2018) 4(12):1721–8. doi: 10.1001/jamaoncol.2018.3923
83. Friedman CF, Proverbs-Singh TA, Postow MA. Treatment of the Immune-Related Adverse Effects of Immune Checkpoint Inhibitors: A Review. *JAMA Oncol* (2016) 2(10):1346–53. doi: 10.1001/jamaoncol.2016.1051
84. Society for Immunotherapy of Cancer Toxicity Management Working Group, Puzanov I, Diab A, Abdallah K, Bingham CO, Brogdon C, et al. Managing toxicities associated with immune checkpoint inhibitors: consensus recommendations from the Society for Immunotherapy of Cancer (SITC) Toxicity Management Working Group. *J Immunother Cancer* (2017) 5(1):95. doi: 10.1186/s40425-017-0300-z
85. Haanen JBAG, Carbonnel F, Robert C, Kerr KM, Peters S, Larkin J, et al. Management of toxicities from immunotherapy: ESMO Clinical Practice

Guidelines for diagnosis, treatment and follow-up. *Ann Oncol* (2017) 28: iv119–42. doi: 10.1093/annonc/mdx225
86. Gardner V, Madu CO, Lu Y. Anti-VEGF Therapy in Cancer: A Double-Edged Sword. In: *Physiologic and Pathologic Angiogenesis - Signaling Mechanisms and Targeted Therapy*. London, UK: InTechOpen (2017).
87. Kamba T, McDonald DM. Mechanisms of adverse effects of anti-VEGF therapy for cancer. *Br J Cancer* (2007) 96(12):1788–95. doi: 10.1038/sj.bjc.6603813
88. Finn RS, Qin S, Ikeda M, Galle PR, Ducreux M, Kim T-Y, et al. Atezolizumab plus Bevacizumab in Unresectable Hepatocellular Carcinoma. *N Engl J Med* (2020) 382(20):1894–905. doi: 10.1056/NEJMoa1915745

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Endocrinology

ORIGINAL RESEARCH
published: 09 December 2020
doi: 10.3389/fendo.2020.566761

SDF-1α/MicroRNA-134 Axis Regulates Nonfunctioning Pituitary Neuroendocrine Tumor Growth *via* Targeting VEGFA

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Specialty section:
This article was submitted to Pituitary Endocrinology, a section of the journal Frontiers in Endocrinology

Received: 28 May 2020
Accepted: 09 November 2020
Published: 09 December 2020

Citation:
Wang X, Fang Y, Zhou Y, Guo X, Xu K, Li C, Zhang J and Hong Y (2020) SDF-1α/MicroRNA-134 Axis Regulates Nonfunctioning Pituitary Neuroendocrine Tumor Growth via Targeting VEGFA. Front. Endocrinol. 11:566761. doi: 10.3389/fendo.2020.566761

Background: Nonfunctioning pituitary neuroendocrine tumor (NF-PitNET) is difficult to resect. Except for surgery, there is no effective treatment for NF-PitNET. MicroRNA-134 (miR-134) has been reported to inhibit proliferation and invasion ability of tumor cells. Herein, the mechanism underlying the effect of miR-134 on alleviating NF-PitNET tumor cells growth is explored.

Methods: Mouse pituitary αT3-1 cells were transfected with miR-134 mimics and inhibitor, followed by treatment with stromal cell-derived factor-1α (SDF-1α) *in vitro*. MiR-134 expression level: we used quantitative real-time PCR (qRT-PCR) to detect the expression of miR-134. Cell behavior level: cell viability and invasion ability were assessed using a cell counting kit-8 (CCK8) assay and Transwell invasion assay respectively. Cytomolecular level: tumor cell proliferation was evaluated by Ki-67 staining; propidium iodide (PI) staining analyzed the effect of miR-134 on cell cycle arrest; western blot analysis and immunofluorescence staining evaluated tumor migration and invasive ability. Additionally, we collected 27 NF-PitNET tumor specimens and related clinical data. The specimens were subjected to qRT-PCR to obtain the relative miR-134 expression level of each specimen; linear regression analysis was used to analyze the miR-134 expression level in tumor specimens and the age of the NF-PitNET population, gender, tumor invasion, prognosis, and other indicators.

Results: *In vitro* experiment, miR-134 was observed to significantly inhibit αT3-1 cells proliferation characterized by inhibited cell viability and expressions of vascular endothelial growth factor A (VEGFA) and cell cycle transition from G1 to S phase ($P < 0.01$). VEGFA was verified as a target of miR-134. Additionally, miR-134-induced inhibition of αT3-1 cell proliferation and invasion was attenuated by SDF-1α and VEGFA overexpression ($P < 0.01$). In primary NF-PitNET tumor analysis, miR-134 expression level was negatively correlated with tumor invasion ($P = 0.003$).

Conclusion: The regulation of the SDF-1α/miR-134/VEGFA axis represents a novel mechanism in the pathogenesis of NF-PitNETs and may serve as a potential therapeutic target for the treatment of NF-PitNETs.

Keywords: SDF-1α (CXCL12), vascular endothelial growth factor A (VEGFA), proliferation, invasion, microRNA-134 (miR-134), nonfunctioning pituitary neuroendocrine tumor (NF-PitNET)

INTRODUCTION

Pituitary tumors are common and account for 10–15% of primary intracranial neoplasms, and 30% originate from gonadotroph (1). Nonfunctioning pituitary adenomas are usually of gonadotroph origin (2) and cannot secrete biologically active hormones (3); however, silent corticotroph, poorly differentiated PIT1-lineage, true null cell tumors, or other silent tumors have also been described, and they often have a more aggressive behavior (4). Recently, a new terminology, pituitary neuroendocrine tumor (PitNET), which is proposed by the International Pituitary Pathology Club, proposed to replace adenoma to better reflect the similarities between adenohypophyseal and neuroendocrine tumors of other organs (5). Generally, patients with NF-PitNET often present with hypopituitarism and visual field defects, due to the compression effect of development of large adenomas (6, 7). Compared with small pituitary tumors, large and invasive pituitary tumors are more difficult to resect, and may be companied with high risks of complications, such as hypothalamic injury, visual impairment, vascular injury, cerebrospinal fluid leakage, pituitary dysfunction, infection, and reoperation or radiation therapy due to recurrence. Currently, surgical resection remains the major treatment for NF-PitNET (8). No effective drug is available for the treatment of NF-PitNET, which seriously affects the outcome of NF-PitNET patients. Therefore, it is urgent to further explore the underlying mechanisms of pituitary tumorigenesis, and provide a theoretical basis for finding new therapeutic targets for NF-PitNET.

MicroRNAs (miRNAs) are a class of 20–25 base-pair (bp)-long noncoding RNAs that regulate gene expression by severing the mRNA to target gene or inhibiting translation of target genes (9). Cheunsuchon et al. (10) have confirmed that the specific expression of six microRNAs (miR-134, miR-299-5p, miR-329, miR-370, miR-377-5p, and miR-432) downstream of delta-like homologue 1–maternally-expressed gene 3 (DLK1-MEG3) gene cluster were lost or reduced in NF-PitNET, but expressed in normal pituitary and other types of pituitary tumors. This finding suggests that the loss or reduction of expression of these six types of miRNAs may play a key role in the occurrence of NF-PitNET.

Previous studies have shown that overexpression and interaction of stromal cell derived factor-1α (SDF-1α) and its receptor CXCR4 play an important role in pituitary cell proliferation and tumorigenesis (11). SDF-1α secreted by fibroblasts plays a key role in the enhancement of NF-PitNET cell proliferation, invasion, migration, transformation, and tumor formation (12). The molecular mechanism of action of SDF-1α in NF-PitNET is still not fully understood. We hypothesize that SDF-1α may play a role in promoting tumorigenesis by affecting the expression of miRNAs in NF-PitNET.

By stimulating the αT3-1 cells with exogenous recombinant SDF1α, we previously found that the expression level of miR-134 in αT3-1 cells was significantly decreased by qRT-PCR (13). MiR-134 can regulate the proliferative activity of pituitary folliculostellate cells and lung cancer tumor cell lines, and arrest cell cycle (14, 15). VEGFA is one of the downstream target proteins of miR-134 (13). It can chemoattract endothelial progenitor cells in tumor stroma or other sites, and stimulate the survival of neovascularization in tumor tissues, thereby promoting tumor cell proliferation and distant migration. Also, clinical studies have shown a significant negative correlation between VEGFA expression and tumor prognosis (16). Shan et al. (17) found pituitary tumor cells increased HIF-1α expressing under hypoxic conditions, and stimulate VEGFA generation, thus promoting the development of pituitary tumor. Meanwhile, Samuel et al. (18) found that SDF-1α/CXCR4 can activate VEGFA to mediate the formation of new blood vessels in tumor tissues through HIF-1α, thereby promoting tumor growth in human colorectal cancer cells.

Therefore, based on results of previous studies and our preliminary experiment, we hypothesized that in NF-PitNET, SDF-1α may regulate the production of its target protein VEGFA by regulating the level of miR-134, and subsequently mediate tumor cell proliferation, migration, and invasion. In this study, because of the high conservation of miR-134 between human and mouse, mouse pituitary αT3-1 cells were used to determine the effect of SDF-1α on miR-134 expression *in vitro* model, and detect the regulation of miR-134 on VEGFA synthesis, proliferation, migration, and invasion in tumor cells treated with SDF-1α. In addition, by collecting human NF-PitNET specimens, the relationship between miR-134 expression level and tumor invasiveness and prognosis were analyzed to reveal the mechanism of NF-PitNET development under the action of SDF-1α which may serve as a potential therapeutic target for the treatment of NF-PitNET patients.

METHODS

Human Tissue Samples

With approval of Ethics Committee, and written informed consent by patients or their guardians, a total of 29 human NF-PitNETs specimens were obtained from the Department of Neurosurgery, the Second Affiliated Hospital, School of

Medicine, Zhejiang University from December 2015 to January 2019. All specimens were obtained from patients who underwent tumor resection or biopsy in our hospital, and Pituitary tumors are diagnosed as "nonfunctioning" in the absence of clinical or biochemical evidence of tumor-related hormone excess. One part of the tissue fragments was frozen in liquid nitrogen and stored at −80°C for qRT-PCR. Another part was formalin-fixed, paraffin-embedded for pathological analysis. The clinical features of patients including age, sex, expression of Ki-67, and invasion. Radiological signs and/or surgical detection of cavernous or sphenoid sinus invasion, or diaphragm or floor of sellar invasion should be highly suspected of invasive NF-PitNET. Resistance to medical treatment and multiple recurrences despite standard therapies were defined to invasive NF-PitNET (19). Ki-67 ≥3% was considered high proliferation. All patients were first time for the surgery and no patients received medical therapy before the specimens' collection.

Cell Culture

The mouse gonadotroph αT3-1 cell line was cultured in Dulbecco's modified Eagle medium (DMEM) (Gibco, Shanghai, China) supplemented with 10% fetal bovine serum (FBS) with 100 U/ml penicillin and 100 μg/ml streptomycin (Invitrogen, Shanghai, China). All cell lines were incubated at 37° in a standard mixture of 95% air and 5% CO2. The cells were observed as adherent cells under the microscope, and the live cell rate of trypan blue staining was over 95%.

Quantitative Real-Time PCR Analysis

Total RNA was extracted from tissue samples cells by using TRIzol reagent (Invitrogen, USA). Then cDNA synthesis from the isolated total 1 μg RNA was performed using NCode VILO miRNA cDNA synthesis Kit (Invitrogen, USA). Primer sequences and PCR conditions were described below: hsa-miR-134a: forward, 5′-TGTGACTGGTTGACCAGAGGGG-3′; reverse primers are provided by NCode EXPRESS SYBR GreenER miRNA qRT-PCR kit (Invitrogen, A11193-052, Shanghai, China). mmu-miR-134: forward, 5' ACACTCCA GCTGGGTGTGACTGGTTGACCA3'; reverse, 5'CTCAAC TGGTGTCGTGGAGTCGGCAATTCAGTTGAGCCCCTC3'; U6: forward, CTCGCTTCGGCAGCACA; reverse, FCTCGCTT CGGCAGCACA. Quantitative real-time PCR was performed with NCode EXPRESS SYBR GreenER miRNA qRT-PCR Kit (Invitrogen, Shanghai, China), according to the manufacturer's instructions, on the ABI Prism 7300 SDS system (ABI, USA). Small nuclear RNA U6 was used as the endogenous control. The expression levels of miRNAs were determined using the 2-ΔΔCt method (20). All samples were analyzed in triplicate. The data was analyzed by the ABI Prism 7300 SDS Software.

Plasmid Construction, Transfection, and SDF-1α Treatment

mmu-miR-134–5p mimic, mmu-miR-134–5p inhibitor and blank vector control were synthesized by GenePharma (Shanghai, China). These constructs were transfected into cells by using Lipofectamine 2000 according to manufacturer instructions (Thermo Scientific, Waltham, USA). Exogenous recombinant SDF-1α cytokine (PeproTech Ltd. China) was added to the SDF-1α stimulation group at a final concentration of 20 ng/ml, and an equal volume of phosphate buffer saline (PBS) containing 0.1% BSA was added to the control group (for preparation of SDF-1α solution). Mouse cell lines were grouped into: mmu-miR-134–5p inhibitor + SDF-1α, mmu-miR-134–5p inhibitor + PBS, mmu-miR-134–5p mimic + SDF-1α, mmu-miR-134–5p mimic + PBS, blank vector control + SDF-1α, and blank vector control + PBS.

Cell Cycle and Cell Proliferation Assay

After treatment, cells were collected and incubated with RNase A (50 μg/ml) and propidium iodide (PI) (50 μg/ml) (MultiSciences, China). Flow detection was completed within 24 h after dyeing was completed. The fluorescence was detected by a FACSCalibur flow cytometer (BD, Shanghai, China) and subsequent cell cycle analysis using FLOWJO software (Treestar, USA). Cell proliferation analysis was performed with the Cell Counting Kit-8 (CCK8) (Beyotime, Shanghai, China). A total 3×10^3 cells cultured in each well of 96-well plates for 24, 48, and 72 h. Then, 10 μl of CCK8 was added to each well and cells were incubated at 37°C for 1 h. The absorbance was detected at 450 nm and measured using an automatic plate analyzer (Bio-Rad Lab, Hercules, CA, USA).

Transwell Invasion Assay

Cell invasion assay was performed using Transwell plates (Corning, USA) with 8-μm-pore size membranes with Matrigel used to measure the invasion ability of cells. NF-PitNET cells with appropriate transfection treatments were harvested after 48 h and seeded with serum-free medium (5×10^5 cells in 300 μl) into the upper chamber. Bottom wells were filled with complete medium. After 24 h, the cells on the upper surface of the membrane were removed by cotton swabs. The cells that moved through the membrane were fixed with 4% paraformaldehyde and stained with 1% crystal violet. Images of the invasion and migration cells were photographed under a microscope. All of the experiments were performed in triplicate.

Western Blot Analysis

All cells were lysed in RIPA-Buffer (Beyotime Biotechnology, Shanghai, China) supplemented with protease and phosphatase inhibitors (Thermo Scientific, USA) on ice for 30 min, followed by centrifugation at 12,000 g for 15 min. Protein concentrations were calculated using the Pierce BCA protein assay kit (Thermo Scientific, USA). Equivalent amounts of protein samples (25 μg/lane) were separated on 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and subsequently transferred to polyvinylidene fluoride (PVDF) membranes. Membranes were blocked with 5% nonfat milk in Tris-buffered saline and 0.1% Tween 20 buffer and then incubated with primary antibody overnight at 4°C. Primary antibody including anti-VEGFA (Proteintech, MA5-13182), anti-ABCC1 (Abcam, ab91451), anti-MMP2 (Abcam, ab97779), anti-MMP9 (Abcam, ab38898), anti-Vimentin (Abcam, ab28151), anti-SNAIL (Cell Signaling Technology, #3879), GAPDH (Cell Signaling Technology, #5174), β-actin

(ab179467). Membranes were incubated with goat anti-rabbit or anti-mouse HRP-conjugated secondary antibody (Beyotime Biotechnology, Shanghai, China) for 1 h at room temperature. Signal detection was performed using the ultrasensitive ECL plus Detection Reagent (Millipore, USA) and a ChemiDoc Touch Imaging System. The intensity of the bands was analyzed using the Quantity One software (Bio-Rad, Hercules, CA, USA).

Immunohistochemistry and Immunocytochemistry

The tissues were routinely fixed in 10% formalin for 8 to 24 h and embedded in paraffin. Five microns serial sections were deparaffinized in xylene and dehydrated in graded ethanol. Endogenous peroxidase activity was blocked with 0.3% hydrogen peroxide in methanol for 30 min. The tissues were incubated with anti-SDF-1α primary antibodies (1:100; Abcam) and anti-VEGFA primary antibodies (1:100; Abcam) at 4°C overnight. Brain tissue from epileptic patients were stained with anti-SDF-1α and anti-VEGFA were used for the negative control. The images were captured under a SP5/Leica confocal microscope with LAS AF Lite software. The cells grown on glass coverslips were fixed with 4% formaldehyde with for 10 min, and washed with PBS. Cells were permeabilized with PBS containing Triton X-100 (0.1–0.25%) for 10 min, and blocked with 1% BSA for 30 min. Then, cells were incubated with rabbit diluted primary antibody overnight at 4°C. After three washes in PBS, cells were incubated for 1 h with secondary antibody and incubated with hematoxylin and diaminobenzidine (DAB). Images were captured with an inverted microscope.

Statistical Analysis

All the experiments were performed at least in technical triplicate and in three independent biological repeats. Results are represented as the mean ± SD. Two tailed Student's t-test or Pearson's analysis (for comparing data from two groups) or one-way analysis of variance (ANOVA) with Tukey's *post hoc* test (for comparing data from multiple groups) was used to calculate statistical significance. All values were obtained using GraphPad Prism 5.0 and SPSS 17.0 software. Data are expressed as means ± SD. A value of $P < 0.05$ was considered to be statistically significant.

RESULTS

Participant Characteristics

Of the 29 NF-PitNET patients, 19 (65.5%) were male and 10 (34.5%) were female. Age was 41.6 ± 3.6 years. miR-134 relative expression value was 5.0 ± 0.7. Twenty (69.0%) patients showed low Ki-67 expression with 9 (31.0%) patients showing high Ki-67 expression. Based on the criteria by Raverot et al. (19), "invasiveness" was defined resistance to medical treatment and multiple recurrences despite standard therapies. Seventeen (58.6%) NF-PitNETs were aggressive growth. All surgical specimens were clinically and pathologically confirmed as hormone-negative tumors. According to the median value of miR-134 (2.5), patients were divided into low miR-134 expression (n = 15, 51.7%) and high miR-134 expression groups (n = 14, 48.3%). The Ki-67 level and invasiveness were inversely correlated with the miR-134 expression ($P < 0.001$ and $P = 0.003$, respectively, **Table 1**). In addition, we compared the expression of SDF-1α and VEGFA in invasive and non-invasive NF-PitNET tissue samples, and the results suggested that the expression levels of SDF-1α and VEGFA in invasive NF-PitNET are relatively high ($P < 0.0001$, **Figures 1A, B**).

SDF-1α Treatment Attenuates the Expression of miR-134

Mouse pituitary αT3-1 cells were used to verify the effect of SDF-1α on the miR-134. QRT-PCR indicated that the expression level of miR-134 in the group treated with SDF-1α was significantly lower than that in the control group ($P < 0.001$, **Figure 2A**). Furthermore, the expression of miR-134 was similarly downregulated by SDF-1α in miR-134–5p inhibitor, miR-134–5p mimic, and blank vector control groups ($P < 0.05$, **Figure 2B**). The results indicated that SDF-1α was able to alleviate miR-134 expression.

SDF-1α Promotes Cell Cycle Transition, Viability, and Proliferation *via* Suppressing the Expression of miR-134

As presented by cell cycle analysis, αT3-1 cells treated with miR-134 mimics seemed to get trapped in G0/G1phase, and this effect was attenuated by SDF-1α treatment ($P < 0.01$). In contrast, cells treated with miR-134 inhibitor and SDF-1α had more cells in S phase, followed by cells treated with miR-134 inhibitor only ($P < 0.01$, **Figure 3A**). The results of CCK8 test to compare the proliferation ability of each group showed treatment with miR-134 inhibitor and SDF-1α increased the cell viability at 24, 48, and 72 h after treatment compared to other groups. Meanwhile, miR-134 mimics decreased the cell viability at 24, 48, and 72 h after treatment. Though the significance shown between all groups at 24, 48, and 72 h, it was found to be most remarkable at 72 h ($P < 0.01$, **Figure 3B**). Similar with the results of cell cycle analysis and CCK8 assay, treatment with SDF-1α and miR-134

TABLE 1 | The correlation between clinical features of NF-PitNETs and the expression of miR-134.

Clinical features	Number (%)	Low miR-134 expression	
		Number (%)	*p* value
Age, years			
<42	13 (44.8)	7 (53.9)	NS
≥42	16 (55.2)	8 (50.0)	
Sex			
Male	19 (65.5)	10 (52.6)	NS
Female	10 (34.5)	5 (50.0)	
Ki-67 (%)			
<3	20 (69.0)	7 (35.0)	<0.001
≥3	9 (31.0)	8 (88.9)	
Aggressive			
Yes	17 (58.6)	11 (73.3)	0.003
No	12 (41.4)	4 (33.3)	

NS, No Significance.

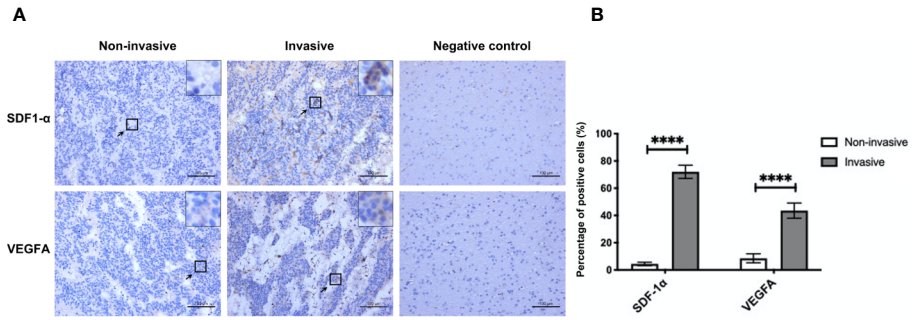

FIGURE 1 | The expression of SDF-1α and VEGFA in invasive and non-invasive NF-PitNET. **(A)** Immunohistochemical staining assay of SDF-1α and VEGFA in invasive NF-PitNET and non-invasive NF-PitNET. Brain tissues from epileptic were used for the negative control. **(B)** The quantification of SDF-1α and VEGFA expression are shown between invasive and non-invasive NF-PitNET. Data were represented as means ± SD (n = 6). ****p < 0.0001 *vs.* non-invasive NF-PitNET group.

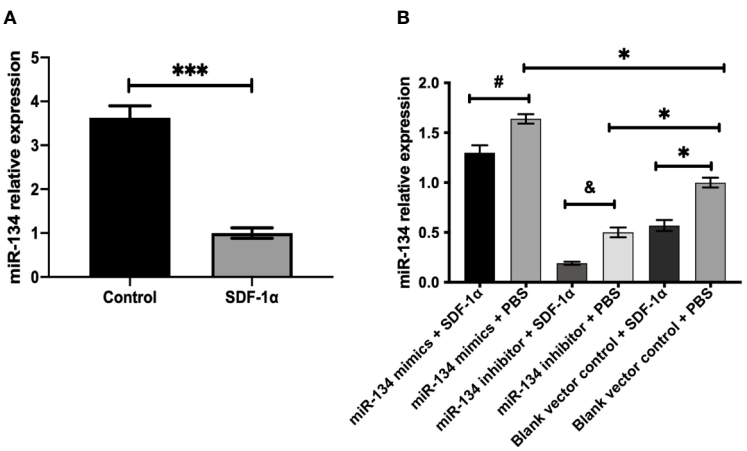

FIGURE 2 | SDF-1α inhibits the expression of miR-134. αT3-1 cells were treated with SDF-1α, control, miR-134 mimics + SDF-1α, miR-134 mimics + PBS, miR-134 inhibitor + SDF-1α, miR-134 inhibitor + PBS, black vector control + SDF-1α, black vector control + PBS, respectively. **(A, B)** MiR-134/U6 relative level in αT3-1 cells by qRT-PCR. Data were represented as means ± SD (n = 3). ***p < 0.001 *vs.* control group. #p < 0.05 *vs.* miR-134 mimics + PBS group. & p < 0.05 *vs.* miR-134 inhibitor + PBS group. *p < 0.05 *vs.* black vector control + PBS group.

inhibitor resulted in the most invasive cells, with SDF-1α or miR-134 inhibitor only following, which indicated that SDF-1α or miR-134 inhibitor increased the cell invasion ability ($P < 0.01$). While miR-134 mimics significantly decreased the cell invasion ability compared to other groups ($P < 0.01$, **Figure 3C**). Furthermore, the data of immunohistochemistry (Ki-67) confirmed that the miR-134 expression was negatively related with NF-PitNET cell proliferation, and SDF-1α can inhibit miR-134 expression which subsequently promoted proliferation ($P < 0.05$, **Figures 3D, E**).

SDF-1α Boosts the Expression of VEGFA and Proliferation/Invasion-Related Downstream Protein of αT3-1 Cells *via* Repressing miR-134 Expression

Previous study showed that MMP2/9, Snail, and Vimentin were considered as the proliferation invasion markers in pituitary growth (21–23). In the analysis of western blot result, we found miR-134 inhibitor + SDF-1α group has the highest expression of VEGFA in αT3-1 cells, followed by miR-134 inhibitor + PBS group and blank vector control + SDF-1α group, the lowest is miR-134 mimics + PBS group ($P < 0.01$, **Figure 4A**). In addition, other proteins related to tumor proliferation/invasion were similar to VEGFA ($P < 0.01$, **Figures 4B–E**). As a consequence, SDF-1α inhibited the expression of the tumor suppressor gene miR-134 by promoting the expression of miR-134 target gene VEGFA to stimulate the proliferation and invasion ability of αT3-1 cells.

DISCUSSION

In this study, we investigated the role of miR-134 in NF-PitNET tumorigenesis and its interaction with tumor growth regulator,

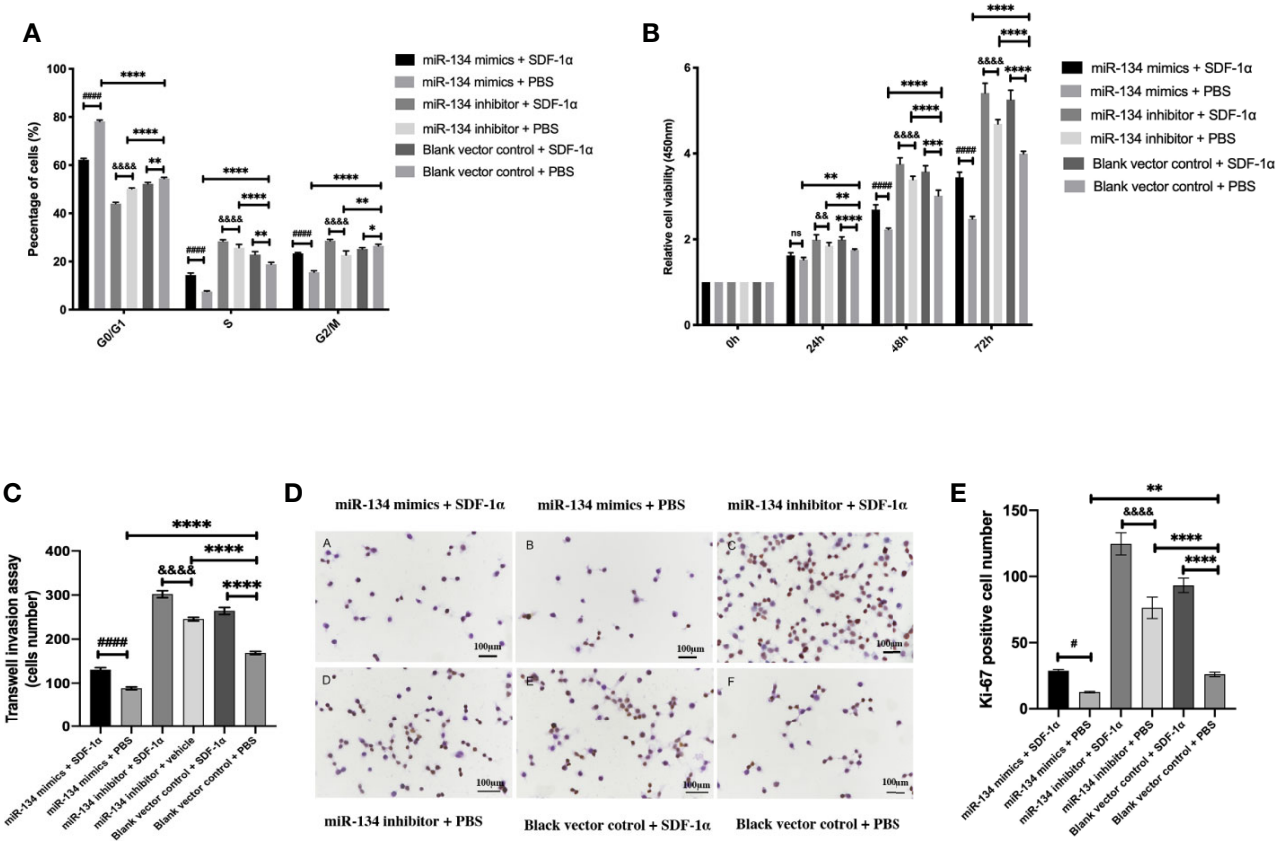

FIGURE 3 | Effects of SDF-1α/miR-134 on cell cycle transition, proliferation, and viability. MiR-134 mimics or inhibitor was transiently transfected into αT3-1 cells. After 48 h transfection, αT3-1 cells were treated with 20 ng/ml SDF-1α, equal volume of PBS, and black vector control respectively. **(A)** Cell cycle distribution using flow cytometry. **(B)** Cell viability using CCK-8 assay. **(C)** Analysis of cell invasion using transwell assay. **(D)** Immunohistochemical staining assay of Ki-67. **(E)** Positive Ki-67 cell was counted manually by Image J. Data were represented as means ± SD (n = 3). $^{\#\#\#\#}p < 0.0001$ and $^{\#}p < 0.05$ *vs.* miR-134 mimics + PBS group. $^{\&\&\&\&}p < 0.0001$ and $^{\&\&}p < 0.01$ *vs.* miR-134 inhibitor + PBS group. $^{****}p < 0.0001$, $^{**}p < 0.01$ and $^{*}p < 0.05$ *vs.* black vector control + PBS group.

SDF-1α. The miR-134 expression was downregulated in mouse pituitary αT3-1 cells after SDF-1α treatment, with upregulation of its target gene VEGFA and proliferation and invasiveness. Also, miR-134 expression was found to be negatively correlated with proliferation and invasiveness in 29 human NF-PitNET tissues, with high expression level of SDF-1α and VEGFA in the invasive NF-PitNET. These findings indicate that miR-134 may function as a tumor suppressor in NF-PitNETs by suppressing the expression of VEGFA. Increased SDF-1α in NF-PitNETs inhibits miR-134 effect on VEGFA and plays an important role in tumor cell growth and invasiveness of NF-PitNETs (**Figure 5**).

MiR-134 is one of the microRNAs downstream of MEG3 in the DLK1-MEG3 gene cluster (14). Previous studies have shown that MEG3 is expressed in normal human pituitary gonadotrophs, but expression is lost in NF-PitNETs. Also, transfection and ectopic expression of MEG3 in human cancer cell lines can inhibit tumor cell proliferation (24). Further genetic analysis indicated that MEG3 is located in the DLK1-MEG3 gene cluster in human chromosome 14q32, and at least 80 imprinted genes have been found on this gene cluster so far. It contains three paternally expressed genes (PEGs), which are not normally expressed, and the rest belong to the MEGs, including long-chain non-coding RNAs such as MEG3, MEG8 and several microRNAs (25). The entire DLK1-MEG3 gene cluster is regulated by the upper-regulator of MEG3, intergenic differentially methylated region (IG-DMR) (26). While it was found that the IG-DMR was in a high methylation status in human NF-PitNET tissues, which suggests that it may be the main reason for the low specific expression of MEG3 in human NF-PitNET (27). The microRNAs in DLK1-MEG3 gene cluster located in the downstream of MEG3 were transcribed in the same direction as MEG3 (28). Subsequently, the following study proved the expression of six microRNAs (miR-134, miR-299-5p, miR-329, miR-370, miR-377-5p, and miR-432) was lost or reduced in NF-PitNETs, but normally expressed in normal pituitary and other types of pituitary tumors (14). Our previous study suggested miR-370–3p functions as a tumor suppressor gene by targeting HMGA2 in NF-PitNET and the tumor suppressor effect was regulated by SDF-1α (13). Also, our *in vitro* study demonstrated that SDF-1α exclusively silenced the expression of miR-134 and upregulated the target gene VEGFA in NF-PitNET.

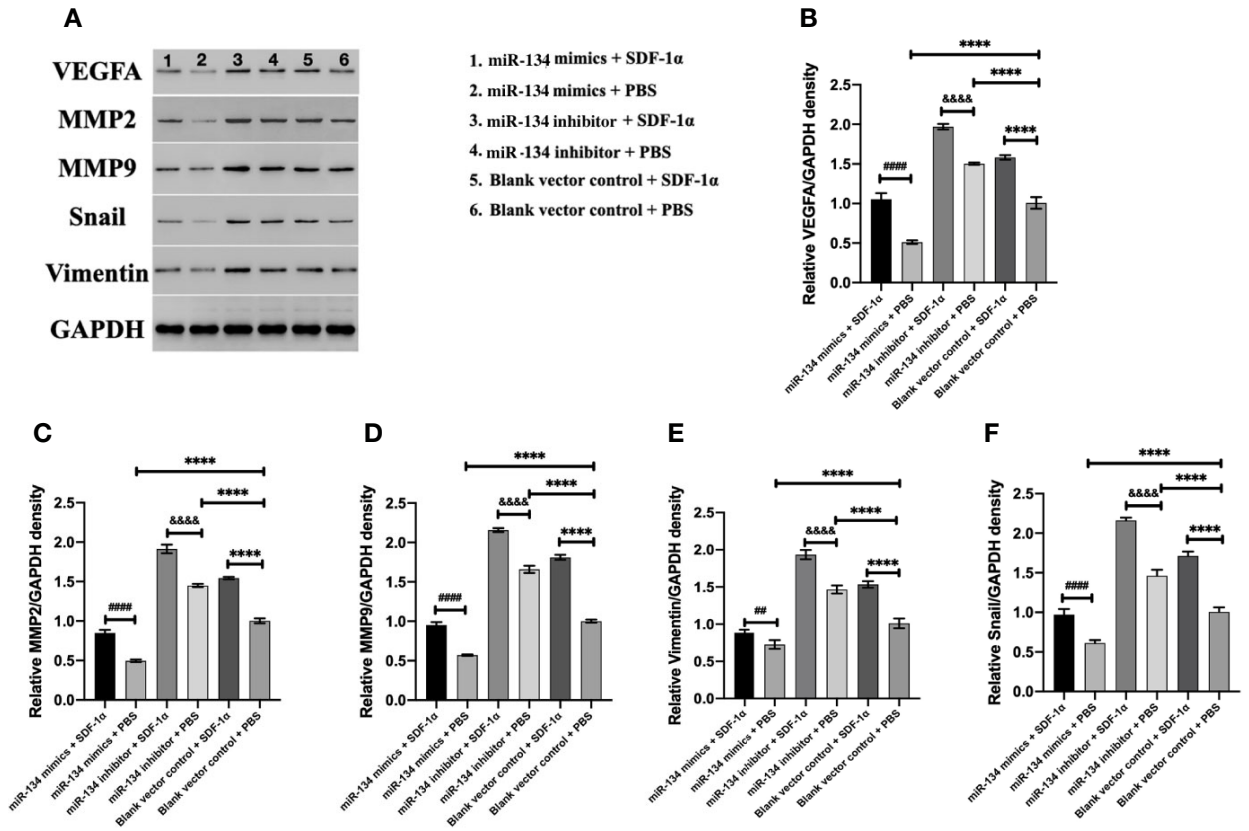

FIGURE 4 | The expression of VEGFA and proliferation/invasion-related downstream protein of αT3-1 cells. **(A)** Schematic diagram of the target protein band. **(B)** The quantification of VEGFA expressions using western blot analysis. **(C)** The quantification of MMP2 expressions using western blot analysis. **(D)** The quantification of MMP9 expressions using western blot analysis. **(E)** The quantification of Vimentin expressions using western blot analysis. **(F)** The quantification of Snail expressions using western blot analysis. Data were represented as means ± SD (n = 5). $^{\#\#\#\#}p < 0.0001$ and $^{\#\#}p < 0.01$ *vs.* miR-134 mimics + PBS group. $^{\&\&\&\&}p < 0.0001$ *vs.* miR-134 inhibitor + PBS group. ****$p < 0.0001$ *vs.* black vector control + PBS group.

Moreover, VEGFA plays an important role among the mechanisms that occur in pituitary tumorigenesis, angiogenesis for tumor growth, and was proposed as pituitary proangiogenic factor and possible therapeutic target (29). Tumor cells with upregulate VEGFA indicates high-grade malignancy and poor outcome in different types of cancer. Patients with overexpression of VEGFA are prone to have larger tumor volumes, vascular hyperpermeability, and hemorrhage (30). We confirmed this by immunohistochemical staining (**Figure 1A**), the invasiveness was correlated with the VEGFA in the NF-PitNET. Meanwhile, we observed increased expression of Snail and Vimentin in cells treated with SDF-1 and miR-134 inhibitor in the western blot experiments. This result perhaps linking it up with the results from Transwell invasion assays considering that Snail and Vimentin overexpression are markers of the activation of epithelial-to-mesenchymal (EMT) (31), as well as the overexpression of MMPs, so the overexpression of these markers would suggest activation of EMT (a crucial pathway for cell migration and invasion) and therefore further support the increased invasiveness observed in Transwell invasion assay experiments (32, 33).

SDF-1α, also named chemokine (C-X-C motif) ligand 12 (CXCL12), is the single natural ligand of C-X-C chemokine receptor type (CXCR) 4 and CXCR7, plays an important roles in cancer cell proliferation, migration, and invasion (34). CXCL12 mRNA is expressed in about 2/3 of GH-secreting pituitary tumors and NF-PitNETs, with CXCR4 mRNA expressed in almost all these tumors (34). Further study proved that CXCL12/CXCR4 mediate the DNA synthesis and cell proliferation by human pituitary tumor primary cultures (35). And the expression of CXCR4 and CXCL12 were significantly higher in invasive PitNET compared with non-invasive PitNET specimens, evaluated by flow cytometry and immunohistochemical staining (36). Previous studies suggested SDF-1α contributes to vasculogenesis and mainly expresses in the areas with angiogenic activity (37). Several studies suggested that SDF-1α could upregulate the expression of VEGFA *via* SDF-1α/CXCR4 pathway (38, 39). Liang Z et al. (40) have reported that activation of PI3K/Akt pathway was also a critical part of CXCL12/CXCR4 signaling axis induced VEGFA expression increase. Interestingly, VEGFA can upregulate CXCL12 and CXCR4 mRNA expression, and contributes to U251 cell invasion (41). Kim et al. (42) use

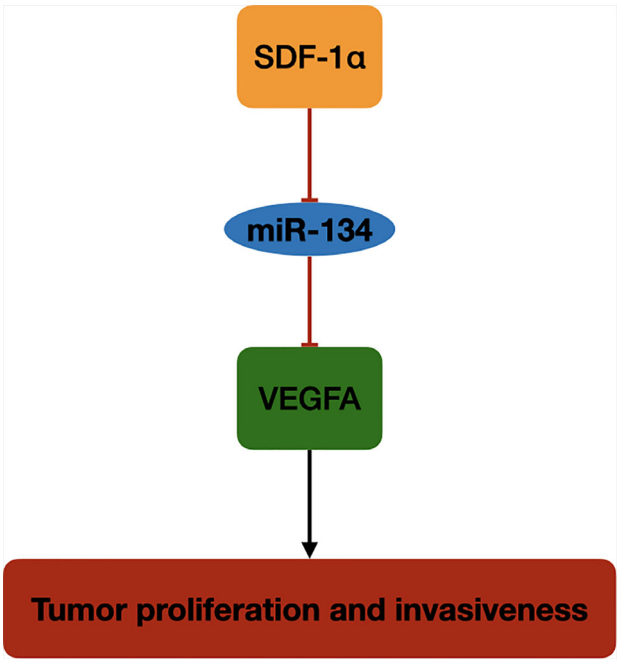

FIGURE 5 | The schematic diagram of SDF-1α/miR-134/VEGFA axis in NF-PitNET. SDF-1α promotes VEGFA expression by inhibiting miR-134 expression in NF-PitNETs proliferation and invasiveness.

CXCR4 antagonists to inhibit GH secretion and also inhibit proliferation of GH-secreting pituitary tumor cells, which indicates targeting CXCL12 might be potentially also useful to NF-PitNETs. It was identified in our study by mouse cell lines that VEGFA expression and cell proliferation and invasiveness were significantly increased after SDF-1α treatment. In addition to the PI3K/Akt pathway, we used mmu-miR-134–5p inhibitor and mmu-miR-134–5p mimic proved the miR-134 was also an important part in SDF-1 α/VEGFA pathway.

LIMITATIONS

Several limitations to our study deserve mention. Firstly, although our NF-PitNET samples were all negative for the detection of the GH, PRL, ACTH, FSH, LH by immunohistochemistry, we did not exclude the possibility that some of these tumors were not of gonadotroph lineage such as silent corticotroph, poorly differentiated PIT1-lineage, true null cell tumors, or other silent tumors of known cytodifferentiation. Secondly, SDF-1α/miR-134/VEGFA signaling pathway needs to be further clarified by luciferase assay. We have not performed *in vivo* experiments and other pituitary tumor cell line to further verify our result in NF-PitNET and we did not evaluate the effect of SDF-1 α on the methylation status of IG-DMR. Thirdly, the VEGFA expression level was only measured on protein level, thus, it remains uncover the molecular network among miR134 to mRNA of VEGFA. Future studies should include *in vivo* analyses with evaluation of methylation status of IG-DMR and mRNA alteration of SDF-1 α/miR-134/VEGFA signal pathway.

CONCLUSION

The SDF-1α and VEGFA function important role in NF-PitNETs proliferation and invasiveness. The miR-134 expression was downregulated after SDF-1α treatment in mouse pituitary αT3-1 cells. Lower expression of miR-134 upregulated its target gene VEGFA expression and subsequently induced PitNET cell proliferation and invasiveness. The regulation of the SDF-1α/miR-134/VEGFA axis represents a novel mechanism in the pathogenesis of NF-PitNETs and may serve as a potential therapeutic target for the treatment of NF-PitNETs.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding authors.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of the Second Affiliated Hospital of Zhejiang University Medical College. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

XW and YF performed the experiments and analyzed the data. YF wrote the manuscript. XW edited the manuscript. YZ revised the manuscript. XG helped with the statistical analysis. KX and CL collected the samples. JZ and YH designed the study and revised the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

This work was funded by the National Natural Science Foundation of China (No.81870964), Natural Science Foundation of Zhejiang Province (Nos. LY17H090012 and Q17H090013), Public Welfare Project of Zhejiang Provincial Department of Science and Technology (No. 2015C33192), and Rookie in the medical field of Zhejiang Province.

REFERENCES

1. Alsumali A, Cote DJ, Regestein QR, Crocker E, Alzarea A, Zaidi HA, et al. The impact of transsphenoidal surgery on neurocognitive function: A systematic review. *J Clin Neurosci* (2017) 42:1–6. doi: 10.1016/j.jocn.2017.01.015
2. Greenman Y, Stern N. Non-functioning pituitary adenomas. *Best Pract Res Clin Endocrinol Metab* (2009) 23(5):625–38. doi: 10.1016/j.beem.2009.05.005
3. Askitis D, Tsitlakidis D, Müller N, Waschke A, Wolf G, Müller UA, et al. Complete evaluation of pituitary tumours in a single tertiary care institution. *Endocrine* (2018) 60(2):255–62. doi: 10.1007/s12020-018-1570-z
4. Mete O, Gomez-Hernandez K, Kucharczyk W, Ridout R, Zadeh G, Gentili F, et al. Silent subtype 3 pituitary adenomas are not always silent and represent poorly differentiated monomorphous plurihormonal Pit-1 lineage adenomas. *Mod Pathol* (2016) 29(2):131–42. doi: 10.1038/modpathol.2015.151
5. Asa SL, Casar-Borota O, Chanson P, Delgrange E, Earls P, Ezzat S, et al. From pituitary adenoma to pituitary neuroendocrine tumor (PitNET): an International Pituitary Pathology Club proposal. *Endocr Relat Cancer* (2017) 24(4):C5–c8. doi: 10.1530/erc-17-0004
6. Greenman Y. MANAGEMENT OF ENDOCRINE DISEASE: Present and future perspectives for medical therapy of nonfunctioning pituitary adenomas. *Eur J Endocrinol* (2017) 177(3):R113–r124. doi: 10.1530/eje-17-0216
7. Cámara Gómez R. Non-functioning pituitary tumors: 2012 update. *Endocrinol Nutr* (2014) 61(3):160–70. doi: 10.1016/j.endonu.2013.04.009
8. Greenman Y, Stern N. Optimal management of non-functioning pituitary adenomas. *Endocrine* (2015) 50(1):51–5. doi: 10.1007/s12020-015-0685-8
9. Trivellin G, Butz H, Delhove J, Igreja S, Chahal HS, Zivkovic V, et al. MicroRNA miR-107 is overexpressed in pituitary adenomas and inhibits the expression of aryl hydrocarbon receptor-interacting protein in vitro. *Am J Physiol Endocrinol Metab* (2012) 303(6):E708–19. doi: 10.1152/ajpendo.00546.2011
10. Cheunsuchon P, Zhou Y, Zhang X, Lee H, Chen W, Nakayama Y, et al. Silencing of the imprinted DLK1-MEG3 locus in human clinically nonfunctioning pituitary adenomas. *Am J Pathol* (2011) 179(4):2120–30. doi: 10.1016/j.ajpath.2011.07.002
11. McComb DJ, Ryan N, Horvath E, Kovacs K. Subclinical adenomas of the human pituitary. New light on old problems. *Arch Pathol Lab Med* (1983) 107 (9):488–91.
12. Barbieri F, Bajetto A, Stumm R, Pattarozzi A, Porcile C, Zona G, et al. Overexpression of stromal cell-derived factor 1 and its receptor CXCR4 induces autocrine/paracrine cell proliferation in human pituitary adenomas. *Clin Cancer Res* (2008) 14(16):5022–32. doi: 10.1158/1078-0432.CCR-07-4717
13. Cai F, Dai C, Chen S, Wu Q, Liu X, Hong Y, et al. CXCL12-regulated miR-370-3p functions as a tumor suppressor gene by targeting HMGA2 in nonfunctional pituitary adenomas. *Mol Cell Endocrinol* (2019) 488:25–35. doi: 10.1016/j.mce.2019.02.020
14. Gejman R, Batista DL, Zhong Y, Zhou Y, Zhang X, Swearingen B, et al. Selective loss of MEG3 expression and intergenic differentially methylated region hypermethylation in the MEG3/DLK1 locus in human clinically nonfunctioning pituitary adenomas. *J Clin Endocrinol Metab* (2008) 93 (10):4119–25. doi: 10.1210/jc.2007-2633
15. Patrussi L, Baldari CT. Intracellular mediators of CXCR4-dependent signaling in T cells. *Immunol Lett* (2008) 115(2):75–82. doi: 10.1016/j.imlet.2007.10.012
16. Zeng Z, Shi YX, Samudio IJ, Wang RY, Ling X, Frolova O, et al. Targeting the leukemia microenvironment by CXCR4 inhibition overcomes resistance to kinase inhibitors and chemotherapy in AML. *Blood* (2009) 113(24):6215–24. doi: 10.1182/blood-2008-05-158311
17. Claesson-Welsh L, Welsh M. VEGFA and tumour angiogenesis. *J Intern Med* (2013) 273(2):114–27. doi: 10.1111/joim.12019
18. Lichtenberger BM, Tan PK, Niederleithner H, Ferrara N, Petzelbauer P, Sibilia M. Autocrine VEGF signaling synergizes with EGFR in tumor cells to promote epithelial cancer development. *Cell* (2010) 140(2):268–79. doi: 10.1016/j.cell.2009.12.046
19. Raverot G, Burman P, McCormack A, Heaney A, Petersenn S, Popovic V, et al. European Society of Endocrinology Clinical Practice Guidelines for the management of aggressive pituitary tumours and carcinomas. *Eur J Endocrinol* (2018) 178(1):G1–g24. doi: 10.1530/eje-17-0796
20. Du Q, Hu B, Feng Y, Wang Z, Wang X, Zhu D, et al. CircOMA1 mediated miR-145-5p suppresses tumor growth of nonfunctioning pituitary adenomas by targeting TPT1. *J Clin Endocrinol Metab* (2019) 104(6):2419–34. doi: 10.1210/jc.2018-01851
21. Paez Pereda M, Ledda MF, Goldberg V, Chervin A, Carrizo G, Molina H, et al. High levels of matrix metalloproteinases regulate proliferation and hormone secretion in pituitary cells. *J Clin Endocrinol Metab* (2000) 85(1):263–9. doi: 10.1210/jcem.85.1.6248
22. Chen L, Zhang L, Xian G, Lv Y, Lin Y, Wang Y. 25-Hydroxycholesterol promotes migration and invasion of lung adenocarcinoma cells. *Biochem Biophys Res Commun* (2017) 484(4):857–63. doi: 10.1016/j.bbrc.2017.02.003
23. Lubke D, Saeger W, Ludecke DK. Proliferation Markers and EGF in ACTH-Secreting Adenomas and Carcinomas of the Pituitary. *Endocr Pathol* (1995) 6 (1):45–55. doi: 10.1007/bf02914988
24. Pertuit M, Barlier A, Enjalbert A, Gerard C. Signalling pathway alterations in pituitary adenomas: involvement of Gsalpha, cAMP and mitogen-activated protein kinases. *J Neuroendocrinol* (2009) 21(11):869–77. doi: 10.1111/j.1365-2826.2009.01910.x
25. Simpson DJ, Bicknell JE, McNicol AM, Clayton R N, Farrell WE. Hypermethylation of the p16/CDKN2A/MTSI gene and loss of protein expression is associated with nonfunctional pituitary adenomas but not somatotrophinomas. *Genes Chromosomes Cancer* (1999) 24(4):328–36.
26. Zhang X, Zhou Y, Mehta KR, Danila DC, Scolavino S, Johnson SR, et al. A pituitary-derived MEG3 isoform functions as a growth suppressor in tumor cells. *J Clin Endocrinol Metab* (2003) 88(11):5119–26. doi: 10.1210/jc.2003-030222
27. Royo H, Cavaille J. Non-coding RNAs in imprinted gene clusters. *Biol Cell* (2008) 100(3):149–66. doi: 10.1042/bc20070126
28. Lin SP, Youngson N, Takada S, Seitz H, Reik W, Paulsen M, et al. Asymmetric regulation of imprinting on the maternal and paternal chromosomes at the Dlk1-Gtl2 imprinted cluster on mouse chromosome 12. *Nat Genet* (2003) 35 (1):97–102. doi: 10.1038/ng1233
29. Ortiz LD, Syro LV, Scheithauer BW, Ersen A, Uribe H, Fadul CE, et al. Anti-VEGF therapy in pituitary carcinoma. *Pituitary* (2012) 15(3):445–9. doi: 10.1007/s11102-011-0346-8
30. Turkowski K, Brandenburg S, Mueller A, Kremenetskaia I, Bungert AD, Blank A, et al. VEGF as a modulator of the innate immune response in glioblastoma. *Glia* (2018) 66(1):161–74. doi: 10.1002/glia.23234
31. Nieto MA. The snail superfamily of zinc-finger transcription factors. *Nat Rev Mol Cell Biol* (2002) 3(3):155–66. doi: 10.1038/nrm757
32. Patel NA, Patel P S, Vora HH. Role of PRL-3, Snail, Cytokeratin and Vimentin expression in epithelial mesenchymal transition in breast carcinoma. *Breast Dis* (2015) 35(2):113–27. doi: 10.3233/bd-140395
33. Thiery JP. Epithelial-mesenchymal transitions in tumour progression. *Nat Rev Cancer* (2002) 2(6):442–54. doi: 10.1038/nrc822
34. Barbieri F, Thellung S, Wurth R, Gatto F, Corsaro A, Villa V, et al. Emerging Targets in Pituitary Adenomas: Role of the CXCL12/CXCR4-R7 System. *Int J Endocrinol* (2014) 2014:753524. doi: 10.1155/2014/753524
35. Lee YH, Noh TW, Lee MK, Jameson J L, Lee EJ. Absence of activating mutations of CXCR4 in pituitary tumours. *Clin Endocrinol (Oxf)* (2010) 72 (2):209–13. doi: 10.1111/j.1365-2265.2009.03629.x
36. Xing B, Kong YG, Yao Y, Lian W, Wang R Z, Ren ZY. Study on the expression levels of CXCR4, CXCL12, CD44, and CD147 and their potential correlation with invasive behaviors of pituitary adenomas. *BioMed Environ Sci* (2013) 26 (7):592–8. doi: 10.3967/0895-3988.2013.07.011
37. Aghi M, Cohen KS, Klein RJ, Scadden D T, Chiocca EA. Tumor stromal-derived factor-1 recruits vascular progenitors to mitotic neovasculature, where microenvironment influences their differentiated phenotypes. *Cancer Res* (2006) 66(18):9054–64. doi: 10.1158/0008-5472.can-05-3759
38. Teicher B A, Fricker SP. CXCL12 (SDF-1)/CXCR4 pathway in cancer. *Clin Cancer Res* (2010) 16(11):2927–31. doi: 10.1158/1078-0432.ccr-09-2329
39. Yu D, Lv F, Zhang J, Li H. SDF-1 Expression is Associated with Poor Prognosis in Osteosarcoma. *Ann Clin Lab Sci* (2016) 46(5):508–14.
40. Liang Z, Brooks J, Willard M, Liang K, Yoon Y, Kang S, et al. CXCR4/CXCL12 axis promotes VEGF-mediated tumor angiogenesis through Akt signaling pathway. *Biochem Biophys Res Commun* (2007) 359(3):716–22. doi: 10.1016/j.bbrc.2007.05.182
41. Hong X, Jiang F, Kalkanis SN, Zhang ZG, Zhang XP, DeCarvalho AC, et al. SDF-1 and CXCR4 are up-regulated by VEGF and contribute to glioma cell invasion. *Cancer Lett* (2006) 236(1):39–45. doi: 10.1016/j.canlet.2005.05.011

42. Kim JM, Lee YH, Ku C R, Lee EJ. The cyclic pentapeptide d-Arg3FC131, a CXCR4 antagonist, induces apoptosis of somatotrope tumor and inhibits tumor growth in nude mice. *Endocrinology* (2011) 152(2):536–44. doi: 10.1210/en.2010-0642

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Endocrinology

REVIEW
published: 11 December 2020
doi: 10.3389/fendo.2020.608422

The Progress of Immunotherapy in Refractory Pituitary Adenomas and Pituitary Carcinomas

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Most pituitary adenomas (PAs) are considered benign tumors, but approximately 0.2% can present metastasis and are classified as pituitary carcinomas (PCs). Refractory PAs lie between benign adenomas and true malignant PC and are defined as aggressive-invasive PAs characterized by a high Ki-67 index, rapid growth, frequent recurrence, and resistance to conventional treatments, including temozolomide. It is notoriously difficult to manage refractory PAs and PC because of the limited therapeutic options. As a promising therapeutic approach, cancer immunotherapy has been experimentally used for the treatment of many tumors, including pituitary tumors. The purpose of this review is to report the progress of immunotherapy in pituitary tumors, including refractory PAs and PCs. The tumor immune microenvironment has been recognized as a key contributor to tumorigenesis, progression, and prognosis. One study indicated that the number of CD68 + macrophages was positively correlated with tumor size and Knosp classification grade for tumor invasiveness. The infiltration of CD4+ and CD8+ T cells was relatively scant in these adenomas, but pituitary growth hormone (GH) adenomas exhibited significantly more CD4+ and CD8+ T cells than non-GH adenomas. These results suggest an association of CD68+ macrophage infiltration with an increase in pituitary tumor size and invasiveness. Another study suggested that a lower number of CD8+ lymphocytes is associated with cavernous sinus invasion and resistance to treatment with first-generation somatostatin analogs in acromegaly patients, highlighting a potential role of the tumor immune microenvironment in determining the prognosis of somatotroph pituitary tumors. Preclinical studies have indicated that widely varying degrees of programmed death-ligand 1 (PD-L1) expression and tumor-infiltrating lymphocytes (TILs) are found among different subtypes. Functional PAs and aggressive PAs express significantly higher levels of PD-L1 and TILs than other subtypes, indicating that PD-1 blockade might be a promising alternative therapy for patients with aggressive PAs. PD-L1 transcript and protein levels were found to be significantly increased in functioning (GH and prolactin-expressing) pituitary tumors compared to nonfunctioning (null cell and silent gonadotroph) adenomas. Moreover, primary pituitary tumors harbored higher levels of PD-L1 mRNA

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Specialty section:
This article was submitted to Pituitary Endocrinology, a section of the journal Frontiers in Endocrinology

Received: *20 September 2020*
Accepted: *10 November 2020*
Published: *11 December 2020*

Citation:
Dai C, Liang S, Sun B and Kang J (2020) The Progress of Immunotherapy in Refractory Pituitary Adenomas and Pituitary Carcinomas. Front. Endocrinol. 11:608422. doi: 10.3389/fendo.2020.608422

than recurrent tumors. These findings suggest the possibility of considering checkpoint blockade immunotherapy for functioning pituitary tumors refractory to conventional management. Animal models of Cushing's disease also demonstrated PD-L1 and TIL expression in cultured tumors and murine models, as well as the effectiveness of checkpoint blockade therapy in reducing the tumor mass, decreasing hormone secretion, and increasing the survival rate. Clinical studies show that immunotherapy may be an effective treatment in patients with pituitary tumors. One corticotroph carcinoma patient showed a significant reduction in hormone levels and shrinkage of the tumor size of primary and metastatic lesions immediately after investigational treatment with ipilimumab and nivolumab. However, another patient with corticotroph adenoma progressed rapidly after four cycles of anti-PD-1 (pembrolizumab) treatment. To date, there are two registered clinical trials of immunotherapy for pituitary tumors. One of them is the phase II clinical trial of nivolumab combined with ipilimumab for patients with aggressive pituitary tumors (NCT04042753). The other one is also a phase II clinical trial of the combination of nivolumab and ipilimumab for rare tumors, including pituitary tumors (NCT02834013). Both clinical trials are in the stage of recruiting patients and have not been completed. In summary, the results from preclinical research and clinical studies indicated that immunotherapy might be a promising alternative therapy for PCs and refractory PAs resistant to conventional treatments. The combination of immunotherapy and radiotherapy or temozolomide may have synergistic effects compared to a single treatment. More preclinical and clinical studies are needed to further indicate the exact efficacy of immunotherapy in pituitary tumors.

Keywords: pituitary carcinomas, refractory pituitary adenomas, immunotherapy, programmed death-ligand 1, tumor-infiltrating lymphocytes

INTRODUCTION

Pituitary tumors represent approximately 10%–15% of intracranial tumors and are the second most common primary brain tumor in humans (1, 2). Most pituitary tumors are noninvasive benign tumors that grow slowly and remain within the sella and/or displace the surrounding tissues. However, up to 35% of them are invasive adenomas and infiltrate adjacent tissues, including the cavernous sinuses, bone, sphenoid sinuses, and nerve sheaths (3). Approximately 10% of pituitary adenomas (PAs) show aggressive clinical behavior and are refractory to conventional therapy (4–6). According to the current WHO classification (2017), aggressive PAs were defined in patients with a radiologically invasive tumor and unusually rapid tumor growth rate, or clinically relevant tumor growth despite optimal standard therapies (surgery, radiotherapy, and medical treatments) (5). Pituitary carcinomas (PCs) are defined as tumors of adenohypophyseal origin that show metastatic spread by either craniospinal dissemination or systemic metastases; PCs are very rare and represent only 0.1%–0.2% of all pituitary tumors (7–9). Although the understanding of the molecular pathogenesis of PCs has progressed rapidly, the early identification of PC is difficult until the presence of metastatic lesions. Refractory PAs lie between benign adenomas and true malignant PC and are defined as aggressive-invasive PAs characterized by a high Ki-67 index, rapid growth, frequent recurrence, and resistance to conventional treatments, including temozolomide (TMZ) (10, 11). Therefore, both refractory PAs and PCs are very difficult to manage due to the lack of effective treatment, and they are associated with increased morbidity and mortality (12).

Recently, TMZ has shown promising efficacy for pituitary tumors and has been recommended as a first-line medication for refractory PAs and PCs by the European Society of Endocrinology (5). However, previous studies showed that only approximately 60% of pituitary tumors are responsive to TMZ treatment, and some of them are resistant to TMZ (13, 14). Emerging targeted therapies, including those targeting vascular endothelial growth factor, epidermal growth factor, fibroblast growth factor, the Raf/Mek/ErK pathway, the PI3K/Akt/mTOR pathway, the notch signaling pathway, the hedgehog signaling pathway, and CDK 4/6, have been studied preclinically and/or have been experimentally used for refractory PAs and PCs, but their efficacy is limited (15–17). Therefore, more effective and novel treatment approaches are needed. As a promising therapeutic approach, cancer immunotherapy has been experimentally used for the treatment of many tumors, including pituitary tumors, and has been proposed as a potential treatment option for refractory PAs and PCs. The purpose of this review is to summarize the recent advances of immunotherapy in pituitary tumors, including refractory PAs and PCs.

MECHANISMS OF IMMUNOTHERAPY

The human immune system is responsible for discriminating self from non-self, thereby protecting the body from tumors recognized as non-self or altered self. The immune system implements surveillance against cancer by generating innate and adaptive immune responses against tumor antigens. Early efforts to strengthen the immune response provide a critical foundation for tumor immunotherapy, despite the products of this era, such as tumor necrosis factor and interleukin-2, being no longer used routinely for tumors due to unacceptable systemic side effects (18). Immune responses are capable of suppressing tumor growth and achieving tumor regression; nevertheless, tumors can evolve to evade immune system elimination for further proliferation, infiltration, and metastasis. The upregulation of T cell coinhibitory ligands plays a key role in tumor immune escape and provides a solid basis for the clinical evaluation of therapeutic strategies that target immune checkpoints (19, 20).

Immune checkpoints, the negative regulators of immune activation, are essential for controlling the strength of the immune response, maintaining self-tolerance, and reducing tissue damage. Tumors exploit immune checkpoints negatively regulate the activation of T cells. The blockade of the coinhibitory cytotoxic T-lymphocyte–associated protein 4 (CTLA-4) and programmed cell death 1 (PD-1) allows the activation of T cell stimulatory signaling, thereby enhancing antitumor T cell cytotoxicity, proinflammatory cytokine production, and proliferation and promoting tumor destruction (21–24).

To date, several monoclonal antibodies that block immune checkpoints have been developed and have achieved medium to high response rate. However, checkpoint inhibitors have not been approved for the treatment of PAs and PCs by the Food and Drug Administration (FDA). Interestingly, patients treated with checkpoint inhibitors can develop severe hypophysitis (25–27). This immune-related adverse event (irAE) provides compelling evidence that checkpoint blockade stimulates an immune response readily within the pituitary gland. However, checkpoint inhibitors are not effective for all; therefore, a variety of directions have emerged in current immunotherapy. The combination of CTLA-4 and PD-1/PD-L1 has achieved higher clinical remission due to the nonredundant coinhibitory roles of these two pathways, yet with a higher frequency of immune-related toxicities (28). Although usually well tolerated, combination therapy increases the risk of high-grade irAEs. The establishment of clinical biomarkers helps to identify patients who will benefit from checkpoint inhibitors and guide individualized treatment with optimal doses. It has demonstrated that immune checkpoint inhibitor efficacy is affected by a combination of factors involving tumor genomics, host germline genetics, PD1 ligand 1 (PDL1) levels, and other features of the tumor microenvironment, as well as the gut microbiome (29). Several possible candidates, including tumor-infiltrating T lymphocyte quantification, immunostaining-based PD-L1, and sequencing-based mutational burden and neoantigen burden biomarkers, have been proposed for further evaluation (29, 30).

TUMOR IMMUNE MICROENVIRONMENT OF PITUITARY TUMORS

The tumor immune microenvironment (TIM) plays a critical role in tumorigenesis and progression. It has demonstrated that certain features of the degree of tumor infiltration by cytotoxic T cells can predict clinical outcome of patients (31). Tumor-infiltrating lymphocytes (TILs) and other mononuclear cells, such as tumor-associated macrophages, are central components of the TIM, participate, and regulate the tumor immune response and have been used as prognostic factors in numerous solid tumors, such as melanomas and ovarian, breast, colorectal, and urothelial carcinomas (31–38). It has been possible to identify different subclasses of immune environment that have an influence on tumor initiation and response and therapy (37). Although PAs are a heterogeneous group of lesions with different clinical behaviors, TILs were found to be present in all subtypes of PAs, albeit at low levels (**Figure 1**) (39–43). The penetration of more than 5% CD8+ cells was observed in 66 of 191 PA patients (44). A retrospective study defined lymphocyte infiltration as the presence of 15 to 20 lymphocytes in the 400 times magnified field. They reported a TIL prevalence of 2.9% in all 1,400 PAs, including both functional and nonfunctional types (41). A case-control study scored CD45 expression on a scale ranging from 0 to 5 to obtain a semiquantitative measure of lymphocyte infiltration. The infiltration of CD45+ cells was mild, with a score of 1 or 0.5 in the majority of PAs, TILs are more abundant in adenoma patients than in healthy controls (40). TILs were first quantitated in 35 cases by counting the numbers of macrophages and lymphocytes in 10 to 30 consecutive high-power fields (HPFs) at 400× magnification. CD68+ macrophages were sparse, with an average number of 4–8 per HPF, and CD4+ and CD8+ cells were relatively rare, with an average number of lower than 4 per HPF in different secretory subtypes (42).

It is unclear whether the features of PAs are associated with immune cell infiltration. CD45+ infiltration was similar among different subtypes of PAs (40, 41). However, CD4+ and CD8+ staining was closely correlated with higher growth hormone (GH) levels (42–44). CD68 expression was higher in sparsely granulated GH adenomas and null cell adenomas than in densely granulated GH-secreting adenomas and adrenocorticotropic hormone (ACTH)-secreting adenomas (42).

The phenotype and functional consequences of TILs are complex; however, the overall presence of TILs and other mononuclear cells is associated with a high risk of recurrence and poor prognosis in patients with PAs. After a mean follow-up period of 34 ± 2 months, a poor clinical outcome was more common in PA patients with CD45 immunostaining than in those without CD45 immunostaining. The odds of a poor clinical outcome were 3.3-fold greater than that without CD45+ cells observed in adenomas (40). This may be due to the correlation between positive CD45 staining and a higher proliferative index. No correlations were found between CD8+ T cells and tumor size (42, 44) or Knosp classification grade (42). One study showed that the number of CD8+ lymphocytes tended to be higher in the cavernous sinus invasion group than in the cavernous sinus non-invasion group, but no significant difference was observed (45).

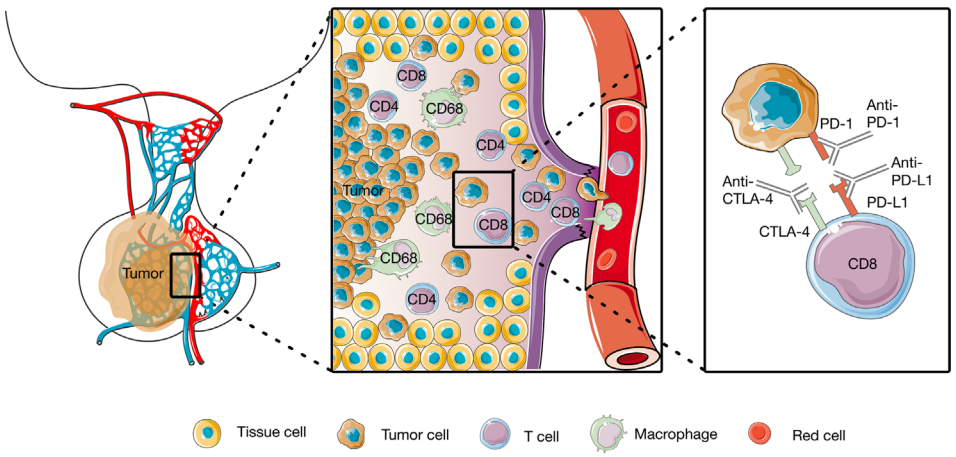

FIGURE 1 | Tumor immune microenvironment of pituitary tumors and of Working model of anti-PD1 and anti-anti-CTLA4. The CD4+, CD8+, and CD68+ cells were identified in pituitary tumors, and anti-PD1 and anti-anti-CTLA4 rescue T cell anti-tumor effector functions.

The number of CD68+ cells was positively correlated with tumor size and Knosp classification grade for tumor invasiveness (42). An increased macrophage (CD68+ cells) infiltration was also observed in aryl hydrocarbon receptor interacting protein (AIP)-mutated PAs and was associated with the invasiveness of AIP-mutated adenomas (46). Together, these TILs and other mononuclear cells not only are related to the tumor characteristics and progression of pituitary tumors, but also offer for both prognosis and targeted therapeutics (47).

PD-1/PD-L1 Expression in Pituitary Tumors

PD-L1 expression is observed to varying degrees in different subtypes (43–45, 48–50). PD-L1 is expressed on both the cell membrane and cytoplasm of tumor cells and occasionally in endothelial cells (45). PD-L1 staining is mild to moderate in most cases (43). One study found that in 36.6% of 191 cases, PD-L1 expression was observed in more than 5% of tumor cells (44), but another study detected the same PD-L1 levels in only 7.9% of 139 cases (48). The concordance of PD-L1 immunostaining between assays may be poor.

The tumor features affected by PD-L1 expression remain controversial. One study showed that PD-L1 expression significantly increased with elevated levels of serum GH, PRL, ACTH and cortisol (44). Consistent with their finding, others further found significantly higher PD-L1 expression in adenomas with clinical functioning and a high Ki-67 or MIB-1 proliferative index (43, 44, 51). Another study revealed an increased tendency of cavernous sinus invasion with higher PD-L1 expression (45). In addition, PD-L1 expression is positively correlated with increased TILs (43, 44). However, a retrospective series of 139 cases found that none of these features, including hormone secretion, aggressiveness, and invasiveness, correlated with PD-L1 expression (48). The findings of this study are inconsistent with those of previous studies, indicating that PD-L1 expression should be further studied among the diverse biological characteristics or behaviors of pituitary tumors.

The PD-L1 RNA transcript is significantly increased not only in GH-secreting and PRL-secreting adenomas compared to null cell and silent gonadotroph adenomas but also in primary pituitary tumors compared to recurrent tumors (43). However, the correlation between PD-L1 mRNA and protein levels was poor at the individual sample level in this study. In nonfunctioning PAs, PD-L1 mRNA transcription was significantly higher in gonadotroph adenomas than in null cell and silent corticotroph adenomas and was accompanied by a more than 3% MIB-1 proliferative index (49). This finding suggests that PD-L1 mRNA levels are associated with aggressiveness in nonfunctioning PAs, but PD-L1 expression was not examined in this study. Although data are limited, it is possible that such tumor features influence PD-L1 expression levels, potentially affecting checkpoint inhibitor effectiveness.

The upregulated coinhibitory ligands, represented by PD-L1, indicate that PAs can evade immune surveillance through the suppression of the immune response (52–54). Functional PAs and aggressive PAs may express higher levels of PD-L1 than other subtypes, indicating that PD-1 blockade might be a promising alternative therapy for patients with aggressive PAs. These findings suggest the possibility of considering checkpoint blockade immunotherapy for pituitary tumors that resist conventional management.

PRECLINICAL MODELS OF PITUITARY TUMORS

The establishment of preclinical models helps to improve our understanding of the mechanism of immunotherapy in PA patients. PD-L1 is prominently expressed in a murine pituitary ACTH adenoma cell line *in vitro*, second only to a melanoma cell line, and higher than that in breast adenocarcinoma, lung carcinoma, and glioma cell lines (51). This pattern of PD-L1 expression is still maintained *in vivo* after 8 weeks of growth in

tumor implantation. Moreover, TILs are present in cultured tumors and have been confirmed to have a high expression of PD-1, TIM-3, and LAG-3, which marked the upregulation of coinhibitory molecules and may further cause TIL exhaustion. It has shown that the effect of anti PD-L1 on the preclinical model is only partially dependent on the T cells function. On the contrary, it seems that CD11b+ myeloid cells, which have high PD-L1 expression, may play a more important role in the response to anti-PD-L1 treatment. Thus, this finding will suggest that interaction between CD11b+ myeloid cells and cancer cells are critical for the survival of cancer cells, further reenforce the notion that myeloid cells are important therapeutic target (51). These findings of preclinical results provided important information for human tumors. However, there are some essential differences between these preclinical models and human tumors, more research related to human tumors are needed.

Given the presence of lymphocytes in PAs and the expression of coinhibitory ligands in the tumor microenvironment, PAs may be sensitive to checkpoint inhibitors. The efficacy of anti-PD-L1 antibodies has been examined in both subcutaneous and intracranial murine models of Cushing disease (51). Following subcutaneous tumor implantation, mice were treated intraperitoneally with either anti-PD-L1 antibody or isotype control antibody every 3 days for a total of 12 doses in 36 days. After 8 weeks, anti-PD-L1 treatment significantly inhibited tumor growth and suppressed serum ACTH secretion compared with untreated tumor-bearing mice. Some mice achieved complete tumor regression. To study the therapeutic capacity of anti-PD-L1 on intracranial lesions, the tumor was placed in the right frontal lobe, where the tumor uncontrollably grows, resulting in 100% mortality after approximately 3 weeks. Following intracranial tumor implantation, mice were given the same treatment as above every 3 days for a total of 15 doses or until the endpoint. The median overall survival was extended to 29.5 days in the anti-PD-L1 treatment group compared with a median survival of 21.5 days in the control group. Additionally, anti-PD-L1 therapy can improve long-term survival. In the treatment group, the 30-day survival rate was estimated to be 50%, and the 60-day survival rate was estimated to be 40%, while in the control group, the survival rate was 0% at both time points.

Preclinical models of Cushing disease demonstrate PD-L1 expression in cell lines and cultured tumors as well as the effectiveness of anti-PD-L1 immunotherapy in reducing tumor mass, decreasing hormone secretion, and arresting tumor proliferation. Although only a few data points are available, murine and cell line studies indicate that PD-L1 is a potential target in PAs.

CLINICAL STUDIES OF IMMUNOTHERAPY IN PITUITARY TUMORS

Pituitary tumors are usually benign and slow growing, but a subset has a more aggressive clinical behavior. PCs, defined by the presence of cerebrospinal or distant metastasis of a pituitary neuroendocrine tumor, are particularly rare and have mortality rates of up to 66% at 1 year after diagnosis. For many patients with refractory PAs and PCs, effective targeted therapy is still lacking (17). PCs have a very limited response to previous therapies, including somatostatin analogs, external beam radiotherapy, chemotherapies including TMZ, capecitabine, everolimus, sunitinib, and bevacizumab, and peptide receptor radionuclide therapy (55). TMZ has been established as a first-line chemotherapeutic treatment for aggressive PAs or PCs. However, in a large cohort including 157 patients treated with TMZ, complete response was observed in only a median of 6% of patients after a median treatment period of 9 cycles. Thirty-one percent achieved partial response, 33% achieved disease stabilization, but 33% had disease progression (56). Of note, of the patients who had complete response, partial response and stable disease, 25%, 40%, and 48%, respectively, further progressed after a median of 12 months of follow-up. The limited long-term effect of TMZ highlights the need to identify additional effective therapies.

The increased understanding of the TIM has significantly improved the effort to enhance the immune response and has brought about advances in cancer therapies over recent years. Similarly, recent data have shown the expression of PD-L1 and the presence of TILs in PA, as well as the possible association of the immune microenvironment with the biological and clinical phenotypes of PA, including hormone secretion, invasiveness, and aggressiveness. These findings provide a rationale to attempt the use of checkpoint inhibitors in some clinical scenarios, although strong support for the use of checkpoint inhibitors in PA is still lacking.

It has been proved that PD1/PDL1 blockade significantly rescue T cell anti-tumor effector functions by interfering with T cell activation and the acquisition of effector capacities (57). The CTLA-4 is a surface molecule expressed by activated T cells, inhibition of CTLA-4 may increase the regulation of the immune response to cancer cells (58). Combination therapy with anti-CTLA4 plus anti-PD1 monocolonal antibodies not only leads to an increased frequency of ICOS+ CD4+ effector T cells, but also leads to unexpected and unique changes including a decreased frequency of exhausted CD8+ T cells and the expansion of activated CD8+ effector T cells (24). Only one patient with ACTH-secreting PC has been reported to be successfully treated with immunotherapy until now (59). A 35-year-old woman with aggressive ACTH-secreting PA received medical therapies, including pasireotide, ketoconazole, and ketoconazole combined with cabergoline, followed by several surgical resections and radiation therapies due to tumor enlargement and incomplete hormonal control. Afterwards, she was treated with TMZ and capecitabine but discontinued after four cycles due to poor tolerance, despite the decrease in tumor volume and ACTH level observed during this period. Sixty-eight months after the initial diagnosis, the patient presented with liver metastatic lesions. Disease progressed intracranially and extracranially during two additional cycles of TMZ combined with capecitabine. She then started immunotherapy with an anti-CTLA-4 agent (ipilimumab) combined with an anti-PD-1 antibody (nivolumab). Following five cycles, the patient yielded a 92% regression in the dominant

hepatic metastasis, a 59% decrease in the recurrent intracranial lesions, and a normalization in plasma ACTH levels to 66 pg/ml from the previous 45,550 pg/ml, with acceptable drug side effects. It is worth noting that subsequent analysis of the hepatic metastasis demonstrated an MSH6 mutation and 1% PD-L1 expression. This case revealed that checkpoint inhibition should be a treatment consideration for refractory PAs and PCs, especially for tumors that have developed resistance to TMZ. It was supposed that TMZ-induced hypermutated tumors may be more sensitive to checkpoint inhibitors. TMZ can induce alterations in the mismatch repair system and, consequently, generate a greater number of neoantigens, which results in a greater efficacy of treatment with checkpoint inhibitors (60).

However, the generalizability of checkpoint inhibitors needs further study. Another case reported that pembrolizumab had poor efficacy in the treatment of refractory ACTH-secreting PA (61). A 47-year-old man had partial response to TMZ for the initial 6 weeks, and then treatment was changed to pasireotide monotherapy until disease progression. Further immunohistochemical analysis revealed a complete loss of MSH2 and MSH6. Notably, no PD-L1 expression was found in PA immunostaining. The patient then received immunotherapy with an anti-PD-1 antibody (pembrolizumab). After four cycles, the patient showed radiological progression of disease and an increase in serum ACTH and urinary cortisol levels. The failure may be explained by the weakened efficacy of anti-PD-L1 and the decreased immune response, which may result from the consistently high levels of serum cortisol in tumors. Further investigation is needed to find reliable biomarkers to predict the response to immunotherapy. Although many studies have explored measurements of PD-L1 expression as a predictor of response to checkpoint inhibitors, it remains primarily a research tool. In current clinical practice, PD-L1 expression is limited as a predictor of the response to immunotherapy. PD-L1 staining may not be used to accurately select patients for PD-1/PD-L1 pathway blockade due to the low prediction accuracy and dynamic changes (62). In some studies, the expression of PD-L1 appeared to strongly predict the efficacy of anti-PD-L1, whereas in other studies, poor predictive value has been found. Although the immune checkpoint inhibitors have shown good efficacy in some cases, anti-PD1 alone may not be potent enough. A strategy to combine agent targeting the immunosuppressive TME with immune checkpoint blockade may overcome the limitation.

Immunotherapy appears to have some clinical benefit in patients with PAs and may be an option for medical therapy in refractory PAs and PCs. Nevertheless, the limitations of immunotherapy should be acknowledged, as the clinical response is variable and predictive efficacy is challenging. Further studies are still needed to identify the optimal use of this treatment in different clinical scenarios.

FUTURE PERSPECTIVES

The Need for Further Improvement of Potential Biomarkers

Although no immunotherapy for PAs has been approved yet, various studies have been carried out to explore immune-related biomarkers or therapeutic targets. In addition to PD-L1 and TILs, which have been detailed above, other important aspects have also been investigated. A range of transcriptomics analyses have been conducted to identify genes expressed differentially between PA and normal samples (63–66). However, high inconsistency was found among different analyses because of the limited number of both the patients and controls in each analysis. Using a novel strategy of data integration, one bioinformatics study integrated available PA microarray datasets to identify more robust differentially expressed genes and to annotate immune-related genes (67). Based on the analysis of the human protein-protein interaction network, some promising target candidates, including GAL, LMO4, STAT3, PD-L1, TGFB, and TGFBR3, were proposed for PA immunotherapy. This study provides useful guidance for the development of novel biomarkers for PA immunotherapy. To better understand the composition of the TIM of pituitary tumors, more novel technologies like single cell RNA-seq and imaging mass cytometry are needed for new immunotherapy development.

The Need for the Standardization of Assessment Methods and Verification in Animal Models

Previous studies used different antibody clones and cut-off values (48). The lack of consensus in assessment criteria partly limited studies on PD-L1 and other ligands as markers of response to checkpoint inhibitors in patients with refractory or TMZ unresponsive PAs and PCs. Therefore, the standardization of methodologies to verify the prognostic value in a large cohort is a prerequisite for the future routine application of immunotherapy (68).

Over 40 animal models for PAs have been generated. Many of these models can represent human syndromes (69). Some models can even simulate drug resistance (70). Establishment and use of patient-derived xenograft models has been widely used for drug testing in many cancers (71), which may offer a perfect mouse model for immunotherapy of pituitary tumors. Evaluation in these models will increase our understanding of the immune microenvironment in PAs and of the roles of immunotherapy.

The Need for Prospective Clinical Studies on Immunotherapy

Case reports revealed that checkpoint inhibitors represent a possible therapy for PAs and adenocarcinoma. Further prospective clinical studies are warranted. To date, there are two registered clinical trials of immunotherapy for pituitary tumors (72, 73). One of them is the phase II clinical trial entitled Nivolumab and Ipilimumab in People with Aggressive Pituitary Tumors (Memorial Sloan Kettering Cancer Center, United States, NCT04042753). The other one is also a phase II clinical trial called Nivolumab and Ipilimumab in Treating Patients with Rare Tumors (National Cancer Institute, United States, NCT02834013), which includes pituitary tumors. Both clinical trials are in the stage of recruiting patients and have not been completed.

CONCLUSION

In summary, it is notoriously difficult to manage refractory PAs and PCs due to the limited effective therapeutic options. As a promising therapeutic approach, cancer immunotherapy in pituitary tumors has recently attracted increasing attention. The TIM has been recognized as a key contributor to the tumorigenesis, progression, invasion, and prognosis of pituitary tumors. The expression of macrophages, lymphocytes and PD-L1 varied greatly in different pituitary tumors, and these immune factors are associated with the clinicopathological characteristics of pituitary tumors. Clinical case studies show that immunotherapy appears to have some clinical benefit in patients with refractory PAs or PCs. However, although these data suggest that cancer immunotherapy may be an effective therapeutic target for patients with refractory PAs and PCs, further basic research and clinical trials are needed to verify these findings.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work, and approved it for publication.

FUNDING

The financial support for this study was provided by the Scientific Research Project of Capital Health Development in 2020 (grant number: 2020-2-2058), and Beijing Natural Science Foundation (grant number: 7172057). The funding institutions had no role in the design of the study, data collection and analysis, the decision to publish, or the preparation of the manuscript.

REFERENCES

1. Molitch ME. Diagnosis and Treatment of Pituitary Adenomas: A Review. *JAMA* (2017) 317(5):516–24. doi: 10.1001/jama.2016.19699
2. Melmed S. Pathogenesis of pituitary tumors. *Nat Rev Endocrinol* (2011) 7 (5):257–66. doi: 10.1038/nrendo.2011.40
3. Hansen TM, Batra S, Lim M, Gallia GL, Burger PC, Salvatori R, et al. Invasive adenoma and pituitary carcinoma: a SEER database analysis. *Neurosurg Rev* (2014) 37(2):279–85; discussion 85-6. doi: 10.1007/s10143-014-0525-y
4. Kasuki L, Raverot G. Definition and diagnosis of aggressive pituitary tumors. *Rev Endocr Metab Disord* (2020) 21(2):203–8. doi: 10.1007/s11154-019-09531-x
5. Raverot G, Burman P, McCormack A, Heaney A, Petersenn S, Popovic V, et al. European Society of Endocrinology Clinical Practice Guidelines for the management of aggressive pituitary tumours and carcinomas. *Eur J Endocrinol* (2018) 178(1):G1–G24. doi: 10.1530/EJE-17-0796
6. Lopes MBS. The 2017 World Health Organization classification of tumors of the pituitary gland: a summary. *Acta Neuropathol* (2017) 134(4):521–35. doi: 10.1007/s00401-017-1769-8
7. Kaltsas GA, Nomikos P, Kontogeorgos G, Buchfelder M, Grossman AB. Clinical review: Diagnosis and management of pituitary carcinomas. *J Clin Endocrinol Metab* (2005) 90(5):3089–99. doi: 10.1210/jc.2004-2231
8. Daly AF, Tichomirowa MA, Beckers A. The epidemiology and genetics of pituitary adenomas. *Best Pract Res Clin Endocrinol Metab* (2009) 23(5):543–54. doi: 10.1016/j.beem.2009.05.008
9. Heaney AP. Clinical review: Pituitary carcinoma: difficult diagnosis and treatment. *J Clin Endocrinol Metab* (2011) 96(12):3649–60. doi: 10.1210/jc.2011-2031
10. Dai C, Liu X, Ma W, Wang R. The Treatment of Refractory Pituitary Adenomas. *Front Endocrinol (Lausanne)* (2019) 10:334. doi: 10.3389/fendo.2019.00334
11. Syro LV, Rotondo F, Camargo M, Ortiz LD, Serna CA, Kovacs K. Temozolomide and Pituitary Tumors: Current Understanding, Unresolved Issues, and Future Directions. *Front Endocrinol (Lausanne)* (2018) 9:318. doi: 10.3389/fendo.2018.00318
12. Ilie MD, Jouanneau E, Raverot G. Aggressive Pituitary Adenomas and Carcinomas. *Endocrinol Metab Clin North Am* (2020) 49(3):505–15. doi: 10.1016/j.ecl.2020.05.008
13. Ji Y, Vogel RI, Lou E. Temozolomide treatment of pituitary carcinomas and atypical adenomas: systematic review of case reports. *Neurooncol Pract* (2016) 3(3):188–95. doi: 10.1093/nop/npv059
14. McCormack AI, Wass JA, Grossman AB. Aggressive pituitary tumours: the role of temozolomide and the assessment of MGMT status. *Eur J Clin Invest* (2011) 41(10):1133–48. doi: 10.1111/j.1365-2362.2011.02520.x
15. Araki T, Cooper O, Fukuoka H. Editorial: Targeted Therapy for Pituitary Adenomas. *Front Endocrinol (Lausanne)* (2019) 10:358. doi: 10.3389/fendo.2019.00358
16. Dai C, Zhang B, Liu X, Ma S, Yang Y, Yao Y, et al. Inhibition of PI3K/AKT/mTOR pathway enhances temozolomide-induced cytotoxicity in pituitary adenoma cell lines in vitro and xenografted pituitary adenoma in female nude mice. *Endocrinology* (2013) 154(3):1247–59. doi: 10.1210/en.2012-1908
17. Lamb LS, Sim H-W, McCormack AI. Exploring the Role of Novel Medical Therapies for Aggressive Pituitary Tumors: A Review of the Literature-"Are We There Yet?". *Cancers (Basel)* (2020) 12(2):E308. doi: 10.3390/cancers12020308
18. Abbott M, Ustoyev Y. Cancer and the Immune System: The History and Background of Immunotherapy. *Semin Oncol Nurs* (2019) 35(5):150923. doi: 10.1016/j.soncn.2019.08.002
19. O'Donnell JS, Teng MWL, Smyth MJ. Cancer immunoediting and resistance to T cell-based immunotherapy. *Nat Rev Clin Oncol* (2019) 16(3):151–67. doi: 10.1038/s41571-018-0142-8
20. Corrales L, Matson V, Flood B, Spranger S, Gajewski TF. Innate immune signaling and regulation in cancer immunotherapy. *Cell Res* (2017) 27(1):96–108. doi: 10.1038/cr.2016.149
21. Ribas A, Wolchok JD. Cancer immunotherapy using checkpoint blockade. *Science* (2018) 359(6382):1350–5. doi: 10.1126/science.aar4060
22. McLane LM, Abdel-Hakeem MS, Wherry EJ. CD8 T Cell Exhaustion During Chronic Viral Infection and Cancer. *Annu Rev Immunol* (2019) 37:457–95. doi: 10.1146/annurev-immunol-041015-055318
23. Baumeister SH, Freeman GJ, Dranoff G, Sharpe AH. Coinhibitory pathways in immunotherapy for cancer. *Annu Rev Immunol* (2016) 34:539–73. doi: 10.1146/annurev-immunol-032414-112049
24. Sharma P, Allison JP. Dissecting the mechanisms of immune checkpoint therapy. *Nat Rev Immunol* (2020) 20(2):75–6. doi: 10.1038/s41577-020-0275-8
25. Atkins P, Ur E. Primary and Ipilimumab-induced Hypophysitis: A Single-center Case Series. *Endocr Res* (2020) 45(4):246–53. doi: 10.1080/07435800.2020.1817064
26. Faje A. Immunotherapy and hypophysitis: clinical presentation, treatment, and biologic insights. *Pituitary* (2016) 19(1):82–92. doi: 10.1007/s11102-015-0671-4
27. Kennedy LB, Salama AKS. A review of cancer immunotherapy toxicity. *CA Cancer J Clin* (2020) 70(2):86–104. doi: 10.3322/caac.21596
28. Larkin J, Chiarion-Sileni V, Gonzalez R, Grob JJ, Cowey CL, Lao CD, et al. Combined Nivolumab and Ipilimumab or Monotherapy in Untreated Melanoma. *N Engl J Med* (2015) 373(1):23–34. doi: 10.1056/NEJMoa1504030
29. Havel JJ, Chowell D, Chan TA. The evolving landscape of biomarkers for checkpoint inhibitor immunotherapy. *Nat Rev Cancer* (2019) 19(3):133–50. doi: 10.1038/s41568-019-0116-x
30. Gibney GT, Weiner LM, Atkins MB. Predictive biomarkers for checkpoint inhibitor-based immunotherapy. *Lancet Oncol* (2016) 17(12):e542–e51. doi: 10.1016/S1470-2045(16)30406-5
31. Giraldo NA, Sanchez-Salas R, Peske JD, Vano Y, Becht E, Petitprez F, et al. The clinical role of the TME in solid cancer. *Br J Cancer* (2019) 120(1):45–53. doi: 10.1038/s41416-018-0327-z

32. Clemente CG, Mihm MCJr., Bufalino R, Zurrida S, Collini P, Cascinelli N. Prognostic value of tumor infiltrating lymphocytes in the vertical growth phase of primary cutaneous melanoma. *Cancer* (1996) 77(7):1303–10. doi: 10.1002/(SICI)1097-0142(19960401)77:7<1303::AID-CNCR12>3.0.CO;2-5
33. Sato E, Olson SH, Ahn J, Bundy B, Nishikawa H, Qian F, et al. Intraepithelial CD8+ tumor-infiltrating lymphocytes and a high CD8+/regulatory T cell ratio are associated with favorable prognosis in ovarian cancer. *Proc Natl Acad Sci U S A* (2005) 102(51):18538–43. doi: 10.1073/pnas.0509182102
34. Smyrk TC, Watson P, Kaul K, Lynch HT. Tumor-infiltrating lymphocytes are a marker for microsatellite instability in colorectal carcinoma. *Cancer* (2001) 91 (12):2417–22. doi: 10.1002/1097-0142(20010615)91:12<2417::aid-cncr1276>3.0.co;2-u
35. Sharma P, Shen Y, Wen S, Yamada S, Jungbluth AA, Gnjatic S, et al. CD8 tumor-infiltrating lymphocytes are predictive of survival in muscle-invasive urothelial carcinoma. *Proc Natl Acad Sci U S A* (2007) 104(10):3967–72. doi: 10.1073/pnas.0611618104
36. Zhang QW, Liu L, Gong CY, Shi HS, Zeng YH, Wang XZ, et al. Prognostic significance of tumor-associated macrophages in solid tumor: a meta-analysis of the literature. *PLoS One* (2012) 7(12):e50946. doi: 10.1371/journal.pone.0050946
37. Binnewies M, Roberts EW, Kersten K, Chan V, Fearon DF, Merad M, et al. Understanding the tumor immune microenvironment (TIME) for effective therapy. *Nat Med* (2018) 24(5):541–50. doi: 10.1038/s41591-018-0014-x
38. Ilie MD, Vasiljevic A, Raverot G, Bertolino P. The Microenvironment of Pituitary Tumors-Biological and Therapeutic Implications. *Cancers (Basel)* (2019) 11(10):1605. doi: 10.3390/cancers11101605
39. Rossi ML, Jones NR, Esiri MM, Havas L, Izzi M, Coakham HB. Mononuclear cell infiltrate and HLA-Dr expression in 28 pituitary adenomas. *Tumori* (1990) 76(6):543–7. doi: 10.1177/030089169007600605
40. Lupi I, Manetti L, Caturegli P, Menicagli M, Cosottini M, Iannelli A, et al. Tumor infiltrating lymphocytes but not serum pituitary antibodies are associated with poor clinical outcome after surgery in patients with pituitary adenoma. *J Clin Endocrinol Metab* (2010) 95(1):289–96. doi: 10.1210/jc.2009-1583
41. Heshmati HM, Kujas M, Casanova S, Wollan PC, Racadot J, Van Effenterre R, et al. Prevalence of lymphocytic infiltrate in 1400 pituitary adenomas. *Endocr J* (1998) 45(3):357–61. doi: 10.1507/endocrj.45.357
42. Lu J-Q, Adam B, Jack AS, Lam A, Broad RW, Chik CL. Immune Cell Infiltrates in Pituitary Adenomas: More Macrophages in Larger Adenomas and More T Cells in Growth Hormone Adenomas. *Endocr Pathol* (2015) 26 (3):263–72. doi: 10.1007/s12022-015-9383-6
43. Mei Y, Bi WL, Greenwald NF, Du Z, Agar NYR, Kaiser UB, et al. Increased expression of programmed death ligand 1 (PD-L1) in human pituitary tumors. *Oncotarget* (2016) 7(47):76565–76. doi: 10.18632/oncotarget.12088
44. Wang P-F, Wang T-J, Yang Y-K, Yao K, Li Z, Li YM, et al. The expression profile of PD-L1 and CD8(+) lymphocyte in pituitary adenomas indicating for immunotherapy. *J Neuro Oncol* (2018) 139(1):89–95. doi: 10.1007/s11060-018-2844-2
45. Sato M, Tamura R, Tamura H, Mase T, Kosugi K, Morimoto Y, et al. Analysis of Tumor Angiogenesis and Immune Microenvironment in Non-Functional Pituitary Endocrine Tumors. *J Clin Med* (2019) 8(5):695. doi: 10.3390/jcm8050695
46. Barry S, Carlsen E, Marques P, Stiles CE, Gadaleta E, Berney DM, et al. Tumor microenvironment defines the invasive phenotype of AIP-mutation-positive pituitary tumors. *Oncogene* (2019) 38(27):5381–95. doi: 10.1038/s41388-019-0779-5
47. DeNardo DG, Ruffell B. Macrophages as regulators of tumour immunity and immunotherapy. *Nat Rev Immunol* (2019) 19(6):369–82. doi: 10.1038/s41577-019-0127-6
48. Suteau V, Collin A, Menei P, Rodien P, Rousselet MC, Briet C. Expression of programmed death-ligand 1 (PD-L1) in human pituitary neuroendocrine tumor. *Cancer Immunol Immunother* (2020) 69(10):2053–61. doi: 10.1007/s00262-020-02611-x
49. Uraki S, Ariyasu H, Doi A, Takeshima K, Morita S, Inaba H, et al. MSH6/2 and PD-L1 Expressions Are Associated with Tumor Growth and Invasiveness in Silent Pituitary Adenoma Subtypes. *Int J Mol Sci* (2020) 21(8):2831. doi: 10.3390/ijms21082831
50. Salomon MP, Wang X, Marzese DM, Hsu SC, Nelson N, Zhang X, et al. The epigenomic landscape of pituitary adenomas reveals specific alterations and differentiates among acromegaly, Cushing's disease and endocrine-inactive subtypes. *Clin Cancer Res* (2018) 24(17):4126–36. doi: 10.1158/1078-0432
51. Kemeny HR, Elsamadicy AA, Farber SH, Champion CD, Lorrey SJ, Chongsathidkiet P, et al. Targeting PD-L1 Initiates Effective Antitumor Immunity in a Murine Model of Cushing Disease. *Clin Cancer Res* (2020) 26(5):1141–51. doi: 10.1158/1078-0432.CCR-18-3486
52. Kim YH, Kim JH. Transcriptome Analysis Identifies an Attenuated Local Immune Response in Invasive Nonfunctioning Pituitary Adenomas. *Endocrinol Metab (Seoul)* (2019) 34(3):314–22. doi: 10.3803/EnM.2019.34.3.314
53. Srirangam Nadhamuni V, Korbonits M. Novel insights into Pituitary Tumorigenesis: Genetic and Epigenetic Mechanisms. *Endocr Rev* (2020) 41 (6):bnaa006. doi: 10.1210/endrev/bnaa006
54. Elsarrag M, Patel PD, Chatrath A, Taylor D, Jane JA. Genomic and molecular characterization of pituitary adenoma pathogenesis: review and translational opportunities. *Neurosurg Focus* (2020) 48(6):E11. doi: 10.3171/2020.3.FOCUS20104
55. Alshaikh OM, Asa SL, Mete O, Ezzat S. An Institutional Experience of Tumor Progression to Pituitary Carcinoma in a 15-Year Cohort of 1055 Consecutive Pituitary Neuroendocrine Tumors. *Endocr Pathol* (2019) 30(2):118–27. doi: 10.1007/s12022-019-9568-5
56. McCormack A, Dekkers OM, Petersenn S, Popovic V, Trouillas J, Raverot G, et al. Treatment of aggressive pituitary tumours and carcinomas: results of a European Society of Endocrinology (ESE) survey 2016. *Eur J Endocrinol* (2018) 178(3):265–76. doi: 10.1530/EJE-17-0933
57. Hui E. Immune checkpoint inhibitors. *J Cell Biol* (2019) 218(3):740–1. doi: 10.1083/jcb.201810035
58. de Miguel M, Calvo E. Clinical Challenges of Immune Checkpoint Inhibitors. *Cancer Cell* (2020) 38(3):326–33. doi: 10.1016/j.ccell.2020.07.004
59. Lin AL, Jonsson P, Tabar V, Yang TJ, Cuaron J, Beal K, et al. Marked Response of a Hypermutated ACTH-Secreting Pituitary Carcinoma to Ipilimumab and Nivolumab. *J Clin Endocrinol Metab* (2018) 103(10):3925–30. doi: 10.1210/jc.2018-01347
60. Cahill DP, Levine KK, Betensky RA, Codd PJ, Romany CA, Reavie LB, et al. Loss of the mismatch repair protein MSH6 in human glioblastomas is associated with tumor progression during temozolomide treatment. *Clin Cancer Res* (2007) 13(7):2038–45. doi: 10.1158/1078-0432.CCR-06-2149
61. Caccese M, Barbot M, Ceccato F, Padovan M, Gardiman MP, Fassan M, et al. Rapid disease progression in patient with mismatch-repair deficiency pituitary ACTH-secreting adenoma treated with checkpoint inhibitor pembrolizumab. *Anti Cancer Drugs* (2020) 31(2):199–204. doi: 10.1097/CAD.0000000000000856
62. Meng X, Huang Z, Teng F, Xing L, Yu J. Predictive biomarkers in PD-1/PD-L1 checkpoint blockade immunotherapy. *Cancer Treat Rev* (2015) 41(10):868–76. doi: 10.1016/j.ctrv.2015.11.001
63. Morris DG, Musat M, Czirjak S, Hanzely Z, Lillington DM, Korbonits M, et al. Differential gene expression in pituitary adenomas by oligonucleotide array analysis. *Eur J Endocrinol* (2005) 153(1):143–51. doi: 10.1530/eje.1.01937
64. Tong Y, Zheng Y, Zhou J, Oyesiku NM, Koeffler HP, Melmed S. Genomic characterization of human and rat prolactinomas. *Endocrinology* (2012) 153 (8):3679–91. doi: 10.1210/en.2012-1056
65. Yu SY, Hong LC, Feng J, Wu YT, Zhang YZ. Integrative proteomics and transcriptomics identify novel invasive-related biomarkers of non-functioning pituitary adenomas. *Tumour Biol* (2016) 37(7):8923–30. doi: 10.1007/s13277-015-4767-2
66. Michaelis KA, Knox AJ, Xu M, Kiseljak-Vassiliades K, Edwards MG, Geraci M, et al. Identification of growth arrest and DNA-damage-inducible gene beta (GADD45beta) as a novel tumor suppressor in pituitary gonadotrope tumors. *Endocrinology* (2011) 152(10):3603–13. doi: 10.1210/en.2011-0109
67. Yang Q, Wang Y, Zhang S, Tang J, Li F, Yin J, et al. Biomarker Discovery for Immunotherapy of Pituitary Adenomas: Enhanced Robustness and Prediction Ability by Modern Computational Tools. *Int J Mol Sci* (2019) 20(1):151. doi: 10.3390/ijms20010151
68. Kasuki L, Wildemberg LE, Gadelha MR. MANAGEMENT OF ENDOCRINE DISEASE: Personalized medicine in the treatment of acromegaly. *Eur J Endocrinol* (2018) 178(3):R89–R100. doi: 10.1530/EJE-17-1006
69. Lines KE, Stevenson M, Thakker RV. Animal models of pituitary neoplasia. *Mol Cell Endocrinol* (2016) 421:68–81. doi: 10.1016/j.mce.2015.08.024
70. Cristina C, Luque GM, Demarchi G, Lopez Vicchi F, Zubeldia-Brenner L, Perez Millan MI, et al. Angiogenesis in pituitary adenomas: human studies and new mutant mouse models. *Int J Endocrinol* (2014) 2014:608497. doi: 10.1155/2014/608497

71. Karamboulas C, Meens J, Ailles L. Establishment and Use of Patient-Derived Xenograft Models for Drug Testing in Head and Neck Squamous Cell Carcinoma. *STAR Protoc* (2020) 1(1):100024. doi: 10.1016/j.xpro.2020.100024
72. *Nivolumab and Ipilimumab in People With Aggressive Pituitary Tumors*. Available at: https://ClinicalTrials.gov/show/NCT04042753 (Accessed September 20, 2020).
73. *Nivolumab and Ipilimumab in Treating Patients With Rare Tumors*. Available at: https://ClinicalTrials.gov/show/NCT02834013 (Accessed September 20, 2020).

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Endocrinology

CASE REPORT
published: 17 December 2020
doi: 10.3389/fendo.2020.608886

Fluconazole for Hypercortisolism in Cushing's Disease: A Case Report and Literature Review

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Background: Cushing's disease is associated with an increased risk of pulmonary fungal infection, which could be a relative contraindication for pituitary adenoma excision surgery.

Case: We report a case of a patient with Cushing's disease and pulmonary *Cryptococcus neoformans*. A 48-year-old woman was admitted to our hospital because of moon face and edema. Laboratory and radiological findings suggested a diagnosis of Cushing's disease and pulmonary cryptococcus infection. Fluconazole 400 mg per day was administered intravenously and continued orally for 3 months. Both cryptococcus infection and hypercortisolism relieved and transsphenoidal resection was performed.

Conclusion: Cushing's disease can be effectively treated with fluconazole to normalize cortisol concentration prior to pituitary surgery. Fluconazole is an alternative treatment especially in Cushing's disease patients with cryptococcal pneumonia.

Keywords: Cushing's syndrome, Cushing's disease, fluconazole, hypercortisolism, medical treatment

OPEN ACCESS

Edited by:
Renzhi Wang, Peking Union Medical College Hospital (CAMS), China

Reviewed by:
Mauro Antonio Czepielewski, Federal University of Rio Grande do Sul, Brazil
Nina Rosa De Castro Musolino, Universidade de São Paulo, Brazil

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Specialty section:
This article was submitted to Pituitary Endocrinology, a section of the journal Frontiers in Endocrinology

Received: *21 September 2020*
Accepted: *12 November 2020*
Published: *17 December 2020*

Citation:
Zhao Y, Liang W, Cai F, Wu Q and Wang Y (2020) Fluconazole for Hypercortisolism in Cushing's Disease: A Case Report and Literature Review. Front. Endocrinol. 11:608886. doi: 10.3389/fendo.2020.608886

INTRODUCTION

Cushing's syndrome (CS) is a rare disorder caused by chronic hypercortisolism with multisystem morbidity, increased mortality and decreased quality of life (1). Surgical excision of the pituitary, adrenal or ectopic lesion is recommended as the first-line therapy of CS, but not all patients are eligible for surgery (2). Guideline recommends medical treatment in patients who are not candidates for surgery, or have recurrent disease, and in patients awaiting the effects of radiotherapy (2). Medication used in patients with CS can be classified into steroidogenesis inhibitors, pituitary-targeting agents, and glucocorticoid receptor antagonists (3). Steroidogenesis inhibitors can block various steps of the steroid synthesis pathway. Ketoconazole, the most widely used medication, is unavailable in most countries and regions because of the risk of severe hepatotoxicity (4). New steroidogenesis inhibitors, including osilodrostat and levoketoconazole, have shown efficacy and acceptable safety profiles in clinical trials (5, 6). Though osilodrostat is approved by US Food and Drug Administration (FDA), it has not been used widely due to unavailability (7). The present work demonstrates the effect of fluconazole in controlling hypercortisolism in a patient with Cushing's disease (CD) and pulmonary cryptococcus infection. All cases previously reported in the literature are also reviewed. In addition, a

comprehensive bioinformatics analysis has been done to identify the potential targets of fluconazole in CD.

CASE PRESENTATION

A 48-year-old woman visited the hospital for evaluation of facial swelling and fatigue lasting for more than one year. She reported 5 kg of weight gain, hypertension, insomnia, weakness, and easy bruising. She denied any fever, oligouria, chest distress or shortness of breath. She went to the local hospital and the examinations were unremarkable except "hypokalemia." She had been treated with irbesartan at a daily dose of 150 mg for 1 year. The social history and family history was unremarkable.

On examination, the blood pressure was 164/84 mm Hg, the pulse 56 beats per minute, the weight 53.7 kg, the height 146 cm and the body mass index (BMI) 25.19 kg/m^2. She had moon face, dorsal fat pad, abdominal obesity and ecchymosis, but no striae. The remainder of the examination was normal.

Initial investigations were summarized in **Table 1**. A midnight serum cortisol was 16.2 ng/dl and adrenocorticotropic hormone (ACTH) was 94.9 pg/ml 24-h urinary free cortisol (UFC) was 813.5 μg/day. Cortisol failed to suppress during a 48-h low-dose dexamethasone suppression test (16.1 ng/dl). These findings were consistent with an ACTH-dependent CS. No suppression was seen with high-dose dexamethasone test. A pituitary magnetic resonance imaging (MRI) demonstrated a 1.2 cm adenoma (**Figure 1A**). Inferior petrosal sinus (IPS) sampling confirmed a pituitary source of ACTH secretion. At the same time, the chest computed tomography revealed multiple lung nodules (**Figure 1B**).

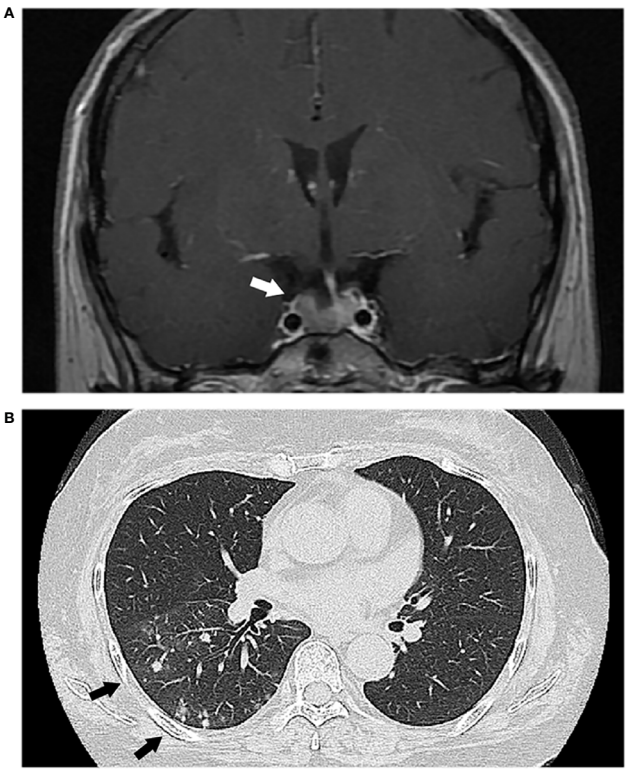

FIGURE 1 | **(A)** Magnetic resonance imaging showed a pituitary adenoma (white arrow). **(B)** Chest computed tomography revealed multiple lung nodules (black arrows).

TABLE 1 | Laboratory data.

Variables	Reference Range, Adults	At admission	Follow up (3 months after admission)	Follow up (3 months after neurosurgery)
24-h UFC (μg)	28.5–213.7	813.5	42.2	NA
8:00 AM cortisol (μg/dl)	6.7–22.5	17.0	1.4	18.7
4:00 PM cortisol (μg/dl)		23.2	1.2	11.3
Midnight cortisol (μg/dl)		16.2	NA	NA
8:00 AM ACTH (pg/ml)	7.2–63.3	85.9	53.2	56.9
4:00 PM ACTH (pg/ml)		109.1	43.3	33.9
Midnight ACTH(pg/ml)		94.9	NA	NA
Potassium (mmol/L)	3.5–5.5	3.34	3.52	4.24
ALT (U/L)	<35	23	21	NA
AST (U/L)	<35	22	22	NA
Aldosterone (pg/ml)	30–353	<30	NA	NA
Renin (uIU/ml)	4.4–46.1	11.6	NA	NA
Testosterone (nmol/L)	<2.53	1.88	1.52	NA
Low-dose dexamethasone suppression test cortisol (μg/dl)	<1.8	16.1	NA	NA
High-dose dexamethasone suppression test cortisol (μg/dl)		9.9	NA	NA
% Change from baseline	Expect >50% suppression for a positive test for Cushing's disease	41.9	NA	NA
IPS: peripheral ACTH ratio	Expect >2.0 in Cushing's disease	3.4	NA	NA
IPS: peripheral prolactin ratio	Expect >1.8 in successful catheterization	2.9	NA	NA

UFC, urinary free cortisol; ACTH, adrenocorticotropic hormone; AST, aspartate transaminase; ALT, alanine aminotransferase; IPS, Inferior petrosal sinus; NA, not available.

Bronchoalveolar lavage fluid (BALF) and serum cryptococcal antigen was positive. Lung biopsy histology confirmed pulmonary *Cryptococcus neoformans*. Cerebrospinal fluid (CSF) cryptococcal antigen was negative.

Immediate transsphenoidal selective adenomectomy (TSS) was not an option due to cryptococcus infection and possible postoperative cryptococcal meningitis. Intravenous fluconazole was started with 400 mg daily. One week later, the patient was discharged and continued with oral fluconazole 400 mg per day. At 3 months follow-up the pulmonary infection improved and the UFC was 42.2 μg/day. Fluconazole was withdrawn and TSS was performed. Histopathology confirmed ACTH-secreting pituitary adenoma.

Three months after TSS, the patient was in good general health and her serum cortisol and UFC normalized.

DISCUSSION

TSS performed by an experienced neurosurgeon is the first-line treatment for CD in most patients (2). Patients with CD have an increased risk of cyrptococcal infection (8). Undiagnosed cryptococcal pneumonia can lead to postoperative cryptococcal meningitis and poor prognosis (9). Medical therapy is needed in patients with hypercortisolism when surgery is not possible. Ketoconazole, which is used to control both hypercortisolism and fungal infections, is unavailable due to severe adverse effect. Fluconazole, another azole antifungal, is an alternative to ketoconazole with less hepatotoxicity.

The present work revealed only 5 other CS patients treated successfully by fluconazole when surgery was not possible or was noncurative (as showed in **Table 2**) (10–14). All of patients were female, which could be attributed to the female predominant prevalence of CS (female-to-male ratio 3:1) (1). Three of them were diagnosed as CD, two with ectopic ATCH-secreting syndrome (EAS), and one had adrenal carcinoma. The median UFC levels was 310 μg/day (range, 112–813.45 μg/day). Hypocortisolism was more prone to occur in CD patients instead of other kinds of CS. The reported dose of fluconazole ranged from 200 to 1200 mg/day. Two of them developed liver dysfunction with the daily dose of fluconazole more than 400 mg. Liver enzymes normalized after the dose decreased to 400 mg/day.

In previous reports, inhibitory effect of fluconazole on glucocorticoid production was controversial and less potent than ketoconazole (10, 15, 16). Some case reports showed adrenal dysfunction with fluconazole in patients with server comorbidities (17–20). However, another study suggested that fluconazole was not associated with adrenal insufficiency compared with placebo (21).

The different in vitro effect of fluconazole could be attributed to the different experimental cell lines and different expression of steroid synthesizing enzymes of the cells. Previous studies demonstrated potent inhibitory effect of fluconazole on cortisol production in several human cell culture lines, but only a weak effect in rat cell culture lines (10, 15, 16). A previous study has revealed that the polymorphism in the CYP17A1 gene is

TABLE 2 | Clinical features of 6 patients with CS successfully treated with fluconazole.

Patient	Age	Race	CS	24 h UFC (μg)	ACTH (pg/ml)	Serum cortisol (μg/dl)	Comorbidities	Treatment of CS	Dose of fluconazole	AE	FU 24 h UFC (μg)	Outcome
1 (our patient)	48	Asian	CD	813.5	85.9	17.1	Cryptococcal pneumonia	Fluconazole and pituitary adenoma excision	400 mg QD	NA	42.2	Relieved
2 (10)	83	NA	Adrenal carcinoma	295	NA	19.4	Sepsis	Adrenalectomy and fluconazole	200–400 mg QD	NA	8.4	Stable
3 (11)	61	Asian	CD	273	99.0	34.0	NA	Pituitary adenoma excision, metyrapone, ketoconazole and fluconazole	400 mg QD	NA	Normal	Stable
4 (12)	50	Asian	CD	325	NA	27.5	Hypertensive basal ganglia hemorrhage	Ketoconazole, cabergoline and fluconazole	400 mg QD	NA	56	Relieved
5 (13)	39	NA	EAS	775	572.9	175.8	Gastrointestinal tract bleeding	Fluconazole and bilateral adrenalectomy	400–600–400 mg QD	Increased liver enzyme levels	NA	Relieved
6 (14)	80	NA	EAS	112	378.0	6.0	Myelodysplasia	Fluconazole	200–1,200–400 mg QD	Increased liver enzyme levels	NA*	Died

**Normalization of cortisol levels.*

CS, Cushing's syndrome; UFC, urinary free cortisol; ACTH, adrenocorticotropic hormone; AE, adverse effect; FU, follow-up; CD = Cushing's disease; EAS, ectopic ATCH-secreting syndrome; NA, not available.

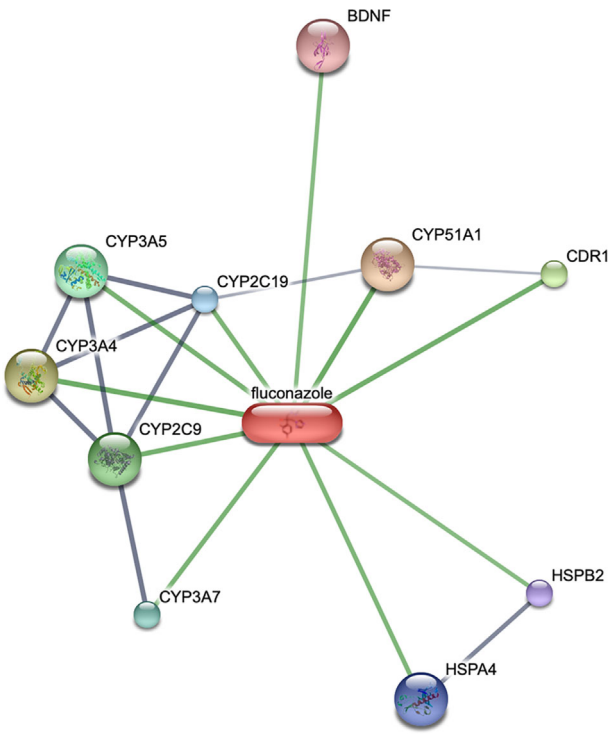

FIGURE 2 | Potential targets of fluconazole and Cushing's disease from STITCH. The node size reflects the degree of relationship: the smaller the degree value, the smaller the node size is.

associated with the responsiveness to steroidogenesis inhibitors in CS patients (22). However, the relationship of genetic variants with the efficacy of fluconazole is unknown.

We predict potential targets of fluconazole and obtained a network from STITCH (**Figure 2**). STITCH (http://stitch.embl.de) is a unique system pharmacological database describing the relationships between drugs, targets, and diseases (23). The results showed brain-derived neurotrophic factor (BDNF) was a target of fluconazole. BDNF is the neurotrophin mediating pro-neuronal survival and plasticity. Fiocco's study showed BDNF regulated the activity of hypothalamic-pituitary-adrenal axis (24). Similar studies showed BDNF polymorphism had an influence on individual cortisol response to stress (25, 26). In Cushing disease patients, decreased BDNF level was observed after remission indicating a potential role of BDNF in Cushing disease (27). Over the past two decades, studies showed that BDNF and its receptor tropomyosin receptor kinase B (TrkB) were up-regulated in many types of cancers such as breast cancer and cervical cancer (28–30). The activated BDNF/TrkB signal stimulates a series of downstream pathways, including phosphoinositide 3-kinase/protein kinase (PI3K), Ras-Raf-mitogen activated protein kinase kinase-extracellular signal-regulated kinases, the phospholipase-C-γ pathway and the transactivation of epidermal growth factor receptor (28). Dworakowska's study showed PI3K pathway was upregulated in ACTH- pituitary adenomas (31). Song's study showed PI3K/AKT signaling pathway could affect migration and invasion of pituitary adenoma (32). In this series, two patients with fluconazole therapy showed decreased ACTH levers, which could not be attributed to effects on adrenocortical steroidogenesis (14). We propose the following hypothesis, BDNF/TrkB and PI3K pathways are potent therapeutic mechanisms for fluconazole in Cushing disease. In the future, more in vitro and in vivo studies are needed to verify our findings.

CONCLUDING REMARKS

In summary, this case report and the bioinformatics analysis suggest that fluconazole might be effective in controlling hypercorticolism in CD patients. Further studies on the mechanism by which fluconazole inhibits cortisol production are needed to develop more potent and less toxic agents.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The patient provided written informed consent for research participation as well as for the publication of indirectly identifiable data (age, gender, and medical history).

AUTHOR CONTRIBUTIONS

YZ and WL wrote the first draft of the manuscript. YZ, QW, FC, and YW made contributions to the acquisition of the clinical data. YZ and YW made critical revisions and approved final version. All authors contributed to the article and approved the submitted version.

FUNDING

This study was supported by the Natural Science Foundation of Zhejiang Province (LQ19H160024).

REFERENCES

1. Lacroix A, Feelders RA, Stratakis CA, Nieman LK. Cushing's syndrome. *Lancet* (2015) 386(9996):913–27. doi: 10.1016/s0140-6736(14)61375-1

2. Nieman LK, Biller BM, Findling JW, Murad MH, Newell-Price J, Savage MO, et al. Treatment of Cushing's Syndrome: An Endocrine Society Clinical Practice Guideline. *J Clin Endocrinol Metab* (2015) 100(8):2807–31. doi: 10.1210/jc.2015-1818

3. Feelders RA, Newell-Price J, Pivonello R, Nieman LK, Hofland LJ, Lacroix A. Advances in the medical treatment of Cushing's syndrome. *Lancet Diabetes Endocrinol* (2019) 7(4):300–12. doi: 10.1016/s2213-8587(18)30155-4
4. McCance DR, Ritchie CM, Sheridan B, Atkinson AB. Acute hypoadrenalism and hepatotoxicity after treatment with ketoconazole. *Lancet* (1987) 1 (8532):573. doi: 10.1016/s0140-6736(87)90222-4
5. Biller BMK, Newell-Price J, Fleseriu M, Bertagna X, Findling J, Shimatsu A, et al. OR16-2 Osilodrostat Treatment in Cushing's Disease (CD): Results from a Phase III, Multicenter, Double-Blind, Randomized Withdrawal Study (LINC 3). *J Endocr Soc* (2019) 3(Suppl 1):OR16–2. doi: 10.1210/js.2019-OR16-2
6. Fleseriu M, Pivonello R, Elenkova A, Salvatori R, Auchus RJ, Feelders RA, et al. Efficacy and safety of levoketoconazole in the treatment of endogenous Cushing's syndrome (SONICS): a phase 3, multicentre, open-label, single-arm trial. *Lancet Diabetes Endocrinol* (2019) 7(11):855–65. doi: 10.1016/s2213-8587(19)30313-4
7. Voelker R. New Therapy Offers Direct Cortisol Control for Cushing Disease. *Jama* (2020) 323(15):1436. doi: 10.1001/jama.2020.5157
8. Lu L, Zhao YY, Yang HB, Tian XL, Xu ZJ, Lu ZL. Cushing's disease with pulmonary Cryptococcus neoformans infection in a single center in Beijing, China: A retrospective study and literature review. *J Formosan Med Assoc = Taiwan yi zhi* (2019) 118(1 Pt 2):285–90. doi: 10.1016/j.jfma.2018.05.005
9. Liu Y, Feng M, Yao Y, Deng K, Bao X, Liu X, et al. Cryptococcal meningitis after transnasal transsphenoidal pituitary microsurgery of ACTH-secreting pituitary adenoma: A case report. *Med (Baltimore)* (2017) 96(28):e7124. doi: 10.1097/md.0000000000007124
10. Riedl M, Maier C, Zettinig G, Nowotny P, Schima W, Luger A. Long term control of hypercortisolism with fluconazole: case report and in vitro studies. *Eur J Endocrinol* (2006) 154(4):519–24. doi: 10.1530/eje.1.02120
11. Burns K, Christie-David D, Gunton JE. Fluconazole in the treatment of Cushing's disease. *Endocrinol Diabetes Metab Case Rep* (2016) 2016:150115. doi: 10.1530/edm-15-0115
12. Teng Chai S, Haydar Ali Tajuddin A, N AW, Mustafa N, Sukor N, Kamaruddin NA. Fluconazole as a Safe and Effective Alternative to Ketoconazole in Controlling Hypercortisolism of Recurrent Cushing's Disease: A Case Report. *Int J Endocrinol Metab* (2018) 16(3):e65233. doi: 10.5812/ijem.65233
13. Canteros TM, De Miguel V, Fainstein-Day P. Fluconazole treatment in severe ectopic Cushing syndrome. *Endocrinol Diabetes Metab Case Rep* (2019) 2019 (1). doi: 10.1530/edm-19-0020
14. Schwetz V, Aberer F, Stiegler C, RP T, Obermayer-Pietsch B, Pilz S. Fluconazole and acetazolamide in the treatment of ectopic Cushing's syndrome with severe metabolic alkalosis. *Endocrinol Diabetes Metab Case Rep* (2015) 2015:150027. doi: 10.1530/edm-15-0027
15. Eckhoff C, Oelkers W, Bähr V. Effects of two oral antimycotics, ketoconazole and fluconazole, upon steroidogenesis in rat adrenal cells in vitro. *J Steroid Biochem* (1988) 31(5):819–23. doi: 10.1016/0022-4731(88)90291-9
16. van der Pas R, Hofland LJ, Hofland J, Taylor AE, Arlt W, Steenbergen J, et al. Fluconazole inhibits human adrenocortical steroidogenesis in vitro. *J Endocrinol* (2012) 215(3):403–12. doi: 10.1530/joe-12-0310
17. Albert SG, DeLeon MJ, Silverberg AB. Possible association between high-dose fluconazole and adrenal insufficiency in critically ill patients. *Crit Care Med* (2001) 29(3):668–70. doi: 10.1097/00003246-200103000-00039
18. Shibata S, Kami M, Kanda Y, Machida U, Iwata H, Kishi Y, et al. Acute adrenal failure associated with fluconazole after administration of high-dose cyclophosphamide. *Am J Hematol* (2001) 66(4):303–5. doi: 10.1002/ajh.1063
19. Huang YW, Chang CC, Sun HY, Chen MY, Hung CC, Chang SC. Primary adrenal insufficiency in patients with acquired immunodeficiency syndrome: report of four cases. *J Microbiol Immunol Infect = Wei mian yu gan ran za zhi* (2004) 37(4):250–3.
20. Santhana Krishnan SG, Cobbs RK. Reversible acute adrenal insufficiency caused by fluconazole in a critically ill patient. *Postgrad Med J* (2006) 82(971): e23. doi: 10.1136/pgmj.2006.047258
21. Magill SS, Puthanakit T, Swoboda SM, Carson KA, Salvatori R, Lipsett PA, et al. Impact of fluconazole prophylaxis on cortisol levels in critically ill surgical patients. *Antimicrob Agents Chemother* (2004) 48(7):2471–6. doi: 10.1128/aac.48.7.2471-2476.2004
22. Valassi E, Aulinas A, Glad CA, Johannsson G, Ragnarsson O, Webb SM. A polymorphism in the CYP17A1 gene influences the therapeutic response to steroidogenesis inhibitors in Cushing's syndrome. *Clin Endocrinol (Oxf)* (2017) 87(5):433–9. doi: 10.1111/cen.13414
23. Szklarczyk D, Santos A, von Mering C, Jensen LJ, Bork P, Kuhn M. STITCH 5: augmenting protein-chemical interaction networks with tissue and affinity data. *Nucleic Acids Res* (2016) 44(D1):D380–4. doi: 10.1093/nar/gkv1277
24. Fiocco AJ, D'Amico D, de Beaumont L, Poirier J, Lupien S. Association between BDNF Polymorphism and Hypothalamic-Pituitary-Adrenal Activity in Later Adulthood. *Gerontology* (2020) 66(2):131–7. doi: 10.1159/000502143
25. de Assis GG, Gasanov EV. BDNF and Cortisol integrative system—Plasticity vs. degeneration: Implications of the Val66Met polymorphism. *Front Neuroendocrinol* (2019) 55:100784. doi: 10.1016/j.yfrne.2019.100784
26. Tsuru J, Tanaka Y, Ishitobi Y, Maruyama Y, Inoue A, Kawano A, et al. Association of BDNF Val66Met polymorphism with HPA and SAM axis reactivity to psychological and physical stress. *Neuropsychiatr Dis Treat* (2014) 10:2123–33. doi: 10.2147/NDT.S68629
27. Valassi E, Crespo I, Keevil BG, Aulinas A, Urgell E, Santos A, et al. Affective alterations in patients with Cushing's syndrome in remission are associated with decreased BDNF and cortisone levels. *Eur J Endocrinol* (2017) 176 (2):221–31. doi: 10.1530/EJE-16-0779
28. Meng L, Liu B, Ji R, Jiang X, Yan X, Xin Y. Targeting the BDNF/TrkB pathway for the treatment of tumors. *Oncol Lett* (2019) 17(2):2031–9. doi: 10.3892/ol.2018.9854
29. Yuan Y, Ye HQ, Ren QC. Proliferative role of BDNF/TrkB signaling is associated with anoikis resistance in cervical cancer. *Oncol Rep* (2018) 40 (2):621–34. doi: 10.3892/or.2018.6515
30. Tajbakhsh A, Mokhtari-Zaer A, Rezaee M, Afzaljavan F, Rivandi M, Hassanian SM, et al. Therapeutic Potentials of BDNF/TrkB in Breast Cancer; Current Status and Perspectives. *J Cell Biochem* (2017) 118 (9):2502–15. doi: 10.1002/jcb.25943
31. Dworakowska D, Wlodek E, Leontiou CA, Igreja S, Cakir M, Teng M, et al. Activation of RAF/MEK/ERK and PI3K/AKT/mTOR pathways in pituitary adenomas and their effects on downstream effectors. *Endocr Relat Cancer* (2009) 16(4):1329–38. doi: 10.1677/ERC-09-0101
32. Zheng Z, Zhang Y, Zhang Z, Yang Y, Song T. Effect of miR-106b on Invasiveness of Pituitary Adenoma via PTEN-PI3K/AKT. *Med Sci Monit* (2017) 23:1277–85. doi: 10.12659/msm.900092

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Endocrinology

OPINION
published: 05 January 2021
doi: 10.3389/fendo.2020.607405

Is Seed and Soil Theory Suitable for Metastatic Spread of Pituitary Carcinomas?

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Keywords: pituitary carcinoma, metastatic spread, seed and soil theory, systemical spread, craniospinal spread

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Specialty section:
This article was submitted to Pituitary Endocrinology, a section of the journal Frontiers in Endocrinology

Received: *17 September 2020*
Accepted: *19 November 2020*
Published: *05 January 2021*

Citation:
Dai C, Sun B and Kang J (2021) Is Seed and Soil Theory Suitable for Metastatic Spread of Pituitary Carcinomas? Front. Endocrinol. 11:607405. doi: 10.3389/fendo.2020.607405

Pituitary carcinomas (PCs) are defined as tumors of adenohypophyseal origin that show craniospinal and/or systemic metastases (1). PCs are extremely rare, making up only 0.1% to 0.2% of all pituitary tumors, but represent a challenge to clinical practice (2). Although research on PCs has progressed rapidly, the pathogenesis and biology of pituitary carcinomas have not yet been well understood due to their rarity. Although some hypotheses have been postulated, they remain controversial. To date, the patterns of metastatic spread in PCs are complex and not yet fully elucidated, needing further research.

The "seed and soil" theory of metastasis was proposed by Paget in 1889. Based on analysis of autopsies in 735 fatal cases of breast cancer, Paget suggested that the spread of metastatic cells was organ-specific and not merely anatomical and involved interaction between the cancer cells and the host organ and that metastases developed only when the seed and soil were compatible (3). After proposal of the "seed and soil" theory, it was validated and supported by abundant animal studies (4–6). In the past 130 years, it has been proven that organ-specific patterns of metastases exist in many cancers (7). However, the concept of "seed and soil" has not yet been discussed in the metastasis of PCs thus far. The purpose of this commentary is to provide a brief perspective on our understanding of the mechanisms of metastasis of PCs and discuss whether the "seed and soil" theory is suitable for PCs.

Currently, there are three possible mechanisms of metastatic spread in PCs, including drop metastasis (occurrence in surgical tract), blood-borne dissemination, and central venous dissemination (8). Although tumor cells dropping from the surgical site and drifting *via* the cerebrospinal fluid pathway occurs mostly during surgery, overall PCs tend toward systemic metastasis rather than craniospinal metastasis. The frequency of systemic metastasis is approximately 47% in overall PCs, the frequency of craniospinal metastases in PCs is approximately 40%, and the other 13% of PCs exhibit both (9). Most PCs (88%) are functional and commonly include adrenocorticotropic hormone (ACTH)- (42%) and prolactin (PRL)-secreting tumors (33%), whereas different types of PCs prefer different patterns of metastatic spread. In one series, PRL-secreting PCs spread systemically more often (71%) than ACTH-secreting PCs (57%); in contrast, growth hormone (GH)-secreting carcinomas present more commonly with cerebrospinal metastasis (10). The sites of PC metastasis include the cerebral cortex, cerebellum, spinal cord, leptomeninges, eyes, heart, lung, cervical lymph nodes, pancreas, liver, kidney, pelvic lymph nodes, ovary, myometrium, and bone. The most common locations of

Abbreviations: PCs, pituitary carcinomas; PAs, pituitary adenomas; ACTH, adrenocorticotropic hormone; PRL, prolactin; GH, growth hormone.

metastasis are extracranial or intracranial and spinal spread, but the preferential specific site for metastasis remains uncertain due to the limited number of reported cases (11).

The "seed and soil" theory was proposed based on large-scale autopsy of patients with fatal breast cancer, whereas PCs are very rare tumors, with only 170 cases reported in the literature (9). Thus, it is difficult to verify whether this theory is suitable for metastatic spread of PCs *via* large-scale clinical case analysis. However, we can obtain some useful information from limited cases and surmise some possible conclusions.

First, the vast majority of reported PCs are endocrinologically active, including ACTH-, and PRL-secreting carcinomas, which are the most common. Although dural invasion is most frequently found in nonfunctioning PAs at 54.2%, the incidence of nonfunctioning PCs is very low (12). A literature review of PCs reported that the most common tumors were ACTH-secreting (34.7%), PRL-secreting (23.6%), and Null Cell (15.3%) respectively (13). In this review, the most common site of metastasis in total 25 cases of ACTH-secreting PCs were intracranial, liver, and spinal respectively, whereas the most common site of metastasis in 17 cases of PRL-secreting PCs was intracranial, lymph nodes, and bones respectively. This review indicated more CNS metastases noted in the cases reviewed than systemic metastases. However, the other previous reviews reported that systemic metastases appear more common than CNS metastases in PCs (9, 14). Another large retrospective series also reported that ACTH-secreting and PRL-secreting PCs showed a greater tendency toward systemic metastasis than central nervous system metastasis (10). In addition to intracranial, the metastasis of ACTH-secreting carcinomas has a predilection for the liver and spinal (10, 15, 16), and the metastasis of PRL-secreting carcinomas exhibits preferences for lymph nodes and bones (9–11, 16, 17). A possible explanation is that the different hormone-secreting tumor cells have their own suitable microenvironment and favorable host organ, thus selectively invading compatible organs and growing in a proper environment. Therefore, these results indicate that the spread of metastatic PC cells is organ-specific and involves interactions between cancer cells and the host organ, not merely through the anatomic structure. However, this conclusion needs to be further validated by more cases of PCs and animal studies.

Second, almost all PCs present initially with an invasive pituitary macroadenoma, and metastatic PC cells have also been isolated from both cerebrospinal and pleural effusion fluid (9). Interestingly, most PCs tend to disseminate systemically *via* hematogenous and lymphatic spread rather than *via* craniospinal spread. In addition, there is no genetic, molecular, or histologic distinction between PCs and invasive or aggressive PAs. Moreover, the PRL-secreting tumors transformed to PCs twice as fast as the ACTH-secreting tumors (4.7 vs 9.5 years, respectively) (10).

Therefore, these data demonstrate that the process of metastasis starts with the release of malignant cells (seed) with metastatic potential from primary tumors, and different types of metastatic PC cells need different durations of metastatic latency to invade favorable host tissues (soil).

Together, these data support that different types of PCs prefer different patterns of metastatic spread and have their own favorable host organs. Therefore, as a viable mechanism, the seed and soil theory may be suitable for the metastatic spread of PCs. However, this conclusion was obtained based on limited cases of PCs reported in the literature and needs to be validated and supported by more cases and animal studies.

AUTHOR CONTRIBUTIONS

CD and BS drafted the manuscript and carried out the literature search. JK reviewed the manuscript. All authors contributed to the article and approved the submitted version.

REFERENCES

1. Ilie MD, Jouanneau E, Raverot G. Aggressive Pituitary Adenomas and Carcinomas. *Endocrinol Metab Clin* (2020) 49(3):505–15. doi: 10.1016/j.ecl.2020.05.008
2. Raverot G, Burman P, McCormack A, Heaney A, Petersenn S, Popovic V, et al. European Society of Endocrinology Clinical Practice Guidelines for the management of aggressive pituitary tumours and carcinomas. *Eur J Endocrinol* (2017) 178(1):G1–G24. doi: 10.1530/EJE-17-0796
3. Paget S. The distribution of secondary growths in cancer of the breast. 1889. *Cancer Metastasis Rev* (1989) 8(2):98–101. doi: 10.00000/2673568
4. Hart IR, Fidler IJ. Role of organ selectivity in the determination of metastatic patterns of B16 melanoma. *Cancer Res* (1980) 40(7):2281–7. doi: 10.00000/7388794
5. KINSEY DL. An experimental study of preferential metastasis. *Cancer* (1960) 13:674–6. doi: 10.1002/1097-0142(196007/08)13:4<674::aid-cncr2820130405>3.0.co;2-q
6. SUGARBAKER ED. The organ selectivity of experimentally induced metastases in rats. *Cancer* (1952) 5(3):606–12. doi: 10.1002/1097-0142(195205)5:3<606::aid-cncr2820050324>3.0.co;2-u
7. Akhtar M, Haider A, Rashid S, Al-Nabet ADMH. Paget's "Seed and Soil" Theory of Cancer Metastasis: An Idea Whose Time has Come. *Adv Anat Pathol* (2019) 26(1):69–74. doi: 10.1097/PAP.0000000000000219
8. KOYAMA J, IKEDA K, SHOSE Y, KIMURA M, OBORA Y, KOHMURA E. Long-term Survival With Non-functioning Pituitary Carcinoma-Case Report. *Neurol Med Chir* (2007) 47(10):475–8. doi: 10.2176/nmc.47.475
9. Heaney AP. Pituitary Carcinoma: Difficult Diagnosis and Treatment. *J Clin Endocrinol Metab* (2011) 96(12):3649–60. doi: 10.1210/jc.2011-2031
10. Pernicone PJ, Scheithauer BW, Sebo TJ, Kovacs KT, Horvath E, Young WJ, et al. Pituitary carcinoma: a clinicopathologic study of 15 cases. *Cancer* (1997) 79(4):804–12. doi: 10.1002/(sici)1097-0142(19970215)79:4<804::aid-cncr18>3.0.co;2-3
11. Lamas C, Nunez R, Garcia-Uria J, Salas C, Saucedo G, Estrada J, et al. Malignant prolactinoma with multiple bone and pulmonary metastases. Case report. *J Neurosurg* (2004) 101(1 Suppl):116–21. doi: 10.3171/ped.2004.101.2.0116
12. Oh MC, Tihan T, Kunwar S, Blevins L, Aghi MK. Clinical Management of Pituitary Carcinomas. *Neurosurg Clin N Am* (2012) 23(4):595–606. doi: 10.1016/j.nec.2012.06.009
13. Yoo F, Kuan EC, Heaney AP, Bergsneider M, Wang MB. Corticotrophic pituitary carcinoma with cervical metastases: case series and literature review. *Pituitary* (2018) 21(3):290–301. doi: 10.1007/s11102-018-0872-8
14. Kaltsas GA, Nomikos P, Kontogeorgos G, Buchfelder M, Grossman AB. Diagnosis and Management of Pituitary Carcinomas. *J Clin Endocrinol Metab* (2005) 90(5):3089–99. doi: 10.1210/jc.2004-2231

15. Venable ER, Kerr SE, Lopes MBS, Jones KA, Bellizzi AM, Mounajjed T, et al. Liver metastases from pituitary carcinomas mimicking visceral well-differentiated neuroendocrine tumors: a series of four cases. *Diagn Pathol* (2020) 15(1):81–8. doi: 10.1186/s13000-020-00997-x
16. Alshaikh OM, Asa SL, Mete O, Ezzat S. An Institutional Experience of Tumor Progression to Pituitary Carcinoma in a 15-Year Cohort of 1055 Consecutive Pituitary Neuroendocrine Tumors. *Endocr Pathol* (2019) 30(2):118–27. doi: 10.1007/s12022-019-9568-5
17. Moscote-Salazar LR, Satyarthee GD, Calderon-Miranda WG, Matus JA, Pacheco-Hernandez A, Puac-Polanco PC, et al. Prolactin Secreting Pituitary Carcinoma with Extracranial Spread Presenting with Pathological Fracture of Femur. *J Neurosci Rural Pract* (2018) 9(1):170–3. doi: 10.4103/jnrp.jnrp_325_17

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The reviewer CY declared a shared affiliation with the authors to the handling editor at time of review.

frontiers in Endocrinology

ORIGINAL RESEARCH
published: 18 January 2021
doi: 10.3389/fendo.2020.601984

Regulation of the EGFR Pathway by HSP90 Is Involved in the Pathogenesis of Cushing's Disease

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OPEN ACCESS

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Reviewed by:
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Specialty section:
This article was submitted to Pituitary Endocrinology, a section of the journal Frontiers in Endocrinology

Received: 02 September 2020
Accepted: 01 December 2020
Published: 18 January 2021

Citation:
Shen Y, Ji C, Jian X, Zhou J, Zhang Q, Qiao N, Zhang Y, Shou X, Zhou X and Ma Z (2021) Regulation of the EGFR Pathway by HSP90 Is Involved in the Pathogenesis of Cushing's Disease. Front. Endocrinol. 11:601984. doi: 10.3389/fendo.2020.601984

Purpose: To investigate the role of heat-shock protein Hsp90 in adrenocorticotropic hormone (ACTH)-secreting cells, and to explore the potential clinical application of an inhibitor of Hsp90, 17-N-allylamino-17-demethoxygeldanamycin(17-AAG) in corticotropinomas [also known as "Cushing's disease" (CD)].

Methods: Culture of mouse pituitary tumor [AtT-20/D16v-F2 (ATCC® CRL-1795™)] cells and human pituitary ACTH-secreting tumor cells were employed. Hepatocellular carcinoma cell line (HLE) was used to evaluate EGFR inhibition by 17-AAG. Cell viability was evaluated using a commercial kit. The ACTH level was measured by a radioimmunoassay. Reverse transcription quantitative polymerase chain reaction (RT-qPCR) was used to measure expression of proopiomelanocortin (POMC) mRNA. Western blotting was done to measure protein levels.

Results: 17-AAG suppressed the viability and proliferation, and promoted the apoptosis, of AtT-20/D16v-F2 cells. 17-AAG suppressed the synthesis and secretion of ACTH in AtT-20/D16v-F2 cells and down-regulated POMC transcription. 17-AAG acted in a similar pattern upon treatment with human pituitary ACTH-secreting tumor cells. Inhibition by 17-AAG was stronger in human pituitary ACTH-secreting tumor cells carrying the ubiquitin-specific protease-8 (*USP8*) mutant in comparison with cells carrying wild-type *USP8*.

Conclusions: The HSP90 inhibitor 17-AAG reduced the viability and secretory function of human pituitary ACTH-secreting tumor cells, and tumor cells carrying the *USP8* mutant were more sensitive to 17-AAG than tumor cells carrying wild-type *USP8*. 17-AAG could be a potential treatment option for CD.

Keywords: epidermal growth factor receptor, Cushing's disease, Hsp90, 17-N-allylamino-17-demethoxygeldanamycin/tanespimycin, pathogenesis

INTRODUCTION

Pituitary adenoma is one of the most common tumors appearing at sella. Corticotropinoma accounts for about 2%–6% of pituitary adenomas (1). Corticotropinoma (Cushing disease) is responsible for ~85% of cases of Cushing's syndrome (2). The clinical manifestations of hypercortisolemia are weight gain, fat redistribution, central obesity, purple striae, ecchymoses, muscle atrophy, and related complications [e.g., hypertension, diabetes mellitus, hyperlipidemia, mental/psychological disorders, osteoporosis, deep-vein thrombosis (2)].

First-line treatment for CD is transsphenoidal surgery, which carries a remission rate of 80%–90% and recurrence of approximately 10–20% (3). Patients with persistent disease can undergo secondary procedure, which carries a remission rate of ≤50% (4). Radiotherapy or medication are options for patients who cannot achieve remission by surgery (5).

Pharmacologic therapy includes drugs that target pituitary tumors, such as dopamine-receptor agonists [e.g., cabergoline (6), octreotide (7)], the adrenal gland [e.g., ketoconazole, metoprolol, metyrapone (5, 8, 9)], or cortisol receptors in peripheral tissues [e.g., mifepristone (10)]. Patients who do not respond to such treatment can undergo bilateral adrenalectomy, which results in permanent adrenal insufficiency (3).

In 2015, we revealed a highly frequent somatic mutation of *USP8* (which encodes ubiquitin-specific protease-8); nearly 60% of CD patients carried this gain-of-function mutation (11). This mutant interfered with the interaction between USP8 and the 14-3-3 motif, resulting in the reduction of the degradation of epidermal growth factor receptors (EGFRs). Therefore, ACTH-secreting adenomas with a *USP8* mutation displayed higher expression of the EGFR, and increased mRNA transcription of proopiomelanocortin (POMC), which is the precursor of ACTH (11, 12). ACTH-secreting adenomas carrying the *USP8* mutation were significantly smaller than genome-wild adenomas, which indicated greater secretion of ACTH (11). Research on primary tumor cells demonstrated that knockdown of USP8 expression or EGFR blockade could inhibit ACTH secretion effectively. Inhibition of expression of USP8 or EGFR could be a potential therapeutic target for patients with corticotropinomas carrying the *USP8* mutation (11, 13).

Heat-shock protein Hsp90 is an important "molecular chaperone" that serves as a key regulator of protein function in eukaryotic cells during stress. Hundreds of "client" proteins interfere with HSP90 and regulate conformational changes in Hsp90 during DNA repair, the immune response, and neurodegenerative changes. Because HSP90 plays an important part in several intracellular processes, it has become a potential target for treatment of various tumor types, as well as neurodegenerative diseases and autoimmune diseases (14).

17-N-allylamino-17-demethoxygeldanamycin (17-AAG) is a derivative of the antibiotic geldanamycin. 17-AAG is an HSP inhibitor, and binds to the N-terminus of HSP90; 17-AAG is being studied as an anti-tumor antibiotic (15). Several studies have shown that HSP90 inhibitors display certain therapeutic effects on immunogenic tumors (e.g., lymphomas, melanomas) and some solid tumors [e.g., liver cancer, breast cancer, non-small-cell lung cancer (16, 17)]. Studies have also suggested that inhibition of HSP90 expression can down-regulate expression of EGFR mutants (18–20) and become potential second-line treatment for non-small-cell lung cancers (21–23).

Based on the previous research of high-frequency mutations USP8 in corticotropinoma, this study tended to explore the regulation of the EGFR pathway by HSP90 involved in the pathogenesis of the tumor, discover potential treatment for Cushing's disease, and provide evidence for precise treatment.

METHODS

Cell Culture

Mouse pituitary tumor [AtT-20/D16v-F2 (ATCC® CRL-1795™)] cells (American Type Culture Collection Manassas, VA, USA) were cultured in 75 cm^2 flasks with Dulbecco's modified Eagle's medium (DMEM; Gibco, Billings, MT, USA), 10% fetal bovine serum (Gibco), and 100 U/ml of penicillin–streptomycin (Invitrogen, Carlsbad, CA, USA) in an atmosphere of 5% CO_2/95% air at 37°C. AtT-20/D16v-F2 cells were incubated with 17-AAG(Cat. No. HY-10211 MedChenExpress Co.) for 72 h before cell assays.

HLE cell line, adherent growing, were cultured in 75 cm^2 flasks with Dulbecco's modified Eagle's medium (DMEM; Gibco, Billings, MT, USA), 10% fetal bovine serum (Gibco), and 100 U/ml of penicillin–streptomycin (Invitrogen, Carlsbad, CA, USA) in an atmosphere of 5% CO_2/95% air at 37°C.

Freshly resected human pituitary ACTH-secreting tumors from the Department of Neurosurgery at Huashan Hospital (Shanghai Pituitary Tumor Center, Shanghai, China) were acquired and transferred to 0.5% fetal bovine serum-containing DMEM. Tumor tissue was minced into pieces (1–2 mm) and digested with DMEM containing 0.3% collagenase (Sigma–Aldrich, Saint Louis, MD, USA) and 0.15% hyaluronidase for 60 min at 37°C. The mixture was filtered with a cell strainer to remove undigested tissues and centrifuged at 180 g for 5 min at room temperature. The cell pellet was resuspended in fresh growth medium. Primary tumor cells were incubated with 17-AAG for 48 h before analyses. Clinical manifestations of the patients were showed in **Table 1**.

Colony-Formation Assays

Colony-formation assays were based on clone proliferation. Briefly, AtT-20/D16v-F2 cells were seeded in 6-well plates at low density (~1000 cells per well) The plates were treated 1 μM 17-AAG and DMSO as control, and cultured for 7 days. Then, the plates were washed with phosphate-buffered saline and stained with Crystal Violet. The images of each well were scanned, and individual clones identified. The number of clones that re-generated (each colony >50 cells) was counted to determine colony formation.

Cell-Viability Assay

AtT-20/D16v-F2 cells were seeded in 96-well plates (Nunc, Roskilde, Denmark) at 10^4 cell/ml, and treated with 17-AAG

TABLE 1 | The clinical information on the six human pituitary tumors collected.

Patients	Age	Gender	USP8 Mutant	mUFC μg/24H	Serum ACTH pg/ml	Cortisol Circadian Rhythm	Tumor Size cm^3	Pathology
1#	35	M	+	1293.30	95.1	–	0.4*0.3*0.3	Pituitary ACTHoma
2#	29	F	+	298.52	75.1	–	0.4*0.3*0.3	Pituitary ACTHoma
3#	48	F	+	748.70	50.4	–	1.0*0.5*0.5	Pituitary ACTHoma
4#	45	F	–	341.25	45.7	–	1.0*1.0*1.0	Pituitary ACTHoma
5#	22	F	–	1132.5	25.94	–	1.0*0.75*0.75	Pituitary ACTHoma
6#	31	F	–	486.42	63.05	–	1.0*0.75*0.75	Pituitary ACTHoma

with concentration at 1, 2, 5, and 10 μM for 24, 48, 72, and 96 h. Cells incubated with DMSO(control) or a series of 2-fold-diluted concentrations of 17-AAG for 72 h to evaluate IC_{50} Cell viability was measured using the CellTiter-Glo® Luminescent Cell Viability Assay kit according to manufacturer (Promega, Madison, WI, USA) instructions.

Western Blotting

Cells were lysed in RIPA lysis buffer (Sigma–Aldrich) complemented with a protease inhibitor cocktail (Roche, Basel, Switzerland) to obtain whole-cell lysates. Proteins were separated by electrophoresis on 4%–12% Bis-Tris gels and electroblotted onto polyvinylidene difluoride membranes using the Trans-Blot® Turbo™ Transfer System (Bio-Rad Laboratories, Hercules, CA, USA). Several primary antibodies were used: anti-caspase 3 (Cell Signaling Technology, Danvers, MA, USA), anti-cleaved caspase 3 (Cell Signaling Technology), anti-EGFR (Abcam, Cambridge, UK), anti-phosphorylated extracellular signal-regulated kinase (pERK)1/2 (Cell Signaling Technology) and anti-ERK1/2 (Cell Signaling Technology).

Reverse Transcription Quantitative Polymerase Chain Reaction

To determine mRNA expression of POMC, RT-qPCR was carried out using SYBR Premix Ex Taq (Tli RNaseH Plus) (Takara Biotechnology, Shiga, Japan). Hypoxanthine-guanine phosphoribosyltransferase (HPRT) was used as an internal control to normalize expression. The following primers (forward and reverse, respectively) were used: 5′-ACCACGGA AAGCAACCTG-3′ and 5′-GTGGCCCATGACGTACTTC-3′ for POMC; 5′-ACCAGTCAACAGGGGACATAAA-3′ and 5′-CTGACCAAGGAAAGCAAAGTCT-3′ for HPRT.

Sanger Sequencing

Detection of gene mutations was completed by Sanger sequencing。 The oligopeptide primers were designed by the Primer3 program. The primers were: Forward: 5'-ATTCACCCACCAACACTGTTCATA-3', Reverse: 5'-TTGTTTTCCCGATTAACTGTTGGA-3'. Dual 384-well GeneAmp PCR System9700 (Applied Biosystems) was used. DNA template 20ng, and 2* Taq PCR Master Mix (Lifefeng Biotech) were added to each system to complete PCR amplification. Sequencing was tested by Big Dye Terminator v.3.1 kit (Applied Biosystems), and analyzed by Chromas Software (Technelysium).

Radioimmunoassay

We adopted RIA to measure ACTH secreted by primary corticotropinoma tumor cells. Before plasma ACTH measurement, we cultured primary tumor cells in a 6-well plate with a normalized cell count of 10^6 per well. The plasma concentration of ACTH was measured by a sensitive and specific commercial radioimmunoassay, Immulite One (Siemens Medical Solutions, Malvern, PA) The sensitivity of the radioimmunoassay was 4.13 nmol/L for ACTH, and the inter- and intra-assay coefficient of variation was 5%–7% and 6%–7.9%, respectively. The final results are expressed as pictograms per milliliter.

ELISA

We measured *in-vitro* ACTH from AtT20/D16v-F2 cell line by ELISA. We cultured AtT20/D16v-F2 cells in a 96-well palte with a normalized cell count of 5000 per well, 3 templates for each sample. We used a commercial kit (ALPCO, ACTH ELISA) in this experiment. First configure different concentrations of standards for testing to draw a standard curve. After adding sample to the provided enzyme-labeled well plate has been coated with streptavidin, add biotin-labeled antibody to each well, and then add the enzyme-labeled antibody. Allow 4 h + -30 min reaction time at room temperature, wash the plate 5 times, then add tetramethylbenzidine (color developer), and check the result with a spectrophotometer.

Statistical Analyses

Statistical analyses were undertaken using SPSS v25.0 (IBM, Armonk, NY, USA). Prism 7.0 (GraphPad, San Diego, CA, USA) was used to prepare graphs. The Student's *t*-test was used for continuous variables. The Pearson chi-square test was employed for categorical variables. $P < 0.05$ was considered significant.

RESULTS

17-AAG Inhibits the Viability of AtT-20/D16v-F2 Cells

First, we demonstrated that 17-AAG could inhibit cell survival for a long time using colony-formation assays. No colony was formed in the petri dish treated with 17-AAG, whereas the mock petri dish presented with 77.8% colony formation (**Figure 1**).

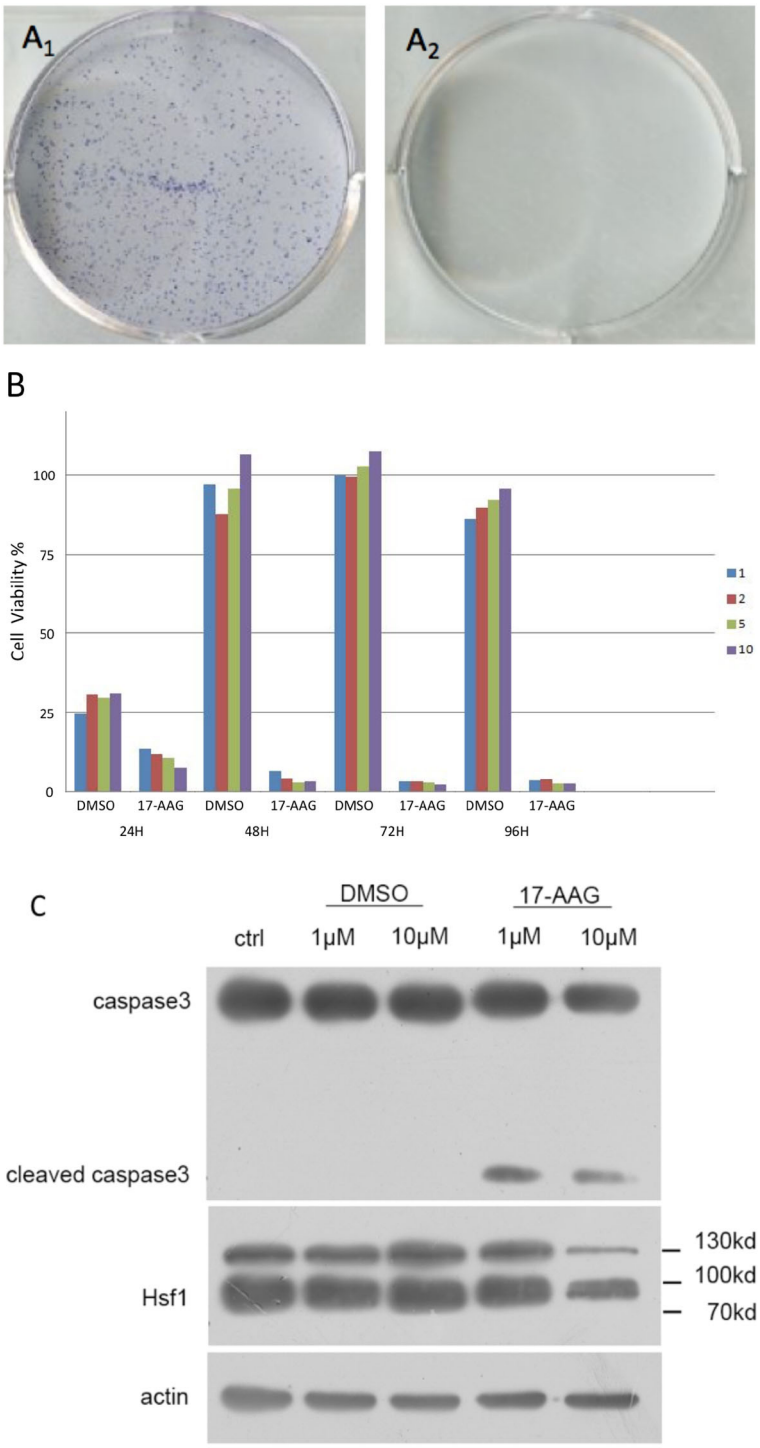

FIGURE 1 | 17-AAG inhibits the viability of AtT-20/D16v-F2 cells. In the latter, colony formation was suppressed by 17-AAG (1μM) **(A)**. Caspase-3 expression demonstrated that 17-AAG promoted apoptosis of AtT-20/D16v-F2 cells **(C)**. Proliferation of AtT-20/D16v-F2 cells was suppressed by 17-AAG remarkably in a dose- and time-dependent manner **(B)**. All the experiments were repeated three times with consistent results.

Caspase-3 expression measured by immunoblotting showed 17-AAG promoted apoptosis of AtT-20/D16v-F2 cells (**Figure 1C**). Caspase-3 expression was decreased in 17-AAG-treated AtT-20/D16v-F2 cells, whereas expression of cleaved caspase-3 was increased, suggesting that 17-AAG activated programmed cell death. Hence, 17-AAG not only

inhibited proliferation of AtT-20/D16v-F2 cells, it also promoted their apoptosis.

We treated AtT-20/D16v-F2 cells with 17-AAG (1, 2, 5, and 10 μM) for 24, 48, 72, and 96 h (**Figure1B**). Viability of AtT-20/D16v-F2 cells was suppressed according to drug dose and exposure time. Upon treatment for >72 h, proliferation stopped, so we chose 72 h as the treatment time to measure the half-maximal inhibitory concentration (IC_{50}).

17-AAG Suppressed of Proliferation of AtT-20/D16v-F2 Cells and ACTH Secretion

proliferation of AtT-20/D16v-F2 cells was found to be effectively inhibited by 17-AAG in a dose-dependent manner (**Figure 2A**). AtT-20/D16v-F2 cells were treated for 72 h with a maximum dose of 20 μM of 17-AAG, and their viability was assessed. The IC_{50} was calculated to be 2.33 μM.

AtT-20/D16v-F2 cells were treated with different concentrations of 17-AGG. Cell supernatants were collected for ACTH level measurement(**Figure 2B**). ACTH secretion from AtT-20/D16v-F2 cells was restrained after 17-AAG treatment, and the inhibitory effect of 17-AAG was dose-dependent. ACTH secretion from AtT-20/D16v-F2 cells was impaired more remarkably after 48 h than 24 h, and secretion was similar upon treatment with higher doses of 17-AGG. When treated with a high dosage of 10 μM for 48 h, ACTH level would reduce approximately 40% from baseline.

Further experiments were conducted to investigate the effect of 17-AAG on expression of POMC mRNA in AtT-20/D16v-F2 cells (**Figure 2C**). The latter were treated with different doses of 17-AAG for 24 and 48 h, respectively, and mRNA expression of POMC was determined by RT-qPCR. 17-AAG restrained transcription of POMC in AtT-20/D16v-F2 cells in a dose-related manner. POMC transcription level reduced approximately 75% from baseline when treated with a high dosage of 10 μM for 48 h.

17-AAG Inhibited Cell Viability and Secretion of Human Pituitary ACTH-Secreting Tumor Cells

After 48 h exposure to 17-AAG at dosage of 0.5, 2, and 10 μM, we measured the ACTH concentration in cell supernatants (**Figure 3A**). We found reduced levels of ACTH secretion from primary human ACTH-secreting pituitary tumor cells in a dose-related manner compared with that in the blank control group. In *USP8* mutant group, the ACTH level was suppressed to about 39% from baseline, while in wild type group just to 82% when primary cells treated with 10 μM. Therefore, we examined the mechanism by which 17-AAG may affect ACTH secretion. Cell-viability tests showed that decreased secretion of ACTH might be related to the reduced number of living cells (**Figure 3B**), which suggested that the antitumor effect of 17-AAG partially through inhibition of cytostatic activity. In *USP8* mutant group, the cell viability was suppressed to about 54% from baseline, while in wild type group just to 84% when primary cells treated with 10 μM. Furthermore, qRT-PCR detected reduced transcription of POMC by 17-AAG treatment. (**Figure 3C**). In the *USP8* mutant group, 17-AAG inhibited POMC translation significantly (74% inhibition, p=0.02); While a trend towards reduced POMC expression was noted in the wild type group (63%inhibition, p=0.06), which meant the therapeutic validity manifested on function damage in tumor cells carrying the *USP8* mutant were more sensitive.

EGFR and Its Downstream Pathway Is Suppressed by 17-AAG in a Model Cell Line

AtT-20/D16v-F2 cells showed negative EGFR expression (**Figure 4A**), so we used the HLE instead. 17-AAG (0.2 μM) could inhibit EGFR expression in our model cell line. Therefore, we continue to increase the drug concentration. We found that the EGFR expression in model cells decreased after 17-AAG treatment, as well as HSP90. We further used EGF to activated cells, and discovered that the p-ERK1/2 was lower in the 17-AAG treated group compared to the blank control. This indicated that HSP90 inhibition arrested the downstream mitogen-activated protein kinase (MAPK) pathway, which was activated to generate ACTH (**Figure 4B**). From **Figure 4C**, we found that at the dosage of 0.5 nM and 1.0 nM, 17-AAG could limit the inhibition of EGFR expression significantly. After autophosphorylation, EGFR bound directly or indirectly to Grb-2 to activate Ras, then activated the downstream silk/threonine protein kinase Raf, thereby phosphorylating ERK1/2. Deficiency of EGFR expression led to shortage of downstream p-ERK1/2. Therefore, we treated HLE cells with 17-AAG and EGF for further experiment (**Figures 4B, C**). We found that the EGFR expression in model cells decreased after 17-AAG treatment, as well as HSP90. We further used EGF to activated cells, and discovered that the p-ERK1/2 was lower in the 17-AAG treated group compared to the blank control. This indicated that HSP90 inhibition arrested the downstream mitogen-activated protein kinase (MAPK) pathway, which was activated to generate ACTH. At the same time we found, even treated with high concentration of 17-AAG, the restrain to USP8 expression were still slightly. Thus, HSP90 inhibitors were not capable to intervene USP8 expression.

DISCUSSION

Unlike the dopamine-receptor agonists acting sensitively on prolactinomas, or somatostatin analogs acting on growth hormone-secreting adenomas, pharmacologic options for ACTH-secreting adenomas are limited to drugs targeting pituitary tumors, adrenal glands, or peripheral glucocorticoid receptors. Ketoconazole can inhibit cortisol synthesis, leading to a normal level of cortisol in 50% of patients, but its use is accompanied with hepatotoxicity.

Our research team had revealed a high prevalence (~60%) of *USP8* mutations in ACTH-secreting adenomas (11). This mutation exists only in corticotropinomas, suggesting that USP8 may play an important part in CD pathogenesis. Immunohistochemical studies have confirmed strong overexpression of HSP90 in AtT-20/D16v-F2 cells, as well as the ACTH-secreting tumor tissue (24). 17-AAG inhibit HSP90 function to induce anti-tumor activity.

Studies have revealed the mechanism of USP8-related secretion in ACTH-secreting adenomas (11, 12). Different from genome-wild USP8 Deubiquitinating enzymes(DUBS), *USP8* mutants cannot be phosphorylated or bind to 14-3-3 protein to activate USP8.

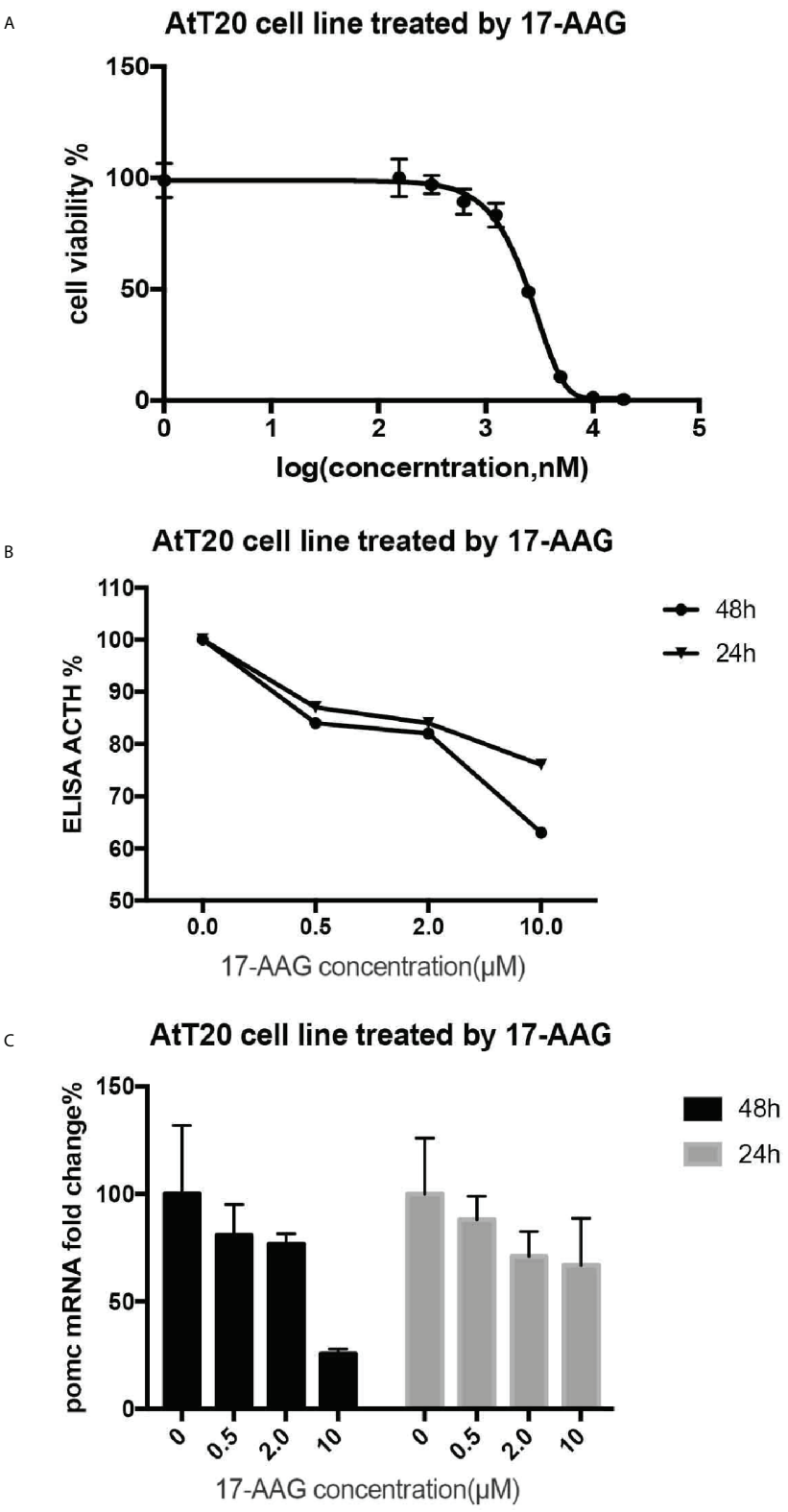

FIGURE 2 | 17-AAG suppressed proliferation of AtT-20/D16v-F2 cells and ACTH secretion. The IC_{50} of 17-AAG on AtT-20/D16v-F2 cells was restrained significantly after 17-AAG treatment in a dose-related manner **(B)**. 17-AAG restrained transcription of POMC in AtT-20/D16v-F2 cells and exhibited dose-related effects **(C)**. Three independent experiments were repeated for each result and three replicates were studied in each single experiment.

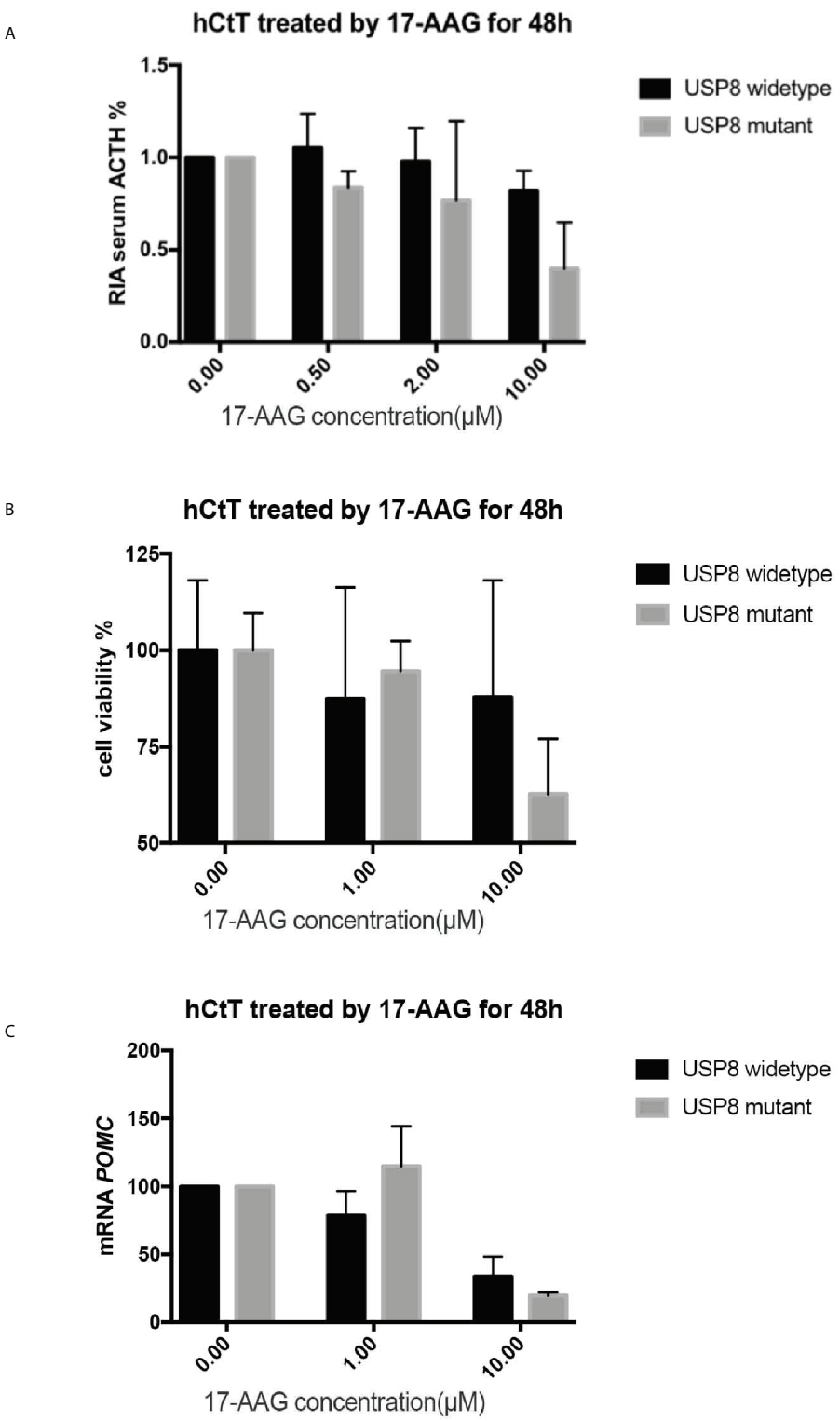

FIGURE 3 | 17-AAG inhibited proliferationof primary ACTH-secreting tumor cells and ACTH secretion. Such inhibition was more remarkable in tumor cells carrying the *USP8* mutant. Reduced secretion of ACTH from primary ACTH-secreting tumor cells occurred in a dose-related manner compared with that in the blank control group **(A)**. The viability **(B)** and POMC transcription **(C)** were reduced when primary ACTH-secreting tumor cells were treated with 17-AAG, especially in the *USP8*-mutant group. Three independent experiments were repeated for each result and three replicates were studied in each single experiment.

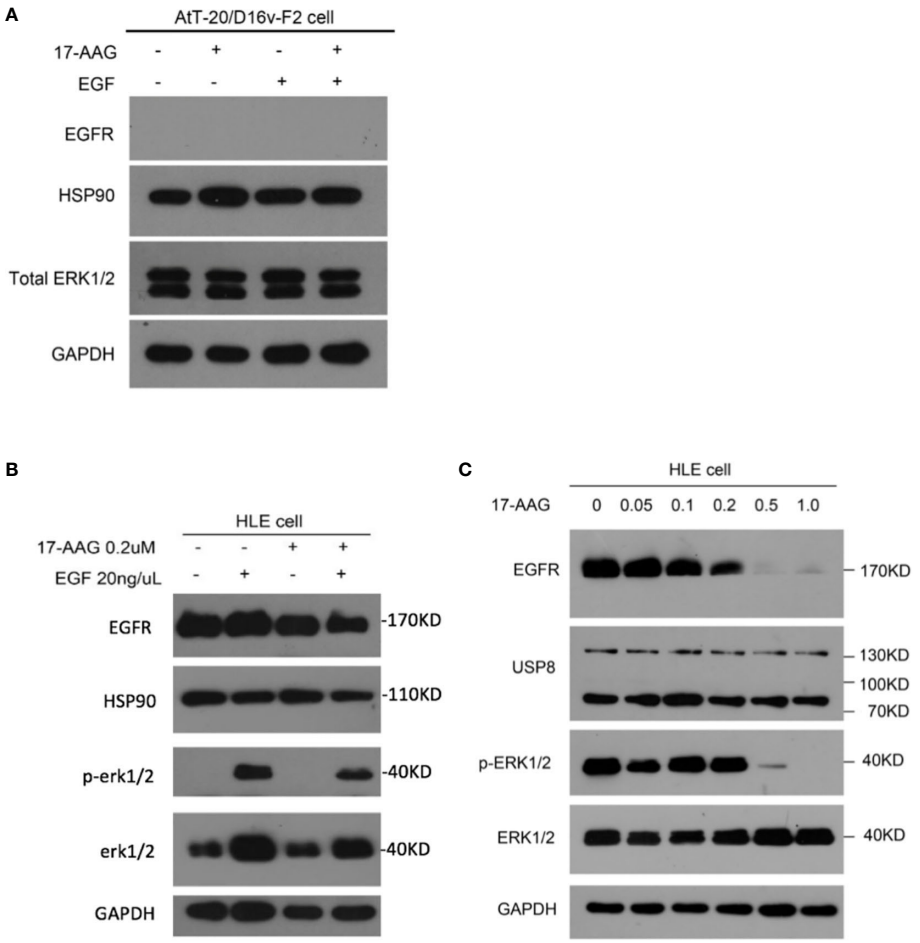

FIGURE 4 | EGFR and its downstream pathway was suppressed by 17-AAG in HLE cells.AtT-20/D16v-F2 cells did not have EGFRS **(A)**, so we used HLE cells instead. Western blotting demonstrated expression of EFGRs and p-ERK1/2 to be down-regulated by 17-AAG treatment **(B, C)**, which suggested that EGFR and its downstream MAPK pathway were suppressed by the HSP90 inhibitor 17-AAG. All the experiments were repeated three times with consistent results.

The function of USP8 is to deubiquitinate molecules and prevent client proteins from degradation. Accumulation of the EGFR causes up-regulation of signaling pathways and thereby accelerates POMC transcription by inducing the deregulation of p27 (25, 26), or degradation of other regulators such as histone deacetylase. Hence, inhibition of USP8 and/or EGFRs represents a potential remedy for CD. Studies in primary tumor cells with USP8 mutants have shown that gefitinib treatment (EGFR inhibitor) can reduce ACTH secretion (11, 13).

When 17-AAG acted upon AtT-20/D16v-F2 cells, it suppressed their colony formation and viability remarkably. Cell proliferation was constrained considerably, with an IC_{50} of 2.33 μM. Connections between apoptosis and ACTH and 17-AAG were found by measurement of caspase-3 expression. Further analyses showed the positive correlation between the 17-AAG concentration and ACTH secretion in AtT-20/D16v-F2 cells was based on the drug dose and exposure time. RT-qPCR also confirmed the conclusion with POMC transcription result. Experiments on AtT-20/D16v-F2 cells suggested that 17-AAG could suppress their viability and ACTH secretion.

Because of the deficiency of EGFR expression in AtT-20/D16v-F2 cells and difficulty in obtaining human corticotropinomas cell line, we used a model cell line HLE which delivered EGFR and human primary tumor cells. In primary ACTH-secreting tumor cells we verified those findings and, interestingly, mutant tumor cells exhibited more sensitivity towards 17-AAG in terms of cell viability and POMC transcription. Expression of EGFRs and p-ERK1/2, which activated in the downstream MAPK pathway, was reduced distinctly by 17-AAG within dosage-related manner. Geldanamycin(GA), a specific inhibitors of the cytosolic chaperone HSP 90, were shown to accelerate degradation of the EGFR (27). GA blocks processing of newly synthesized EGFR. The effects of GA on receptor degradation are mediated by the cytosolic portion of EGFR and could be conferred to the erythropoietin receptor (EPO-R), by employing the respective chimera. But GA didn't affect stability of newly synthesized EGFR lacking the cytosolic domain (Ex EGFR) (27).However, Geldanamycin has not been used in clinical scenario because of liver toxicity. 17-AAG is a new derivative of geldanamycin that

shares its important biological activities but shows less toxicity (28). Hence, EGFR function could be restrained and POMC transcription mediated by the MAPK pathway reduced after inhibition of HSP90 expression. These actions would contribute to tumor-growth inhibition in signaling pathways involving ACTH secretion.

While, in the context of *USP8* mutated, the deregulation of EGFR led to improve MAPK signaling and subsequently promoted POMC transcription, which further result to the oversecretion of ACTH. *In-vitro* model cell experiments also confirmed that HSP90 inhibitor could significantly reduce the expression level of EGFR and lower down-regulate the MAPK pathway. We believed that overexpressed EGFR provided greater targets for HSP90 inhibitors, therefore 17-AAG manifested a more significant effect on *USP8* mutant tumor cells.

HSP90, as a molecular chaperone, plays a key role in the conformational maturation of oncogenic signaling proteins. HSP90 inhibitors selectively kill cancer cells compared to normal cells, and have also demonstrated antitumor effects towards lymphomas, melanomas, as well as some solid tumors (e.g., breast cancer, non-small-cell lung cancer) (16, 17). To date the majority of pharmaceutical research and development focused on targeting the N-domain ATP binding site of HSP90, C-terminal of the protein on the heels. Silibinin, as well as novobiocin, were the prototypic inhibitors that interact at the C-domain site. And the therapeutic effect in cancer had been demonstrated. One conceivable benefit of these medication is that certain C-terminal inhibitors appear to have weaker HSF1 activation capacity. The ongoing study support further optimization of medicinal chemistry and preclinical evaluation of C-terminal Hsp90 inhibitors. The four Hsp90 subtypes (Hsp90α and Hsp90β in the cytoplasm and nucleus, the relative importance of GRP94 and TRAP1 in the endoplasmic reticulum in cancer) and mitochondria were still under research (29). Our present study provides a foundation for potential clinical treatment of CD by HSP90 inhibitor. Further research will continue to build animal models to improve related *in-vivo* experiments. It will provide more detailed research basis for the clinical application of HSP90 inhibitors in the treatment of Cushing's disease.

CONCLUSIONS

The HSP90 inhibitor 17-AAG reduced the viability and secretory function of human pituitary ACTH-secreting tumor cells, and tumor cells carrying the *USP8* mutant were more sensitive to 17-AAG than tumor cells carrying wild-type *USP8*. 17-AAG could be potential clinical treatment for CD.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

Conception or design of the work: ZM and XZ. Financial support: ZM, QZ, and YZ. Surgical specimen recruitment: NQ, YZ, XS, XZ, ZM. Laboratory Practice: YS, CJ. Data analysis and interpretation: YS, ZM, QZ. Drafting the article: YS, CJ. Critical revision of the article: ZM, XZ. All authors contributed to the article and approved the submitted version.

FUNDING

The research was supported by the National Science Fund for Distinguished Young Scholars (81725011), Clinical Research Plan of SHDC(SHDC2020CR2004A) to YZ; the National Natural Science Funds of China (81702467), Shanghai Sailing program (17YF1401500) to ZM; National Natural Science Funds of China (81802495), Shanghai Sailing program (18YF1403400) to QZ.

ACKNOWLEDGMENTS

We gratefully acknowledge Prof Yao Zhao for funding support. We thank Mrs. Yun Zhang, Mrs. Ye Wang, Mrs. Qiuyue Wu and Mrs. Lan Lai for sample collection.

REFERENCES

1. Clayton RN, Jones PW, Reulen RC, Stewart PM, Hassan-Smith ZK, Ntali G, et al. Mortality in patients with Cushing's disease more than 10 years after remission: a multicentre, multinational, retrospective cohort study. *Lancet Diabetes Endocrinol* (2016) 4(7):569–76.
2. Pivonello R, Isidori AM, De Martino MC, Newell-Price J, Biller BMK, Colao A. Complications of Cushing's syndrome: state of the art. *Lancet Diabetes Endocrinol* (2016) 4(7):611–29.
3. Bertagna X, Guignat L. Approach to the Cushing's disease patient with persistent/recurrent hypercortisolism after pituitary surgery. *J Clin Endocrinol Metab* (2013) 98(4):1307–18.
4. Ram Z, Nieman LK, Cutler GB, Chrousos GP, Doppman JL, Oldfield EH. Early repeat surgery for persistent Cushing's disease. *J Neurosurg* (1994), 37–45, 80(1).
5. Castinetti F, Guignat L, Giraud P, Muller M, Kamenicky P, Drui D, et al. Ketoconazole in Cushing's disease: is it worth a try? *J Clin Endocrinol Metab* (2014) 99(5):1623–30.
6. Molitch ME. Current approaches to the pharmacological management of Cushing's disease. *Mol Cell Endocrinol* (2015) 408:185–9.
7. Colao A, Petersenn S, Newell-Price J, Findling JW, Gu F, Maldonado M, et al. A 12-month phase 3 study of pasireotide in Cushing's disease. *New Engl J Med* (2012) 366(10):914–24.
8. Preda VA, Sen J, Karavitaki N, Grossman AB. Therapy In Endocrine Disease: Etomidate in the management of hypercortisolaemia in Cushing's syndrome: a review. *Eur J Endocrinol* (2012) 167(2):137–43.

9. Daniel E, Aylwin S, Mustafa O, Ball S, Munir A, Boelaert K, et al. Effectiveness of metyrapone in treating Cushing's syndrome: a retrospective multicenter study in 195 patients. *J Clin Endocrinol Metab* (2015) 100(11):4146–54.
10. Fleseriu M, Biller BM, Findling JW, Molitch ME, Schteingart DE, Gross C, et al. Mifepristone, a glucocorticoid receptor antagonist, produces clinical and metabolic benefits in patients with Cushing's syndrome. *J Clin Endocrinol Metab* (2012) 97(6):2039–49. doi: 10.1210/jc.2011-3350
11. Ma ZY, Song ZJ, Chen JH, Wang YF, Li SQ, Zhou LF, et al. Recurrent gain-of-function USP8 mutations in Cushing's disease. *Cell Res* (2015) 25(3):306. doi: 10.1038/cr.2015.20
12. Reincke M, Sbiera S, Hayakawa A, Theodoropoulou M, Osswald A, Beuschlein F, et al. Mutations in the deubiquitinase gene USP8 cause Cushing's disease. *Nat Genet* (2015) 47(1):31.
13. Fukuoka H, Cooper O, Ben-Shlomo A, Mamelak A, Ren SG, Bruyette D, et al. EGFR as a therapeutic target for human, canine, and mouse ACTH-secreting pituitary adenomas. *J Clin Invest* (2011) 121(12):4712–21.
14. Taldone T, Gozman A, Maharaj R, Chiosis G. Targeting Hsp90: small-molecule inhibitors and their clinical development. *Curr Opin Pharmacol* (2008) 8(4):370–4.
15. Dimopoulos MA, Mitsiades CS, Anderson KC, Richardson PG. Tanespimycin as antitumor therapy. *Clin Lymphoma Myeloma Leukemia* (2011) 11(1):17–22.
16. Usmani SZ, Chiosis G. HSP90 inhibitors as therapy for multiple myeloma. *Clin Lymphoma Myeloma Leukemia* (2011) 11:S77–81.
17. J haveri K, Chandarlapaty S, Lake D, Gilewski T, Robson M, Goldfarb S, et al. A phase II open-label study of ganetespib, a novel heat shock protein 90 inhibitor for patients with metastatic breast cancer. *Clin Breast Cancer* (2014) 14(3):154–60.
18. Johnson ML, Yu HA, Hart EM, Weitner BB, Rademaker AW, Patel JD, et al. Phase I/II study of HSP90 inhibitor AUY922 and erlotinib for EGFR-mutant lung cancer with acquired resistance to epidermal growth factor receptor tyrosine kinase inhibitors. *J Clin Oncol* (2015) 33(15):1666.
19. Yang YC, Cheng TY, Huang SM, Su CY, Yang PW, Lee JM, et al. Cytosolic PKM2 stabilizes mutant EGFR protein expression through regulating HSP90-EGFR association. *Oncogene* (2017) 36(29):4234.
20. Jorge SE, Lucena-Araujo AR, Yasuda H, Piotrowska Z, Oxnard GR, Rangachari D, et al. EGFR exon 20 insertion mutations display sensitivity to Hsp90 inhibition in preclinical models and lung adenocarcinomas. *Clin Cancer Res* (2018) 24(24):6548–55.
21. Wang CY, Guo ST, Wang JY, Liu F, Zhang YY, Yari H, et al. Inhibition of HSP90 by AUY922 preferentially kills mutant KRAS colon cancer cells by activating Bim through ER stress. *Mol Cancer Ther* (2016) 15 (3):448–59.
22. Wang CY, Guo ST, Wang JY, Yan XG, Zhang XD. Abstract 3066: inhibition of hsp90 by auy922 preferentially kills mutant kras colon cancer cells by activating bim through er stress. *Cancer Res* (2017) 77(13 Supplement):3066–6.
23. Raverot G, Burman P, Mccormack A, Heaney A, Petersenn S, Popovic V, et al. European Society of Endocrinology Clinical Practice Guidelines for the management of aggressive pituitary tumours and carcinomas. *Eur J Endocrinol* (2018) 178(1):G1–G24.
24. Riebold M, Kozany C, Freiburger L, Sattler M, Buchfelder M, Hausch F, et al. A C-terminal HSP90 inhibitor restores glucocorticoid sensitivity and relieves a mouse allograft model of Cushing disease. *Nat Med* (2015) 21(3):276.
25. Lidhar K, Korbonits M, Jordan S, Khalimova Z, Kaltsas G, Lu X, et al. Low expression of the cell cycle inhibitor p27Kip1 in normal corticotroph cells, corticotroph tumors, and malignant pituitary tumors. *J Clin Endocrinol Metab* (1999) 84(10):3823–30.
26. Chu IM, Hengst L, Slingerland JM. The Cdk inhibitor p27 in human cancer: prognostic potential and relevance to anticancer therapy. *Nat Rev Cancer* (2008) 8(4):253.
27. Supino-Rosin L, Yoshimura A, Yarden Y, Elazar Z, Neumann D. Intracellular Retention and Degradation of the Epidermal Growth Factor Receptor, Two Distinct Processes Mediated by Benzoquinone Ansamycins. *J Biol Chem* (2000) 275(29):21850–5.
28. Kamal A, Thao L, Sensintaffar J, Zhang L, Boehm MF, Fritz LC, et al. A high-affinity conformation of Hsp90 confers tumour selectivity on Hsp90 inhibitors. *Nature* (2003) 425(6956):407–10.
29. Neckers L, Workman P. Hsp90 molecular chaperone inhibitors: are we there yet? *Clin Cancer Res: an Off J Am Assoc Cancer Res* (2012) 18(1):64–76. doi: 10.1158/1078-0432.CCR-11-1000

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Endocrinology

ORIGINAL RESEARCH
published: 18 January 2021
doi: 10.3389/fendo.2020.608691

Clinical Parameters of Silent Corticotroph Adenomas With Positive and Negative Adrenocorticotropic Hormone Immunostaining: A Large Retrospective Single-Center Study of 105 Cases

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Purpose: To investigate the different clinical characteristics of silent corticotroph adenomas (SCAs) with positive and negative adrenocorticotropic hormone (ACTH) immunostaining, and to explore the value of pituitary-restricted transcription factor (Tpit) immunostaining for diagnosing SCAs.

Methods: The clinical materials of patients with SCAs who had a typical pathological feature with positive Tpit immunostaining and positive/negative ACTH immunostaining, and without clinical features and biochemical evidence for Cushing's Syndrome in our center from April 2018 to March 2019 were analyzed retrospectively. The differences in clinical characteristics and surgical results between ACTH-positive and -negative SCAs were explored.

Results: A total of one hundred and five patients (94.3% female) with SCAs were included. There were 66 SCAs with ACTH-negative (66/105, 62.9%), and 39 SCAs with ACTH-positive (39/105, 37.1%). Cases with ACTH-negative SCAs were more likely to have lower ACTH levels (27.5 ± 24.0 vs. 54.4 ± 58.6, P = 0.011), more multiple microcysts (81.8% vs. 61.5%, P = 0.022) and lower levels of Ki-67 expression (low expression rate 90.9% vs. 74.4%, P = 0.023). No statistical significant differences were observed between patients with ACTH-positive and -negative SCAs regarding gender (97.0% vs. 89.7%, P = 0.192), age (50.3 ± 10.3 vs. 49.0 ± 11.2, P = 0.543), surgical history (16.7% vs. 23.1%, P = 0.419), suprasellar extension (66.7% vs. 74.4%, P = 0.408), sphenoid sinus extension (51.5% vs. 56.4%, P = 0.627), cavernous sinus invasion (75.8% vs. 66.7%, P = 0.314), large cyst on Magnetic Resonance Imaging (MRI) (47.0% vs. 61.5%, P = 0.149), or gross total resection rate (42.4% vs. 51.3%, P = 0.379).

OPEN ACCESS

Edited by:
Renzhi Wang, Peking Union Medical College Hospital (CAMS), China

Reviewed by:
Odelia Cooper, Cedars Sinai Medical Center, United States
Hiroshi Nishioka, Toranomon Hospital, Japan

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Specialty section:
This article was submitted to Pituitary Endocrinology, a section of the journal Frontiers in Endocrinology

Received: *21 September 2020*
Accepted: *01 December 2020*
Published: *18 January 2021*

Citation:
Zhang K, Shou X, Chen H, Qiao N, He W, Chen Z, Shen M, Li S, Zhao Y, Zhang Z, Li Y, Ye H and Wang Y (2021) Clinical Parameters of Silent Corticotroph Adenomas With Positive and Negative Adrenocorticotropic Hormone Immunostaining: A Large Retrospective Single-Center Study of 105 Cases. Front. Endocrinol. 11:608691. doi: 10.3389/fendo.2020.608691

Conclusions: ACTH-negative SCAs were observed to be more clinically silent and more likely to demonstrate multiple microcysts on MRI. The prevalence of SCAs, especially ACTH-negative SCAs, proved to be substantially underestimated and thus they should be given enough attention in consideration of the high aggressiveness of this subtype of refractory pituitary adenoma (PA).

Keywords: silent corticotroph adenoma, Tpit immunostaining, refractory pituitary adenoma, non-functioning pituitary adenoma, pituitary incidentaloma

INTRODUCTION

Silent corticotroph adenomas (SCAs) were defined as pituitary adenomas (PAs) with positive adrenocorticotropic hormone (ACTH) immunostaining but present as clinically nonfunctioning adenomas (NFPAs) in most studies in the literature. Some SCAs are completely silent, without Cushingoid features or elevated cortisol levels. Some are clinically silent, with excess ACTH but absent of related clinical manifestations. All NFPAs lack specific clinical manifestations related to hormonal hypersecretion, including SCAs, which leads to the fact that most patients were only diagnosed and operated on when the tumor caused mass effects.

However, the 2017 World Health Organization (WHO) classification of PAs redefined a corticotroph adenoma as a PA that expresses ACTH and other proopiomelanocortin (POMC)–derived peptides and arises from adenohypophyseal cells of pituitary-restricted transcription factor (Tpit) lineage (1). With the addition of Tpit immunostaining, the criteria of SCA has changed completely. SCAs were redefined as PAs clinically diagnosed as NFPA and had typical pathological feature with positive Tpit immunostaining and positive/negative ACTH immunostaining.

The goals of this study were to investigate the different clinical characteristics between SCAs with positive and negative ACTH immunostaining and to determine the value of Tpit immunostaining for diagnosing SCAs.

MATERIALS AND METHODS

Patients

We retrospectively reviewed the medical records of 757 cases with NFPAs who underwent pituitary surgery between April 2018 and March 2019 in our center and identified 184 patients with equivocal SCAs. A total of 105 patients with SCAs (99 women and 6 men, sex ratio 16.5:1) were included in the analysis for clinical characteristics, with a mean age of 49.8 ± 10.6 years. The inclusion criteria were: image evidence of a pituitary tumor, clinically diagnosed as NFPA, without Cushingoid features, and a typical pathological feature with positive Tpit immunostaining and positive/negative ACTH immunostaining. NFPAs are characterized by the lack of hormonal expression or secretion or any related hormonal syndrome and include a variety of pathological subtypes, as described in the latest WHO classification of PAs (1). Cases without accessible radiographs and cases without an explicit pathological diagnosis were excluded. The study was approved by the Institutional Review Board of Huashan Hospital, Shanghai Medical School, Fudan University. All patients were given informed consent when they were admitted to our center.

Radiological Evaluation

All patients underwent conventional magnetic resonance imaging (MRI) with and without contrast before the surgery. PA smaller than 10 mm and larger than 10 mm in diameter was defined as microadenomas and macroadenomas, respectively. Suprasellar extension and sphenoid sinus extension of tumors were evaluated according to the Hardy-Wilson classification. Cavernous sinus invasion was evaluated according to the Knosp grading system. Multiple microcysts were confirmed if typical microcystic changes covered over 25% of the solid portion of the adenoma (**Figure 1**). Visual examination and visual field examination were checked in patients with radiographic compression of the optic chiasm.

Biochemical Evaluation

A series of biochemical examinations were performed routinely on patients before the surgery to screen for hormone hypersecretion and hypopituitarism, including serum cortisol, ACTH, prolactin (PRL), testosterone or estrogen, growth hormone (GH), insulin-like growth factor-1 (IGF-1), thyroid-stimulating hormone (TSH), and thyroid hormone.

Surgical Procedure

All operations were performed in our medical center, using either a transnasal approach or transcranial approach depending on the location and aggressiveness of the tumor (**Table 1**). To transnasal approach, intraoperative cerebrospinal fluid (CSF) leakage was evaluated according to the Kelly grading system.

Immunohistochemical Staining

The specimen retained during the operation was fixed in 10% formalin and then gradient dehydrated by ethanol and immersed in paraffin. Section the paraffin-embedded tissue block at 2-mm thickness for immunohistochemistry. After conventional xylene and ethanol gradient dewaxing and dehydration, the sections were incubated in 3% H_2O_2 solution in methanol to block endogenous peroxidase activity and washed by phosphate buffer.

Subsequently, synaptophysin (Syn), ACTH, GH, PRL, TSH, follicle-stimulating hormone (FSH), luteinizing hormone (LH), Tpit, pituitary specific transcription factor-1 (PIT-1), CAM5.2, P53, SSTR2a, SF-1, and Ki-67 monoclonal antibody (Abcam Trading (Shanghai) Company Ltd., Shanghai, China) (1:50) was added, incubated at 37°C for two h and washed with phosphate buffer. Next, second antibody was applied to the slides and

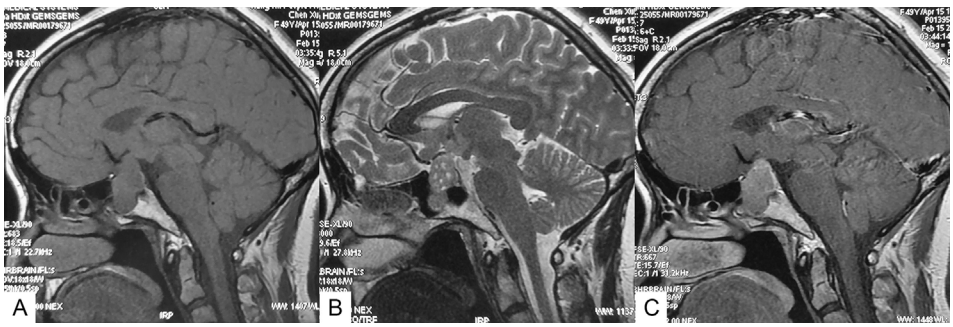

FIGURE 1 | Sagittal MR images of an SCA with typical multiple microcysts. **(A)** T1-weighted image. **(B)** T2-weighted image, showing multiple microcysts. **(C)** Contrast-enhanced image.

TABLE 1 | Different surgical approaches used for silent corticotroph adenomas (n = 105).

Surgical approach	Number (%)
Transsphenoidal	98 (93.3%)
Pterional	3 (2.9%)
Subfrontal	2 (1.9%)
Supraorbital	2 (1.9%)

incubated for 30 min. Finally, the color was revealed by DAB substrate solution (Abcam Trading (Shanghai) Company Ltd., Shanghai, China) and counterstained with hematoxylin.

Statistical Analysis

Data are reported as means standard deviations (SD) for continuous variables normally or not normally distributed, and as frequencies for categorical variables. Normality was tested using the Kolmogorov-Smirnov test. Means were compared using the unpaired t-test when data distribution was normal, or by the Wilcoxon rank-sum (Mann-Whitney) test when variables were not normally distributed. Categorical variables were compared using the chi-square test or Fisher's exact test as appropriate. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated using univariate logistic regression. Covariates with $P < 0.1$ were entered into the multivariable logistic regression analysis (2). Statistical analysis was performed using the SPSS 23.0 statistical analysis software (IBM Corp., Armonk, NY). A two-tailed P value < 0.05 was considered statistically significant.

RESULTS

Epidemiology

Among the 757 cases with NFPAs who underwent pituitary surgery from April 2018 to March 2019 in our center, Tpit immunostaining was performed in 66.7% of cases (505/757). We divided all cases into 2 groups based on whether Tpit immunostaining was performed. In the 505 cases with Tpit immunostaining, 138 adenomas (138/505, 27.3%) were identified as SCAs. In the 252 cases without Tpit immunostaining, 46 adenomas (46/252, 18.3%) were identified as SCAs. Therefore, the calculated proportion of SCAs in NFPAs was 24.3%.

Clinical Features

105 patients (6 male and 99 female) with SCAs were included in the analysis for clinical parameters (**Table 2**). 33 cases with incomplete clinical data were excluded in the study. The age of the patients ranged from 17 to 74, with an average of 49.8 ± 10.6 years old. Eighty-five patients were having initial operations, and 20 patients (2 male and 18 female) had a previous pituitary surgical history.

Before surgery, 58 patients (58/105, 55.2%) had vision loss or visual field defects. Twenty-four patients (24/105, 22.9%) showed frequent headaches. Among 58 female patients of childbearing age (15–49 years old), 18 (18/48, 37.5%) showed galactorrhea, oligomenorrhoea, or amenorrhea.

Preoperative Evaluation

All 105 cases were clearly confirmed by MRI, 103 cases among which were macroadenomas, and 2 were microadenomas. Seventy-three adenomas (73/105, 69.5%) had suprasellar extension (Wilson-Hardy Grade B-C) and 56 adenomas (56/105, 53.3%) had sphenoid sinus extension (Wilson-Hardy Grade III-IV). Cavernous sinus invasion occurred in 76 cases (76/105, 72.4%) (Knosp grade 3-4). Cystic degeneration appeared in 98 cases (98/105, 93.3%), and 78 cases (78/105, 74.3%) among which were found with typical multiple microcysts.

Among all 105 cases included, hyperprolactinemia was presented in 41 patients (41/105, 39.0%). 38 patients (38/105, 36.2% had varying degrees of hypopituitarism. Twenty-four patients failed to test for serum ACTH levels due to personal reasons. In the remaining 81 cases, the serum ACTH level ranged from 2.3 to 285.6, with an average of 40.1 ± 45.5. All cases were assessed by the senior endocrinologists, confirmed with normal morning serum cortisol and no Cushingoid symptoms. Low dose dexamethasone suppression test (LDDST) was not performed preoperatively on every patient due to the limited length of hospital stay.

Pathological Findings

Tpit immunostaining was performed in all 105 cases, and all cases were Tpit-positive. Among them, 39 cases (39/105, 37.1%) showed positive staining for ACTH and 66 cases (66/105, 62.9%) showed negative staining for ACTH (**Figure 2**). None of the adenomas were found ACTH positive but Tpit negative in our study.

TABLE 2 | Baseline characteristics of patients (n = 105).

Characteristic	Number (%) or Mean ± S.D.
Age	49.8 ± 10.6
Gender	
Female	99 (94.3%)
Clinical Feature	
Vision loss or visual field defect	58 (55.2%)
Frequent headaches	24 (22.9%)
Galactorrhea, oligomenorrhoea or amenorrhea	18 (37.5%)*
Polydipsia and polyuria	4 (3.8%)
Previous history	
Pituitary surgery	16 (15.2%)
Pituitary surgery + radiotherapy	4 (3.8%)
Size of tumor	
Microadenoma	2 (1.9%)
Macroadenoma	103 (98.1%)
Invasiveness of tumor	
Suprasellar extension	73 (69.5%)
Sphenoid sinus invasion	56 (53.3%)
Cavernous sinus invasion	76 (72.4%)
Cystic change of tumor	
Large cyst	55 (52.4%)
Multiple microcysts	78 (74.3%)
Pituitary function	
High levels of ACTH	13 (12.4%)†
Hyperprolactinemia	41 (39.0%)
Central hypothyroidism	23 (21.9%)
Low levels of cortisol	19 (18.1%)
Low levels of ACTH	2 (1.9%)†
Low levels of testosterone	5 (83.3%)†
Pathology result	
ACTH (-), Tpit (+)	66 (62.9%)
ACTH (+), Tpit (+)	39 (37.1%)
Ki-67 index	
Ki-67<3%	89 (84.8%)
3%≤Ki-67<10%	16 (15.2%)
Ki-67≥10%	0 (0%)

*Only female patients of childbearing age (age 15–49) were included, n = 8.
†Only cases in which blood ACTH levels were tested were included, n = 81.
‡Only male patients were included, n = 6.

All 105 cases were stained negative for TSH, FSH, LH and PIT-1. One case was stained slightly positive for GH and PRL. Immunostaining for Syn, CAM5.2, P53, SSTR2a, and SF-1 were positive in 105 (105/105, 100.0%), 88 (88/100, 88.0%), 22 (22/103, 21.4%), 24 (24/99, 24.2%), and 3 (3/105, 2.9%) cases, respectively. Ki-67 expression was lower than 3% in 89 cases (89/105, 84.8%), between 3% and 10% (≥3%, <10%) in 16 cases (16/105, 15.2%), and no more higher than 10%.

Surgical Outcomes

The extent of resection by microscope or endoscope were gross total resection (GTR) for 48 patients (48/105, 45.7%), subtotal resection (STR) for 50 patients (50/105, 47.6%) and partial resection (PR) for 7 patients (7/105, 6.7%). Among the 98 cases operated *via* the transnasal approach, intraoperative CSF leakage occurred in 36 cases (36/98, 36.7%). Nineteen cases (19/98, 19.4%) had grade 1 CSF leakage, 10 cases (10/98, 10.2%) had grade 2 leakage, and 7 cases (7/98, 7.1%) had grade 3 leakage.

Transient diabetes insipidus (DI) was observed in four (4/105, 3.8%) patients. Hypoadrenalism, hypothyroidism, and panhypopituitarism (≥2 endocrinological axes) occurred in 61.9% (65/105), 1.0% (1/105), and 15.2% (16/105) of all patients, respectively. An appropriate hormonal replacement was given. One patient reported decreased vision after surgery. An optic nerve decompression surgery was performed, and the patient's vision recovered after surgery. No postoperative CSF leakage, meningitis, or permanent DI was observed.

The mean follow-up time was 17.2 ± 7.6 months (range 3-29), with three cases lost to follow-up (2 GTR, 1 STR). Forty-four patients who received GTR (44/46, 95.7%) had no recurrence at last follow-up. The 2 patients who had a recurrence both received stereotactic radiotherapy (gamma knife) immediately after recurrence were detected (3 months and 24 months after surgery, respectively).

Group Comparison

We compared 66 (66/105, 62.9%) cases with ACTH-negative SCAs with 39 (39/105, 37.1%) cases with ACTH-positive SCAs (**Table 3**). No statistical differences were observed between the two groups regarding gender (97.0% vs. 89.7%, P = 0.192), age (50.3 ± 10.3 vs. 49.0 ± 11.2, P = 0.543), surgical history (16.7% vs. 23.1%, P = 0.419), suprasellar extension (66.7% vs. 74.4%, P = 0.408), sphenoid sinus extension (51.5% vs. 56.4%, P = 0.627), cavernous sinus invasion (75.8% vs. 66.7%, P = 0.314), large cyst on MRI (47.0% vs. 61.5%, P = 0.149) or gross total resection rate (42.4% vs. 51.3%, P = 0.379). We found that patients with ACTH-negative SCAs were more likely to have lower ACTH levels (27.5 ± 24.0 vs. 54.4 ± 58.6, P = 0.011) and multiple microcysts on MRI (81.8% vs. 61.5%, P = 0.022). ACTH-positive SCAs were more likely to have a higher level of Ki-67 expression (low expression rate 74.4% vs. 90.9%, P = 0.023).

Following univariate analysis (**Table 4**), the ACTH levels, multiple microcysts on MRI, and Ki-67 expression were included in the multivariate analysis (**Table 5**). The ACTH levels (OR = 1.019; 95% CI 1.001–1.037; P = 0.036) were found as an independent factor.

DISCUSSION

In this study, we have identified the different manifestations between ACTH-positive and ACTH-negative SCAs and calculated the proportion of SCA in all NFPAs based on whether Tpit immunostaining was performed. Most previous studies about SCAs had no ACTH-negative cases included, and in existing researches about ACTH-negative SCAs, the numbers of cases were rather smaller. 2017 WHO classification of PAs defined SCA as a subtype of PA that belongs to the TPIT lineage (1) and Tpit immunostaining was suggested to be added to the diagnostic protocol (3). After Tpit immunostaining was added, the diagnostic rate of SCA in all NFPAs improved significantly. Our results suggested that without performing Tpit immunostaining, up to 62.9% of cases (66/105) could be missed, which proved the necessity to give more attention to this substantially underestimated subtype of PA.

In our study, serum ACTH levels were found significantly higher in ACTH-positive SCAs (54.4 ± 58.6 vs. 27.5 ± 24.0., P = 0.011) than in ACTH-negative SCAs, and clearly above the

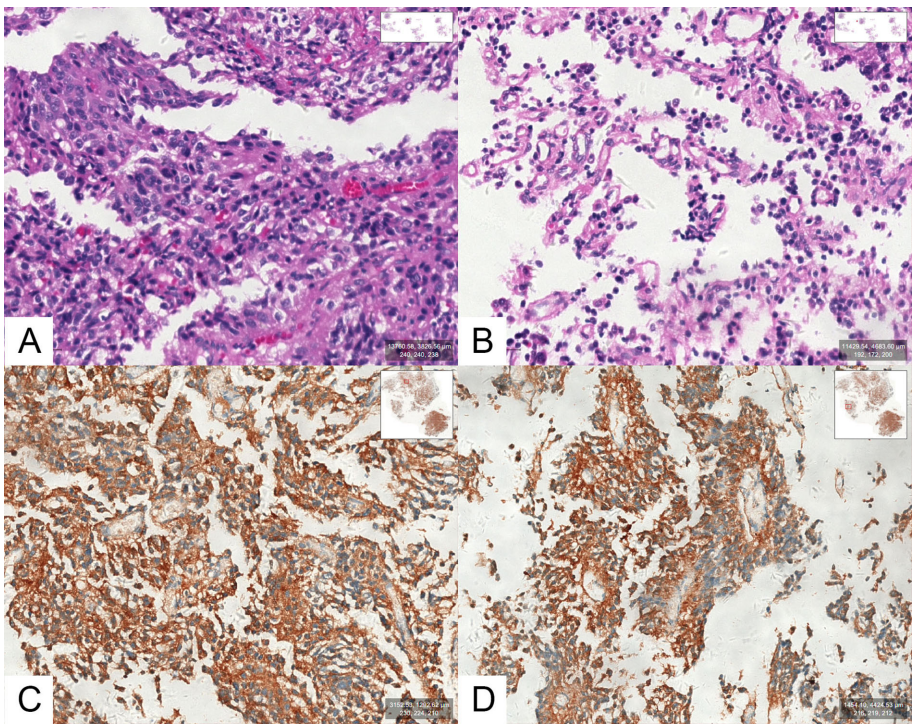

FIGURE 2 | **(A, B)** Optical photographs for hematoxylin and eosin (H&E) staining in an SCA. **(C, D)** Optical photographs for ACTH staining in an SCA.

normal range. In previous studies, most series found that SCAs (only ACTH-positive cases included) had elevated ACTH levels and normal cortisol levels (4, 5), which agrees with our findings. Several hypotheses have been proposed to explain the lack of clinical hypercortisolism in SCAs despite the high expression of ACTH in ACTH-positive SCAs. However, recent studies about cell origin and molecular mechanisms still need further experiments to be proven. In ACTH-negative SCAs, the serum ACTH levels were mostly normal (average ± std.). Because the commercially available ACTH antibodies cannot separate bioactive or bio-inactive ACTH from pro-ACTH or POMC (6), presumably none of the three is elevated in the circulation of patients with ACTH-negative SCAs. Thus, ACTH-negative SCAs were completely clinically SCAs, which present with no hypersecretion of bioactive ACTH, bio-inactive ACTH, pro-ACTH, or POMC.

We observed that multiple microcysts appeared more often in ACTH-negative SCAs (81.8% vs. 61.5%, P = 0.022) than in ACTH-positive cases. According to the literature, more cysts were observed in SCAs (only ACTH-positive cases included)

TABLE 3 | Comparison of clinical features between ACTH-negative SCAs and ACTH-positive SCAs (n = 105).

Feature	ACTH-negative (n = 66)	ACTH-positive (n = 39)	p value
Age	50.3 ± 10.3	49.0 ± 11.2	0.543
Female gender	64 (97.0%)	35 (89.7%)	0.192
Blood ACTH levels	27.5 ± 24.0†	54.4 ± 58.6‡	0.011*
Pituitary surgical history	11 (16.7%)	9 (23.1%)	0.419
Invasiveness of tumor			
Cavernous sinus invasion	50 (75.8%)	26 (66.7%)	0.314
Suprasellar extension	44 (66.7%)	29 (74.4%)	0.408
Sphenoid sinus invasion	34 (51.5%)	22 (56.4%)	0.627
Cystic change of tumor			
Large cyst	31 (47.0%)	24 (61.5%)	0.149
Multiple microcysts	54 (81.8%)	24 (61.5%)	0.022*
Gross total resection	28 (42.4%)	20 (51.3%)	0.379
Ki-67 expression (<3%)	60 (90.9%)	29 (74.4%)	0.023*

ACTH, adrenocorticotropic hormone; SCA, silent corticotroph adenoma.

**P < 0.05 was considered statistically significant.*

†Only cases in which blood ACTH levels were tested were included, n = 43.

‡Only cases in which blood ACTH levels were tested were included, n = 38.

TABLE 4 | Univariate analysis results for the difference in clinical features between ACTH-negative SCAs and ACTH-positive SCAs (n = 105).

Feature	OR (95% CI)	p value
Age	0.988 (0.952–1.026)	0.539
Female gender	0.273 (0.048–1.568)	0.146
Blood ACTH levels	1.020 (1.003–1.039)	0.025*
Pituitary surgical history	1.500 (0.559–4.024)	0.421
Cavernous sinus invasion	0.640 (0.268–1.530)	0.316
Suprasellar extension	1.450 (0.600–3.504)	0.409
Sphenoid sinus invasion	1.218 (0.549–2.700)	0.627
Large cyst	1.806 (0.807–4.045)	0.150
Multiple microcysts	0.356 (0.145–0.873)	0.024*
Gross total resection	0.700 (0.316–1.551)	0.379
Ki-67 expression (<3%)	3.448 (1.142–10.410)	0.028*

ACTH, adrenocorticotropic hormone; SCA, silent corticotroph adenoma; OR, odds ratio; CI, confidence interval.
**P < 0.05 was considered statistically significant.*

TABLE 5 | Multivariate analysis results for the difference in clinical features between ACTH-negative SCAs and ACTH-positive SCAs (n = 105).

Feature	OR (95% CI)	p value
Blood ACTH levels	1.019 (1.001–1.037)	0.036*
Multiple microcysts	0.378 (0.130–1.096)	0.073
Ki-67 expression (<3%)	3.528 (0.907–13.730)	0.069

ACTH, adrenocorticotropic hormone; SCA, silent corticotroph adenoma; OR, odds ratio; CI, confidence interval.

than NFPAs, with 75% of them showing multiple microcysts, which agrees with our results (61.5% of ACTH-positive SCAs and 81.8% of ACTH-negative SCAs showed multiple microcysts) (7, 8). However, this imaging manifestation also appeared in most null cell adenomas and gonadotroph adenomas according to some series (8). In other words, ACTH-negative SCAs and null cell adenomas were similar in lack of hormone secretion and imaging findings, though differ greatly in recurrence rate (9).

In order to check and predict the possible SCAs, several studies have investigated the difference in baseline characteristics between SCAs and NFPAs. SCAs were found more prevalent in females compared with NFPAs in most series (3, 7, 10, 11), while the male-female ratio in our study was 1:16.5; Age at presentation was mostly similar in patients with SCAs and NFPAs (7, 10, 12–14). Patients with SCAs (only ACTH-positive cases included) secreted ACTH higher than normal (4, 5, 10). SCAs were found with more microcysts and intratumoral hemorrhage than NFPAs (7, 10, 12, 15). Cavernous sinus invasion observed in SCAs compared to NFPAs was either higher or similar in the literature (3, 4, 7, 14, 16). Thus, female gender, high blood ACTH levels (only for ACTH-positive SCAs), and cystic degeneration were found as relatively reliable predictors for SCAs, and the predictive significance of tumor invasiveness remains to be confirmed.

Possible mechanisms of the "silence" feature of SCAs include two main hypotheses. Kovacs et al. first introduced this type of PA in 1978 and put forward a hypothesis that ACTH was not discharged from the adenoma cells due to abnormal lysosomal function or abnormal hormone synthesis (17). Another hypothesis suggested that the low expression level of prohormone convertase (PC) 1/3 in SCAs caused the lack of post-transcriptional regulation of POMC, which led to the silence feature of SCAs (18–20). Moreover, we also found that in a patient who had a pituitary surgical history, the ACTH immunostaining was positive for the previous operation and negative for the current operation, which needs further research to be explained.

Limitations in this study included: 1) We did not perform a comprehensive endocrine assessment for every patient before the surgery. Considering almost all of the cases in our study (103/105) were nonfunctioning pituitary macroadenomas, and that the Cushingoid symptoms of cases with ACTH-secreting pituitary macroadenomas can be considerably serious, the possibility of Cushing's Disease can be roughly ruled out. 2) The follow-up period was relatively short for estimating the long-term recurrence rate in our series. Most of our patients come from other cities outside Shanghai. It is difficult for them to visit us regularly after surgery if they were feeling no sign of recurrence.

CONCLUSIONS

The ACTH-negative SCAs appeared to be more clinically silent, and more likely to show multiple microcysts on MRI. The addition of Tpit immunostaining significantly improved the diagnostic rate of SCA. The prevalence of SCA was substantially underestimated, and sufficient attention should be paid to this high-risk, aggressive type of refractory PA.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

The protocol was approved by the Huashan Hospital, Shanghai Medical School, Fudan University. All subjects gave written informed consent in accordance with the Declaration of Helsinki.

AUTHOR CONTRIBUTIONS

KZ, XS, HY, and YW contributed conception and design of the study. KZ organized the database and performed the statistical analysis. KZ and XS wrote the first draft of the manuscript. HC, NQ, WH, and ZC contributed to the acquisition of data for the work. MS, SL, YZ, ZZ, YL, HY, and YW revised the work critically for important intellectual content. All authors contributed to the article and approved the submitted version.

FUNDING

The present work was supported by the Science and Technology Commission of Shanghai Municipality (Grant no. 17441901400). The present work was supported by the National Natural Science Foundation of China (Grant no. 81602191).

REFERENCES

1. Mete O, Lopes MB. Overview of the 2017 WHO Classification of Pituitary Tumors. *Endocr Pathol* (2017) 28(3):228–43. doi: 10.1007/s12022-017-9498-z
2. Altman DG. *Practical statistics for medical research*. New York: Chapman and Hall/CRC (1990).
3. Nishioka H, Inoshita N, Mete O, Asa SL, Hayashi K, Takeshita A, et al. The Complementary Role of Transcription Factors in the Accurate Diagnosis of Clinically Nonfunctioning Pituitary Adenomas. *Endocr Pathol* (2015) 26 (4):349–55. doi: 10.1007/s12022-015-9398-z
4. Langlois F, Lim DST, Yedinak CG, Cetas I, McCartney S, Cetas J, et al. Predictors of silent corticotroph adenoma recurrence; a large retrospective single center study and systematic literature review. *Pituitary* (2018) 21 (1):32–40. doi: 10.1007/s11102-017-0844-4
5. Raverot G, Wierinckx A, Jouanneau E, Auger C, Borson-Chazot F, Lachuer J, et al. Clinical, hormonal and molecular characterization of pituitary ACTH adenomas without (silent corticotroph adenomas) and with Cushing's disease. *Eur J Endocrinol* (2010) 163(1):35–43. doi: 10.1530/eje-10-0076
6. Ben-Shlomo A, Cooper O. Silent corticotroph adenomas. *Pituitary* (2018) 21 (2):183–93. doi: 10.1007/s11102-018-0864-8
7. Cho HY, Cho SW, Kim SW, Shin CS, Park KS, Kim SY. Silent corticotroph adenomas have unique recurrence characteristics compared with other nonfunctioning pituitary adenomas. *Clin Endocrinol (Oxf)* (2010) 72 (5):648–53. doi: 10.1111/j.1365-2265.2009.03673.x
8. Guttenberg KB, Mayson SE, Sawan C, Kharlip J, Lee JY, Martinez-Lage M, et al. Prevalence of clinically silent corticotroph macroadenomas. *Clin Endocrinol (Oxf)* (2016) 85(6):874–80. doi: 10.1111/cen.13146
9. Ntali G, Wass JA. Epidemiology, clinical presentation and diagnosis of non-functioning pituitary adenomas. *Pituitary* (2018) 21(2):111–8. doi: 10.1007/s11102-018-0869-3
10. Kim D, Ku CR, Park SH, Moon JH, Kim EH, Kim SH, et al. Clinical Parameters to Distinguish Silent Corticotroph Adenomas from Other Nonfunctioning Pituitary Adenomas. *World Neurosurg* (2018) 115:e464–71. doi: 10.1016/j.wneu.2018.04.070
11. Kim J, Yoon SJ, Moon JH, Ku CR, Kim SH, Lee EJ, et al. Clinical Significance of Radical Surgery in the Treatment of Silent Corticotroph Adenoma. *J Kor Neurosurg Soc* (2019) 62(1):114–22. doi: 10.3340/jkns.2018.0027
12. Alahmadi H, Lee D, Wilson JR, Hayhurst C, Mete O, Gentili F, et al. Clinical features of silent corticotroph adenomas. *Acta Neurochir (Wien)* (2012) 154 (8):1493–8. doi: 10.1007/s00701-012-1378-1
13. Ioachimescu AG, Eiland L, Chhabra VS, Mastrogianakis GM, Schniederjan MJ, Brat D, et al. Silent corticotroph adenomas: Emory University cohort and comparison with ACTH-negative nonfunctioning pituitary adenomas. *Neurosurgery* (2012) 71(2):296–303; discussion 4. doi: 10.1227/NEU.0b013e318257c1f0
14. Jahangiri A, Wagner JR, Pekmezci M, Hiniker A, Chang EF, Kunwar S, et al. A comprehensive long-term retrospective analysis of silent corticotrophic adenomas vs hormone-negative adenomas. *Neurosurgery* (2013) 73(1):8–17; discussion -8. doi: 10.1227/01.neu.0000429858.96652.1e
15. Kasuki L, Antunes X, Coelho MCA, Lamback EB, Galvao S, Silva Camacho AH, et al. Accuracy of microcystic aspect on T2-weighted MRI for the diagnosis of silent corticotroph adenomas. *Clin Endocrinol (Oxf)* (2020) 92 (2):145–9. doi: 10.1111/cen.14133
16. Cohen-Inbar O, Xu Z, Lee CC, Wu CC, Chytka T, Silva D, et al. Prognostic significance of corticotroph staining in radiosurgery for non-functioning pituitary adenomas: a multicenter study. *J Neurooncol* (2017) 135(1):67–74. doi: 10.1007/s11060-017-2520-y
17. Kovacs K, Horvath E, Bayley TA, Hassaram ST, Ezrin C. Silent corticotroph cell adenoma with lysosomal accumulation and crinophagy. A distinct clinicopathologic entity. *Am J Med* (1978) 64(3):492–9. doi: 10.1016/0002-9343(78)90236-x
18. Ohta S, Nishizawa S, Oki Y, Yokoyama T, Namba H. Significance of absent prohormone convertase 1/3 in inducing clinically silent corticotroph pituitary adenoma of subtype I–immunohistochemical study. *Pituitary* (2002) 5 (4):221–3. doi: 10.1023/a:1025321731790
19. Righi A, Faustini-Fustini M, Morandi L, Monti V, Asioli S, Mazzatenta D, et al. The changing faces of corticotroph cell adenomas: the role of prohormone convertase 1/3. *Endocrine* (2017) 56(2):286–97. doi: 10.1007/s12020-016-1028-0
20. Tateno T, Izumiyama H, Doi M, Akashi T, Ohno K, Hirata Y. Defective expression of prohormone convertase 1/3 in silent corticotroph adenoma. *Endocr J* (2007) 54(5):777–82. doi: 10.1507/endocrj.k07-059

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Endocrinology

CASE REPORT
published: 12 March 2021
doi: 10.3389/fendo.2021.616339

Case Report: Temozolomide Treatment of Refractory Prolactinoma Resistant to Dopamine Agonists

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OPEN ACCESS

Edited by:
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Reviewed by:
Luiz Augusto Casulari, University of Brasilia, Brazil
Odelia Cooper, Cedars Sinai Medical Center, United States

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Specialty section:
This article was submitted to Pituitary Endocrinology, a section of the journal Frontiers in Endocrinology

Received: *12 October 2020*
Accepted: *12 February 2021*
Published: *12 March 2021*

Citation:
Tang H, Cheng Y, Huang J, Li J, Zhang B and Wu ZB (2021) Case Report: Temozolomide Treatment of Refractory Prolactinoma Resistant to Dopamine Agonists. Front. Endocrinol. 12:616339. doi: 10.3389/fendo.2021.616339

Therapeutic agents for refractory prolactinomas that are resistant to dopamine agonists (DAs) are troublesome, and surgery often only removes a large part of the tumor without complete remission. Among the various second-line treatment regimens, the treatment effect of the alkylating agent temozolomide (TMZ) is only effective for approximately half of patients; however, complete remission is rare. Here we report a patient with prolactinoma who was resistant to high-dose cabergoline (CAB) treatment, demonstrating a continuous increase in both the tumor volume and the prolactin (PRL) level. Given that this case is a refractory prolactinoma, the patient underwent two transsphenoidal approach (TSA) surgeries. The pathological analysis indicated that the Ki-67 index increased significantly from 3% to 30%, and the expression levels of DRD2 and MGMT were low. Finally, TMZ treatment was recommended. A total of six cycles of TMZ standard chemotherapy shrank the tumor volume and the tumor disappeared completely. During the 6-month follow-up period, the tumor did not relapse again, and the PRL level was also normal. RNA sequencing and DNA whole genome sequencing were performed on this prolactinoma specimen, revealing 16 possible gene mutations, including a missense mutation of the PABPC1 gene. Additionally, the copy number variation analysis results showed that several chromosomes had copy number gains compared to the matched peripheral blood sample. In this case, low expression of DRD2 and high proliferation led to resistance to CAB, whereas low MGMT expression contributed to sensitivity to TMZ treatment. The results of genome sequencing still need further investigation at the molecular level to explain the tumor aggressiveness and high sensitivity to TMZ.

Keywords: resistance, dopamine agonist, PABPC1, temozolomide, prolactinoma

INTRODUCTION

Pituitary adenomas are the third most common intracranial tumor. The majority are considered to be benign, but approximately 35% are invasive (1, 2). Prolactinoma accounts for approximately 40% of pituitary adenomas, the majority of which can be treated with dopamine agonists (DAs), such as cabergoline (CAB) and bromocriptine (BRC). However, approximately 10-20% are resistant and refractory cases (3, 4), lacking effective treatments. A small number of tumors continue to enlarge significantly under high-dose DA treatment. However, these tumors are rarely reported, and the underlying mechanism is still unknown. To date, major studies have surmised that the resistance of prolactinoma to DAs may be due to the decrease in dopamine 2 receptor (DRD2) expression in tumor cells (5).

Temozolomide (TMZ) is a DNA alkylating agent that can cause base mismatches, which can trigger a futile DNA repair cycle. Eventually, the DNA strand breaks, which leads to cell death (6). TMZ has an effective stabilization rate of 27% for invasive and refractory pituitary tumors (7). TMZ is especially effective for 50% of patients with refractory prolactinomas who have failed conventional treatments, and increases the overall survival rate (7). However, there are fewer patients who achieve complete remission, and drug resistance and recurrence rates remain high (8, 9). In a systematic review of 42 patients with prolactinoma (or carcinoma) using TMZ treatment, only one patient with prolactin-secreting carcinoma and two patients with prolactinoma exhibited complete remission (10).

This study presents a case report of prolactinoma that is resistant to DAs and exhibits aggressive growth. Treatment with TMZ has achieved good and even significant antitumor effects. In addition, genomics and other research methods were used to investigate the underlying mechanism of drug therapy.

CASE DESCRIPTION

History

A 40-year-old woman attended the endocrinology department due to menstrual disorders and lactation. Hormonal examination revealed a prolactin (PRL) of 105 ng/ml. A microadenoma in the sellar region was found on MR images with a size of approximately 0.5 * 0.5 * 0.5 cm. She was advised to take BRC 7.5 mg/d for the first 18 months, and the symptoms disappeared. Later, due to the incomplete control of PRL, she switched to CAB, gradually increasing and maintaining the dose at 2 mg/w. Two years after regular medication, the PRL level was reduced to 39 ng/ml (**Figure S1**), and the tumor appeared to shrink (0.4 * 0.4 * 0.3 cm in size) on MR images. However, over the next three years, she took the drugs, and the patient found that the PRL level gradually increased (133 ng/ml). However, the microadenoma exhibited an increasing trend (0.9 * 0.8 * 0.7 cm, **Figures 1A, B**), although the dose of CAB increased to 4 mg/w (**Figure S1**). Unfortunately, this patient often experienced dizziness and gastrointestinal discomfort with increased drug doses, so she was transferred to our Center of Pituitary Tumor for surgery. The center diagnosed this case of prolactin microadenoma resistant to DAs.

After careful consideration, at the age of 45 years, the patient underwent neuroendoscopic transsphenoidal pituitary adenoma resection. One week after the operation, the PRL level dropped to 54 ng/ml. The pathology confirmed pure PRL adenoma. Six months after the operation, the patient was re-examined by MR scan, and no obvious residual tumor was found. However, the PRL level increased to 89 ng/ml (**Figure S1**). After this follow-up visit, it was recommended that the patient continued to take CAB at the original tolerable dose (2 mg/w).

Unfortunately, the drugs were no longer able to inhibit tumor growth. Two years after regular medication, the dose of CAB was

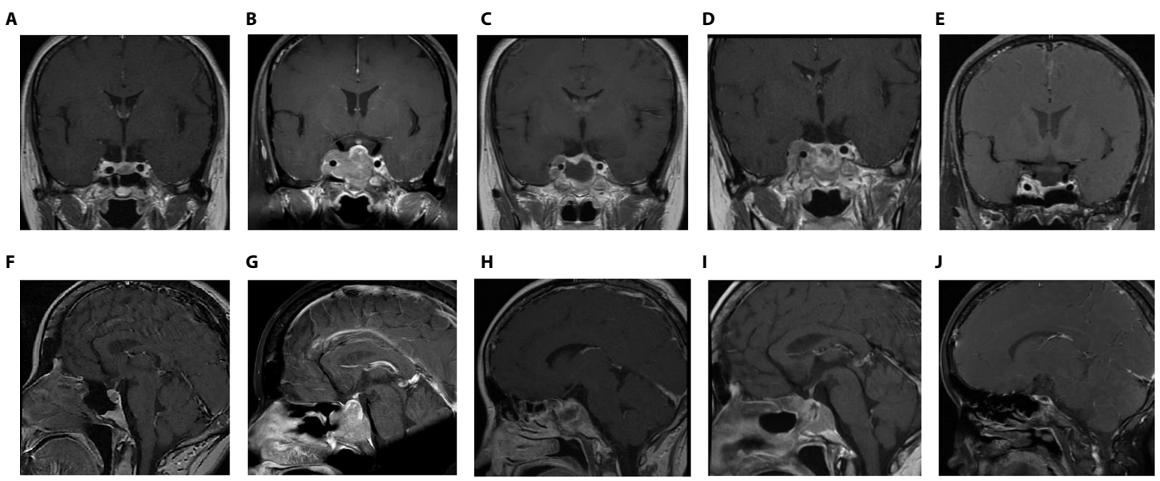

FIGURE 1 | Coronal- and sagittal- enhanced MR images in various periods **(A, B)**: Before the first operation (5 years after DA treatment); **(C, D)**: 2 years after the first operation (before the second operation); **(E, F)**: The day after the second operation; **(G, H)**: 1 month after the second operation (the eve of TMZ treatment); **(I, J)**: 6 months after TMZ treatment.

gradually increased to 4 mg/w. The patient was re-examined by MR, which revealed that the tumor continued to increase to approximately 3 * 3.2 * 2.6 cm in size with right cavernous sinus invasion (**Figures 1C, D**). The PRL level continued to increase with a peak level of 4902 ng/ml (**Figure S1**). In addition, she complained of visual field defects. After discussion with a multidisciplinary team (MDT), a diagnosis of refractory and aggressive prolactinoma was made. The second transsphenoidal pituitary tumor resection was performed to decompress the optic nerve.

New MR was conducted one day and one month after the second operation, showing remnant and rapidly relapsed tumors in the right cavernous sinus and sellar region, respectively (**Figures 1E–H**). The tumor size increased from 1 * 1.2 * 2 cm to 1.8 * 2 * 2.3 cm. The serum PRL level remained high (2066 ng/ml) (**Figure S1**). Based on the immunohistochemical (IHC) staining results of both tumor specimens collected from two operations subsequently (DRD2, MGMT and the change of Ki67, detailed in the "Pathology" section below), with consent, the patient was recommended to stop CAB and switch to standard chemotherapy with TMZ (240 mg × 5 d for the first month, 320 mg × 5 d per month for 2-6 months) for a total of 6 months. After 3 months of taking TMZ medicine, the tumor was significantly suppressed (0.8 * 0.7 * 0.4 cm). By the 6-month point of TMZ treatment, the tumor had completely disappeared (**Figures 1I, J**) and the PRL level returned to the normal level (18 ng/ml) (**Figure S1**).

The patient was followed up for another 6 months. The tumor did not reappear and the PRL level was also in the normal range (19 ng/ml).

Pathology

Pathology of the first operation specimen: pituitary adenoma (**Figures 2A–D**, partial display). HE staining (**Figure 2A**); immunohistochemical (IHC) staining: Ki67 (3%+) (**Figure 2B**), PRL (+) (**Figure 2C**), ACTH (-), TSH (-), LH (-), FSH (-), GH (-), DRD2 (-) (**Figure 2D**), reticular fiber (-).

Pathology of the second operation specimen (**Figure 2E-K**, partial display): HE staining (**Figure 2 E**); IHC: Ki67 (30%+) (**Figure 2F**), PRL (+) (**Figure 2G**), ACTH (-), GH (-) (**Figure 2H**), FSH (-), LH (-), TSH (-), pit-1 (+) (**Figure 2 I**), MGMT (-) (**Figure 2J**), DRD2 (-) (**Figure 2K**), reticular fiber (-).

The pathological results have several characteristics. One was pit-1 positive (**Figure 2I**) and PRL positive (**Figure 2G**) but GH negative (**Figure 2H**), indicating that it is a single prolactin cell source, not a mixed adenoma. Another is to compare the active mitosis and high proliferation index of the two specimens. Ki67 increased from 3% (**Figure 2B**) in the first operation to 30% in the second operation (**Figure 2F**). Finally, the expression of DRD2 (**Figures 2D, K**) and MGMT was very low or even nonexistent (**Figure 2J**).

Genome Sequencing

In view of the patient's response to drugs, that is, resistance to DAs and the high sensitivity to TMZ, the pharmacotherapeutic mechanism had to be illustrated. RNA sequencing and DNA whole genome sequencing (WGS) on specimens from the second operation were performed. Genomic DNA and total RNA obtained from the patient were extracted using an AllPrep DNA/RNA Mini Kit (Qiagen) and subjected to paired-end (2 × 150 bp) sequencing on a NovaSeq platform (Illumina) according to the manufacturer's protocol. WGS with an average read depth of 60× was analyzed using GATK (11) and VarScan2 (12). RNA-seq data were analyzed using STAR (13), salmon (14) and DESeq2 (15). RNA sequencing found that the number of DRD2 mRNA transcripts was very low (only 5 cycles, data not shown) compared to the other 23 prolactinoma specimens (15 resistant and 8 sensitive to DAs). WGS confirmed somatic mutations in FAM160B1, MYBPC1, CSPG4, GAN, CIRBP, PRX, SF3B1, PABPC1, CABS1, TENM3, DMXL1, HOXA3, CSGALNACT, PLEC, RBL2, and KIR2DL3 (**Table 1**). Among them, the missense mutation in PABPC1 (poly (A) binding protein cytoplasmic 1) is located at base 1424, exon 10. The mutation from guanine (G) to adenine (A) results in the conversion of the 475th amino acid of the expression protein from arginine (R) to glutamine (Q).

On the other hand, copy number variation analysis results showed that several chromosomes had copy number gains compared to the matched peripheral blood sample, e.g., chr1, chr3, chr7, chr8, chr18 and chr19. Most of them (chr1, chr3, chr8 and chr19) had copy number gains on the long arm (q) or short arm (p), whereas there was almost one whole chromosome copy number gain on chr7 and chr18 (**Figure 3**).

DISCUSSION

It is well known that the effectiveness of DAs on prolactinoma is determined by the expression of DRD2 and that a decrease in DRD2 expression may play a critical role in DA-resistant prolactinoma (16–18). Our previous study (19) found that DRD2 expression was significantly different between resistant and sensitive prolactinomas, and even between aggressive and nonaggressive prolactinomas. In this study, we used immunohistochemical (IHC) staining, which is commonly used in pathology, to identify the expression level of specific protein molecules in cells. IHC staining of the patient's tumor specimens revealed very low expression of DRD2, which probably explained the tumor's resistance to high-dose CAB treatment. In addition, this tumor resistance might be related to the high degree of tumor aggressiveness. Ki67 expression obviously increased, reaching 30%, which combined with invasion of the right cavernous sinus, indicating that the tumor exhibited significant aggressiveness. The tumor volume even increased under high-dose CAB treatment. All the above results indicated that this was a refractory prolactinoma resistant to DA treatment.

TMZ has some effect on refractory prolactinomas or carcinomas, but the proportion of patients with complete remission is very low (10, 20). In the TMZ treatment cohort study of 166 aggressive pituitary tumors initiated by the European Society of Endocrinology (ESE) (20), 40 cases were aggressive, refractory prolactinoma or carcinoma, and 2 of these cases (5%) achieved complete remission after treatment. The article did not disclose the

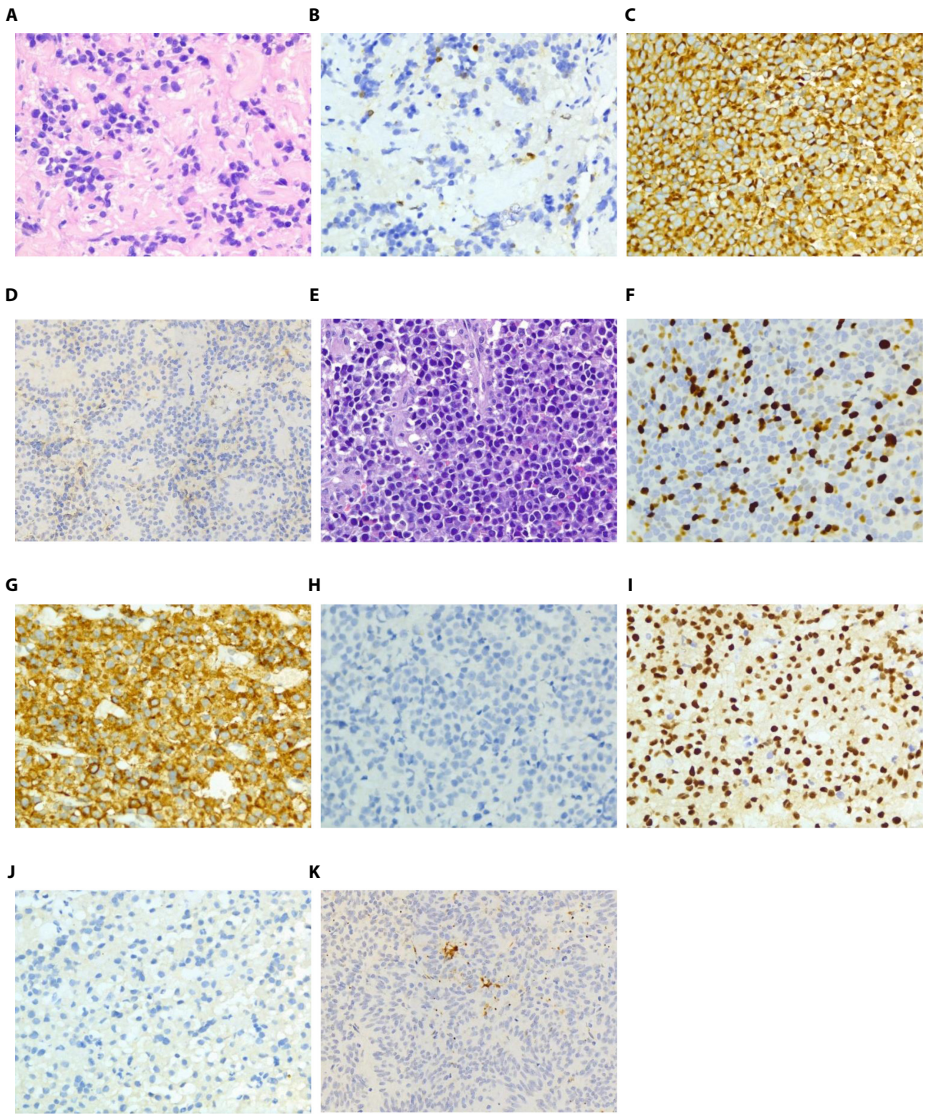

FIGURE 2 | Pathological images of specimens after the first and second operations **(A)**: HE staining of specimens after the first operation; **(B)**: Ki-67 immunohistochemical staining of specimens after the first operation; **(C)**: PRL immunohistochemical staining of specimens after the first operation; **(D)**: DRD2 immunohistochemical staining of specimens after the first operation; **(E)**: HE staining of specimens after the second operation; **(F)**: Ki-67 immunohistochemical staining of specimens after the second operation; **(G)**: PRL immunohistochemical staining of specimens after the second operation; **(H)**: GH immunohistochemical staining of specimens after the second operation; **(I)**: Pit-1 immunohistochemical staining of specimens after the second operation; **(J)**: MGMT immunohistochemical staining of specimens after the second operation; **(K)**: DRD2 immunohistochemical staining of specimens after the second operation. Magnification: **(A–C, E–J)**: ×400; D&K: ×200.

detailed PRL or imaging changes of the cases. In another review (10), among the 42 cases included, there were 23 patients with prolactinoma and 19 patients with prolactin-secreting pituitary carcinoma, of whom 3 patients (3/42, 7%) had their PRL levels reduced to normal after TMZ treatment. However, the original reports of the two prolactinoma cases with complete remission did not show the detailed size changes and imaging data of the tumors before and after treatment (21, 22). In addition, a patient with prolactin-secreting pituitary carcinoma who achieved complete remission was reported in detail (23). He was an elderly male (>70 y) and had metastatic lesions in the cerebellopontine angle and cervical spine in addition to a primary tumor in the sellar region. Three tumor lesions disappeared completely after 18 months of TMZ treatment. Our present case cannot be defined as prolactin-secreting pituitary carcinoma because there was no intracranial metastasis. This tumor exhibited a complete response to TMZ treatment with a biological cure.

Obviously, the effect of TMZ treatment has a strong correlation with the expression of MGMT in glioma (24, 25). Specifically, the lower the degree of MGMT expression, the

TABLE 1 | 16 possible gene mutations identified with whole genome sequencing.

Gene	Chromosome	Location	Ref → Seq	Tumor variant frequency	Coding	Amino acid change
FAM160B1	chr10	114846067	A → G	0.2903	c.1183A>G	p.M395V
MYBPC1	chr12	101652686	A → C	0.2075	c.1460A>C	p.K487T
CSPG4	chr15	75689920	A → C	0.25	c.1145T>G	p.L382R
GAN	chr16	81362563	G → T	0.45	c.1038G>T	p.K346N
CIRBP	chr19	1272068	G → C	0.4444	c.519G>C	p.E173D
PRX	chr19	40394057	C → A	0.4348	c.4295G>T	p.G1432V
SF3B1	chr2	197402759	C → T	0.5	c.1874G>A	p.R625H
CABS1	chr4	70335766	G → A	0.2927	c.727G>A	p.D243N
TENM3	chr4	182751942	G → A	0.2895	c.3772G>A	p.A1258T
DMXL1	chr5	119129367	C → T	0.2031	c.1259C>T	p.P420L
PABPC1	chr8	100706893	G → A	0.3227	c.1424G>A	p.R475Q
HOXA3	chr7	27110570	A → C	0.2034	c.71T>G	p.F24C
CSGALNACT1	chr8	19406045	C → A	0.3871	c.1334G>T	p.G445V
PLEC	chr8	143917661	C → T	0.3438	c.12571G>A	p.D4191N
RBL2	chr16	53490280	A → C	0.25	c.3400A>C	p.N1134H
RBL2	chr16	53490281	A → C	0.2703	c.3401A>C	p.N1134T
KIR2DL3	chr19	54752514	C → T	0.6087	c.1021C>T	p.P341S

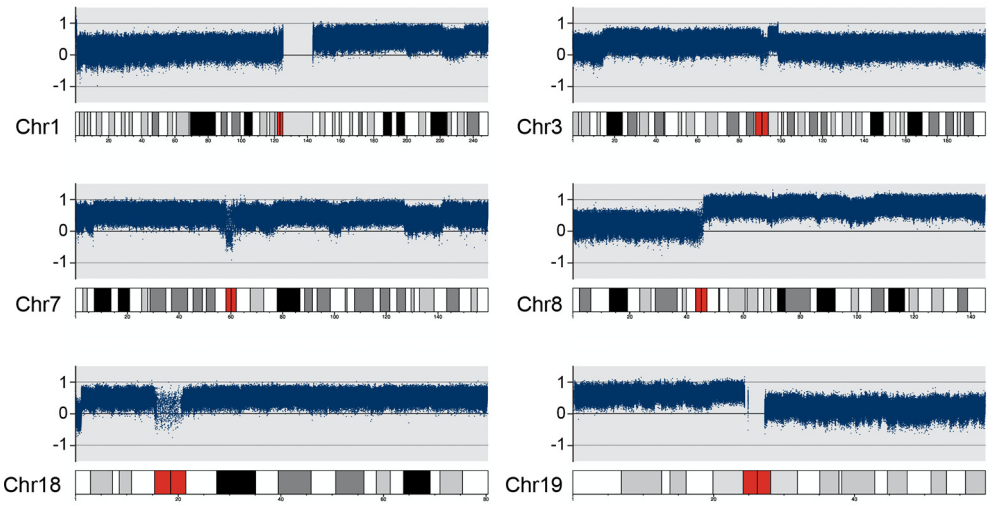

FIGURE 3 | DNA copy number variation analysis results on chr1, chr3, chr7, chr8, chr18 and chr19 Several chromosomes had copy number gains compared to the matched PB (peripheral blood) sample. Chr1&8 have copy number gains on the long arm (q); Chr3&19 have copy number gains on the short arm (p); Chr7&18 have copy number gain on almost the entire chromosome.

better the tumor's response to TMZ treatment. However, in pituitary tumors, there is controversy regarding the relationship between MGMT expression and tumor response to TMZ treatment. MGMT protein expression was very low in this case, indicating that refractory tumor cells were highly sensitive to TMZ treatment.

DNA whole genome sequencing showed 16 possible gene mutations, including the missense mutation of the PABPC1 gene. Wang Q *et al.* (26) reported that PABPC1 is downregulated in highly malignant gliomas and that high expression of PABPC1 is significantly related to a better prognosis and is related to the biological process of translation. It is reasonable to believe that the aggressiveness and proliferation index of pituitary tumors may also be negatively correlated with the biological activity of PABPC1 protein. Of course, the specific mechanism still needs further exploration in *in vitro* and *in vivo* experiments.

CONCLUSION

The above reports on a case of refractory prolactinoma that was significantly aggressive and resistant to DA treatment, but was highly sensitive to the alkylating agent TMZ. Low DRD2 expression and high proliferation lead to resistance to BRC and CAB, while low expression of MGMT contributes to sensitivity to TMZ treatment. The results of genome sequencing still requires further investigations to explain the underlying molecular mechanisms of tumor aggressiveness and high sensitivity to the alkylating agent TMZ.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

Ethical review and approval were not required for the study on human participants in accordance with the local legislation and institutional requirements. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

HT wrote the first draft of the manuscript. YC and ZBW contributed to the design and writing. JH and JL provided the genome sequencing analysis. BZ provided the pathological figures. HT provided the other figures. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by the Shanghai Municipal Science and Technology Commission 18XD1403400 (ZBW), Program of Shanghai Academic Research Leader (ZBW), Shanghai Training 641 and Support Program for Outstanding Young Medical Talents 642 (ZBW), and Ruijin Youth NSFC Cultivation Fund (2019QNPY01048).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2021.616339/full#supplementary-material

REFERENCES

1. Scheithauer BW, Kovacs KT, Laws ER Jr., Randall RV. Pathology of invasive pituitary tumors with special reference to functional classification. *J Neurosurg* (1986) 65(6):733–44. doi: 10.3171/jns.1986.65.6.0733
2. Yang Q, Li X. Molecular Network Basis of Invasive Pituitary Adenoma: A Review. *Front Endocrinol (Lausanne)* (2019) 10:7:7. doi: 10.3389/fendo.2019.00007
3. Oh MC, Aghi MK. Dopamine agonist-resistant prolactinomas. *J Neurosurg* (2011) 114(5):1369–79. doi: 10.3171/2010.11.JNS101369
4. Lin S, Zhang A, Zhang X, Wu ZB. Treatment of Pituitary and Other Tumours with Cabergoline: New Mechanisms and Potential Broader Applications. *Neuroendocrinology* (2020) 110(6):477–88. doi: 10.1159/000504000
5. Liu YT, Liu F, Cao L, Xue L, Gu WT, Zheng YZ, et al. The KBTBD6/7-DRD2 axis regulates pituitary adenoma sensitivity to dopamine agonist treatment. *Acta Neuropathol* (2020) 140(3):377–96. doi: 10.1007/s00401-020-02180-4
6. Strobel H, Baisch T, Fitzel R, Schilberg K, Siegelin MD, Karpel-Massler G, et al. Temozolomide and Other Alkylating Agents in Glioblastoma Therapy. *Biomedicines* (2019) 7(3):69. doi: 10.3390/biomedicines7030069
7. Syro LV, Rotondo F, Camargo M, Ortiz LD, Serna CA, Kovacs K. Temozolomide and Pituitary Tumors: Current Understanding, Unresolved Issues, and Future Directions. *Front Endocrinol (Lausanne)* (2018) 9:318. doi: 10.3389/fendo.2018.00318
8. Halevy C, Whitelaw BC. How effective is temozolomide for treating pituitary tumours and when should it be used? *Pituitary* (2017) 20(2):261–6. doi: 10.1007/s11102-016-0745-y
9. Lasolle H, Ilie MD, Raverot G. Aggressive prolactinomas: how to manage? *Pituitary* (2020) 23(1):70–7. doi: 10.1007/s11102-019-01000-7
10. Almalki MH, Aljoaib NN, Alotaibi MJ, Aldabas BS, Wahedi TS, Ahmad MM, et al. Temozolomide therapy for resistant prolactin-secreting pituitary adenomas and carcinomas: a systematic review. *Hormones (Athens)* (2017) 16(2):139–49. doi: 10.14310/horm.2002.1729
11. McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytsky A, et al. The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Res* (2010) 20(9):1297–303. doi: 10.1101/gr.107524.110
12. Koboldt DC, Zhang Q, Larson DE, Shen D, McLellan MD, Lin L, et al. VarScan 2: somatic mutation and copy number alteration discovery in cancer by exome sequencing. *Genome Res* (2012) 22(3):568–76. doi: 10.1101/gr.129684.111
13. Dobin A, Davis CA, Schlesinger F, Drenkow J, Zaleski C, Jha S, et al. STAR: ultrafast universal RNA-seq aligner. *Bioinformatics* (2013) 29(1):15–21. doi: 10.1093/bioinformatics/bts635
14. Patro R, Duggal G, Love MI, Irizarry RA, Kingsford C. Salmon provides fast and bias-aware quantification of transcript expression. *Nat Methods* (2017) 14(4):417–9. doi: 10.1038/nmeth.4197
15. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol* (2014) 15(12):550. doi: 10.1186/s13059-014-0550-8
16. Passos VQ, Fortes MA, Giannella-Neto D, Bronstein MD. Genes differentially expressed in prolactinomas responsive and resistant to dopamine agonists. *Neuroendocrinology* (2009) 89(2):163–70. doi: 10.1159/000156116
17. Radl DB, Ferraris J, Boti V, Seilicovich A, Sarkar DK, Pisera D. Dopamine- induced apoptosis of lactotropes is mediated by the short isoform of D2 receptor. *PloS One* (2011) 6(3):e18097. doi: 10.1371/journal.pone.0018097
18. Liu X, Tang C, Wen G, Zhong C, Yang J, Zhu J, et al. The Mechanism and Pathways of Dopamine and Dopamine Agonists in Prolactinomas. *Front Endocrinol (Lausanne)* (2018) 9:768. doi: 10.3389/fendo.2018.00768
19. Wu ZB, Zheng WM, Su ZP, Chen Y, Wu JS, Wang CD, et al. Expression of D2RmRNA isoforms and ERmRNA isoforms in prolactinomas: correlation with the response to bromocriptine and with tumor biological behavior. *J Neurooncol* (2010) 99(1):25–32. doi: 10.1007/s11060-009-0107-y
20. McCormack A, Dekkers OM, Petersenn S, Popovic V, Trouillas J, Raverot G, et al. Treatment of aggressive pituitary tumours and carcinomas: results of a European Society of Endocrinology (ESE) survey 2016. *Eur J Endocrinol* (2018) 178(3):265–76. doi: 10.1530/EJE-17-0933
21. Losa M, Mazza E, Terreni MR, McCormack A, Gill AJ, Motta M, et al. Salvage therapy with temozolomide in patients with aggressive or metastatic pituitary adenomas: experience in six cases. *Eur J Endocrinol* (2010) 163(6):843–51. doi: 10.1530/EJE-10-0629
22. Hirohata T, Asano K, Ogawa Y, Takano S, Amano K, Isozaki O, et al. DNA ypical pituitary adenomas and pituitary carcinomas to temozolomide: the national cooperative study by the Japan Society for Hypothalamic and Pituitary Tumors. *J Clin Endocrinol Metab* (2013) 98(3):1130–6. doi: 10.1210/jc.2012-2924

23. Lim S, Shahinian H, Maya MM, Yong W, Heaney AP. Temozolomide: a novel treatment for pituitary carcinoma. *Lancet Oncol* (2006) 7(6):518–20. doi: 10.1016/S1470-2045(06)70728-8
24. Thomas A, Tanaka M, Trepel J, Reinhold WC, Rajapakse VN, Pommier Y. Temozolomide in the Era of Precision Medicine. *Cancer Res* (2017) 77 (4):823–6. doi: 10.1158/0008-5472.CAN-16-2983
25. Whitelaw BC. How and when to use temozolomide to treat aggressive pituitary tumours. *Endocr Relat Cancer* (2019) 26(9):R545–52. doi: 10.1530/ERC-19-0083
26. Wang Q, Wang Z, Bao Z, Zhang C, Wang Z, Jiang T. PABPC1 relevant bioinformatic profiling and prognostic value in gliomas. *Future Oncol* (2020) 16(1):4279–88. doi: 10.2217/fon-2019-0268

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Endocrinology

MINI REVIEW
published: 17 March 2021
doi: 10.3389/fendo.2021.604644

How to Classify and Define Pituitary Tumors: Recent Advances and Current Controversies

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Specialty section:
This article was submitted to Pituitary Endocrinology, a section of the journal Frontiers in Endocrinology

Received: 10 September 2020
Accepted: 22 February 2021
Published: 17 March 2021

Citation:
Dai C, Kang J, Liu X, Yao Y, Wang H and Wang R (2021) How to Classify and Define Pituitary Tumors: Recent Advances and Current Controversies. Front. Endocrinol. 12:604644. doi: 10.3389/fendo.2021.604644

Pituitary tumors are very complex and heterogeneous and have a very wide range of proliferative and aggressive behaviors, and how to define and classify these tumors remains controversial. This review summarizes the epidemiology and progress in the classification and definition of pituitary tumors, as well as controversial issues. Based on the results of radiologic and autopsy studies, the prevalence of pituitary tumors has recently increased significantly. However, the majority of pituitary tumors are incidentally discovered and asymptomatic, and such tumors are called pituitary incidentalomas. Most of these incidentalomas do not induce symptoms, remain stable in size, and do not need treatment. The recent revised classification strategies mainly depend on immunohistochemistry (IHC) to detect pituitary hormones and pituitary transcription factors; therefore, the accuracy of diagnosing pituitary tumors has improved. Although new classification strategies and definitions for pituitary tumors have been presented, there are still some controversies. The term pituitary neuroendocrine tumor (PitNET) was proposed by the International Pituitary Pathology Club, and this terminology can encompass the unpredictable malignant behavior seen in the subset of aggressive pituitary adenomas (PAs). However, some endocrinologists who oppose this change in terminology have argued that the use of tumor in the terminology is misleading, as it gives PAs a harmful connotation when the majority are not aggressive. Such terminology may add new ambiguity to the origin of PAs and unnecessary anxiety and frustration for the majority of patients with benign PAs. The classification of aggressive PAs mainly relies on subjective judgment of clinical behavior and lacks objective biomarkers and unified diagnostic criteria. However, the term "refractory" could more accurately represent the characteristics of these tumors, including their clinical behaviors, radiological features, and pathologic characteristics. Moreover, the diagnostic criteria for refractory PAs are stricter, more objective, and more accurate than those for aggressive PAs. Early identification of

patients with these tumors through recognition and increased awareness of the definition of refractory PAs will encourage the early use of aggressive therapeutic strategies.

Keywords: pituitary tumors, definition, classification, management, China Pituitary Adenoma Specialist Council

INTRODUCTION

Pituitary tumors arise from anterior pituitary cells, most of which are generally benign tumors and are classified as pituitary adenomas (PAs); only 0.1–0.2% of tumors present craniospinal or systemic metastasis, and these tumors are known as pituitary carcinomas (PCs) (1). Although the classification of pituitary tumors has been modified significantly in recent years, there are still some controversies (2, 3). The China Pituitary Adenoma Specialist Council (CPASC) established in 2012 includes experienced neurosurgeons, endocrinologists, pathologists, radiologists, and scientists. To introduce newer concepts into the classification and definition of pituitary tumors, the CPASC has summarized the recent advances and controversial issues in the classification and definition of pituitary tumors. This short review presents the epidemiology, new classification strategies, and new definitions of pituitary tumors, as well as controversial issues.

PREVALENCE AND INCIDENCE OF PITUITARY TUMORS

Pituitary tumors account for approximately 10–15% of all intracranial neoplasms and are the second most common primary brain tumors in humans (4). A meta-analysis of autopsy and imaging evaluation studies found an overall estimated prevalence of PAs of 16.7% (14.4% in autopsy studies and 22.5% in radiologic studies) (5). With the wide application of magnetic resonance imaging (MRI), the prevalence of PAs has increased significantly in the last 10 years. According to the results from several cross-sectional community-based studies, the prevalence of PAs varies from 78 to 116 per 100,000 individuals (6–9). However, a majority of adenomas are incidentally discovered and asymptomatic, and such tumors are called pituitary incidentalomas. Currently, the accepted definition for a pituitary incidentaloma is an unexpected pituitary/sellar lesion discovered on an imaging study performed for an unrelated reason (10). The prevalence of pituitary incidentalomas is rather high, and a previous autopsy series revealed a mean prevalence of 10.7%, with the frequency of identified pituitary incidentalomas ranging from 1.5 to 31% (11). A retrospective analysis of 353 patients from one institution reported that pituitary incidentalomas represented 12% of all pituitary tumors (12). The majority of pituitary incidentalomas are non-functioning adenomas that do not induce symptoms and remain stable in size over long-term follow-up and therefore do not need treatment (13). Only approximately 11–13.3% of pituitary incidentalomas will increase in size and need therapeutic intervention (13, 14). Within one million people, approximately 100,000 harbor PAs; however, only 0.1% of these PAs cause clinically significant health problems (15). Among patients with PAs who experience significant clinical health issues, 68.4% of them (range, 62.2–77.3%) are female, and 47.8% (range, 41.3–56.9%) have macroadenomas at diagnosis (16). Prolactinomas are the most prevalent (32 to 66%), followed by non-functioning adenomas (NFPAs) (14 to 54%), acromegaly (8 to 16%), Cushing's disease (2 to 6%), thyrotropin-secreting adenomas (less than 1%), and the very rare PCs (0.1 to 0.2%) (17, 18).

ADVANCES IN THE CLASSIFICATION AND DEFINITION OF PITUITARY TUMORS

Pituitary tumors are divided into PAs and PCs based on whether they show cerebrospinal and/or systemic metastasis. Before the release of the 4th edition of the World Health Organization (WHO) guidelines for the classification of pituitary tumors in 2017, PAs were classified as invasive or non-invasive, typical, or atypical, and aggressive or non-aggressive PAs according to their radiological characteristics, pathologic features, and clinical behavior (19). In the 4th edition of the guidelines for the classification of pituitary tumors, the term "atypical adenoma" was abandoned due to the lack of sufficient evidence that poor prognosis can be predicted by only pathological markers (20). Another main change is the introduction of a cell lineage-based classification strategy for PAs, including classification according to pituitary adenohypophyseal cell lineage and hormones production (21). The new classification strategy for PAs has abandoned the concept of "a hormone-producing pituitary adenoma" and adopted a pituitary adenohypophyseal cell lineage-based strategy to classify PAs. In the new classification, in addition to immunohistochemistry (IHC) analysis to detect hormones, IHC to detect transcription factors is required and prioritized to classify PAs, but routine ultrastructural examination is not required for these tumors (2).

Most PAs are considered benign tumors, but approximately 10% of them have aggressive behavior and are refractory to conventional therapy. The definition of aggressive PAs has also been modified in the 4th edition of the classification of pituitary tumors. According to the recent Clinical Practice Guidelines issued by the European Society of Endocrinology, aggressive PAs are tumors that are radiologically invasive and have an unusually rapid growth rate or clinically relevant tumor growth despite optimal standard therapies (surgery, radiotherapy, and medicinal treatments) (22).

This new classification scheme also recognizes some subtypes of pituitary tumors as "high-risk Pas," which include sparsely granulated somatotroph adenomas, lactotroph adenomas in men, silent corticotroph adenomas, Crooke's cell adenomas, and

plurinominal Pit-1-positive adenomas (23). Most of these "high-risk PAs" are invasive, large macroadenomas with a high Ki-67 proliferation index; in addition, they are usually difficult to completely resect and tend to have a high recurrence rate after surgery (24).

Compared with the previous WHO 2004 classification guidelines for pituitary tumors, the 2017 WHO classification guidelines are more practical because they enable an accurate diagnosis mostly based on the levels of pituitary hormones, pituitary transcription factors, and other commonly used markers (detected by IHC). A good correlation between the subtypes and pathological features and clinical outcomes has also been shown, which contributes to our understanding of the clinicopathological characteristics of pituitary tumors.

CONTROVERSIES IN THE CLASSIFICATION AND DEFINITION OF PITUITARY TUMORS

Pituitary tumors are a group of tumors with complex and heterogeneous clinical features, including a very wide range of proliferative and aggressive behaviors. Some pituitary tumors are asymptomatic and remain stable in size for a long time, whereas other aggressive PAs grow rapidly and are refractory to conventional treatments. Some pituitary tumors are small in size but have severe systemic metabolic abnormalities caused by excessive secretion of pituitary hormones, whereas others are massive, invasive adenomas that cause local mass effects but do not show excessive hormone secretion. Thus, it is difficult to classify these complex pituitary tumors. Although the new classification and definition strategies for pituitary tumors have been significantly modified, there are still some controversies.

The term "pituitary adenoma" has been used to define a benign pituitary tumor for approximately a century. However, "adenoma" does not represent the malignant characteristics of aggressive PAs, such as invasion of nearby anatomical structures, rapid growth, non-response to conventional treatments, and early postoperative recurrence. More recently, a proposal to change the terminology from PA to pituitary neuroendocrine tumor (PitNET) was proposed by the International Pituitary Pathology Club (25). They argued that the term "PitNET" instead of "adenoma" permitted variability in biological behavior and aligned these tumors with the terminology used for other neuroendocrine tumors. Subsequently, the European Pituitary Pathology Group endorsed the term PitNETs and developed a practical recommendation for standardized reports and diagnostic algorithms for PitNETs (26). Trouillas and colleagues also believe that PitNETs more closely reflect the variability of pituitary tumor behavior (with invasiveness linked to a higher risk of recurrence) and that the new terminology may suggest new strategies for the early identification and management of the most aggressive tumors (27). Although a large majority of PitNETs behave as well differentiated and benign neoplasms, the terminology of PitNETs not only reflects more closely the variability of behavior of pituitary tumors, but also impact positively on clinical practice. In addition, the current classification of the anterior pituitary adenomas does not accurately reflect the clinical behavior including invasive PAs that cannot be completely resected and aggressive PAs refractory to therapy. Therefore, application of this term may open up new strategies for the early identification and management of the most aggressive forms. Therefore, they endorsed PitNET terminology as well because it could positively impact clinical practice.

However, the PitNET terminology has caused a nomenclature debate. Some endocrinologists who oppose this change in terminology argue that the use of the term tumor is misleading, as it gives PAs a negative connotation when the majority are not aggressive (only a small subset of them are aggressive adenomas), and true malignant PCs are extremely rare (28). They criticize that the PitNET term encompasses tumors with unpredictable malignant behaviors and that the PitNET terminology will add new ambiguity to the origin of PAs and bring unnecessary anxiety and frustration to patients with typical benign Pas (15). Therefore, both the adenoma and PitNET terminologies have their own pros and cons, and more research and discussion are required to verify which is more representative of the characteristics of pituitary tumors. To date, there is still no consensus or consist terminology and definitions used for these complex and heterogeneous pituitary tumors, and more accurate terminology and definitions are needed to appropriately represent the highly variable clinical features of pituitary tumors.

Most PAs are benign and non-invasive adenomas that can be cured by surgery or controlled by medicinal therapy, such as dopamine-agonist therapy for lactotroph tumors. However, approximately 35% of PAs have invaded into surrounding anatomical structures and cannot be completely resected (19). Moreover, approximately 10% of PAs with invasive characteristics and unusually rapid growth rates recur multiple times despite optimal administration of standard therapies, and such tumors are defined as clinically aggressive Pas (29). Before the release of the recent Practice Guidelines for aggressive PAs, the definitions of "aggressive tumor" varied from a large invasive pituitary tumor with rapid growth, to a tumor resistant to conventional treatment, a pituitary tumor with early recurrence despite gross-total resection, to a tumor with malignant potential without metastasis, to a localized pituitary carcinoma (30–32). Trouillas and colleagues demonstrated that aggressive PAs and pituitary carcinomas are clinically and histologically similar. Therefore, they suggested that aggressive PAs are "tumors with malignant potential without metastasis" and proposed that aggressive PAs and carcinomas may be two sides of the same coin (32). Compared with the various previous definitions for aggressive PAs, the version recently proposed by the European Society of Endocrinology is currently the most authoritative and comprehensive (22). Although the definition of aggressive PAs has been revised and modified repeatedly, there is still some controversy and confusion.

Previously, radiological investigation was used to determine the invasiveness of aggressive PAs; however, radiologically

determined invasion, based on preoperative MRI, is not always consistent with intraoperatively observed invasiveness (33). The Knosp classification is based on preoperative MRI and is most widely used system to evaluate the invasiveness of Pas (34). According to this classification system, Knosp grade 0–2 tumors are non-invasive PAs, and Knosp grade 3–4 tumors are invasive PAs. However, the MRI-based Knosp grade is often unreliable for predicting intraoperative invasion in Knosp grade 1–3 microadenomas (35). A subset of MRI-based radiologically invasive tumors is found to exhibit expansive growth without cavernous sinus invasion on intraoperative inspection, whereas some radiologically non-invasive tumors are found to have cavernous sinus invasion during surgery. Therefore, radiologically determined invasion cannot accurately represent the invasiveness of aggressive PAs, and the term invasive should consider both radiological and surgical findings.

Second, there is no uniform objective standard to identify the unusually rapid tumor growth rate required in the definition of aggressive PAs. Currently, physicians determine whether the tumor is growing rapidly mainly based on their own subjective experience, which may result in different clinicians having inconsistent judgments for the same patient. Therefore, the lack of objective and unified standards for identifying an unusually rapid tumor growth rate can easily lead to inconsistent diagnoses for the same patient with an aggressive PA.

Third, the diagnosis of aggressive PAs mainly depends on subjective judgment of clinical characteristics and lacks objective biomarkers and unified diagnostic criteria. It is difficult for inexperienced physicians to diagnose aggressive PAs early and accurately. Therefore, some aggressive PAs are easily missed by inexperienced physicians due to the lack of obvious diagnostic markers and uniform objective criteria.

Therefore, the term "aggressive" is mainly applied based on clinical behaviors rather than histological markers. The definition of aggressive tumors does not accurately convey the precise clinical and pathological features of "aggressive" tumors, and there is no correlation between pathologic findings and the clinical behavior of these tumors. Furthermore, there is no biomarker used in the definition of "aggressive" that predicts the aggressiveness of these tumors. In addition, the term "aggressive" is frequently interpreted differently by individual clinicians due to the lack of objective diagnostic markers and uniform criteria. Moreover, the terms "invasive" and "aggressive" are constantly interchangeably and synonymously used in some studies. Thus, a more reasonable and comprehensive term or definition is needed to properly convey the clinical, radiological, and histological features of these aggressive PAs.

To address this situation, we proposed the term "refractory PAs" to identify invasive-aggressive PAs with a high Ki-67 index, rapid growth, early recurrence, and resistance to conventional treatments (36). This definition was derived by summarizing the clinical characteristics and pathological findings of a group of patients with refractory PAs from Peking Union Medical College Hospital (PUMCH). The diagnostic criteria for refractory PAs were also proposed as follows: 1) tumor infiltration of adjacent structures according to radiological results or intraoperative findings; 2) Ki-67 index greater than 3%, and growth velocity greater than 2% per month; 3) failure of current treatments to control tumor growth and/or hormonal hypersecretion; and 4) tumor recurrence within 6 months after surgery (37). Although the term "refractory" has caused some controversy (38), it does provide a more accurate definition and more objective diagnostic criteria for these invasive-aggressive PAs.

Instead of "aggressive," we prefer to use the term "refractory." Because the definition of "refractory" has several advantages over "aggressive".

First, although both the "aggressive" and "refractory" definitions include invasive tumors, the "aggressive tumor" definition only mentions radiologically invasive tumors, which is not comprehensive or accurate. In the diagnostic criteria for "refractory tumors," tumor infiltration into adjacent structures according to radiological results or intraoperative findings is used to determine the invasiveness of tumor, which is more comprehensive than the term "radiologically invasive tumor" used in the definition of "aggressive tumors."

Second, Ki-67 >3%, as an objective biomarker, is used to diagnose refractory PAs, which provides an objective biomarker for these tumors. Although most studies have demonstrated that Ki-67 >3% predicts more aggressive tumor behavior, no firm consensus on the precise cutoff value has been reached (39). Therefore, the use of a cutoff value >3% for Ki-67 to distinguish refractory PAs from benign PAs is still controversial; however, such a cutoff does help physicians distinguish refractory PAs from benign PAs.

Third, a rapid tumor growth rate is mentioned in both the "aggressive" and "refractory" tumor definitions, but the "aggressive" tumor definition does not provide an objective and precise value indicating rapid growth. In the diagnostic criteria for "refractory" tumors, a growth rate >2% per month is used to define rapid growth of tumors. Although the growth rate >2% per month criterion was determined based on the summarized findings of a series of patients with refractory PAs at PUMCH, and its validity as a marker remains to be confirmed, it indeed provides an objective reference for an unusually rapid tumor growth rate. Further research is needed to explore a more acceptable cutoff value for identifying rapid growth to distinguish refractory PAs from benign PAs.

Fourth, early recurrence (6–12 months postoperatively) is another important feature of aggressiveness characterizing the behavior of aggressive/refractory PAs, whereas typical benign adenomas may develop recurrence within 5–10 years after initial treatment (40). Aggressive/refractory PAs are usually resistant to conventional treatment and recur early and multiple times despite optimal standard therapies. However, early recurrence is not highlighted at all in the definition of "aggressive" tumors. In the definition of "refractory" tumors, tumor recurrence or regrowth within 6 months after surgery is used to distinguish refractory PAs from typical benign adenomas. There is still no definite cutoff value for identifying early tumor recurrence to distinguish refractory PAs from truly benign adenomas, and the cutoff for identifying early recurrence (<6 months postoperatively) is still

controversial (41). However, the use of the <6 months postoperatively time for identifying early recurrence provides an important reference for the early recognition and diagnosis of refractory PAs.

In summary, the prevalence of pituitary tumors has recently increased, but the majority of patients are asymptomatic and do not need treatment. Although the revised classification strategy for pituitary tumors is based on the levels of transcription factors (determined by IHC) and is more practical and reasonable than older strategies, there are still some controversies. The PitNET terminology encompasses the unpredictable malignant behavior seen in the small subset of aggressive adenomas. However, it may add new ambiguity to the origin of PAs and unnecessary anxiety and frustration to the majority of patients with benign PAs. The definition of aggressive PAs mainly relies on clinical behavior but lacks objective biomarkers and unified diagnostic criteria. However, the term "refractory" could accurately represent the characteristics of these tumors, including clinical behaviors, radiological features, and pathologic characteristics. Moreover, the diagnostic criteria for refractory PAs are stricter, more objective, and more accurate than those for aggressive PAs.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work, and approved it for publication.

FUNDING

Financial support for this study was provided by the Scientific Research Project of Capital Health Development in 2020 (grant number: 2020-2-2058) and the Beijing Natural Science Foundation (grant number: 7172057). The funding institutions had no role in the design of the study, data collection and analysis, the decision to publish, or the preparation of the manuscript.

REFERENCES

1. Chatzellis E, Alexandraki KI, Androulakis II, Kaltsas G. Aggressive Pituitary Tumors. *Neuroendocrinology* (2015) 101(2):87–104. doi: 10.1159/000371806
2. Lopes MBS. The 2017 World Health Organization classification of tumors of the pituitary gland: a summary. *Acta Neuropathol* (2017) 134(4):521–35. doi: 10.1007/s00401-017-1769-8
3. Laws ER, Penn DL, Repetti CS. Advances and controversies in the classification and grading of pituitary tumors. *J Endocrinol Invest* (2019) 42 (2):129–35. doi: 10.1007/s40618-018-0901-5
4. Asa SL, Ezzat S. The pathogenesis of pituitary tumours. *Nat Rev Cancer* (2002) 2(11):836–49. doi: 10.1038/nrc926
5. Ezzat S, Asa SL, Couldwell WT, Barr CE, Dodge WE, Vance ML, et al. The prevalence of pituitary adenomas: a systematic review. *Cancer-Am Cancer Soc* (2004) 101(3):613–9. doi: 10.1002/cncr.20412
6. Fernandez A, Karavitaki N, Wass JA. Prevalence of pituitary adenomas: a community-based, cross-sectional study in Banbury (Oxfordshire, UK). *Clin Endocrinol (Oxf)* (2010) 72(3):377–82. doi: 10.1111/j.1365-2265.2009.03667.x
7. Daly AF, Rixhon M, Adam C, Dempegioti A, Tichomirowa MA, Beckers A. High prevalence of pituitary adenomas: a cross-sectional study in the province of Liege, Belgium. *J Clin Endocrinol Metab* (2006) 91(12):4769–75. doi: 10.1210/jc.2006-1668
8. Tjörnstrand A, Gunnarsson K, Evert M, Holmberg E, Ragnarsson O, Rosén T, et al. The incidence rate of pituitary adenomas in western Sweden for the period 2001–2011. *Eur J Endocrinol* (2014) 171(4):519–26. doi: 10.1530/EJE-14-0144
9. Agustsson TT, Baldvinsdottir T, Jonasson JG, Olafsdottir E, Steinthorsdottir V, Sigurdsson G, et al. The epidemiology of pituitary adenomas in Iceland, 1955-2012: a nationwide population-based study. *Eur J Endocrinol* (2015) 173 (5):655–64. doi: 10.1530/EJE-15-0189
10. Freda PU, Beckers AM, Katznelson L, Molitch ME, Montori VM, Post KD, et al. Pituitary Incidentaloma: An Endocrine Society Clinical Practice Guideline. *J Clin Endocrinol Metab* (2011) 96(4):894–904. doi: 10.1210/jc.2010-1048
11. Boguszewski CL, de Castro Musolino NR, Kasuki L. Management of pituitary incidentaloma. *Best Pract Res Cl En* (2019) 33(2):101268. doi: 10.1016/j.beem.2019.04.002
12. Gsponer J, De Tribolet N, Deruaz JP, Janzer R, Uske A, Mirimanoff RO, et al. Diagnosis, treatment, and outcome of pituitary tumors and other abnormal intrasellar masses. Retrospective analysis of 353 patients. *Med (Baltimore)* (1999) 78(4):236–69. doi: 10.1097/00005792-199907000-00004
13. Anagnostis P, Adamidou F, Polyzos SA, Efstathiadou Z, Panagiotou A, Kita M. Pituitary incidentalomas: a single-centre experience. *Int J Clin Pract* (2011) 65(2):172–7. doi: 10.1111/j.1742-1241.2010.02537.x
14. Sanno N, Oyama K, Tahara S, Teramoto A, Kato Y. A survey of pituitary incidentaloma in Japan. *Eur J Endocrinol* (2003) 149(2):123–7. doi: 10.1530/eje.0.1490123
15. Ho KKY, Fleseriu M, Wass J, van der Lely A, Barkan A, Giustina A, et al. A tale of pituitary adenomas: to NET or not to NET. *Pituitary* (2019) 22(6):569–73. doi: 10.1007/s11102-019-00988-2
16. Daly AF, Beckers A. The Epidemiology of Pituitary Adenomas. *Endocrinol Metab Clin North Am* (2020) 49(3):347–55. doi: 10.1016/j.ecl.2020.04.002
17. Molitch ME. Diagnosis and Treatment of Pituitary Adenomas: A Review. *JAMA* (2017) 317(5):516–24. doi: 10.1001/jama.2016.19699
18. Heaney AP. Pituitary Carcinoma: Difficult Diagnosis and Treatment. *J Clin Endocrinol Metab* (2011) 96(12):3649–60. doi: 10.1210/jc.2011-2031
19. Sav A, Rotondo F, Syro LV, Di Ieva A, Cusimano MD, Kovacs K. Invasive, Atypical and Aggressive Pituitary Adenomas and Carcinomas. *Endocr Metab Clin* (2015) 44(1):99–104. doi: 10.1016/j.ecl.2014.10.008
20. Inoshita N, Nishioka H. The 2017 WHO classification of pituitary adenoma: overview and comments. *Brain Tumor Pathol* (2018) 35(2):51–6. doi: 10.1007/s10014-018-0314-3
21. Mete O, Lopes MB. Overview of the 2017 WHO Classification of Pituitary Tumors. *Endocr Pathol* (2017) 28(3):228–43. doi: 10.1007/s12022-017-9498-z
22. Raverot G, Burman P, McCormack A, Heaney A, Petersenn S, Popovic V, et al. European Society of Endocrinology Clinical Practice Guidelines for the management of aggressive pituitary tumours and carcinomas. *Eur J Endocrinol* (2017) 178(1):G1–G24. doi: 10.1530/EJE-17-0796
23. Shibuya M. Welcoming the new WHO classification of pituitary tumors 2017: revolution in TTF-1-positive posterior pituitary tumors. *Brain Tumor Pathol* (2018) 35(2):62–70. doi: 10.1007/s10014-018-0311-6
24. Nishioka H, Inoshita N. New WHO classification of pituitary adenomas (4th edition): assessment of pituitary transcription factors and the prognostic histological factors. *Brain Tumor Pathol* (2018) 35(2):57–61. doi: 10.1007/s10014-017-0307-7
25. Asa SL, Casar-Borota O, Chanson P, Delgrange E, Earls P, Ezzat S, et al. From pituitary adenoma to pituitary neuroendocrine tumor (PitNET): an International Pituitary Pathology Club proposal. *Endocr-Relat Cancer* (2017) 24(4):C5–8. doi: 10.1530/ERC-17-0004
26. Villa C, Vasiljevic A, Jaffrain-Rea ML, Ansorge O, Asioli S, Barresi V, et al. A standardised diagnostic approach to pituitary neuroendocrine tumours (PitNETs): a European Pituitary Pathology Group (EPPG) proposal. *Virchows Arch* (2019) 475(6):687–92. doi: 10.1007/s00428-019-02655-0
27. Trouillas J, Jaffrain-Rea M, Vasiljevic A, Raverot G, Roncaroli F, Villa C. How to Classify Pituitary Neuroendocrine Tumors (PitNET)s in 2020. *Cancers* (2020) 12(2):514. doi: 10.3390/cancers12020514

28. Sathyakumar R, Chacko G. Newer Concepts in the Classification of Pituitary Adenomas. *Neurol India* (2020) 68(Supplement):S7–12. doi: 10.4103/0028-3886.287667
29. Kasuki L, Raverot G. Definition and diagnosis of aggressive pituitary tumors. *Rev Endocr Metab Disord* (2019) 21(2):203–8. doi: 10.1007/s11154-019-09531-x
30. Dehdashti AR, Chakraborty S. Aggressive pituitary adenomas: is pathology the only feature of aggressiveness? *Acta Neurochir* (2018) 160(1):57–8. doi: 10.1007/s00701-017-3398-3
31. Asa SL, Ezzat S. Aggressive Pituitary Tumors or Localized Pituitary Carcinomas: Defining Pituitary Tumors. *Expert Rev Endocrinol Metab* (2016) 11(2):149–62. doi: 10.1586/17446651.2016.1153422
32. Trouillas J, Burman P, McCormack A, Petersenn S, Popovic V, Dekkers O, et al. Aggressive pituitary tumours and carcinomas: two sides of the same coin? *Eur J Endocrinol* (2018) 178(6):C7–9. doi: 10.1530/EJE-18-0250
33. Micko ASG, 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):803–11. doi: 10.3171/2014.12.JNS141083
34. 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):610–7; discussion 617-8. doi: 10.1227/00006123-199310000-00008
35. Dallapiazza R, Bond AE, Grober Y, Louis RG, Payne SC, Oldfield EH, et al. Retrospective analysis of a concurrent series of microscopic versus endoscopic transsphenoidal surgeries for Knosp Grades 0-2 nonfunctioning pituitary macroadenomas at a single institution. *J Neurosurg* (2014) 121(3):511–7. doi: 10.3171/2014.6.JNS131321
36. Dai C, Feng M, Liu X, Ma S, Sun B, Bao X, et al. Refractory pituitary adenoma: a novel classification for pituitary tumors. *Oncotarget* (2016) 7(50):83657–68. doi: 10.18632/oncotarget.13274
37. Dai C, Liu X, Ma W, Wang R. The Treatment of Refractory Pituitary Adenomas. *Front Endocrinol (Lausanne)* (2019) 10:334. doi: 10.3389/fendo.2019.00334
38. Dworakowska D, Grossman AB. Aggressive and malignant pituitary tumours: state-of-the-art. *Endocr Relat Cancer* (2018) 25(11):R559–75. doi: 10.1530/ERC-18-0228
39. Salehi F, Agur A, Scheithauer BW, Kovacs K, Lloyd RV, Cusimano M. Ki-67 in pituitary neoplasms: a review–part I. *Neurosurgery* (2009) 65(3):429. doi: 10.1227/01.NEU.0000349930.66434.82
40. Ilie MD, Jouanneau E, Raverot G. Aggressive Pituitary Adenomas and Carcinomas. *Endocr Metab Clin* (2020) 49(3):505–15. doi: 10.1016/j.ecl.2020.05.008
41. Heaney A. Management of aggressive pituitary adenomas and pituitary carcinomas. *J Neuro-Oncol* (2014) 117(3):459–68. doi: 10.1007/s11060-014-1413-6

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Endocrinology

REVIEW
published: 26 March 2021
doi: 10.3389/fendo.2021.624686

Multimodal Non-Surgical Treatments of Aggressive Pituitary Tumors

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Up to 35% of aggressive pituitary tumors recur and significantly affect mortality and quality of life. Management can be challenging and often requires multimodal treatment. Current treatment options, including surgery, conventional medical therapies such as dopamine agonists, somatostatin receptor agonists and radiotherapy, often fail to inhibit pituitary tumor growth. Recently, anti-tumor effects of chemotherapeutic drugs such as Temozolomide, Capecitabine, and Everolimus, as well as peptide receptor radionuclide therapy on aggressive pituitary tumors have been increasingly investigated and yield mixed, although sometimes promising, outcomes. The purpose of this review is to provide thorough information on non-surgical medical therapies and their efficacies and used protocols for aggressive pituitary adenomas from pre-clinical level to clinical use.

Keywords: non-surgical therapy, Temozolomide, CAPTEM, PRRT (Peptide Receptor Radionuclide Therapy), aggressive pituitary tumors, pituitary carcinomas

OPEN ACCESS

Edited by:
Adam Mamelak, Cedars Sinai Medical Center, United States

Reviewed by:
Thierry C. Brue, Aix-Marseille Université, France
Maria Chiara Zatelli, University of Ferrara, Italy

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Specialty section:
This article was submitted to Pituitary Endocrinology, a section of the journal Frontiers in Endocrinology

Received: *31 October 2020*
Accepted: *12 January 2021*
Published: *26 March 2021*

Citation:
Nakano-Tateno T, Lau KJ, Wang J, McMahon C, Kawakami Y, Tateno T and Araki T (2021) Multimodal Non-Surgical Treatments of Aggressive Pituitary Tumors. Front. Endocrinol. 12:624686. doi: 10.3389/fendo.2021.624686

INTRODUCTION

Pituitary tumors are mostly benign and progress slowly. Most of them are non-invasive and cured by surgery or controlled by long-term pharmacologic treatment. However, some pituitary tumors exhibit continued growth despite conventional therapies, including multiple surgeries, radiotherapy, and medical treatment (1). Recent evidence suggests that Temozolomide (TMZ), an alkylating agent, can be used as a valuable first-line chemotherapy for treatment of aggressive pituitary tumors and carcinomas (1). However, some patients may not respond to TMZ. Moreover, many patients experience disease progression or disease recurrence after completion of TMZ treatment, although tumors and carcinomas regressed during the treatment (1–13). In order to ensure long-term positive outcomes in cases of aggressive pituitary tumors, alternative treatment options are needed. Recent case reports showed that TMZ in combination with capecitabine had an improved progression free survival in aggressive pituitary tumors (14, 15). Moreover, several new therapies have been reported. These novel therapies include peptide receptor radionuclide therapy (PRRT) (16–24) and treatment with mTOR inhibitors (11, 25–27), epidermal growth factor receptor (EGFR) inhibitors (28), immune checkpoint inhibitors (29–31), cyclin dependent kinase (Cdk) inhibitors (32), or vitamin A derivatives (33, 34). In this review, we describe the mechanisms, efficacies, and protocols of each treatment and summarize the studies and cases published in the literature.

TEMOZOLOMIDE (TMZ)

TMZ is an orally-administered alkylating agent that was originally used for treatment of glioblastomas combined with radiation therapy (35). TMZ was first used in 2006 to manage high-risk pituitary tumors (36–38). TMZ induces methylation of guanine residue at the O6 position in the DNA. O6-methylguanine incorrectly pairs with thymine and triggers the mismatch repair system, leading to the formation of double-strand breaks in the genome, which causes cell cycle arrest and induction of apoptosis (39).

TMZ was recommended as first-line chemotherapy for aggressive pituitary tumors and carcinomas by the European Society of Endocrinology in 2018 (1) and usually elicits an immediate response. The standard dose of TMZ is 150–200 mg/m^2 for 5 consecutive days every 28 days (=1 cycle) for at least six cycles (1) (**Figure 1A**). TMZ is a generally well-tolerated therapy. The major side effects are fatigue and nausea. Twelve studies with greater than five patients have been published since the first case of TMZ use in the management of aggressive pituitary tumors in 2006 (2–13) (**Table 1**). The total cumulative number of patients is 366, including 113 with pituitary carcinomas. Although follow-up duration and definition of a response differed among studies, the overall response in the reduction of tumor size ranged from 33%–87%. In the largest study by the European Society of Endocrinology, 166 patients were treated with TMZ as first-line chemotherapy. 37% of the patients showed radiological response and 33% showed stable disease (11). Similarly, the second largest study with 47 patients, including 13 with pituitary carcinomas, reported that 37% of patients exhibited tumor size reduction following TMZ treatment (13). In a meta-analysis of 106 patients from 11 studies of aggressive pituitary tumors, 47% of the patients showed reduction in tumor size (1).

TMZ treatment outcome differs between hormone secreting and non-hormone secreting pituitary tumors. A review of 8 case series encompassing 100 pituitary tumor cases treated with TMZ showed that corticotroph adenomas and prolactinomas have about a 56% and 44% tumor reduction rate, respectively. In contrast, non-functioning pituitary adenomas have only a 22% of tumor response rate (40). Two other studies also showed that patients with functioning tumors exhibited better tumor size reduction after treatment with TMZ compared to those with NFPAs (2, 11).

As mentioned above, immediate responses to TMZ are mostly favorable; however, tumor progression and relapse frequently occur during long-term follow-up (33%–63%) (**Table 1**). In two large-scale studies with long-term follow-up, tumor relapse occurred in 46% (16 month follow up with 5 months after cessation) and 38% (21 months follow-up with 12 months after cessation) of patients, respectively (2, 11). In another study that focused on long-term effects of TMZ, up to 63% of the patients demonstrated disease progression after a median of 16 months following initiation of TMZ (13). Longer TMZ treatment duration (more than 12 cycles) was found to be associated with better survival free of tumor-progression (1, 2, 11, 41). A retrospective analysis showed higher survival rate in the long-term treatment group (longer than 12 months) compared to the short-term (1–12 months) treatment group [5-year overall survival 92% vs 54% (41)], suggesting that longer treatment cycles can increase the likelihood of sustained remission. However, reports from many cases showed that a second course of TMZ failed to induce tumor regression if tumor progression or systemic metastases occurred after an initial treatment (2, 8, 10, 42, 43). Therefore, re-challenging with

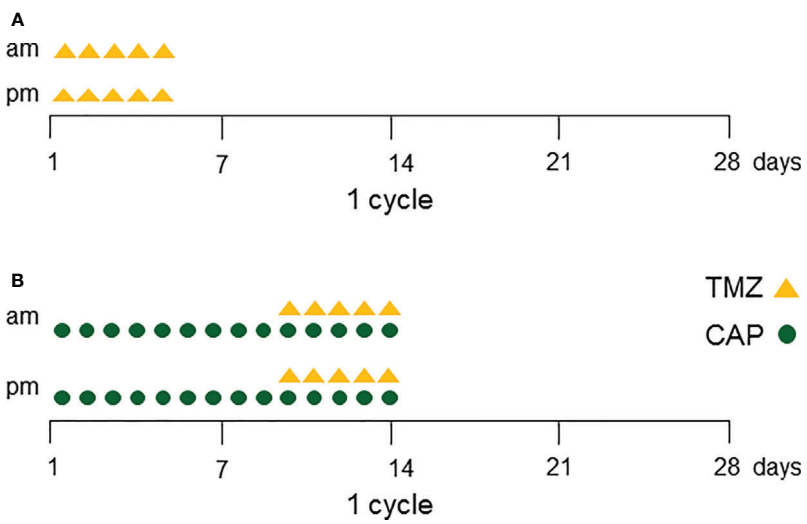

FIGURE 1 | **(A)** One cycle of Temozolomide (TMZ) protocol. TMZ is given twice daily (150–200 mg/m^2/day) for 5 consecutive days every 28 days (=1 cycle). After three cycles of TMZ, MRI is taken for treatment evaluation. Continue TMZ at least six cycles in total for responder patients. **(B)** One cycle of Capecitabine (pro-drug of 5-Fluorouracil) + Temozolomide (CAPTEM) protocol. Oral capecitabine (CAP) is given (1,500 mg/m^2/day) on days 1 through 14 divided into two doses, and TMZ is given twice daily (150 to 200 mg/m^2/day) on days 10 through 14. This 2-week regimen is followed by 2 weeks off treatment. Continue CAPTEM at least 12 cycles in total for responder patients.

TABLE 1 | Temozolomide (TMZ) treatment response in pituitary tumors and carcinomas from published case series of 5 or more patients.

response in tumor growth with medication	TMZ (cycles) follow-up (months)	follow-up (months)	recurrent rate (%) and occurred timing after treatment (month)	MGMT correlations	case numbers (cancer)	ref, year [ref no]
CR/PR;51%	2–24	16	46%; after 5 months	No	43 (14)	Lasolle (2)
PR/CR;29%, SD;58%, PD14%	2–13	ns	ns	No	7 (2)	Bush (3)
CR/PR/SD; 38%	3–24	ns	ns	No	8 (5)	Raverot (4)
CR/PR;33%, SD;33%, PD;33%	3–12	22.5	33%; after 6 months	Yes	6 (1)	Losa (5)
CR/PR;31%, SD;15%, PD;54%	3–24	ns	46%; after 10.5 months	No	13 (10)	Hirohata (6)
CR/PR;33%	3–6	ns	ns	ns	6 (1)	Bruno (7)
CR/PR;43%	1–23	32.5	33%	Yes	21 (8)	Bengtsson (8)
PR;40%, SD;20%, PD;40%	3–24	ns	ns	ns	5 (0)	Ceccato (9)
CR/PR;36%, SD;45%, PD;19%	3–12	43	52%; after cessation	No	31 (6)	Losa (10)
CR;6%, PR;31%, SD;33%, PD;30%	1–36	21	38%; after 12 months	Yes	166 (40)	McCormack (11)
TMZ-based therapy was associated with the longest PFS (71%) and long disease control	–12	28	62%	ns	13 (13)	Santos (12)
CR/PR;20%, SD;17%, PD;63%	1-26	32	63%; after 16 months	No	47 (13)	Elbelt (13)

ns, not stated; CR complete response; PR, partial response; SD, stable disease; PD, progressive disease; PFS, progression free survival.

TMZ is not recommended if an initial treatment fails to control disease progression. One of the reasons for such a relapse might be related to TMZ-induced hypermutations in genes such as *MSH6, CDKN2A/B*, and *PIK3CA*, which can be associated with TMZ resistance (29).

Several biomarkers that may predict response to TMZ have been identified. One such marker is O6-methylguanine DNA methyltransferase (MGMT), a DNA-repair enzyme, which counteracts TMZ-induced DNA damage (44). The expression of MGMT seems to correlate with response of a tumor to the TMZ therapy. A number of studies indicate an association between low expression levels of MGMT (determined by immunostaining) and better treatment response to TMZ (1, 5, 8, 11, 45–51). However, this association was not observed in several other studies (2–4, 6, 10, 13) (**Table 1**). This discrepancy could be, at least in part, derived from a lack of standardized scoring systems for MGMT immunostaining. In addition, the expression level of MGMT may increase during TMZ therapy (45), which could affect interpretation of MGMT expression. At present, the expression of MGMT is still considered a predictive marker for TMZ response. Another biomarker is MutS Homolog 6 (MSH6), a DNA mismatch repair protein. Mutations in the *MSH6* gene in glioblastoma are known to be associated with TMZ resistance (52). One study showed that expression of MSH6 was positively correlated with pituitary tumor regression following treatment with TMZ (6); however, a follow-up study failed to confirm this correlation (8).

TMZ-BASED COMBINATION THERAPIES: CAPECITABINE (PRO-DRUG OF 5-FLUOROURACIL) + TEMOZOLOMIDE (CAPTEM)

Capecitabine (orally administered systemic pro-drug of 5-Fluorouracil) is an antimetabolite that incorporates 5-fluorodeoxyuridine triphosphate into genomic DNA. Capecitabine causes attenuation of MGMT DNA repair activity through thymidylate synthase inhibition and reduction of the thymidine level, which enhances the apoptotic effect of TMZ (53, 54). CAPTEM is a novel combination of capecitabine and TMZ. Two *in vitro* experiments investigating anti-tumor effects of CAPTEM have been reported. One study in human carcinoid cell lines showed synergistic cytotoxicity when 5-fluorouracil (5-FU) exposure was preceded by TMZ (55). The other study, conducted by the authors of this review, used mouse corticotroph tumor cells to demonstrate that 5-FU treatment in combination with TMZ had an additive effect both in decreasing cell viability and reducing the amount of ACTH released by the tumor cells in the culture medium (15).

The clinical protocol of CAPTEM consists of oral capecitabine 1,500 mg/m^2/day (maximum daily dose of 2,500 mg on days 1 through 14 divided into two doses) and TMZ 150 to 200 mg/m^2/day (oral divided into two doses) given on days 10 through 14. This 2-week regimen is followed by 2 weeks off-treatment [**Figure 1B** (14)]. Data obtained from *in vitro* experiments support the efficacy of two separate TMZ treatments (56). Specifically, the first treatment causes partial reduction of MGMT activities, whereas the second treatment is responsible for the methylation of guanine residues once repair activity by MGMT has been attenuated (53).

CAPTEM is generally well tolerated; among 13 patients with aggressive pituitary tumors/carcinomas, 12 patients were able to tolerate the treatment without discontinuing therapies (**Table 2**). Three patients developed thrombocytopenia but only one of these patients had to discontinue CAPTEM due to thrombocytopenia and poor tolerance (29). Another patient developed lymphopenia without discontinuing treatment, and one case reduced to 75% of the maximal dose of CAPTEM due to nausea (15). The first reported use of CAPTEM for pituitary tumors was for an aggressive corticotroph pituitary carcinoma in 2011 (57). To date, 13 TMZ naïve cases of aggressive pituitary tumors/carcinomas treated with CAPTEM (TMZ naïve cases) have been reported, of which seven were carcinomas (7/13) and 11 were ACTH positive (11/13) (11, 14, 15, 26, 29, 57–59) (**Table 2**). Clinical or radiological improvements were observed in 11 out of 13 cases, in which tumor progression stopped for up to 54 months. In seven patients with

TABLE 2 | Published cases of Capecitabine (pro-drug of 5-Fluorouracil) + Temozolomide (CAPTEM) (TMZ naïve) for aggressive pituitary tumors and carcinomas.

Response in tumor growth/PFS (month)	CAPTEM (cycles)	Tumor subtype	Pathology	Previous treatment	Side effects	Age/ sex	Ref, year [ref no]
PR, PFS (22)	12	ACTH	MGMT: ns	ns	ns	44/M	McCormack (11)
PR, PFS (6)	6	ACTH	MGMT: ns	ns	ns	49/M	McCormack (11)
PD	18	PRL-CA	MGMT: ns	ns	ns	38/M	McCormack (11)
SD, PFS (54+)	30	ACTH	MGMT:low, Ki-67: <5%	TSS, BAD, RT	thrombocytopenia	50/M	Zacharia (14)
CR, PFS (32+)	32	ACTH	MGMT:low, Ki-67: 5%	TSS, RT	lymphopenia	46/F	Zacharia (14)
CR, PFS (45+)	45	Silent ACTH	MGMT:low, Ki-67: <5%	TSS, RT	none	44/M	Zacharia (14)
PR, extra-axial met	12 off 27 mon, 12 ongoing	Silent ACTH-CA	MGMT: low, Ki-67: 1-5%	TSS, RT	mild constipation 12	54/M	Nakano-Tateno (15)
PR, PFS (34+)	12	ACTH	MGMT: low, Ki-67: <1%	TSS, RT	nausea	48/M	Nakano-Tateno (15)
PD	3	PIT1-CA	MGMT: ns, Ki-67: 15%	TSS, RT	ns	23/F	Alshaikh (26)
PR for 24 mo, then liver met	4	ACTH-CA	MGMT+ (liver)	TSS, BAD, RT)	thrombocytopenia	35/F	Lin (29)
PR, PFS (5.5)	4	ACTH-CA	MGMT: ns, Ki-67:31%	TSS, BAD, RT	none	50/M	Thearle (57)
PR,spine and pelvic met	8	ACTH-CA	MGMT:ns, Ki-67:19-50%, p53+	TSS, RT	thrombocytopenia	46/F	Donovan (58)
SD, PFS (8)	8	ACTH-CA	MGMT:ns, Ki-67: 4-15%	TSS, RT	ns	49/F	Joehlin-Price (59)

ns, not stated; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; PFS, progression free survival; CA, carcinoma TSS, transsphenoidal surgery; BAD, bilateral adrenalectomy; RT, external beam radiotherapy; mo, months; met, metastasis.

carcinomas (five ACTH positive, one PRL positive, and one PIT-1 positive), five patients (71%) initially showed partial reduction of tumor size in response to CAPTEM treatment and no tumor progression for as long as 39 months. These reports include two cases of pituitary tumors treated with CAPTEM: one is a corticotroph carcinoma and the other is an aggressive corticotroph tumor (15). Both patients had undergone previous surgical and radiologic therapies. The first patient with corticotroph carcinoma started to receive the CAPTEM treatment after developing leptomeningeal spread. Twelve cycles of the treatment resulted in tumor control associated with improvement of clinical and radiological tumor size reduction for 39 months. The patient restarted CAPTEM after recurrence of the tumor, and is currently under treatment. The second patient, with an aggressive corticotroph tumor, was treated with 12 cycles of CAPTEM, which led to tumor shrinkage with no tumor regrowth 22 months after cessation of therapy. As for the side effects of CAPTEM, the second patient had nausea, which was manageable by reducing the dose of CAPTEM to 75% of the maximal after the 6^{th} cycle.

CAPTEM has been used in TMZ resistant cases as well. CAPTEM treatment followed by TMZ monotherapy (TMZ resistant cases) has been reported in eight cases (three ACTH secreting carcinomas, two PRL secreting carcinomas, one null-cell carcinoma, and two cases not stated) (8, 10–12, 42). However, the outcome was unfavorable in seven cases (88%), and only one (null-cell carcinoma) had a partial regression of carcinoma (42). From this limited dataset, CAPTEM seems to be more effective in TMZ naïve cases compared to those with TMZ resistance. Unlike TMZ monotherapy, it is unknown whether MGMT expression in tumors can be a predictive marker for response to CAPTEM. In six aggressive pituitary tumors treated with CAPTEM, five cases (83%) showed low MGMT expression levels, and one case had positive expression in the liver metastasis (14, 15, 29, 57). The outcome of these low MGMT cases varies, including cases of complete regression, partial regression, and stabilization of the tumor. Further studies are required to determine the role of MGMT expression in predicting response to CAPTEM therapy.

Other than CAPTEM, there are a few case reports of TMZ in combination with other therapeutic agents. Among them are the combinations of TMZ with VEGF-targeted therapy (Bevacizumab or Apatanib) (60–62) and a somatostatin receptor ligand (Pasireotide) (9, 63). In a survey by the European Society of Endocrinology, 1 case treated with TMZ in combination with bevacizumab achieved a partial tumor regression, 1 case with thalidomide showed no progression of the disease, and 1 case with Carmustine showed progressive disease (PD) (11). Although a prospective clinical trial is required to determine whether the treatment with CAPTEM is superior to the treatment with TMZ alone, CAPTEM appears to be a promising treatment option for aggressive pituitary tumors and carcinomas based on several case reports and our experimental data.

PEPTIDE RECEPTOR RADIONUCLIDE THERAPY (PRRT)

Peptide Receptor Radionuclide Therapy (PRRT) is a form of targeted therapy that utilizes the delivery of radionuclide-bound

somatostatin agonists to pituitary tumors expressing somatostatin receptors (SSTRs). PRRT has successfully treated neuroendocrine tumors, due to their high levels of SSTR expression (64–66). The use of PRRT for pituitary tumors was introduced after its success in treating neuroendocrine tumors (17). Radionuclides such as Yttrium 90 (Y-90), Lutetium 177 (Lu-177), and Indium-111 (In-111) are combined with peptides or somatostatin agonists (e.g., DOTATOC, DOTATATE) to deliver radiation to tumor cells. Because this is a targeted therapy, the risk of the systemic adverse effects is lower than conventional radiation therapy (18, 38).

There is still limited evidence whether PRRT is effective in the management of aggressive pituitary adenomas or carcinomas (8, 16–24) (**Table 3**). From the review of English literature between the years of 2012 and 2020, we found a total of 15 cases describing PRRT treatment in aggressive pituitary tumors (11 cases) and carcinomas (four cases). The treatment protocols used (dose, type of radionuclide and peptide, timing of radionuclide delivery) and measured outcomes across these cases vary greatly and are summarized in **Table 3**. Broadly, regarding tumor size, among 15 reported cases, six cases (43%) responded to PRRT. Decrease in hormone levels was reported in only three cases (20%, **Table 3**).

In general, PRRT treatment is tolerated well without major side effects. In a study of 30 patients with advanced cancers (mostly neuroendocrine tumors) receiving [111In-DTPA^0] octreotide up to a high cumulative dose of 75Gbq, no major side effects were noted. Transient reduction in platelets and leukocytes can occur (67). From our review of 15 cases of aggressive pituitary tumors treated with PRRT, one patient developed transient grade 2 thrombocytopenia (20).

There is no clear evidence as to whether dosage or type of radionucleotide affects outcomes (23). From our review of 15 cases, total average radiation dose seems similar, with an average of 15.4 GBq in responders versus an average dose of 14.5 GBq in non-responders. However, dosages varied widely in both groups (from 0.4 GBq to 37 GBq), therefore it is difficult to assess whether dosage affects outcomes or not. It is also unknown whether the pre-treatment radioisotope uptake scan corresponds with treatment outcomes (18, 22, 23). In well-differentiated neuroendocrine tumors and medullary thyroid cancers, the total amount of pre-treatment radioisotope uptake did not reflect prognosis well, but heterogeneous uptake may correspond with poor prognosis after PRRT treatment (68, 69). In pituitary tumors, dosimetry analysis was reported in only

TABLE 3 | Published cases of peptide receptor radionuclide therapy (PRRT) for pituitary tumors and carcinomas.

Response in tumor growth	Hormone reduction	PFS (month)	Tumor subtype	Initial tumor volume (ml)	Total radiation dose/ (number of cycles)	Type of radionuclide	Previous treatment	Age/ sex	Ref, year [ref no]
n/a	ns	5	NFPA	ns	ns	177Lu-DOTATE	TMZ	59/F	Bengtsson (8)
PD	ns	8	GH-CA	ns	ns	90Y-DOTATE	TMZ	46/ M	Bengtsson (8)
PD	ns	8	PRL	ns	ns	68Gallium DOTATE	TMZ	23/ M	Bengtsson (8)
n/a	ns	ns	NFPA-CA	4.1ml	0.15 GBq (one dose)	177Lu-DOTATE	none	71/F	Kumar (16)
decreased 95% over 8 years	PRL decreased	ns	PRL	63 mL	37 GBq (5)	111In-DTPA-octreotide	TSS, RT, octreotide	58/F	Baldari (17)
SD over 8 years	ns	96	NFPA	ns	0.6 GBq (3)	177Lu-DOTATE	TSS	55/ M	Komor (18)
Decreased 60.5% over 12 months	IGF decreased	12	GH	23.1ml	0.4 GBq (0.1 GBq every 3 mo)	90Y-DOTATE	TSS, RT, octreotide, lanreotide	26/ M	Waligórska-Stachura (19)
Pituitary: SD, met volume: decrease	ns	40	NFPA-CA	ns	29.6 GBq (one dose)	177Lu-DOTATE	TSS, RT	63/ M	Maclean (20)
PD	ns	ns	GH/PRL	ns	15.3 GBq (2)	177Lu-DOTATE	TSS, RT, TMZ, lanreotide	42/ M	Maclean (20)
PD	ns	ns	ACTH	ns	ns (one dose)	177Lu-DOTATE	TSS, RT, TMZ	32/ M	Maclean (20)
SD over 1 year, then pituitary apoplexy	GH decreased, IGF persistently high	12	GH	31.8ml	22.2 GBq (3)	177Lu-DOTATE	surgical attempt	48/ M	Assadi (21)
SD over 4 years	ns	48	NFPA-CA	ns	22.2 GBq (3)	177Lu-DOTATE	TSS, RT	68/ M	Novruzov (22)
PD	ns	ns	PRL	20.2 ml	12.6 GBq (2)	177Lu-DOTATOC	TSS, RT, TMZ	54/ M	Giuffrida (23)
PD	ns	ns	NFPA	7.7 mL	29.8 GBq (5)	177Lu-DOTATOC	TSS, RT, TMZ	53/F	Giuffrida (23)
PD	ns	<12	ACTH-CA	ns	0.2 GBq (one dose)	90Y-DOTATOC	TSS, RT	16/F	Kovács (24)

ns, not stated; NFPA, non-functioning pituitary adenoma; CA, carcinoma; TSS, transsphenoidal surgery; RT, radiation therapy; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; PFS, progression free survival; mo, months.

1 case, which showed a heterogeneous uptake pattern with progressed disease (20).

It is of interest to know the difference in efficacy between somatostatin analog (SSA) therapy and PRRT, however, at this point, there is no direct comparison study. At this stage, PRRT is a treatment option for aggressive pituitary tumors that express SSTR and are resistant to other therapies. The 2018 guidelines of the European Society of Endocrinology listed PRRT as an alternative treatment option for aggressive pituitary tumors (1). Taken together, current evidence does not suggest that PRRT is an impressively effective treatment for aggressive pituitary tumors, however it is still premature to conclude that PRRT is not an important treatment strategy since half of the reported cases were TMZ resistant which are usually difficult to control disease progressions.

mTOR INHIBITORS

The phosphatidylinositol 3-kinase/mammalian target of rapamycin (mTOR) pathway is involved in the regulation of survival, growth, protein synthesis, and cellular metabolism (70–73). Everolimus (EVE) is an orally active mTOR inhibitor FDA-approved to treat neuroendocrine tumors. EVE forms a complex with mTORC1, affecting downstream cellular activities, and causes cell cycle arrest and protein synthesis inhibition. Many studies have shown that EVE is effective in human pituitary tumor cultures and murine cell lines *in vitro* (74–77). It is also effective *in vivo* in intracranial GH4 cell xenograft mouse models and reduced transplanted tumor cell viability and proliferation (78). Moreover, EVE synergized with SSAs, such as octreotide or pasireotide, and exhibited anti-proliferative effects in primary human pituitary tumor cells (79, 80).

Although EVE has been widely used in the management of pancreatic and other neuroendocrine neoplasms, there are only seven published cases of pituitary tumors treated with EVE combination therapy (three ACTH secreting carcinomas, one PRL secreting adenoma, and three not stated) (11, 25–27, 58) (**Table 4**). Of note, five out of seven (71%) cases had disease progression, and tumors in all the failed cases were TMZ resistant. The protocol used for EVE treatment is variable. The reported dose of EVE is 5 to 10 mg daily for several months, either as a monotherapy or in combination with other therapies. Common side effects include stomatitis, rash, fatigue, diarrhea, infections, anemia, and hyperglycemia (25). Initial reduction in hormone secretion was observed in only one out of seven patients treated with EVE (25). A patient with a PRL secreting tumor treated with EVE 10mg + octreotide showed decreased PRL levels (454 ng/ml to 253 ng/ml; 55% reduction) after an initial 3 months of EVE therapy; however, PRL level gradually increased to its previous level by 5 months following initiation of EVE therapy (25). Among the seven published cases, only two out of seven cases (29%) reported no progression of tumor growth: one corticotroph carcinoma treated with EVE (7.5 mg) + Capecitabine followed by EVE (7.5–10 mg) + RT showed no increase in tumor volume for 5 months (58), and an aggressive PRL secreting tumor treated with EVE 10mg + octreotide showed stable tumor volume for 12 months (25). Currently, there is not a sufficient number of cases with EVE treatment for pituitary tumors to determine its effectiveness. In addition, the complexity of the reported cases may have biased the outcomes of EVE treatment. For example, three out of seven reported cases were pituitary carcinomas and six out of seven EVE treated cases were TMZ refractory cases. Further work will be required to define the role and effectiveness of EVE in management of pituitary tumors.

IMMUNOTHERAPY

Immune checkpoint inhibitors upregulate the body's immune response to fight against malignancy and have been used to treat melanomas, lung cancers, renal cancers and Hodgkin lymphomas (81). Pembrolizumab (PEM) and nivolumab (NIV) are checkpoint inhibitors that inhibit programmed cell death 1 (PD-1), which is a transmembrane protein expressed on immune cells that inhibits T-cell destruction of tumor cells. Ipilimumab (IPI) is a checkpoint inhibitor that inhibits the action of Cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), which downregulates immune

TABLE 4 | Published cases of Everolimus (EVE) treatments for pituitary tumors and carcinomas.

Response in tumor growth	EVE treatment	Duration (month)	Tumor subtype	Pathology	Previous treatments	Age/ sex	Ref, year [ref no]
PD	EVE	ns	ns	ns	TMZ	ns	McCormack (11)
PD	EVE	ns	ns	ns	TMZ	ns	McCormack (11)
PD	EVE	ns	ns	ns	TMZ	ns	McCormack (11)
tumor size: SD for 12+mon, hormone reduction: PR for 8 mon	EVE+OCT	12+	PRL	Ki-67: 30%, p53+	Surgery, RT, CAB	62/M	Zhang (25)
PD	EVE	ns	ACTH-CA	Ki-67: 10%	Surgery, BAD, RT, TMZ	49/F	Alshaikh (26)
PD	EVE+OCT	1	ACTH-CA	Ki-67: low, mitoses+	TMZ	45/M	Jouanneau (27)
SD for 5 mon then PD	EVE+paliative RT, EVE+Capecitabine	8	ACTH-CA	Ki-67: 19-50%, p53+	Surgery, BAD, RT, CAB, CAPTEM	46/F	Donovan (58)

ns, not stated; CA, carcinoma; BAD, bilateral adrenalectomy; RT, radiation therapy; SD, stable disease; PD, progressive disease.

response (82). CTLA-4 and PD-1 are both expressed in pituitary adenomas (83–85). This is thought to be a mechanism by which immunotherapy can contribute to the treatment of pituitary tumors. However, the use of checkpoint inhibitors is associated with several adverse effects, including hypophysitis (86). For example, single-agent anti-PD-1/PD-L1 monoclonal antibody therapy is associated with incidence rate of hypophysitis between 1% to 6% (87). Combination of IPI (CTLA-4) plus NIV (PD-1) is associated with hypophysitis rate as high as 7.7% to 11.7 percent (88, 89). Other adverse effects by ICIs include thyroiditis (1%–6%) (90), primary adrenal insufficiency (0.7%) (91), dermatitis (34%–39%) (92), and hepatotoxicity (5%–10% with PD-1) (93).

Thus far, checkpoint inhibitors have been used to treat pituitary adenomas in five cases (29–31, 94) (**Table 5**). Of these five cases, three cases were ACTH-producing carcinomas, one case was an ACTH-producing adenoma, and one case was a prolactinoma. All ACTH-producing carcinoma cases received a combination of IPI and NIV and showed decreased to stable sizes of pituitary carcinomas, ACTH levels, and the liver metastatic volume in two cases (29, 30, 94). However, one of these positively responding cases of pituitary carcinoma recurred over 1 year. Of note, two other cases of non-carcinomas [ACTH-producing adenoma (treated with PEM) and prolactinoma (treated with IPI and NIV)] had disease progression despite immunotherapy (31, 94).

CONVENTIONAL THERAPIES

DAs Based Combination Therapies for Aggressive Prolactinomas

Although the dopamine agonists (DAs) (bromocriptine (BRC) and cabergoline (CAB)) are established first-line treatments for prolactinomas (95), DA resistance in prolactinomas occurs in 20%–30% of patients treated with BRC and 10% of patients treated with CAB (96). Possible mechanisms include decreased expression of dopamine receptor D2 (D2R), changes upstream or downstream of D2Rs signaling, increased angiogenic markers, and disruptions in the TGF- β1 pathway (97). It has been reported that higher dose of CAB therapy is successful in treating BRC resistant prolactinomas (96). Patients are generally able to tolerate high doses of DAs (98), but periodic echocardiogram may be required for prolonged high-dose use since cumulative dosage increases the risk of valvular heart disease (99). In addition to monotherapy, DA therapies in combination with tamoxifen (TAM) or octreotide (OCT) have been reported to treat DA-resistant prolactinomas with various response rates, summarized in **Table 6** (100–105). Metformin was also used in combination therapies (103). A case series with DAs and metformin over 8-14 months have noted both PRL normalization and significant tumor reduction (103).

DAs Based Therapies for Recurrent or Residual NFPAs

CAB monotherapy has been also used for recurrent or residual NFPAs (187 cases in six studies) (106–111). In these studies, in 33% of patients, CAB monotherapy resulted in more than 25% of tumor volume reduction or reduction of tumor size less than 2 mm in diameter, although the tumor reduction rate in these studies varies. The average dose of CAB was 2.95 mg/week and the average treatment duration was 8 months. The largest study, including 79 patients treated with DAs, demonstrated 35% reduction of tumor size on average when used against recurrent or residual NFPAs (111). It remains to be determined whether combinations of DAs with other drugs will be effective against refractory NFPAs.

SSA Based Combination Therapies for SSA-Resistant Acromegaly

OCT resistance is estimated to occur in approximately 30% of GH-secreting adenomas and is thought to be mediated by

TABLE 5 | Published cases of Immunotherapy treatments for pituitary tumors and carcinomas.

Response in tumor growth	Hormone reduction	Treatment	Tumor subtype	Pathology	Previous treatment	Age/sex	Ref, year [ref no]
intracranial: decreased by 59%, liver met: decreased by 98%. SD after 6 months	Decreased ACTH by 100%	IPI and NIV 5 cycles followed by NIV only	ACTH-CA	Liver: mitotic index 50%, PDL-1 <1%	TSS, RT BAD, TMZ	41/F	Lin (29)
SD	Decreased ACTH by 30%, am cortisol by 64%, UFC by 74%	IPI and NIV 4 cycles, then NIV maintenance and ketoconazole	ACTH-CA	Ki-67:<1%	TSS, RT, TMZ	41/M	Sol (30)
PD	PD	PEM 4 cycles	ACTH	MIB>3%, PDL-1 negative	TSS, RT, TMZ	66/M	Caccese (31)
Pituitary: decreased by 15%, liver met: decreased by 57–69%. PD after 12 months	Decreased ACTH by >93% then PD	IPI and NIV 5 cycles followed by NIV 21 cycles	ACTH-CA	Ki-67: 5%, Liver: Ki-67 10%, PDL-1 negative	TSS, RT, TMZ	60/F	Duhamel (94)
PD	PD	IPI and NIV 2 cycles	PRL	Ki-67: 25%	TSS, RT, TMZ	68/M	Duhamel (94)

NFPA, non-functioning pituitary adenoma; CA, carcinoma; TSS, transsphenoidal surgery; RT, radiation therapy; BAD, bilateral adrenalectomy; PEM, pembrolizumab; IPI, ipilimumab; NIV, nivolumab; HR, hormonal response; SD, stable disease; PD, progressive disease; UFC, urinary free cortisol.

TABLE 6 | Summary of conventional therapies for aggressive prolactinomas, Cushing's disease, and acromegaly and non-functional pituitary adenomas.

Tumor subtype	Response in tumor growth	Hormone reduction	Treatment	Duration	Case numbers	Ref, year [ref no]
PRL	38%	0%	2.5 mg/day BRC, 20 mg/day TAM	5 days	8	Lamberts (100)
	ns	58%	2.5–7.5 mg/day BRC, 10–20 mg/day TAM	4 weeks	12	Volker (101)
	100%	100%	4 mg/week CAB, 20 mg three times daily TAM	8 months	1	Christian (102)
	100%	100%	15 mg/day BRC, 1.5g/day MET	12 to 14 months	2	Liu (103)
	0%	100%	3 mg/week CAB, 20 mg/month OCT	12 months	1	Fusco (104)
	40%	0%	3–7.5 mg/week CAB, 20 mg/month OCT	6 to 13 months	5	Sosa-Eroza (105)
NFPA	32%	ns	2 mg/week CAB	6 months	19	Garcia (106)
	0%	ns	1 mg/week CAB	12 months	12	Lohmann (107)
	44%	ns	1–3 mg/week CAB	12 months	9	Pivonello (108)
	67%	ns	3 mg/week CAB	6 months	9	Vieira Neto (109)
	29%	ns	3.5 mg/week CAB	24 months	59	Batista (110)
	35%	ns	0.5–3.5 mg/week CAB or 2.5–10 mg/day BRC	ns	79	Greenman (111)
GH	ns	39%	1 to 3.5 mg/week CAB, 30 mg/28 days OCT	3 to 18 months	13	Cozzi (112)
	ns	50%	1 to 3.5 mg/week CAB, 60 mg/28 days LAN	3 to 18 months	6	Cozzi (112)
	0%	56%	1.5 or 3.5 mg/week CAB, 20–30 mg/28 days OCT	2 to 12 months	34	Jallad (113)
	ns	40%	1 to 3 mg/week CAB, 30 mg/28 days OCT	6 months	52	Vilar (114)
	11%	22%	0.25–2 mg/week CAB, 20–40 mg/month OCT	8.1 to 80.5 months	9	Suda (115)
	ns	9%	0.5–3.5 mg/week CAB, 40 mg/28 days OCT	8 months	32	Colao (116)
	0%	50%	1.5–3 mg/week CAB, 60-90 mg/month LAN	3 months	10	Marzullo (117)
	ns	44%	1.8 mg/week on average CAB, 30 mg/month OCT	8.44 months average	9	Gatta (118)
	ns	40%	1–10 mg three times daily BRC, 30 µg/month depot OCT	7 to 29 months	5	Selvarajah (119)
	ns	50%	1–1.5 mg/week CAB, 30 µg/month depot OCT	7 to 29 months	4	Selvarajah (119)
	ns	37%	2 or 3.5 mg/week CAB, 30 mg/month OCT	18 weeks	19	Mattar (120)
	0%	100%	30 mg/month OCT, 40 mg/day PEG	18 months	1	van der Lely (121)
	5%	58%	120 mg/month LAN, 40–80 mg/week or 40 or 60 mg twice/week PEG	7 months	57	van der Lely (122)
	0%	62%	median 30 mg/28 days OCT, median 15 mg/day PEG	9 months	29	Trainer (123)
	0%	56%	30 mg/28 days OCT, average 17.9 mg/day PEG	median 30 months	27	Bianchi (124)
	12%	97%	30 mg/28 days OCT or 120 mg/28 days LAN, median 80 mg/week PEG	median 59 months	112	Neggers (125)
	0%	100%	2.25 mg/week CAB, 60 mg/month PAS, 20 mg six times a week PEG	6 months	1	Ciresi (126)
ACTH	0%	67%	0.5 to 3mg/week CAB, 200–600 mg/day KCZ	12 months	6	Barbot (127)
	ns	67%	2–3 mg/week CAB, 200–400 mg/day KCZ	6 months	9	Vilar (128)
	ns	33%	0.5 mg every other day CAB, 250 µg three times daily PAS	33 days	12	Feelders (129)
	ns	75%	0.5 mg every other day CAB, 250 µg three times daily PAS, 200 mg three times daily KCZ	21 days	8	Feelders (129)

NFPA, non-functioning pituitary adenoma; ns, not stated; BRC, bromocriptine; TAM, tamoxifen; CAB, cabergoline; MET, metformin; OCT, octreotide; LAN, lanreotide; PEG, pegvisomant; KCZ, ketoconazole; PAS, pasireotide.

reduced expression of SSTRs (130). It is found that three combination therapies (DA, pegvisomant or both) with SSA are mildly to moderately effective against these SSA-resistant GH-secreting tumors. As summarized in **Table 6**, these conventional combination therapies have shown a wide range of efficacies for controlling hormone levels (9%–100%) (112–126). The combination of PEG and SSA seems more effective than CAB and SSA to control IGF1 levels (112–126). None of the combinations are effective in reducing tumor size (0%–12%) (112–120).

Combination Therapies for Aggressive Cushing Disease

Only a limited number of studies report treatment of aggressive Cushing disease with combinations of conventional therapies [**Table 6** (127–129)]. Ketoconazole (KCZ) in combination with CAB or PAS showed a moderate normalizing effect on cortisol levels in KCZ monotherapy resistant cases (129). CAB and PAS combinations were reported, but were less effective for reducing cortisol levels (129). One study showed no tumor reduction and the others did not mention efficacies on tumor size reductions.

EPIDERMAL GROWTH FACTOR RECEPTOR (EGFR) INHIBITORS

EGFR is a transmembrane receptor with the intracellular tyrosine kinase domain that regulates cell proliferation, migration, and survival. The EGFR inhibitors gefitinib and lapatinib are promising treatments for aggressive prolactinomas and aggressive corticotroph adenomas. EGFR signaling is detected in up to 82% of DA resistant prolactinomas. Activating EGFR signaling in rat lactosomatotroph cells causes elevation of *PRL* mRNA expression (131). EGFR signaling also upregulates the *POMC* (proopiomelanocortin) promoter and increases ACTH production in corticotrophs (132). Mutations in the ubiquitin specific peptidase 8 (*USP8*) gene, which causes gain-of-function of USP8 protein, are highly prevalent in Cushing disease (133). It is thought that the mutations in *USP8* cause enhanced EGFR signaling and are closely associated with development of Cushing disease (133–136).

Gefitinib has been implemented for treatment of both aggressive prolactinomas and corticotroph adenomas (137). Gefitinib suppressed PRL secretion by approximately 50% in human prolactinoma primary cultures *in vitro*. Moreover, gefitinib suppressed serum PRL levels by 40%–50% and tumor volume by 30% in somatotroph adenoma xenografted rodent models (131, 138). In primary cultures of ACTH-secreting human, canine, and murine adenomas, *POMC* mRNA was significantly suppressed by 63%–95% following treatment with gefitinib. In corticotroph adenoma xenografted mice, a 10-day course of treatment with gefitinib resulted in 40% inhibition of tumor growth (132).

Lapatinib is a dual EGFR inhibitor that inhibits both EGFR and human epidermal growth factor receptor 2 (HER2) (139). It has mostly been used to treat aggressive prolactinomas. A comparative study between lapatinib and gefitinib has found that lapatinib suppressed PRL levels more strongly in human prolactinoma primary cultures (60% with lapatinib and 40% with gefitinib) (138). It has also been reported that lapatinib suppresses PRL secretion (by 72%) and cell proliferation (by 80%) in EGFR and HER2-expressing transgenic mice, which are models of prolactinomas (140). In addition, a recent clinical trial treating two patients with DA-resistant prolactinomas with lapatinib at a dosage of 1,250 mg/day has reported 78% and 42% PRL suppression respectively with a 22% tumor reduction in the former and a stabilized tumor in the latter (28). These studies suggest that targeting EGFR signaling is a promising therapeutic strategy to, at least, populations of pituitary tumors with elevated EGFR signaling.

CYCLIN DEPENDENT KINASE 2 (CDK2) INHIBITOR

CDK2 interacts with Cyclin E to facilitate entry into the S phase of the cell cycle and protects cells against apoptosis (141). The CDK2 inhibitor, roscovitine, has been tested in phase I and II clinical trials against various malignancies, including non-small cell lung cancer and nasopharyngeal and hepatocellular carcinomas (142). Roscovitine is currently being evaluated in preclinical studies for its ability to treat Cushing disease. Roscovitine targets the CDK2/Cyclin E complexes that are highly expressed specifically in corticotroph tumors (143). Roscovitine has dual effects; inhibition of corticotroph tumor growth and suppression of transcription of *POMC*, which encodes a precursor of ACTH (32, 143). It has been demonstrated that roscovitine can reduce *pomc* expression by over 50% in pituitary tumor transforming gene (PTTG) zebrafish model and reduced ACTH and corticosterone levels by 50% in corticotroph xenografted mice (143). In primary cells derived from human corticotroph tumors, roscovitine suppressed ACTH levels in five out of six tumors (83%) (32). These reports suggest promising results and may become a new avenue of treatment of Cushing disease.

RETINOIC ACID (RA)

RA is a vitamin A derivative that interacts with RA receptors and retinoid X receptors and regulates transcription of downstream genes (144, 145). In terms of the effects on pituitary hormone genes, studies have found that the all-trans and 9-cis isomers of RA reduce the DNA-binding affinity of NURR1, a nuclear orphan receptor important for the transcription of *POMC* (146). 9-cis RA has also been found to activate the transcription of *D2Rs*, suggesting that RA may be effective against both corticotroph adenomas and prolactinomas (147). As of the publication of this review, studies of the application of RA in the treatment of pituitary tumors have focused on the treatment of corticotroph adenomas.

The majority of studies utilizing RA are pre-clinical. It has been shown that RA treatment inhibits ACTH production and tumor growth in murine and canine models of Cushing disease (148, 149). Two other clinical studies have been published using RA to treat aggressive Cushing disease (33, 34). In one prospective study, urinary free cortisol levels normalized in 4 of 16 patients after treatment with RA (33). In a recent clinical trial, over 50% cortisol suppression was observed in five of seven recurrent pituitary tumors, with normalization in three patients (34). Of note, one patient who was resistant to CAB therapy had normalized urinary free cortisol levels following the addition of RA in the treatment (33). Moreover, a study utilizing primary

culture derived from 11 corticotroph adenomas found that the combination of RA+BRC inhibited *POMC* transcription and ACTH production at higher levels than either alone in five of 11 cultures, indicating that RA+DA combination therapy may be a possible treatment for Cushing disease (33, 150). At this point, the use of RA remains at pre-clinical level, and further evidence is required to determine its efficacy.

CONCLUSION

Aggressive pituitary tumors can be resistant to conventional therapies, including surgery, radiotherapy, and medical treatment. TMZ is recommended as the first-line chemotherapy for treatment of aggressive pituitary tumors. However, TMZ has a high rate of relapse in the long term. Several lines of evidence suggest the use of novel therapy, such as CAPTEM, PRRT, EVE, immunotherapy, EGFR inhibitors, CDK2 inhibitors, and RA, could be valuable strategies for long-term tumor control. These novel therapies could improve inhibition of pituitary tumor growth and/or control of excess hormone(s) compared to established treatment methods. Although information about the efficacy of such treatments is limited and few cases have utilized them so far, some treatments are showing promising outcomes. Further research will establish new treatment options and optimize treatment sequencing for aggressive pituitary tumors.

AUTHOR CONTRIBUTIONS

TA and TT designed the project. TN-T, KL, and JW did literature searches and analyzed data. TA, TT, TN-T, KL, JW, CM, and YK wrote the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

This study is supported by grants from the Graduate School, University of Minnesota (UMF0011528 to TA, Grant-in-Aid 212588 to TA), the University of Alberta (Start-up to TT), the University Hospital Foundation (Medical Research Competition 2019 to TT), and the US National Institutes of Health (AR064195 to YK).

REFERENCES

1. Raverot G, Burman P, McCormack A, Heaney A, Petersenn S, Popovic V, et al. European Society of Endocrinology Clinical Practice Guidelines for the management of aggressive pituitary tumours and carcinomas. *Eur J Endocrinol* (2018) 178(1):G1–g24. doi: 10.1530/EJE-17-0796
2. Lasolle H, Cortet C, Castinetti F, Cloix L, Caron P, Delemer B, et al. Temozolomide treatment can improve overall survival in aggressive pituitary tumors and pituitary carcinomas. *Eur J Endocrinol* (2017) 176 (6):769–77. doi: 10.1530/EJE-16-0979
3. Bush ZM, Longtine JA, Cunningham T, Schiff D, Jane JA Jr, Vance ML, et al. Temozolomide treatment for aggressive pituitary tumors: correlation of clinical outcome with O(6)-methylguanine methyltransferase (MGMT) promoter methylation and expression. *J Clin Endocrinol Metab* (2010) 95 (11):E280–90. doi: 10.1210/jc.2010-0441
4. Raverot G, Sturm N, de Fraipont F, Muller M, Salenave S, Caron P, et al. Temozolomide treatment in aggressive pituitary tumors and pituitary carcinomas: a French multicenter experience. *J Clin Endocrinol Metab* (2010) 95(10):4592–9. doi: 10.1210/jc.2010-0644
5. Losa M, Mazza E, Terreni MR, McCormack A, Gill AJ, Motta M, et al. Salvage therapy with temozolomide in patients with aggressive or metastatic pituitary adenomas: experience in six cases. *Eur J Endocrinol* (2010) 163 (6):843–51. doi: 10.1530/EJE-10-0629
6. Hirohata T, Asano K, Ogawa Y, Takano S, Amano K, Isozaki O, et al. DNA mismatch repair protein (MSH6) correlated with the responses ofatypical pituitary adenomas and pituitary carcinomas to temozolomide: the national cooperative studyby the Japan Society for Hypothalamic and Pituitary Tumors. *J Clin Endocrinol Metab* (2013) 98(3):1130–6. doi: 10.1210/jc.2012-2924
7. Bruno OD, Juárez-Allen L, Christiansen SB, Manavela M, Danilowicz K, Vigovich C, et al. Temozolomide Therapy for Aggressive Pituitary Tumors: Results in a Small Series of Patients from Argentina. *Int J Endocrinol* (2015) 2015:587893. doi: 10.1155/2015/587893
8. Bengtsson D, Schrøder HD, Andersen M, Maiter D, Berinder K, Feldt Rasmussen U, et al. Long-term outcome and MGMT as a predictive marker in 24 patients with atypical pituitary adenomas and pituitary carcinomas given treatment with temozolomide. *J Clin Endocrinol Metab* (2015) 100 (4):1689–98. doi: 10.1210/jc.2014-4350
9. Ceccato F, Lombardi G, Manara R, Emanuelli E, Denaro L, Milanese L, et al. Temozolomide and pasireotide treatment for aggressive pituitary adenoma: expertise at a tertiary care center. *J Neurooncol* (2015) 122(1):189–96. doi: 10.1007/s11060-014-1702-0
10. Losa M, Bogazzi F, Cannavo S, Ceccato F, Curtò L, De Marinis L, et al. Temozolomide therapy in patients with aggressive pituitary adenomas or carcinomas. *J Neurooncol* (2016) 126(3):519–25. doi: 10.1007/s11060-015-1991-y
11. McCormack A, Dekkers OM, Petersenn S, Popovic V, Trouillas J, Raverot G, et al. Treatment of aggressive pituitary tumours and carcinomas: results ofa European Society of Endocrinology (ESE) survey 2016. *Eur J Endocrinol* (2018) 178(3):265–76. doi: 10.1530/eje-17-0933
12. Santos-Pinheiro F, Prado M, Kamiya-Matsuoka C, Waguespack SG, Mahajan A, Brown PD, et al. Treatment and long-term outcomes in pituitary carcinoma: a cohort study. *Eur J Endocrinol* (2019) 181(4):397–407. doi: 10.1530/EJE-18-0795
13. Elbelt U, Schlaffer SM, Buchfelder M, Knappe UJ, Vila G, Micko A, et al. Efficacy of Temozolomide Therapy in Patients With Aggressive Pituitary Adenomas and Carcinomas-A German Survey. *J Clin Endocrinol Metab* (2020) 105(3). doi: 10.1210/clinem/dgz211
14. Zacharia BE, Gulati AP, Bruce JN, Carminucci AS, Wardlaw SL, Siegelin M, et al. High response rates and prolonged survival in patients with corticotroph pituitary tumors and refractory Cushing disease from capecitabine and temozolomide (CAPTEM): a case series. *Neurosurgery* (2014) 74(4):E447–55; discussion E455. doi: 10.1227/NEU.0000000000000251
15. Nakano-Tateno T, Satou M, Inoshita N, van Landeghem FKH, Easaw J, Mehta V, et al. Effects of CAPTEM (Capecitabine and Temozolomide) on a Corticotroph Carcinoma and an Aggressive Corticotroph Tumor. *Endocr Pathol* (2020). doi: 10.1007/s12022-020-09647-w
16. Kumar Gupta S, Singla S, Damle NA, Agarwal K, Bal C. Diagnosis of Men-I Syndrome on (68)Ga-DOTANOC PET-CT and Role of Peptide Receptor Radionuclide Therapy With (177)Lu-DOTATATE. *Int J Endocrinol Metab* (2012) 10(4):629–33. doi: 10.5812/ijem.4313

17. Baldari S, Ferraù F, Alafaci C, Herberg A, Granata F, Militano V, et al. First demonstration of the effectiveness of peptide receptor radionuclide therapy (PRRT) with 111In-DTPA-octreotide in a giant PRL-secreting pituitary adenoma resistant to conventional treatment. *Pituitary* (2012) 15 Suppl 1: S57–60. doi: 10.1007/s11102-011-0373-5
18. Komor J, Reubi JC, Christ ER. Peptide receptor radionuclide therapy in a patient with disabling non-functioning pituitary adenoma. *Pituitary* (2014) 17(3):227–31. doi: 10.1007/s11102-013-0494-0
19. Waligórska-Stachura J, Gut P, Sawicka-Gutaj N, Liebert W, Gryczyńska M, Baszko-Blaszyk D, et al. Growth hormone-secreting macroadenoma of the pituitary gland successfully treated with the radiolabeled somatostatin analog (90)Y-DOTATATE: case report. *J Neurosurg* (2016) 125(2):346–9. doi: 10.3171/2015.6.JNS15363
20. Maclean J, Aldridge M, Bomanji J, Short S, Fersht N. Peptide receptor radionuclide therapy for aggressive atypical pituitary adenoma/carcinoma: variable clinical response in preliminary evaluation. *Pituitary* (2014) 17 (6):530–8. doi: 10.1007/s11102-013-0540-y
21. Assadi M, Nemati R, Shooli H, Rekabpour SJ, Nabipour I, Jafari E, et al. An aggressive functioning pituitary adenoma treated with peptide receptor radionuclide therapy. *Eur J Nucl Med Mol Imaging* (2020) 47(4):1015–6. doi: 10.1007/s00259-019-04578-z
22. Novruzov F, Aliyev JA, Jaunmuktane F, Bomanji JB, Kayani I. The use of (68)Ga DOTATATE PET/CT for diagnostic assessment and monitoring of (177)Lu DOTATATE therapy in pituitary carcinoma. *Clin Nucl Med* (2015) 40(1):47–9. doi: 10.1097/RLU.0000000000000589
23. Giuffrida G, Ferraù F, Laudicella R, Cotta O, Messina E, Granata F, et al. Peptide receptor radionuclide therapy for aggressive pituitary tumors: a monocentric experience. *Endocr Connect* (2019) 8(5):528–35. doi: 10.1530/EC-19-0065
24. Kovács GL, Góth M, Rotondo F, Scheithauer BW, Carlsen E, Saadia A, et al. ACTH-secreting Crooke cell carcinoma of the pituitary. *Eur J Clin Invest* (2013) 43(1):20–6. doi: 10.1111/eci.12010
25. Zhang D, Way JS, Zhang X, Sergey M, Bergsneider M, Wang MB, et al. Effect of Everolimus in Treatment of Aggressive Prolactin-Secreting Pituitary Adenomas. *J Clin Endocrinol Metab* (2019) 104(6):1929–36. doi: 10.1210/jc.2018-02461
26. Alshaikh OM, Asa SL, Mete O, Ezzat S. An Institutional Experience of Tumor Progression to Pituitary Carcinoma in a 15-Year Cohort of 1055 Consecutive Pituitary Neuroendocrine Tumors. *Endocr Pathol* (2019) 30 (2):118–27. doi: 10.1007/s12022-019-9568-5
27. Jouanneau E, Wierinckx A, Ducray F, Favrel V, Borson-Chazot F, Honnorat J, et al. New targeted therapies in pituitary carcinoma resistant to temozolomide. *Pituitary* (2012) 15(1):37–43. doi: 10.1007/s11102-011-0341-0
28. Cooper O, Mamelak A, Bannykh S, Carmichael J, Bonert V, Lim S, et al. Prolactinoma ErbB receptor expression and targeted therapy for aggressive tumors. *Endocrine* (2014) 46(2):318–27. doi: 10.1007/s12020-013-0093-x
29. Lin AL, Jonsson P, Tabar V, Yang TJ, Cuaron J, Beal K, Lin AL, et al. Marked Response of a Hypermutated ACTH-Secreting Pituitary Carcinoma to Ipilimumab and Nivolumab. *J Clin Endocrinol Metab* (2018) 103 (10):3925–30. doi: 10.1210/jc.2018-01347
30. Sol B, de Filette JMK, Awada G, Raeymaeckers S, Aspeslagh S, Andreescu CE, et al. Immune checkpoint inhibitor therapy for ACTH-secreting pituitary carcinoma: a new emerging treatment? *Eur J Endocrinol* (2021) 184(1):K1–5. doi: 10.1530/EJE-20-0151
31. Caccese M, Barbot M, Ceccato F, Padovan M, Gardiman MP, Fassan M, et al. Rapid disease progression in patient with mismatch-repair deficiency pituitary ACTH-secreting adenoma treated with checkpoint inhibitor pembrolizumab. *Anticancer Drugs* (2020) 31(2):199–204. doi: 10.1097/CAD.0000000000000856
32. Liu NA, Araki T, Cuevas-Ramos D, Hong J, Ben-Shlomo A, Tone Y, et al. Cyclin E-Mediated Human Proopiomelanocortin Regulation as a Therapeutic Target for Cushing Disease. *J Clin Endocrinol Metab* (2015) 100(7):2557–64. doi: 10.1210/jc.2015-1606
33. Vilar L, Albuquerque JL, Lyra R, Trovão L, Diniz E, Rangel Filho F, et al. The Role of Isotretinoin Therapy for Cushing's Disease: Results of a Prospective Study. *Int J Endocrinol* (2016) 2016:8173182. doi: 10.1155/2016/8173182
34. Pecori Giraldi F, Ambrogio AG, Andrioli M, Sanguin F, Karamouzis I, Corsello SM, et al. Potential role for retinoic acid in patients with Cushing's disease. *J Clin Endocrinol Metab* (2012) 97(10):3577–83. doi: 10.1210/jc.2012-2328
35. Stupp R, Mason WP, van den Bent MJ, Weller M, Fisher B, Taphoorn MJ, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N Engl J Med* (2005) 352(10):987–96. doi: 10.1056/NEJMoa043330
36. Lim S, Shahinian H, Maya MM, Yong W. Temozolomide: a novel treatment for pituitary carcinoma. *Lancet Oncol* (2006) 7(6):518–20. doi: 10.1016/S1470-2045(06)70728-8
37. Syro LV, Uribe H, Penagos LC, Ortiz LD, Fadul CE, Horvath E, et al. Antitumour effects of temozolomide in a man with a large, invasive prolactin-producing pituitary neoplasm. *Clin Endocrinol (Oxf)* (2006) 65 (4):552–3. doi: 10.1111/j.1365-2265.2006.02653.x
38. Fadul CE, Kominsky AL, Meyer LP, Kingman LS, Kinlaw WB, Rhodes CH, et al. Long-term response of pituitary carcinoma to temozolomide. Report of two cases. *J Neurosurg* (2006) 105(4):621–6. doi: 10.3171/jns.2006.105.4.621
39. Liu L, Gerson SL. Targeted modulation of MGMT: clinical implications. *Clin Cancer Res* (2006) 12(2):328–31. doi: 10.1158/1078-0432.CCR-05-2543
40. Halevy C, Whitelaw BC. How effective is temozolomide for treating pituitary tumours and when should it be used? *Pituitary* (2017) 20(2):261–6. doi: 10.1007/s11102-016-0745-y
41. Ji Y, Vogel RI, Lou E. Temozolomide treatment of pituitary carcinomas and atypical adenomas: systematic review of case reports. *Neurooncol Pract* (2016) 3(3):188–95. doi: 10.1093/nop/npv059
42. Campderá M, Palacios N, Aller J, Magallón R, Martín P, Saucedo G, et al. Temozolomide for aggressive ACTH pituitary tumors: failure of a second course of treatment. *Pituitary* (2016) 19(2):158–66. doi: 10.1007/s11102-015-0694-x
43. Bilbao I, Egaña N, García C, Olaizola I, et al. Failure of a second temozolomide cycle in a patient with a prolactin-secreting pituitary carcinoma. *Endocrinol Diabetes Nutr* (2017) 64(10):564–6. doi: 10.1016/j.endien.2017.08.007
44. Syro LV, Rotondo F, Camargo M, Ortiz LD, Serna CA, Kovacs K, et al. Temozolomide and Pituitary Tumors: Current Understanding, Unresolved Issues, and Future Directions. *Front Endocrinol (Lausanne)* (2018) 9:318. doi: 10.3389/fendo.2018.00318
45. McCormack AI, Wass JA, Grossman AB. Aggressive pituitary tumours: the role of temozolomide and theassessment of MGMT status. *Eur J Clin Invest* (2011)41:1133–48. doi: 10.1111/j.1365-2362.2011.02520.x
46. Mohammed AI, Cusimano MD, Scheithauer BW, Rotondo F, Horvath E, Kovacs K. O-methylguanine-DNA methyltransferase immunoexpression in a double pituitary adenoma: case report. *Neurosurgery* (2010) 66:E421–2; discussion E422. doi: 10.1227/01.Neu.0000363852.77126.Ad
47. Kovacs K, Scheithauer BW, Lombardero M, McLendon RE, Syro LV, Uribe H, et al. MGMT immunoexpression predicts responsiveness of pituitary tumors to temozolomide therapy. *Acta Neuropathol* (2008) 115:261–2. doi: 10.1007/s00401-007-0279-5
48. McCormack AI, McDonald KL, Gill AJ, Clark SJ, Burt MG, Campbell KA, et al. Low O6-methylguanine-DNA methyltransferase (MGMT) expression and response to temozolomide in aggressive pituitary tumours. *Clin Endocrinol (Oxf)* (2009) 71:226–33. doi: 10.1111/j.1365-2265.2008.03487.x
49. Hagen C, Schroeder HD, Hansen S, Hagen C, Andersen M. Temozolomide treatment of a pituitary carcinoma and two pituitary macroadenomas resistant to conventional therapy. *Eur J Endocrinol* (2009) 161:631–7. doi: 10.1530/eje-09-0389
50. Whitelaw BC, Dworakowska D, Thomas NW, Barazi S, Riordan-Eva P, King AP, et al. Temozolomide in the management of dopamine agonist-resistant prolactinomas. *Clin Endocrinol (Oxf)* (2012) 76:877–86. doi: 10.1111/j.1365-2265.2012.04373.x
51. Annamalai AK, Dean AF, Kandasamy N, Kovacs K, Burton H, Halsall DJ, et al. Temozolomide responsiveness in aggressive corticotroph tumours: a case report and review of the literature. *Pituitary* (2012) 15:276–87. doi: 10.1007/s11102-011-0363-7
52. Yip S, Miao J, Cahill DP, Iafrate AJ, Aldape K, Nutt CL, et al. MSH6 mutations arise in glioblastomas during temozolomide therapy and mediate temozolomide resistance. *Clin Cancer Res* (2009) 15(14):4622–9. doi: 10.1158/1078-0432.CCR-08-3012
53. Murakami J, Lee YJ, Kokeguchi S, Tsujigiwa H, Asaumi J, Nagatsuka H, et al. Depletion of O6-methylguanine-DNA methyltransferase by O6-

benzylguanine enhances 5-FU cytotoxicity in colon and oral cancer cell lines. *Oncol Rep* (2007) 17(6):1461–7. doi: 10.3892/or.17.6.1461
54. Kulke MH, Hornick JL, Frauenhoffer C, Hooshmand S, Ryan DP, Enzinger PC, et al. O6-methylguanine DNA methyltransferase deficiency and response to temozolomide-based therapy in patients with neuroendocrine tumors. *Clin Cancer Res* (2009) 15(1):338–45. doi: 10.1158/1078-0432.CCR-08-1476
55. Fine RL, Gulati AP, Krantz BA, Moss RA, Schreibman S, Tsushima DA, et al. Capecitabine and temozolomide (CAPTEM) for metastatic, well-differentiated neuroendocrine cancers: The Pancreas Center at Columbia University experience. *Cancer Chemother Pharmacol* (2013) 71(3):663–70. doi: 10.1007/s00280-012-2055-z
56. Spiro TP, Liu L, Majka S, Haaga J, Willson JK, Gerson SL. Temozolomide: the effect of once- and twice-a-day dosing on tumor tissue levels of the DNA repair protein O(6)-alkylguanine-DNA-alkyltransferase. *Clin Cancer Res* (2001) 7(8):2309–17.
57. Thearle MS, Freda PU, Bruce JN, Isaacson SR, Lee Y, Fine RL. Temozolomide (Temodar®) and capecitabine (Xeloda®) treatment of an aggressive corticotroph pituitary tumor. *Pituitary* (2011) 14(4):418–24. doi: 10.1007/s11102-009-0211-1
58. Donovan LE, Arnal AV, Wang SH, Odia Y. Widely metastatic atypical pituitary adenoma with mTOR pathway STK11(F298L) mutation treated with everolimus therapy. *CNS Oncol* (2016) 5(4):203–9. doi: 10.2217/cns-2016-0011
59. Joehlin-Price AS, Hardesty DA, Arnold CA, Kirschner LS, Prevedello DM, Lehman NL. Case report: ACTH-secreting pituitary carcinoma metastatic to the liver in a patient with a history of atypical pituitary adenoma and Cushing's disease. *Diagn Pathol* (2017) 12(1):34. doi: 10.1186/s13000-017-0624-5
60. Rotman LE, Vaughan TB, Hackney JR, Riley KO. Long-Term Survival After Transformation of an Adrenocorticotropic Hormone-Secreting Pituitary Macroadenoma to a Silent Corticotroph Pituitary Carcinoma. *World Neurosurg* (2019) 122:417–23. doi: 10.1016/j.wneu.2018.11.011
61. Touma W, Hoostal S, Peterson RA, Wiernik A, SantaCruz KS, Lou E. Successful treatment of pituitary carcinoma with concurrent radiation, temozolomide, and bevacizumab after resection. *J Clin Neurosci* (2017) 41:75–7. doi: 10.1016/j.jocn.2017.02.052
62. Wang Y, He Q, Meng X, Zhou S, Zhu Y, Xu J. Apatinib (YN968D1) and temozolomide in recurrent invasive pituitary adenoma: case report and literature review. *World Neurosurg* (2019) 124:319–22. doi: 10.1016/j.wneu.2018.12.174
63. Bode H, Seiz M, Lammert A, Brockmann MA, Back W, Hammes HP. SOM230 (pasireotide) and temozolomide achieve sustained control of tumour progression and ACTH secretion in pituitary carcinoma with widespread metastases. *Exp Clin Endocrinol Diabetes* (2010) 118(10): 760–3. doi: 10.1055/s-0030-1253419
64. Goglia U, Ferone D, Sidoti M, Spaziante R, Dadati P, Ravetti JL, et al. Treatment of a pituitary metastasis from a neuroendocrine tumour: case report and literature review. *Pituitary* (2008) 11(1):93–102. doi: 10.1007/s11102-007-0038-6
65. Imhof A, Brunner P, Marincek N, Briel M, Schindler C, Rasch H, et al. Response, survival, and long-term toxicity after therapy with the radiolabeled somatostatin analogue [90Y-DOTA]-TOC in metastasized neuroendocrine cancers. *J Clin Oncol* (2011) 29(17):2416–23. doi: 10.1200/JCO.2010.33.7873
66. Kwekkeboom DJ, de Herder WW, Krenning EP. Somatostatin receptor-targeted radionuclide therapy in patients with gastroenteropancreatic neuroendocrine tumors. *Endocrinol Metab Clin North Am* (2011) 40 (1):173–85, ix. doi: 10.1016/j.ecl.2010.12.003
67. Krenning EP, de Jong M, Kooij PPM, Breeman WAP, Bakker WH, de Herder WW, et al. Radiolabelled somatostatin analogue(s) for peptide receptor scintigraphy and radionuclide therapy. *Ann Oncol* (1999) 10:S23–30. doi: 10.1093/annonc/10.suppl_2.S23
68. Lapa C, Werner RA, Schmid JS, Papp L, Zsótér N, Biko J, et al. Prognostic value of positron emission tomography-assessed tumor heterogeneity in patients with thyroid cancer undergoing treatment with radiopeptide therapy. *Nucl Med Biol* (2015) 42(4):349–54. doi: 10.1016/j.nucmedbio.2014.12.006
69. Werner RA, Lapa C, Ilhan H, Higuchi T, Buck AK, Lehner S, et al. Survival prediction in patients undergoing radionuclide therapy based on intratumoral somatostatin-receptor heterogeneity. *Oncotarget* (2017) 8 (4):7039–49. doi: 10.18632/oncotarget.12402
70. Engelman JA, Luo J, Cantley LC. The evolution of phosphatidylinositol 3-kinases as regulators of growth and metabolism. *Nat Rev Genet* (2006) 7 (8):606–19. doi: 10.1038/nrg1879
71. Monsalves E , Juraschka K, Tateno T, Agnihotri S, Asa SL, Ezzat S, et al. The PI3K/AKT/mTOR pathway in the pathophysiology and treatment of pituitary adenomas. *Endocr Relat Cancer* (2014) 21(4):R331–44. doi: 10.1530/ERC-14-0188
72. Musat M, Korbonits M, Kola B, Borboli N, Hanson MR, Nanzer AM, et al. Enhanced protein kinase B/Akt signalling in pituitary tumours. *Endocr Relat Cancer* (2005) 12(2):423–33. doi: 10.1677/erc.1.00949
73. Sajjad EA, Zieliński G, Maksymowicz M, Hutnik Ł, Bednarczuk T, Włodarski P. mTOR is frequently active in GH-secreting pituitary adenomas without influencing their morphopathological features. *Endocr Pathol* (2013) 24(1):11–9. doi: 10.1007/s12022-012-9230-y
74. Gorshtein A, Rubinfeld H, Kendler E, Theodoropoulou M, Cerovac V, Stalla GK, et al. Mammalian target of rapamycin inhibitors rapamycin and RAD001 (everolimus) induce anti-proliferative effects in GH-secreting pituitary tumor cells in vitro. *Endocr Relat Cancer* (2009) 16(3):1017–27. doi: 10.1677/ERC-08-0269
75. Lee M, Wiedemann T, Gross C, Leinhäuser I, Roncaroli F, Braren R, et al. Targeting PI3K/mTOR Signaling Displays Potent Antitumor Efficacy against Nonfunctioning Pituitary Adenomas. *Clin Cancer Res* (2015) 21 (14):3204–15. doi: 10.1158/1078-0432.CCR-15-0288
76. Chanal M, Chevallier P, Raverot V, Fonteneau G, Lucia K, Monteserin Garcia JL, et al. Differential Effects of PI3K and Dual PI3K/mTOR Inhibition in Rat Prolactin-Secreting Pituitary Tumors. *Mol Cancer Ther* (2016) 15 (6):1261–70. doi: 10.1158/1535-7163.MCT-15-0891
77. Pivonello C, Patalano R, Solari D, Auriemma RS, Frio F, Vitulli F, et al. Effect of combined treatment with a pan-PI3K inhibitor or an isoform-specific PI3K inhibitor and everolimus on cell proliferation in GH-secreting pituitary tumour in an experimental setting. *Endocrine* (2018) 62(3):663–80. doi: 10.1007/s12020-018-1677-2
78. Jalali S, Monsalves E, Tateno T, Zadeh G. Role of mTOR Inhibitors in Growth Hormone-Producing Pituitary Adenomas Harboring Different FGFR4 Genotypes. *Endocrinology* (2016) 157(9):3577–87. doi: 10.1210/en.2016-1028
79. Zatelli MC, Minoia M, Filieri C, Tagliati F, Buratto M, Ambrosio MR, et al. Effect of everolimus on cell viability in nonfunctioning pituitary adenomas. *J Clin Endocrinol Metab* (2010) 95(2):968–76. doi: 10.1210/jc.2009-1641
80. Cerovac V, Monteserin-Garcia J, Rubinfeld H, Buchfelder M, Losa M, Florio T, et al. The somatostatin analogue octreotide confers sensitivity to rapamycin treatment on pituitary tumor cells. *Cancer Res* (2010) 70(2):666–74. doi: 10.1158/0008-5472.CAN-09-2951
81. Darvin P, Toor SM, Sasidharan Nair V, Elkord E. Immune checkpoint inhibitors: recent progress and potential biomarkers. *Exp Mol Med* (2018) 50 (12):1–11. doi: 10.1038/s12276-018-0191-1
82. Hui E. Immune checkpoint inhibitors. *J Cell Biol* (2019) 218(3):740–1. doi: 10.1083/jcb.201810035
83. Mei Y, Bi WL, Greenwald NF, Du Z, Agar NY, Kaiser UB, et al. Increased expression of programmed death ligand 1 (PD-L1) in human pituitary tumors. *Oncotarget* (2016) 7(47):76565–76. doi: 10.18632/oncotarget.12088
84. Uraki S, Ariyasu H, Doi A, Takeshima K, Morita S, Inaba H, et al. MSH6/2 and PD-L1 Expressions Are Associated with Tumor Growth and Invasiveness in Silent Pituitary Adenoma Subtypes. *Int J Mol Sci* (2020) 21 (8). doi: 10.3390/ijms21082831
85. Wang Z, Guo X , Gao L, Deng K, Lian W, Bao X, et al. The Immune Profile of Pituitary Adenomas and a Novel Immune Classification for Predicting Immunotherapy Responsiveness. *J Clin Endocrinol Metab* (2020) 105(9): e3207–23. doi: 10.1210/clinem/dgaa449
86. Albarel F, Gaudy C, Castinetti F, Carré T, Morange I, Conte–Devolx B, et al. Long-term follow-up of ipilimumab-induced hypophysitis, a common adverse event of the anti-CTLA-4 antibody in melanoma. *Eur J Endocrinol* (2015) 172(2):195–204. doi: 10.1530/EJE-14-0845
87. Naidoo J, Page DB, Li BT, Connell LC, Schindler K, Lacouture ME, et al. Toxicities of the anti-PD-1 and anti-PD-L1 immune checkpoint antibodies. *Ann Oncol* (2015) 26(12):2375–91. doi: 10.1093/annonc/mdv383

88. Postow MA, Chesney J, Pavlick AC, Robert C, Grossmann K, McDermott D, et al. Nivolumab and ipilimumab versus ipilimumab in untreated melanoma. *N Engl J Med* (2015) 372(21):2006–17. doi: 10.1056/NEJMoa1414428
89. Larkin J, Chiarion-Sileni V, Gonzalez R, Grob JJ, Cowey CL, Lao CD, et al. Combined Nivolumab and Ipilimumab or Monotherapy in Untreated Melanoma. *N Engl J Med* (2015) 373(1):23–34. doi: 10.1056/NEJMoa1504030
90. Ferrari SM, Fallahi P, Galetta F, Citi E, Benvenga S, Antonelli A. Thyroid disorders induced by checkpoint inhibitors. *Rev Endocr Metab Disord* (2018) 19(4):325–33. doi: 10.1007/s11154-018-9463-2
91. Barroso-Sousa R, Barry WT, Garrido-Castro AC, Hodi FS, Min L, Krop IE, et al. Incidence of Endocrine Dysfunction Following the Use of Different Immune Checkpoint Inhibitor Regimens: A Systematic Review and Meta-analysis. *JAMA Oncol* (2018) 4(2):173–82. doi: 10.1001/jamaoncol.2017.3064
92. Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, et al. Improved survival with ipilimumab in patients with metastatic melanoma. *N Engl J Med* (2010) 363(8):711–23. doi: 10.1056/NEJMoa1003466
93. Tian Y, Abu-Sbeih H, Wang Y. Immune Checkpoint Inhibitors-Induced Hepatitis. *Adv Exp Med Biol* (2018) 995:159–64. doi: 10.1007/978-3-030-02505-2_8
94. Duhamel C, Ilie MD, Salle H, Nassouri AS, Gaillard S, Deluche E, et al. Immunotherapy in Corticotroph and Lactotroph Aggressive Tumors and Carcinomas: Two Case Reports and a Review of the Literature. *J Pers Med* (2020) 10(3). doi: 10.3390/jpm10030088
95. Colao A, Savastano S. Medical treatment of prolactinomas. *Nat Rev Endocrinol* (2011) 7(5):267–78. doi: 10.1038/nrendo.2011.37
96. Maiter D. Management of Dopamine Agonist-Resistant Prolactinoma. *Neuroendocrinology* (2019) 109(1):42–50. doi: 10.1159/000495775
97. Recouvreux MV, Camilletti MA, Rifkin DB, Díaz-Torga G. The pituitary TGFβ1 system as a novel target for the treatment of resistant prolactinomas. *J Endocrinol* (2016) 228(3):R73–83. doi: 10.1530/JOE-15-0451
98. Ono M, Miki N, Kawamata T, Makino R, Amano K, Seki T, et al. Prospective study of high-dose cabergoline treatment of prolactinomas in 150 patients. *J Clin Endocrinol Metab* (2008) 93(12):4721–7. doi: 10.1210/jc.2007-2758
99. Zanettini R, Antonini A, Gatto G, Gentile R, Tesei S, Pezzoli G. Valvular heart disease and the use of dopamine agonists for Parkinson's disease. *N Engl J Med* (2007) 356(1):39–46. doi: 10.1056/NEJMoa054830
100. Lamberts SW, Verleun T, Oosterom R. Effect of tamoxifen administration on prolactin release by invasive prolactin-secreting pituitary adenomas. *Neuroendocrinology* (1982) 34(5):339–42. doi: 10.1159/000123324
101. Völker W, Gehring WG, Berning R, Schmidt RC, Schneider J, von zur Mühlen A. Impaired pituitary response to bromocriptine suppression: reversal after bromocriptine plus tamoxifen. *Acta Endocrinol (Copenh)* (1982) 101(4):491–500. doi: 10.1530/acta.0.1010491
102. Christian ZK, Hatanpaa KJ, Auchus RJ, Hammes SR, Patel AR, Mickey BE. Dopamine agonist and tamoxifen combination therapy for a prolactin-secreting pituitary tumor resistant to dopamine agonist monotherapy: Case report and review. *Interdiscip Neurosurg* (2020) 21:100777. doi: 10.1016/j.inat.2020.100777
103. Liu X, Liu Y, Gao J, Feng M, Bao X, Deng K, et al. Combination Treatment with Bromocriptine and Metformin in Patients with Bromocriptine-Resistant Prolactinomas: Pilot Study. *World Neurosurg* (2018) 115:94–8. doi: 10.1016/j.wneu.2018.02.188
104. Fusco A, Lugli F, Sacco E, Tilaro L, Bianchi A, Angelini F, et al. Efficacy of the combined cabergoline and octreotide treatment in a case of a dopamine-agonist resistant macroprolactinoma. *Pituitary* (2011) 14(4):351–7. doi: 10.1007/s11102-008-0162-y
105. Sosa-Eroza E, Espinosa E, Ramírez-Rentería C, Mendoza V, Arreola R, Mercado M. Treatment of multiresistant prolactinomas with a combination of cabergoline and octreotide LAR. *Endocrine* (2018) 61(2):343–8. doi: 10.1007/s12020-018-1638-9
106. Garcia EC, Naves LA, Silva AO, de Castro LF, Casulari LA, Azevedo MF. Short-term treatment with cabergoline can lead to tumor shrinkage in patients with nonfunctioning pituitary adenomas. *Pituitary* (2013) 16 (2):189–94. doi: 10.1007/s11102-012-0403-y
107. Lohmann T, Trantakis C, Biesold M, Prothmann S, Guenzel S, Schober R, et al. Minor tumour shrinkage in nonfunctioning pituitary adenomas by long-term treatment with the dopamine agonist cabergoline. *Pituitary* (2001) 4(3):173–8. doi: 10.1023/a:1015366923810
108. Pivonello R, Matrone C, Filippella M, Cavallo LM, Di Somma C, Cappabianca P, et al. Dopamine receptor expression and function in clinically nonfunctioning pituitary tumors: comparison with the effectiveness of cabergoline treatment. *J Clin Endocrinol Metab* (2004) 89 (4):1674–83. doi: 10.1210/jc.2003-030859
109. Vieira Neto L, Wildemberg LE, Moraes AB, Colli LM, Kasuki L, Marques NV, et al. Dopamine receptor subtype 2 expression profile in nonfunctioning pituitary adenomas and in vivo response to cabergoline therapy. *Clin Endocrinol (Oxf)* (2015) 82(5):739–46. doi: 10.1111/cen.12684
110. Batista RL, Musolino NRC, Cescato VAS, da Silva GO, Medeiros RSS, Herkenhoff CGB, et al. Cabergoline in the Management of Residual Nonfunctioning Pituitary Adenoma: A Single-Center, Open-Label, 2-Year Randomized Clinical Trial. *Am J Clin Oncol* (2019) 42(2):221–7. doi: 10.1097/COC.0000000000000505
111. Greenman Y, Cooper O, Yaish I, Robenshtok E, Sagiv N, Jonas-Kimchi T, et al. Treatment of clinically nonfunctioning pituitary adenomas with dopamine agonists. *Eur J Endocrinol* (2016) 175(1):63–72. doi: 10.1530/EJE-16-0206
112. Cozzi R, Attanasio R, Lodrini S, Lasio G. Cabergoline addition to depot somatostatin analogues in resistant acromegalic patients: efficacy and lack of predictive value of prolactin status. *Clin Endocrinol (Oxf)* (2004) 61(2):209–15. doi: 10.1111/j.1365-2265.2004.02082.x
113. Jallad RS, Bronstein MD. Optimizing medical therapy of acromegaly: beneficial effects of cabergoline in patients uncontrolled with long-acting release octreotide. *Neuroendocrinology* (2009) 90(1):82–92. doi: 10.1159/000218323
114. Vilar L, Azevedo MF, Naves LA, Casulari LA, Albuquerque JL, Montenegro RM, et al. Role of the addition of cabergoline to the management of acromegalic patients resistant to longterm treatment with octreotide LAR. *Pituitary* (2011) 14(2):148–56. doi: 10.1007/s11102-010-0272-1
115. Suda K, Inoshita N, Iguchi G, Fukuoka H, Takahashi M, Nishizawa H, et al. Efficacy of combined octreotide and cabergoline treatment in patients with acromegaly: a retrospective clinical study and review of the literature. *Endocr J* (2013) 60(4):507–15.
116. Colao A, Zgliczyński W, Komorowski J, Kos-Kudla B, Tabarin A, Kerlan V, et al. Efficacy and safety of high-dose long-acting repeatable octreotide as monotherapy or in combination with pegvisomant or cabergoline in patients with acromegaly not adequately controlled by conventional regimens: results of an open-label, multicentre study. *Endokrynol Pol* (2019) 70(4):305–12. doi: 10.5603/EP.a2019.0023
117. Marzullo P, Ferone D, Di Somma C, Pivonello R, Filippella M, Lombardi G, et al. Efficacy of combined treatment with lanreotide and cabergoline in selected therapy-resistant acromegalic patients. *Pituitary* (1999) 1(2):115–20. doi: 10.1023/a:1009932521242
118. Gatta B, Hau DH, Catargi B, Roger P, Tabarin A. Re-evaluation of the efficacy of the association of cabergoline to somatostatin analogues in acromegalic patients. *Clin Endocrinol (Oxf)* (2005) 63(4):477–8. doi: 10.1111/j.1365-2265.2005.02329.x
119. Selvarajah D, Webster J, Ross R, Newell-Price J. Effectiveness of adding dopamine agonist therapy to long-acting somatostatin analogues in the management of acromegaly. *Eur J Endocrinol* (2005) 152(4):569–74. doi: 10.1530/eje.1.01888
120. Mattar P, Alves Martins MR, Abucham J. Short- and long-term efficacy of combined cabergoline and octreotide treatment in controlling igf-I levels in acromegaly. *Neuroendocrinology* (2010) 92(2):120–7. doi: 10.1159/000317314
121. van der Lely AJ, Muller A, Janssen JA, Davis RJ, Zib KA, Scarlett JA, et al. Control of tumor size and disease activity during cotreatment with octreotide and the growth hormone receptor antagonist pegvisomant in an acromegalic patient. *J Clin Endocrinol Metab* (2001) 86(2):478–81. doi: 10.1210/jcem.86.2.7206
122. van der Lely AJ, Bernabeu I, Cap J, Caron P, Colao A, Marek J, et al. Coadministration of lanreotide Autogel and pegvisomant normalizes IGF1 levels and is well tolerated in patients with acromegaly partially controlled by somatostatin analogs alone. *Eur J Endocrinol* (2011) 164(3):325–33. doi: 10.1530/EJE-10-0867

123. Trainer PJ, Ezzat S, D'Souza GA, Layton G, Strasburger CJ. A randomized, controlled, multicentre trial comparing pegvisomant alone with combination therapy of pegvisomant and long-acting octreotide in patients with acromegaly. *Clin Endocrinol (Oxf)* (2009) 71(4):549–57. doi: 10.1111/j.1365-2265.2009.03620.x
124. Bianchi A, Valentini F, Iuorio R, Poggi M, Baldelli R, Passeri M, et al. Long-term treatment of somatostatin analog-refractory growth hormone-secreting pituitary tumors with pegvisomant alone or combined with long-acting somatostatin analogs: a retrospective analysis of clinical practice and outcomes. *J Exp Clin Cancer Res* (2013) 32(1):40. doi: 10.1186/1756-9966-32-40
125. Neggers SJ, Franck SE, de Rooij FW, Dallenga AH, Poublon RM, Feelders RA, et al. Long-term efficacy and safety of pegvisomant in combination with long-acting somatostatin analogs in acromegaly. *J Clin Endocrinol Metab* (2014) 99(10):3644–52. doi: 10.1210/jc.2014-2032
126. Ciresi A, Radellini S, Guarnotta V, Giordano C. Efficacy of combined treatment with pasireotide, pegvisomant and cabergoline in an acromegalic patient resistant to other treatments: a case report. *BMC Endocr Disord* (2018) 18(1):2. doi: 10.1186/s12902-018-0231-9
127. Barbot M, Albiger N, Ceccato F, Zilio M, Frigo AC, Denaro L, et al. Combination therapy for Cushing's disease: effectiveness of two schedules of treatment: should we start with cabergoline or ketoconazole? *Pituitary* (2014) 17(2):109–17. doi: 10.1007/s11102-013-0475-3
128. Vilar L, Naves LA, Azevedo MF, Arruda MJ, Arahata CM, Moura ESL, et al. Effectiveness of cabergoline in monotherapy and combined with ketoconazole in the management of Cushing's disease. *Pituitary* (2010) 13 (2):123–9. doi: 10.1007/s11102-009-0209-8
129. Feelders RA, de Bruin C, Pereira AM, Romijn JA, Netea-Maier RT, Hermus AR, et al. Pasireotide alone or with cabergoline and ketoconazole in Cushing's disease. *N Engl J Med* (2010) 362(19):1846–8. doi: 10.1056/NEJMc1000094
130. Peverelli E, Treppiedi D, Giardino E, Vitali E, Lania AG, Mantovani G. Dopamine and Somatostatin Analogues Resistance of Pituitary Tumors: Focus on Cytoskeleton Involvement. *Front Endocrinol (Lausanne)* (2015) 6:187. doi: 10.3389/fendo.2015.00187
131. Vlotides G, Siegel E, Donangelo I, Gutman S, Ren SG, Melmed S, et al. Rat prolactinoma cell growth regulation by epidermal growth factor receptor ligands. *Cancer Res* (2008) 68(15). doi: 10.1158/0008-5472.CAN-08-0508
132. Fukuoka H, Cooper O, Ben-Shlomo A, Mamelak A, Ren SG, Bruyette D, et al. EGFR as a therapeutic target for human, canine, and mouse ACTH-secreting pituitary adenomas. *J Clin Invest* (2011) 121(12):4712–21. doi: 10.1172/JCI60417
133. Reincke M, Sbiera S, Hayakawa A, Theodoropoulou M, Osswald A, Beuschlein F, et al. Mutations in the deubiquitinase gene USP8 cause Cushing's disease. *Nat Genet* (2015) 47(1):31–8. doi: 10.1038/ng.3166
134. Ma ZY, Song ZJ, Chen JH, Wang YF, Li SQ, Zhou LF, et al. Recurrent gain-of-function USP8 mutations in Cushing's disease. *Cell Res* (2015) 25(3):306–17. doi: 10.1038/cr.2015.20
135. Weigand I, Knobloch L, Flitsch J, Saeger W, Monoranu CM, Höfner K, et al. Impact of USP8 Gene Mutations on Protein Deregulation in Cushing Disease. *J Clin Endocrinol Metab* (2019) 104(7):2535–46. doi: 10.1210/jc.2018-02564
136. Wanichi IQ, de Paula Mariani BM, Frassetto FP, Siqueira SAC, de Castro Musolino NR, Cunha-Neto MBC, et al. Cushing's disease due to somatic USP8 mutations: a systematic review and meta-analysis. *Pituitary* (2019) 22 (4):435–42. doi: 10.1007/s11102-019-00973-9
137. Nurwidya F, Takahashi F, Takahashi K. Gefitinib in the treatment of nonsmall cell lung cancer with activating epidermal growth factor receptor mutation. *J Nat Sci Biol Med* (2016) 7(2):119–23. doi: 10.4103/0976-9668.184695
138. Fukuoka H, Cooper O, Mizutani J, Tong Y, Ren SG, Bannykh S, et al. HER2/ErbB2 receptor signaling in rat and human prolactinoma cells: strategy for targeted prolactinoma therapy. *Mol Endocrinol* (2011) 25(1):92–103. doi: 10.1210/me.2010-0353
139. Opdam FL, Guchelaar HJ, Beijnen JH, Schellens JH. Lapatinib for advanced or metastatic breast cancer. *Oncologist* (2012) 17(4):536–42. doi: 10.1634/theoncologist.2011-0461
140. Liu X, Kano M, Araki T, Cooper O, Fukuoka H, Tone Y, et al. ErbB receptor-driven prolactinomas respond to targeted lapatinib treatment in female transgenic mice. *Endocrinology* (2015) 156(1):71–9. doi: 10.1210/en.2014-1627
141. Tadesse S, Caldon EC, Tilley W, Wang S. Cyclin-Dependent Kinase 2 Inhibitors in Cancer Therapy: An Update. *J Med Chem* (2019) 62(9):4233–51. doi: 10.1021/acs.jmedchem.8b01469
142. Cicenas J, Kalyan K, Sorokinas A, Stankunas E, Levy J, Meskinyte I, et al. Roscovitine in cancer and other diseases. *Ann Transl Med* (2015) 3(10):135. doi: 10.3978/j.issn.2305-5839.2015.03.61
143. Liu NA, Jiang H, Ben-Shlomo A, Wawrowsky K, Fan XM, Lin S, et al. Targeting zebrafish and murine pituitary corticotroph tumors with a cyclin-dependent kinase (CDK) inhibitor. *Proc Natl Acad Sci USA* (2011) 108 (20):8414–9. doi: 10.1073/pnas.1018091108
144. Massaro F, Molica M, Breccia M. Current first- and second-line treatment options in acute promyelocytic leukemia. *Int J Hematol Oncol* (2016) 5 (3):105–18. doi: 10.2217/ijh-2016-0010
145. Labeur M, Paez-Pereda M, Arzt E, Stalla GK. Potential of retinoic acid derivatives for the treatment of corticotroph pituitary adenomas. *Rev Endocr Metab Disord* (2009) 10(2):103–9. doi: 10.1007/s11154-008-9080-6
146. Kang HJ, Song MR, Lee SK, Shin EC, Choi YH, Kim SJ, et al. Retinoic acid and its receptors repress the expression and transactivation functions of Nur77: a possible mechanism for the inhibition of apoptosis by retinoic acid. *Exp Cell Res* (2000) 256(2):545–54. doi: 10.1006/excr.2000.4832
147. Bondioni S, Angioni AR, Corbetta S, Locatelli M, Ferrero S, Ferrante E, et al. Effect of 9-cis retinoic acid on dopamine D2 receptor expression in pituitary adenoma cells. *Exp Biol Med (Maywood)* (2008) 233(4):439–46. doi: 10.3181/0704-RM-94
148. Páez-Pereda M, Kovalovsky D, Hopfner U, Theodoropoulou M, Pagotto U, Uhl E, et al. Retinoic acid prevents experimental Cushing syndrome. *J Clin Invest* (2001) 108(8):1123–31. doi: 10.1172/JCI11098
149. Castillo V, Giacomini D, Páez-Pereda M, Stalla J, Labeur M, Theodoropoulou M, et al. Retinoic acid as a novel medical therapy for Cushing's disease in dogs. *Endocrinology* (2006) 147(9):4438–44. doi: 10.1210/en.2006-0414
150. Occhi G, Regazzo D, Albiger NM, Ceccato F, Ferasin S, Scanarini M, et al. Activation of the dopamine receptor type-2 (DRD2) promoter by 9-cis retinoic acid in a cellular model of Cushing's disease mediates the inhibition of cell proliferation and ACTH secretion without a complete corticotroph-to-melanotroph transdifferentiation. *Endocrinology* (2014) 155(9):3538–49. doi: 10.1210/en.2013-1820

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Endocrinology

REVIEW
published: 16 June 2021
doi: 10.3389/fendo.2021.650791

Aggressive Cushing's Disease: Molecular Pathology and Its Therapeutic Approach

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Cushing's disease is a syndromic pathological condition caused by adrenocorticotropic hormone (ACTH)-secreting pituitary adenomas (ACTHomas) mediated by hypercortisolemia. It may have a severe clinical course, including infection, psychiatric disorders, hypercoagulability, and metabolic abnormalities, despite the generally small, nonaggressive nature of the tumors. Up to 20% of ACTHomas show aggressive behavior, which is related to poor surgical outcomes, postsurgical recurrence, serious clinical course, and high mortality. Although several gene variants have been identified in both germline and somatic changes in Cushing's disease, the pathophysiology of aggressive ACTHomas is poorly understood. In this review, we focused on the aggressiveness of ACTHomas, its pathology, the current status of medical therapy, and future prospects. Crooke's cell adenoma (CCA), Nelson syndrome, and corticotroph pituitary carcinoma are representative refractory pituitary tumors that secrete superphysiological ACTH. Although clinically asymptomatic, silent corticotroph adenoma is an aggressive ACTH-producing pituitary adenoma. In this review, we summarize the current understanding of the pathophysiology of aggressive ACTHomas, including these tumors, from a molecular point of view based on genetic, pathological, and experimental evidence. The treatment of aggressive ACTHomas is clinically challenging and usually resistant to standard treatment, including surgery, radiotherapy, and established medical therapy (e.g., pasireotide and cabergoline). Temozolomide is the most prescribed pharmaceutical treatment for these tumors. Reports have shown that several treatments for patients with refractory ACTHomas include chemotherapy, such as cyclohexyl-chloroethyl-nitrosourea combined with 5-fluorouracil, or targeted therapies against several molecules including vascular endothelial growth factor receptor, cytotoxic T lymphocyte antigen 4, programmed cell death protein 1 (PD-1), and ligand for PD-1. Genetic and experimental evidence indicates that some possible therapeutic candidates are expected, such as epidermal growth factor receptor tyrosine kinase inhibitor, cyclin-dependent kinase inhibitor, and BRAF inhibitor. The development of novel treatment options for aggressive ACTHomas is an emerging task.

Keywords: Cushing's disease (CD), aggressiveness/physiology, pathology, medical treatment/surgical treatment, targeted therapy

OPEN ACCESS

Edited by:
Adam Mamelak, Cedars Sinai Medical Center, United States

Reviewed by:
Ning-Ai Liu, Cedars Sinai Medical Center, United States
Mônica Gadelha, Federal University of Rio de Janeiro, Brazil

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Specialty section:
This article was submitted to Pituitary Endocrinology, a section of the journal Frontiers in Endocrinology

Received: *08 January 2021*
Accepted: *26 May 2021*
Published: *16 June 2021*

Citation:
Yamamoto M, Nakao T, Ogawa W and Fukuoka H (2021) Aggressive Cushing's Disease: Molecular Pathology and Its Therapeutic Approach. Front. Endocrinol. 12:650791. doi: 10.3389/fendo.2021.650791

INTRODUCTION

Cushing's disease is a hypercortisolemic state caused by adrenocorticotropic hormone (ACTH)-secreting pituitary adenomas (ACTHomas). Although most ACTHomas can be successfully resected using the transsphenoidal approach, up to 20% of ACTHomas exhibit aggressive behavior, which is defined on the basis of clinical behavior, with a generally invasive, high rate of recurrence, lack of response to optimal standard therapies, or atypical pathological findings including carcinoma like features (1). These result in poor surgical and hormonal outcomes. Crooke's cell adenomas (CCAs) are one of the well-known aggressive ACTHomas that exhibit characteristic pathological features. ACTHomas can be transformed into an aggressive nature after bilateral adrenalectomy, and such tumors are called Nelson's syndrome. In pituitary carcinomas, the most aggressive tumoral nature, corticotroph carcinomas followed by or along with PRL-secreting pituitary carcinomas are the most common features. Patients frequently have a clinically serious course due to corticotroph carcinomas, and their management is challenging. In contrast, silent corticotroph adenomas (SCAs) exhibit aggressive tumor behavior, whereas hypercortisolemia is not present. In this review, we summarize our current knowledge of the definition, pathophysiology, and treatment of refractory ACTHomas and provide directions for future research.

AGGRESSIVE ACTHOMAS

Crooke's Cell Adenomas

Crooke's changes are characterized by large perinuclear cytokeratin filament accumulation in normal corticotrophs due to long-term exposure to endogenous or exogenous glucocorticoid excess, including Cushing's syndrome. The pathological finding represents an eosinophilic perinuclear hyaline appearance on hematoxylin and eosin staining (2, 3). Crooke's changes were also discovered within corticotroph adenomas by Kovacs et al. in 1981, called Crooke's cell adenomas (CCAs) (4). The frequency of Crooke's changes in ACTHomas varies from 36% to 100% among several reports, and this change is significantly increased in cases with severe hypercortisolism, at least fourfold greater than the upper limit of the normal range of UFC (1, 3, 5). CCA is diagnosed when Crooke's cells account for more than 50% of the tumor cells (1). These tumors exhibit a high frequency of macroadenoma (77.2%) and more aggressive behavior with invasion to the cavernous sinuses (79.2%) than macro-ACTHomas without Crooke's change. They can also transform into metastatic pituitary carcinoma, which was previously shown in 7.5% of cases. Some CCAs (24.4%) are clinically silent (6, 7). The recurrence rate of CCAs after surgery is 66% due to the higher frequency of cavernous sinus invasion (8).

Nelson's Syndrome

The first case of Nelson's syndrome was reported in 1958, in a 33-year-old woman who underwent bilateral adrenalectomy due to refractory Cushing's disease. Three years later, skin hyperpigmentation and visual defects with elevated ACTH levels appeared. These symptoms improved after surgical removal of pituitary tumors, which pathologically exhibited ACTH production (9). Nelson's syndrome is observed in 8–38% of cases after bilateral adrenalectomy for Cushing's disease (10, 11).

Accumulating data suggest several risk factors for this syndrome, including a rapid elevation of plasma ACTH after bilateral adrenalectomy (12–14), insufficient steroid replacement therapy (10, 15), residual corticotroph tumor after transsphenoidal surgery (TSS) (16), younger age (17), and histopathological characteristics of corticotroph tumor specimens. Elevation of plasma ACTH levels of more than 100 pg/mL in the first year after bilateral adrenalectomy is associated with the development of Nelson's syndrome (18).

From a histological point of view, there is no difference between the tumors of Nelson's syndrome and those of Cushing's disease. Despite the low expression of Ki-67 (usually less than 3%), tumor behavior is aggressive and invasive. The most common clinical manifestation of Nelson's syndrome is dark skin hyperpigmentation, with markedly elevated plasma ACTH levels. Bitemporal hemianopia and progressive visual loss caused by aggressive tumors are clinically important issues to be addressed. Therefore, regular follow-up should be monitored using MRI.

Corticotroph Carcinoma

Pituitary carcinomas are currently defined as pituitary tumors with craniospinal dissemination or metastasis to other types of tissues (19). Pituitary carcinomas occupy only 0.1–0.2% of pituitary neoplasms derived from the anterior pituitary (19). Epidemiologically, there is no gender difference in the prevalence of pituitary carcinomas (20). Metastasis is commonly identified in the central nervous system, followed by the liver, bones, and lungs (20). The specific symptoms of pituitary carcinoma are absent and depend on the region of metastasis, such as hearing loss, ataxia, or motor impairment (21). While CT and MRI are most often utilized to identify the metastatic region, ^{18}F-FDG, ^{111}In-labeled octreotide, and ^{68}Ga-DOTANOC in scintigraphy are also useful according to recent case reports (21–23).

There are no pathological criteria to distinguish aggressive adenomas from carcinomas. The following morphological features are not useful in predicting malignant transformation of pituitary adenomas: hypercellularity, nuclear and cellular pleomorphism, increased mitotic activity, necrosis, and dural and/or bony invasion, which are generally associated with malignancy (24, 25).

In endocrinological manifestation, 85–90% of carcinomas express pituitary hormone, and 15–20% of them are clinically nonfunctioning. Prolactin or ACTH-secreting carcinomas are the most frequent, followed by growth hormones and other rare hormones (23, 26–28). In a literature review, corticotroph carcinomas were shown to be the most common (34.7%) among pituitary carcinomas, followed by prolactin-secreting (23.6%) and null cells (15.3%) (20). Corticotroph carcinoma is usually developed from a group that exhibits aggressive phenotypes, such as invasive, rapid growth, and prone to

recurrence. One case series of 31 patients with CCAs exhibited more than 80% with single or multiple recurrences, and two patients developed corticotroph carcinoma (6). Nelson's syndrome occurring after bilateral adrenalectomy has also potentially progressed to carcinomas (21, 29). Furthermore, malignant transformation of SCAs has been reported in rare case reports (30).

Regarding prognosis, one case series of 15 patients with pituitary carcinoma reported that 66% of the patients died within 1 year and 20% were alive at the last follow-up 9–18 months after diagnosis (28). A recent review showed that 34 of 62 patients (55%) died within approximately 10 months after the diagnosis of pituitary carcinoma (31–33).

Silent Corticotroph Adenomas (SCAs)

SCAs are defined as ACTH-expressing pituitary tumors that lack both the clinical symptoms of Cushing's syndrome and evidence of autonomous cortisol secretion, which is diagnosed with nonfunctioning pituitary adenomas (34–36). The clinical importance of differentiating SCAs from other nonfunctioning pituitary adenomas is due to their aggressive nature (37). The prevalence of SCAs ranges from 3% to 6% in all pituitary adenomas and less than 40% in corticotroph adenomas (36–39).

Patients with SCAs are younger and predominantly females and have a higher prevalence of giant adenomas and an association with cavernous sinus invasion than other nonfunctioning pituitary adenomas, such as silent gonadotroph adenomas (SGA) and null cell adenomas (40, 41). In imaging studies of nonfunctioning pituitary adenomas, cystic or hemorrhagic changes on MRI T2WI are observed in SCAs (42). Regardless of tumor size, multiple microcystic changes are more frequently observed within SCAs and are highly specific to SCAs. These multiple microcysts have been correlated with pseudopapillary features of SCA pathological findings (42). According to the WHO classification, SCAs are classified into two subtypes: type 1 (densely granulated) and type 2 (sparsely granulated) (24, 34, 41). Type 1 SCAs show strong ACTH immunoreactivity similar to typical ACTHomas, while type 2 SCAs exhibit weak and focal expression (43). The lack of galectin-3 expression in corticotroph adenomas can be pathologically diagnostic of SCAs rather than functioning corticotroph adenomas (44). In type 2 SCAs, the expression levels of fibroblast growth factor receptor-4, matrix metalloproteinase-1, and β1-integrin which associates with tumor aggressiveness, are higher than that in type 1 SCAs (45), suggesting different tumor pathologies, however it remains unclear whether these two subtypes indeed influence tumor behavior.

The underlying mechanism of the discrepancy between elevated ACTH levels and normocortisolemia remains unclear in these patients. Various hypotheses have been reported to date. First, SCAs are driven from an intermediate lobe, which, in turn, demonstrates a low ACTH secretory capacity (30, 46). However, this concept was not established in a subsequent study (47). As a second hypothesis, SCAs secrete predominantly unprocessed high-molecular-weight ACTH (also known as big ACTH), which causes competition with mature ACTH at the receptor binding level (48). Another mechanism was suggested to be an increased intracellular degradation of ACTH, which resulted in insufficient ACTH exocytosis from the cell membrane (49). As the most widely accepted concept, the expression levels of prohormone convertase (PC), which is a critical enzyme in POMC posttranslational processes, determine the characteristic difference between SCAs and Cushing's disease. POMC is cleaved by PC1/3 and PC2 into biologically active ACTH and alpha-MSH, respectively (50). Several reports have demonstrated that SCAs exhibit decreased protein expression levels of PC1/3 concomitant with PC1/3 mRNA downregulation compared to typical corticotroph adenomas (35, 51).

In a certain portion of corticotroph adenomas, bidirectional transformation of the tumor phenotype between SCA and Cushing's disease has been reported (52) in 3.9% of cases, with a transformation period ranging from 1 to 7 years (52). Interestingly, an altered expression level of PC1/3 has been observed with the lapse of time in identical pituitary adenomas. The clinical phenotype correlates with *PC1/3* mRNA or protein levels in corticotroph adenomas (43), suggesting that PC1/3 expression levels play an important role in determining the characteristics of these tumor phenotypes.

MOLECULAR PATHOLOGY

Typical ACTHomas and Their Aggressiveness

The most common genetic cause of ACTHomas is the somatic *ubiquitin-specific protease 8* (*USP8*) variant within the 14-3-3 binding motif, which accounts for approximately 20–60% of these tumors (53, 54). The underlying mechanisms of *USP8* variants are thought to be mediated by increasing the deubiquitylation activity of this enzyme, leading to epidermal growth factor receptor (EGFR) overexpression (53). EGFR overexpression in corticotrophs has been proven to be a pathogenesis of ACTHomas due to its enhanced proliferation and ACTH hypersecretion (55, 56). In *USP8* wild-type ACTHomas, the p.Met415 variant within the catalytic domain of *USP48* has been identified (57). However, these genetic variants are found in small tumors and are not associated with tumor aggressiveness in ACTHomas. The *BRAF* p.V600E variant, which is also found in other cancers, has been identified in ACTH-secreting macroadenomas (57). However, tumor behavior remains unclear due to its low frequency (58).

Next-generation sequencing of *USP8* wild-type ACTHomas has revealed *TP53* pathogenic variants that are associated with larger and invasive tumors including tumors from patients with Nelson's syndrome or pituitary carcinomas (58, 59). *TP53* variants with wild-type *USP8* are associated with chromosome instability, aneuploidy, and tumor aggressiveness (60). Another possible gene associated with tumor aggressiveness is *CABLES1*, a major glucocorticoid-dependent cell cycle regulator in corticotrophs (61). Recently, mutations in the *ATRX* gene, which is one of the driver mutations in neuroendocrine tumors and regulates chromatin remodeling and telomere maintenance,

have been shown to be associated with aggressive pituitary adenomas, especially in ACTHomas (62). *ATRX* gene mutations are associated with a lack of ATRX expression in tumor specimens. Loss of function somatic mutations have been found in the *CABLES1* gene in children or young adult patients with aggressive corticotroph adenomas (63).

As germline mutations, *MEN1, PRKAR1A, CDKN1B*, and *AIP* genes should be considered as a young age-onset genetic syndrome phenotype (64). In aggressive pediatric Cushing's syndrome, the *DICER1* gene has been reportedly identified to have a causal role in ACTH-producing pituitary blastoma caused by DICER1 syndrome (65–67) (**Table 1**). Since DICER1 is an enzyme required for the cleavage of a precursor into mature microRNA, noncoding RNA, including microRNA, are associated with the pathogenesis of pituitary ACTH-producing tumors and their aggressiveness.

Crooke's Cell Adenomas (CCAs)

The genetic causes of CCAs have not been elucidated. In histopathologic findings, the Ki-67 score has limitations in predicting tumor proliferation and aggressiveness (6, 19). Rather than the Ki-67 labeling index, miR-106b–25 and its host gene *MCM7*, a member of the minichromosome maintenance complex (MCM) family of proteins, have been shown to be novel markers that correlate with tumor recurrence and progression in invasive ACTH-producing pituitary adenomas, including CCAs (68, 69).

Nelson's Syndrome

The underlying mechanisms of pituitary tumorigenesis and autonomous ACTH secretion in Nelson's syndrome are not fully understood. Corticotropin-releasing hormone (CRH) hyperactivity induced by rapid cortisol reduction is thought to be one of the pathogenesis of its marked ACTH elevation and aggressive tumor enlargement. These tumors were originally derived from monoclonal cells (70, 71). Essential transcription factors (e.g., Ptx1, Tpit, NeuroD, Nur77) in corticotroph cells and *POMC* gene posttranscriptional processes are properly conserved, which results in a mature POMC product (72–74). Regarding the molecular function of corticotroph tumors, CRHR1 and AVPR1b receptors on the tumor exhibit good responsiveness to their ligands (72–75). Intriguingly, loss of heterozygosity of the glucocorticoid receptor (GR) gene has been reported in patients with Nelson's syndrome, while GR expression of the corticotroph tumor in Nelson's syndrome is conserved, similar to that in Cushing's disease (76, 77). In some tumors in Nelson's syndrome, TP53 loss of function has been identified after radiation therapy (78). The primary management mode (pituitary surgery and radiotherapy (RT) followed by adrenalectomy) of Cushing's disease before the diagnosis of Nelson's syndrome has been reported as the highest risk and a predictor of tumor progression (79).

TABLE 1 | Causative Genes in Cushing's disease and its association with aggressiveness.

Gene	SIG	Frequency	Aggressive
USP8	Both S>G	20-60% in CD	No
USP48	S	13.3% in CD	No
MEN1	Both G>S	1% in genetic syndrome	No
CDKN1B	G	Rare - 2.6% in pituitary adenomas	No
PRKAR1A	G	Very Rare	No
AIP	Both G>S	5% of FIPA	Possibly No
BRAF	S	Rare	Possibly Yes
CABLES1	Both	2.2% in CD	Yes
DICER1	G	Very rare	Yes
TP53	S	12.5% in CD	Yes
ATRX	S	28% in PC 13% in APT	Yes

S, somatic variants; G, germline variants; CD, Cushing's disease; PC, pituitary carcinomas; APT, aggressive pituitary tumors.

Pituitary Carcinomas

The pathogenesis of pituitary carcinomas is not fully understood due to its low frequency. However, *TP53* variants or *ATRX* variants have been shown in some corticotroph pituitary carcinomas (59, 62). Lynch syndrome, which is caused by *MSH2* gene mutation complicated with pituitary carcinoma, has been reported as a case report, showing an association between this tumor-prone syndrome and pituitary tumors (80). However, further investigations need to clarify the tumor transformation's underlying mechanism to the malignant behavior in pituitary adenomas.

Silent Corticotroph Adenomas

The transformation from functioning ACTHomas into SCAs is very rare (3.9%) (52). From an autopsy pathological study of the human pituitary gland, it has been suggested that SCA originates from pars intermedia POMC-positive cells, while ACTHomas originate from the anterior lobe (46). This hypothesis was confirmed by an animal study using tamoxifen-inducible $Pax7^{CreERp/WT}$ $Rb^{flox/flox}$ mice, which revealed that Rb loss in the Pax7-expressing pituitary intermediate lobe results in cell proliferation leading to tumorigenesis expressing POMC without circulating ACTH elevation (81). Regarding the genetic cause of SCAs, candidate genes have not yet been clarified, and the *USP8* mutation commonly found in functioning and silent corticotroph adenomas (30, 82). Immunohistochemical analysis of SCA has demonstrated that lower expression of some proteins implicated in tumor progression and metastasis, such as galectin-3, a beta-galactoside-binding protein, and KLK10, belonging to the kallikrein family, in SCA than functioning ACTHomas may be one mechanism of its aggressiveness (30). Lower expression of CDKN2A with upregulated cyclin D1 in SCA than functioning ACTHomas has been shown to be another reason for its aggressive behavior (83). Recently, gene and protein expression comparison analysis between SCAs and functioning ACTHomas has been performed using both RNA-seq and mass spectrometry-based proteomics technology, revealing the downregulation of the gene related to protein processing in the endoplasmic reticulum (ER) pathway and upregulation of *PCSK1N*, an inhibitor of PC1/3 coding *PCSK1* gene. These results suggest a reason for the lack of active ACTH secretion from these tumors. Moreover, the extracellular matrix (ECM) protein cluster is downregulated in SCAs compared to functioning ACTHomas, suggesting that this is associated with their invasive behavior (84).

TREATMENT

Surgery is the first-line treatment to control tumor volume for refractory corticotroph tumors, even though the postoperative recurrence rate remains high (8, 85). Radiosurgery is an important option for treating postoperative residual tumor or recurrence and progression of tumors. Stereotactic radiosurgery is especially superior to radiosurgery in terms of a lower incidence of adverse events and earlier remission (86, 87). In aggressive ACTHomas, medical therapy is required, followed by surgery and radiosurgery in most cases. Although hypercortisolemia can be controlled by adrenal or GR-targeted drugs, the effect of targeted therapy on ACTHoma remains a challenge. Medical treatment can be initiated immediately after the diagnosis until hypercortisolemia is collected, including the perioperative period.

Surgery

Surgical treatment remains the first-line treatment choice even for aggressive ACTHomas by a skilled neurosurgeon with extensive experience in pituitary surgery (88). Endoscopic or microscopic TSS can be performed according to the neurosurgeon's preference (89). Preoperative medical treatment to improve hypercortisolemia is recommended, mainly using steroidogenesis inhibitors, including metyrapone, ketoconazole, and osilodrostat with or without hydrocortisone replacement (90–92). Since a higher rate of morbidity, including poorly controlled diabetes mellitus, hypokalemia, venous thromboembolism, gastrointestinal hemorrhage, and osteoporosis, has been complicated in patients with refractory Cushing's disease, several pharmaceutical treatments such as insulin, mineral corticoid antagonists, anticoagulants, proton pump inhibitors, and anti-osteoporotic agents are required during the perioperative period (91, 93). In a recent systematic review, the complete surgical remission and recurrence rates of macro-tumors in primary surgery were 68% (95% confidence interval [CI]; 60–76) and 30% (95% CI; 18–43), and those in revision surgery are 49% (95% CI; 23–75) and 45% (95% CI; 0–98), respectively (94). If the tumor extends into the suprasellar region, a transcranial approach may be needed (88). In a literature review of initial surgery for ACTHomas, a tumor with Knosp grades 3–4 have been identified in 12–20% of all tumors, and 12 of 36 patients (33.3%) have achieved the remission criteria (95), indicating that further treatment option is emergently required for these tumors. In Nelson's syndrome, pituitary surgery is the first-line treatment; however, the complete remission rate depends on whether the pituitary tumor extends to the extrasellar region, similar to an ordinal pituitary tumor (96).

Radiotherapy (RT)

Stereotactic RT (SRT), including the Gamma Knife™ (GK), Cyberknife™, and proton-beam RT, has become the mainstream rather than the conventional fractionated RT (CRT) and could be a second treatment option for aggressive Cushing's disease if residual or recurrent tumors are visible on MRI despite TSS (97). Because of the recent development of drug therapy, the choice of second-line therapy needs to be individualized according to tumor progression speed by MRI, pathological findings, and patient background. In a systematic review from 2000 to 2017, the tumor was controlled in 95% of cases (83.3–100%) with a median follow-up of 56 months (2–17 years). Hormonal control has achieved 54–68% in SRT with a follow-up of 5–10 years, while the definition of biochemical remission is not unified. However, the recurrence rate of RT is 20–32% with a median time of 25.5–37 months (range 6–60) after an initial remission. Adverse radiation effects for patients with Cushing's disease including hypopituitarism [12.3–52% (median 22.6%)], visual toxicity (0–39%), and cranial nerve neuropathy (0–5.5%) have reported, while secondary brain tumors have not occurred yet. The median time to hormonal normalization is 12–25 months (98–102). In aggressive Cushing tumors, the mean time of hormonal control may have taken a longer period than that in those with nonaggressive ones (33.0 ± 5.0 *vs.* 23.5 ± 6.3 months) (101). In patients with CCAs, SRT has shown to be as effective as ACTHomas without Crooke's hyaline changes (87).

Medical Therapy

There are three therapeutic targets for drug therapy in patients with Cushing's disease: pituitary directed therapies, adrenal directed therapies, and cortisol-target tissues. In aggressive Cushing's disease, which is usually accompanied by remarkable hypercortisolemia, adrenal gland-targeted steroidogenesis inhibitors, including ketoconazole, metyrapone, and etomidate, can be the first choice for acute phase intervention or preoperative treatment. Further medical treatment might be required in the chronic phase, or if there are residual tumors that oversecrete ACTH after the operation of the tumors. Pituitary gland-targeted drugs, such as second-generation somatostatin receptor ligands (SRLs), pasireotide, cabergoline is a dopamine receptor agonist, could be the next treatment choice with or without steroidogenesis inhibitors.

Our manuscript mainly focused on pituitary gland-targeted therapies including currently approved and further developing drugs (**Figure 1**), besides described adrenal gland-targeted drugs and peripheral GR blockers.

Somatostatin Receptor Ligands

First-generation SRLs, octreotide and/or lanreotide, which mainly target SSTR2, are not effective in most ACTHomas because of their low expression of membrane SSTR2. Pasireotide, a second-generation SRL that targets SSTR1, 2, 3, and 5 with the highest affinity for SSTR5, has been approved as a promising drug for the treatment of ACTHomas (103, 104). In a recent meta-analysis, pasireotide was shown to be effective in normalizing cortisol in 41.1% (95% CI, 32.7–49.8) of patients (105).

In ACTH-secreting macroadenomas, ACTH reduction and tumor shrinkage have been reported by initial treatment of pasireotide (106). Furthermore, the effectiveness of rapid ACTH and cortisol suppression as preoperative treatment has been shown in several ACTH-secreting macroadenoma cases (107). Conversely, escape from ACTH reduction, or even a

paradoxical rise of ACTH has been reported (108), indicating further investigation to clarify the effect of this drug on aggressive ACTHomas.

The effect of pasireotide on CCAs is still under debate and requires further studies to clarify the efficacy of such challenging aggressive tumors. In Nelson's syndrome, a sufficient effect on ACTH reduction and tumor shrinkage has been reported in a case report of pasireotide (109). A multicenter trial of pasireotide treatment for Nelson's syndrome has been reported (110). In this trial, patients were treated with subcutaneous (s.c.) pasireotide twice daily for 1 month (n=8), followed by treatment with monthly pasireotide LAR for 6 months (n=5). ACTH reduction showed a complete response (CR) in five out of eight patients and partial response (PR) in two out of eight patients by s.c. pasireotide and further exhibited a CR in three out of five patients and PR in one out of five patients treated with pasireotide LAR. However, tumor shrinkage was not observed with hyperglycemia in six patients.

SCAs also exhibit a higher expression of SSTR2 and SSTR5 compared to null cell adenomas and SGAs by immunohistochemical analysis (40, 111). SSTR3 is also abundantly expressed in SCAs (40). Although the efficacy of somatostatin analogs for SCAs has not yet been established, pasireotide LAR (PASSILCORT; ClinicalTrial.gov identifier, NCT02749227) is under a phase II randomized clinical trial for residual or recurrent SCAs.

Dopamine Receptor Agonist

Since dopamine 2 receptor (D2R) is frequently expressed on ACTHomas, the dopamine receptor agonist cabergoline has shown to be an effective drug in approximately 20–30% of patients with Cushing's disease (112–114). In a recent multicenter study, the efficacy of cabergoline in hormone reduction did not differ between microadenomas and macroadenomas (112). The effect of cabergoline on CCAs remains unclear (8). Although some case reports show that Nelson's syndrome has been successfully treated with cabergoline (115, 116), the efficacy of cabergoline for such aggressive tumors is limited (79). In SCAs, a case report revealed that cabergoline has been shown to induce tumor shrinkage in a patient with SCA, in which D2R expression has been proved by *in situ* hybridization (117). However, SCAs have been reported to exhibit lower D2R mRNA levels than ACTH-negative nonfunctioning adenomas (118).

Temozolomide

Temozolomide is the drug with the most developed evidence for the treatment of aggressive pituitary adenomas and pituitary carcinomas. Although insurance is not covered in most countries, temozolomide is a promising therapeutic choice for refractory hormone-secreting and non-secreting pituitary tumors, including Cushing's disease.

This drug was initially used in the treatment of glioblastoma multiforme (GBM) because of its significant clinical benefits. It is an alkylating agent that methylates specific guanine residue, leading to DNA damages by triggering tumor apoptosis. However, the existence of O6-methylguanine-DNA methyltransferase (MGMT), a DNA repair enzyme that can remove the methyl from the O6-methylguanine, works in contrast with temozolomide. Therefore, high MGMT expression levels in GBM tumors are related to drug resistance (119).

The first two cases of pituitary carcinomas were reported in 2006, who were successfully treated with temozolomide after initial therapy including surgery, dopamine agonists, somatostatin analogs, radiation, and chemotherapy (120). Following this report, more than 150 cases with pituitary carcinomas or aggressive pituitary adenomas have been treated with temozolomide, demonstrating a 69% (33–86%) response rate, which is defined as either a complete remission (CR), partial

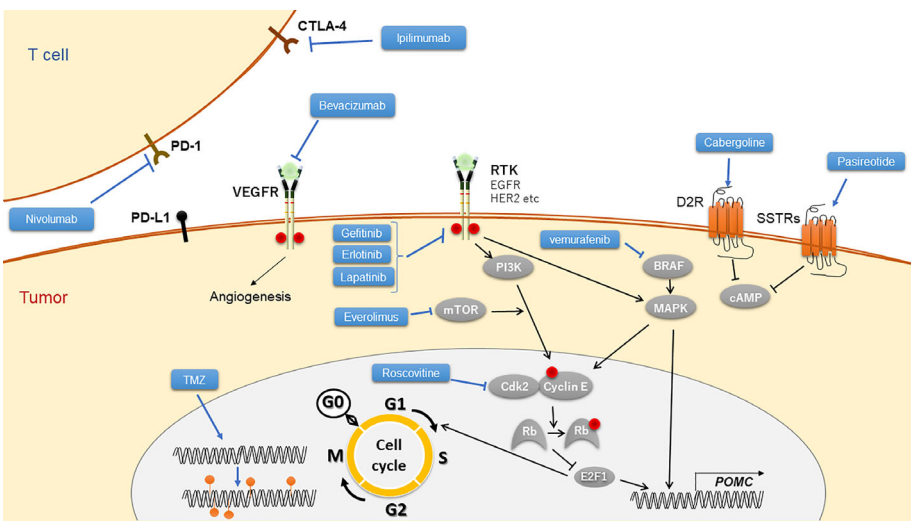

FIGURE 1 | Targeted drugs to Cushing's disease and their mechanistic scheme. TMZ, temozolomide; RTK, receptor of tyrosine kinase.

response (PR), or stable disease (SD). Additionally, 42% (29–69%) of significant tumor volume reduction has been shown to be either a CR or PR (88).

The reduction in tumor size occurred within 1–6 months after initiation of temozolomide therapy. Noting a report from a European cohort, after 3 (6) cycles of temozolomide, 23% (59%) achieved maximal radiological response, indicating that approximately 40% of patients experienced maximal radiological tumor response after 6 months (88, 121). Moreover, a German survey also reported that 52% of corticotroph tumors showed regression, 21% stability, and 26% progression at the end of temozolomide treatment. After a median follow-up of the 32-month radiological evaluation, disease stabilization in 37% and progression in 63% of patients were observed (121). These results are consistent with previous findings in Italy and France (122).

Although randomized prospective trials, or head-to-head studies of temozolomide compared to placebo or other treatment options, have not been performed yet, temozolomide can be a potential recommended therapy of choice for aggressive pituitary adenomas and pituitary carcinomas.

In aggressive pituitary tumors, Cushing's disease is the most common type, with 45% of adenomas and 47% of carcinomas (88). Generally, functioning tumors, especially prolactinomas and corticotroph adenomas, have been reported to have a better response to temozolomide than nonfunctioning tumors. The response rate of temozolomide in corticotroph tumors is estimated to be 56%, compared to 44% in prolactinomas, 38% in somatotroph tumors, and only 22% in nonfunctioning tumors (89), indicating that aggressive corticotroph adenoma and corticotroph carcinomas are good candidates for temozolomide treatment. In fact, five cases of CCAs, which showed lower MGMT expression than noninvasive ACTHomas, treated with temozolomide, were shown to have a partial or complete reduction of tumor size in all cases. Plasma ACTH levels in these cases have also been suppressed, except for one case in which laboratory data were not documented (123). In addition, a German survey reported that ACTH decreased from 42 (9–794) pmol/L at baseline to a minimum of 23 (10–276) pmol/L after a median of 6 (3–10) months on temozolomide and then increased to 182 (12–671) pmol/L at the end of temozolomide treatment (122).

In children, temozolomide treatment for aggressive pituitary adenoma and carcinoma is quite rare, which leads to insufficient treatment data. In limited cases of *DICER1* mutations with ACTH-secreting pituitary tumors, the effect of temozolomide has not been clearly shown (65, 124). Although there is no established course of treatment because of the paucity of data, the ESE guidelines suggest that temozolomide might be beneficial in adults (88). The common adverse events observed were similar to those observed in adults, including diarrhea, constipation, nausea, thrombocytopenia, headaches, syncope, and somnolence (36, 125–127).

In SCAs, temozolomide is considered a possible chemotherapeutic agent because of its low expression of MGMT (36, 126, 127). Several case reports of SCAs treated with temozolomide have been reported. Ceccato et al. reported two SCA patients treated with temozolomide: one associated with pasireotide treatment showed stable disease (SD) with 6% volume reduction and the other showed partial response (PR) with 49% volume reduction (128). A systematic review and meta-analysis reported that the recurrence rate of SCAs with a mean follow-up of <5 years or >5 years was 25% and 31%, respectively, and there was no significant difference in the recurrence rates between SCAs and other nonfunctioning pituitary adenomas (129). However, it should be noted that there are many unresolved points because of the rarity of SCAs.

When pituitary tumors show metastatic spread or are refractory to multiple treatments, temozolomide could be the last resort and salvage therapy. However, some recent studies have suggested that early use of temozolomide in these patients could result in a better outcome (88, 121). In this regard, high-grade tumors on MRI, such as invasiveness and increasing tumor size, and pathological findings, including high MIB1-labeling index, could be a sign of temozolomide initiation after surgery under RT (130, 131). However, patients administered with temozolomide in the early stage are relatively rare. Further clinical investigations are needed to determine whether early administration of temozolomide in patients with aggressive or metastatic pituitary tumors is associated with better outcomes.

Tumors resistant to TMZ chemotherapy have been shown in a certain number of refractory pituitary tumors (130). Thus, a predictive marker for resistance to temozolomide needs to be identified. As shown in GBM, low MGMT expression, a beneficial predictor of the response to temozolomide in glioblastoma (119), has been mostly associated with a positive response to temozolomide in pituitary tumors. However, some discrepancies, such as a high MGMT with a lack of response and no response despite low MGMT expression, have also been reported in pituitary tumors (88). Furthermore, no statistical association between MGMT expression levels and resistance to temozolomide has been shown (121, 122), indicating the limitation of MGMT as a predictive marker in these tumors. In addition to MGMT, several DNA mismatch repair (MMR) pathway proteins have been proposed, including MLH1, MSH2, MSH6, and PMS2, which recognize adducts including O6-methylguanin and remove them, leading to cell death (132). Therefore, the expression levels of MMR proteins may be critical to the cytotoxic effects of temozolomide (88). In fact, MSH6 immunopositivity has been associated with responsiveness to temozolomide in malignant pituitary neoplasms (133). Further analysis of the relationship between MMR pathway protein expression levels and temozolomide responsiveness is required (132). Overall, the expression of DNA repair proteins, including MGMT, may be associated with resistance to temozolomide treatment but is still controversial.

Chemotherapy

There are no established chemotherapy protocols for pituitary carcinomas. Mono- or combination therapy using chemotherapy, including capecitabine, carboplatin, etoposide, cisplatin, doxorubicin, 5-fluorouracil, tamoxifen, cyclophosphamide, lomustine, procarbazine, vincristine,

oxaliplatin, dacarbazine, methotrexate, bleomycin, and cyclohexyl-chloroethyl-nitrosourea, has been used for several pituitary carcinoma cases, with some PR (20, 134–136).

Immune Therapy

As a successful immunotherapeutic strategy, immune checkpoint inhibitors (ICIs) for several cancers, has recently been developed. These targets include cytotoxic T lymphocyte antigen 4 (CTLA-4) and programmed cell death protein 1 (PD-1), located in T cells, and ligand for PD-1 (PD-L1), located in tumor cells. Ipilimumab, the first developed ICIs targeting CTLA-4; nivolumab, pembrolizumab, and cemiplimab targeting PD-1; and atezolizumab, avelumab, and durvalumab targeting PD-L1, have been approved and applied for the treatment of several cancers. For pituitary tumors, the first case treated with ICIs has been reported to have ACTH-secreting pituitary carcinoma exhibiting liver metastasis (137). In this patient, initial treatment, including TSSs, fractionated RT and pasireotide, and cabergoline, was performed followed by TMZ and a combination of TMZ and capecitabine. Since the tumor volume and hormonal hypersecretion were not controlled despite these treatments, the combination of ipilimumab (3 mg/kg every 3 weeks) and nivolumab (1 mg/kg every 3 weeks) was initiated, leading to regression of both sellar tumors and metastatic liver tumors (59% and 92%, respectively) with a 90% reduction in ACTH levels. In this report, genetic analysis of primary and metastatic tumors revealed several pathogenic somatic gene hypermutations possibly induced by medical therapy such as TMZ, which can be neoantigens for the targets of ICIs. Following this report, successful treatment of a second corticotroph carcinoma case derived from Nelson's syndrome, with a combination of ipilimumab (3 mg/kg) and pembrolizumab (1 mg/kg) every 3 weeks, leading to stable disease, has been reported (138). Immunotherapy could be the next possible therapeutic candidate for aggressive ACTHomas.

Possible Targeted Therapy

Drug repositioning from several targeted therapies for cancers, including neuroendocrine tumors to aggressive pituitary tumors, has been investigated using receptors for tyrosine kinases, including EGFR, human epidermal growth factor receptor 2 (HER2), vascular endothelial growth factor receptor (VEGFR), intracellular signal transduction pathway proteins such as the mammalian target of rapamycin (mTOR), BRAF, and nuclear proteins such as cyclin-dependent kinase (CDK) (136, 139).

In ACTHomas, EGFR has been shown to be a tumorigenic factor, especially in *USP8* variants (53, 56, 140). Although ACTHomas with *USP8* mutations have been shown to be small and nonaggressive, EGFR overexpression in ACTHomas has been reported to be associated with aggressive ACTHomas *via* the activated MAPK pathway (141). Since EGFR can induce experimental corticotroph tumor proliferation both *in vitro* and *in vivo*, its tyrosine kinase inhibitor (TKI) gefitinib has been shown to reduce serum corticosterone levels with shrinking pituitary tumors of corticotroph-specific EGFR overexpressing mice, an animal model of Cushing's disease (55, 56). EGFR TKI erlotinib and dual EGFR and HER2 TKI lapatinib have been used to treat aggressive pituitary tumors as a third-line treatment, revealing poor outcomes (142). Recently, lapatinib treatment for aggressive PRLomas has been reported to have a partial effect on tumor shrinkage and hormonal reduction (143). Further investigation of EGFR targets is required for aggressive corticotroph tumors.

VEGF inhibitors, including TKIs for VEGFR2, and monoclonal neutralizing antibodies against VEGF-A have been used for several vascular-rich cancers, including neuroendocrine neoplasms. The rationale of these drugs is to suppress angiogenesis, leading to the suppression of tumor growth and induction of shrinkage. Bevacizumab, a humanized monoclonal antibody for VEGF-A, has been used to treat SCA, leading to stable disease for at least 26 months (144). Eight years of PFS with RT, TMZ, and bevacizumab has been reported (145). Five more cases of ACTHoma treated with bevacizumab have been reported, showing some effectiveness (146) since VEGF can also modulate the tumor microenvironment, their inhibition can act as an antitumor immunity (147).

In pituitary adenomas, mTOR expression has been shown to be higher than those in a normal pituitary gland and is elevated in invasive tumors (148, 149), suggesting a potential therapeutic target of mTOR for aggressive ACTHomas. Everolimus, an mTOR inhibitor, has been approved for several cancer treatments, including neuroendocrine neoplasms (150). Everolimus has been reported as an effective therapy for *STK11* mutated refractory ACTHoma with clinical improvement and stable disease for at least 6 months (151). In contrast, one case of corticotroph carcinoma was treated with everolimus with octreotide, exhibiting resistance in both tumoral growth and hormone secretion. According to the microarray investigation, *regulatory associated protein of mTOR* (*RAPTOR*) mRNA expression was low, suggesting the cause of everolimus resistance in corticotroph carcinoma (152).

In ACTHomas with *BRAF*, V600E mutation, a rare variant, has been shown to be a good candidate for the treatment with the BRAF inhibitor vemurafenib (57).

From the Cushing's disease model of corticotroph-specific pituitary tumor transforming gene (PTTG) transgenic zebrafish, drug screening has been performed, identifying that the CDK2/cyclin E inhibitor, R-roscovitine, could be a potential drug for human ACTHomas (153, 154).

Adrenal Gland-Targeted Drugs

Steroidogenesis Inhibitors

Adrenal steroidogenesis inhibitors block cortisol synthesis by inhibiting various enzymes in steroidogenesis pathway while they have no evidence in corticotroph tumor shrinkage.

Ketoconazole is known as an anti-fungal biotics, which can inhibit cholesterol side-chain cleavage enzyme such as 17α-hydroxylase and 17, 20-lyase and 11β-hydroxylase (155). Ketoconazole which has numerous evidence in treating hypercortisolemia due to Cushing's syndrome, exhibited high remission rate from 45 to 93% (91). Liver enzyme elevation is one of the most common side effects, which was observed in 13.5% of patients. As other adverse events, gastrointestinal disturbances and male hypogonadism should be considered (91).

Mitotane is currently approved for treating adrenocortical carcinoma, also widely used for Cushing's syndrome, by inhibiting not only steroidogenesis but also inducing cell death of adrenocortical cells. According to a recent meta-analysis, mitotane exhibited high remission rate in treating Cushing's syndrome (105), While dyslipidemia, gastrointestinal disturbances and neurological disorders are frequently observed adverse events, mitotane-induced adrenal insufficiency requires special caution, which demand more glucocorticoid dose than physiological setting.

Metyrapone is widely used as a steroidogenesis inhibitor for Cushing's syndrome even though it has been still in off-label use in US. Metyrapone inhibits 11β-hydroxylase and converting from 11-deoxycortisol to cortisol, results in reducing cortisol level. Metyrapone showed a revised estimated average remission rate of 75.9% (105) The frequently reported adverse events were hirsutism in women, dizziness, arthralgias, gastrointestinal disturbances, adrenal insufficiency, hypokalemia and peripheral edema (156).

Novel Steroidogenesis Inhibitors

Levoketoconazole

Levoketoconazole which is an enantiomer of ketoconazole, was developed for achievement of better efficacy and safety. Levoketoconazole inhibits 21-hydroxylase, 17 alpha-hydroxylase, and 11 beta-hydroxylase steroidogenesis enzymes, resulted in exhibiting higher potency than ketoconazole (157). In phase 3 clinical trial, treated 81% patients with levoketoconazole achieved normalization of UFC level. While most of adverse events such as nausea and headache were acceptable, 13% of patients was obliged to discontinue the drug due to serious adverse events such as abnormal liver functio, prolonged QT interval, and adrenal insufficiency (158).

Osilodrostat

Osilodrostat is novel 11 beta-hydroxylase inhibitor that blocks the conversion from deoxycortisol to cortisol, which has similar action mechanism with metyrapone. Osilodrostat exhibited 3-fold higher affinity to 11 beta-hydroxylase and longer half-life than metyrapone. In please II clinical trial, osilodrostat treatment reduced UFC in 78.9% of patients at week 22 (159). Adverse events were very similar with those of other steroidogenesis inhibitors, including nausea, diarrhea, asthenia, adrenal insufficiency, and hirsutism in female (160).

Glucocorticoid Receptor-Directed Drugs

Mifepristone is officially approved non-selective GR antagonist for treating Cushing's syndrome. The data from a multicenter, open-label, prospective clinical trial showed the improvement of clinical features associated with hypercortisolemia, psychiatric symptoms and glucose intolerance (161). On the other hand, specific inhibition of GR action causes hyperaldosteronism-like phenotype due to cortisol binding to MR, such as hypertension and hypokalemia. Apart from that, various adverse events also have been reported as follows: nausea, fatigue, and endometrial thickening in women (162).

CONCLUSIONS

In this review article, we introduced several aggressive types of ACTHomas, including CCAs, Nelson's syndrome, and SCAs. The pathogenesis and treatment of these tumors have been introduced. Although numbers of genetic variants and mutations are implicated in ACTHomas, their mechanistic link to the aggressiveness and, more importantly, to therapeutical targeting are yet to be established. Future targeted drugs and immunotherapy are shown with their potential evidence. Further analysis and investigation are urgently required for this clinically serious disease.

AUTHOR CONTRIBUTIONS

MY wrote the sections "Aggressive ACT Homas" and "Molecular Pathology". TN wrote the section "Temozolomide". The rest was written by HF. HF and WO planned and edited this review. All authors contributed to the article and approved the submitted version.

FUNDING

Grant-in-Aid for Scientific Research from the Japanese Ministry of Education, Culture, Sports, Science and Technology 15K09432, 19K09003.

REFERENCES

1. Di Ieva A, Rotondo F, Syro LV, Cusimano MD, Kovacs K. Aggressive Pituitary Adenomas–Diagnosis and Emerging Treatments. *Nat Rev Endocrinol* (2014) 10(7):423–35. doi: 10.1038/nrendo.2014.64
2. Crooke AC. Change in the Basophil Cells of the Pituitary Gland Common to Conditions Which Exhibit the Syndrome Attributed to Basophil Adenoma. *J Pathol Bacteriol* (1935) 41(2):339–49. doi: 10.1002/path.1700410215
3. Oldfield EH, Vance ML, Louis RG, Pledger CL, Jane JAJr., Lopes MB. Crooke's Changes in Cushing's Syndrome Depends on Degree of Hypercortisolism and Individual Susceptibility. *J Clin Endocrinol Metab* (2015) 100(8):3165–71. doi: 10.1210/JC.2015-2493
4. Felix IA, Horvath E, Kovacs K. Massive Crooke's Hyalinization in Corticotroph Cell Adenomas of the Human Pituitary. A Histological, Immunocytological, and Electron Microscopic Study of Three Cases. *Acta Neurochir (Wien)* (1981) 58(3-4):235–43. doi: 10.1007/BF01407130
5. Saeger W, Ludecke DK, Buchfelder M, Fahlbusch R, Quabbe HJ, Petersenn S. Pathohistological Classification of Pituitary Tumors: 10 Years of Experience With the German Pituitary Tumor Registry. *Eur J Endocrinol* (2007) 156(2):203–16. doi: 10.1530/eje.1.02326
6. George DH, Scheithauer BW, Kovacs K, Horvath E, Young WFJr., Lloyd RV, et al. Crooke's Cell Adenoma of the Pituitary: An Aggressive Variant of Corticotroph Adenoma. *Am J Surg Pathol* (2003) 27(10):1330–6. doi: 10.1097/00000478-200310000-00005
7. Holthouse DJ, Robbins PD, Kahler R, Knuckey N, Pullan P. Corticotroph Pituitary Carcinoma: Case Report and Literature Review. *Endocr Pathol* (2001) 12(3):329–41. doi: 10.1385/EP:12:3:329

8. Di Ieva A, Davidson JM, Syro LV, Rotondo F, Montoya JF, Horvath E, et al. Crooke's Cell Tumors of the Pituitary. *Neurosurgery* (2015) 76(5):616–22. doi: 10.1227/NEU.0000000000000657
9. Nelson DH, Meakin JW, Dealy JB Jr, Matson DD, Emerson KJr., Thorn GW. ACTH-Producing Tumor of the Pituitary Gland. *N Engl J Med* (1958) 259 (4):161–4. doi: 10.1056/NEJM195807242590403
10. Nagesser SK, van Seters AP, Kievit J, Hermans J, Krans HM, van de Velde CJ. Long-Term Results of Total Adrenalectomy for Cushing's Disease. *World J Surg* (2000) 24(1):108–13. doi: 10.1007/s002689910020
11. Kemink SA, Grotenhuis JA, De Vries J, Pieters GF, Hermus AR, Smals AG. Management of Nelson's Syndrome: Observations in Fifteen Patients. *Clin Endocrinol (Oxf)* (2001) 54(1):45–52. doi: 10.1046/j.1365-2265.2001.01187.x
12. Barnett AH, Livesey JH, Friday K, Donald RA, Espiner EA. Comparison of Preoperative and Postoperative ACTH Concentrations After Bilateral Adrenalectomy in Cushing's Disease. *Clin Endocrinol (Oxf)* (1983) 18 (3):301–5. doi: 10.1111/j.1365-2265.1983.tb03215.x
13. Pereira MA, Halpern A, Salgado LR, Mendonca BB, Nery M, Liberman B, et al. A Study of Patients With Nelson's Syndrome. *Clin Endocrinol (Oxf)* (1998) 49(4):533–9. doi: 10.1046/j.1365-2265.1998.00578.x
14. McCance DR, Russell CF, Kennedy TL, Hadden DR, Kennedy L, Atkinson AB. Bilateral Adrenalectomy: Low Mortality and Morbidity in Cushing's Disease. *Clin Endocrinol (Oxf)* (1993) 39(3):315–21. doi: 10.1111/j.1365-2265.1993.tb02371.x
15. Banasiak MJ, Malek AR. Nelson Syndrome: Comprehensive Review of Pathophysiology, Diagnosis, and Management. *Neurosurg Focus* (2007) 23 (3):E13. doi: 10.3171/foc.2007.23.3.15
16. Gil-Cardenas A, Herrera MF, Diaz-Polanco A, Rios JM, Pantoja JP. Nelson's Syndrome After Bilateral Adrenalectomy for Cushing's Disease. *Surgery* (2007) 141(2):147–51; discussion 51-2. doi: 10.1016/j.surg.2006.12.003
17. Leinung MC, Zimmerman D. Cushing's Disease in Children. *Endocrinol Metab Clin North Am* (1994) 23(3):629–39. doi: 10.1016/S0889-8529(18) 30089-6
18. Assie G, Bahurel H, Coste J, Silvera S, Kujas M, Dugue MA, et al. Corticotroph Tumor Progression After Adrenalectomy in Cushing's Disease: A Reappraisal of Nelson's Syndrome. *J Clin Endocrinol Metab* (2007) 92(1):172–9. doi: 10.1210/jc.2006-1328
19. Mete O, Lopes MB. Overview of the 2017 WHO Classification of Pituitary Tumors. *Endocr Pathol* (2017) 28(3):228–43. doi: 10.1007/s12022-017-9498-z
20. Yoo F, Kuan EC, Heaney AP, Bergsneider M, Wang MB. Corticotrophic Pituitary Carcinoma With Cervical Metastases: Case Series and Literature Review. *Pituitary* (2018) 21(3):290–301. doi: 10.1007/s11102-018-0872-8
21. Kaltsas GA, Nomikos P, Kontogeorgos G, Buchfelder M, Grossman AB. Clinical Review: Diagnosis and Management of Pituitary Carcinomas. *J Clin Endocrinol Metab* (2005) 90(5):3089–99. doi: 10.1210/jc.2004-2231
22. Veit JA, Boehm B, Luster M, Scheuerle A, Rotter N, Rettinger G, et al. Detection of Paranasal Ectopic Adrenocorticotropic Hormone-Secreting Pituitary Adenoma by Ga-68-DOTANOC Positron-Emission Tomography-Computed Tomography. *Laryngoscope* (2013) 123(5):1132–5. doi: 10.1002/lary.23867
23. Lopes MB, Scheithauer BW, Schiff D. Pituitary Carcinoma: Diagnosis and Treatment. *Endocrine* (2005) 28(1):115–21. doi: 10.1385/ENDO:28:1:115
24. Mete O, Asa SL. Clinicopathological Correlations in Pituitary Adenomas. *Brain Pathol* (2012) 22(4):443–53. doi: 10.1111/j.1750-3639.2012.00599.x
25. Mete O, Ezzat S, Asa SL. Biomarkers of Aggressive Pituitary Adenomas. *J Mol Endocrinol* (2012) 49(2):R69–78. doi: 10.1530/JME-12-0113
26. Colao A, Ochoa AS, Auriemma RS, Faggiano A, Pivonello R, Lombardi G. Pituitary Carcinomas. *Front Horm Res* (2010) 38:94–108. doi: 10.1159/000318499
27. Ragel BT, Couldwell WT. Pituitary Carcinoma: A Review of the Literature. *Neurosurg Focus* (2004) 16(4):E7. doi: 10.3171/foc.2004.16.4.8
28. Pernicone PJ, Scheithauer BW, Sebo TJ, Kovacs KT, Horvath E, Young WFJr., et al. Pituitary Carcinoma: A Clinicopathologic Study of 15 Cases. *Cancer* (1997) 79(4):804–12. doi: 10.1002/(SICI)1097-0142(19970215)79:4<804::AID-CNCR18>3.0.CO;2-3
29. Heaney A. Management of Aggressive Pituitary Adenomas and Pituitary Carcinomas. *J Neurooncol* (2014) 117(3):459–68. doi: 10.1007/s11060-014-1413-6
30. Ben-Shlomo A, Cooper O. Silent Corticotroph Adenomas. *Pituitary* (2018) 21(2):183–93. doi: 10.1007/s11102-018-0864-8
31. Arnold PM, Ratnasingam D, O'Neil MF, Johnson PL. Pituitary Carcinoma Recurrent to the Lumbar Intradural Extramedullary Space: Case Report. *J Spinal Cord Med* (2012) 35(2):118–21. doi: 10.1179/2045772311Y.0000000055
32. Scheithauer BW, Kovacs K, Nose V, Lombardero M, Osamura YR, Lloyd RV, et al. Multiple Endocrine Neoplasia Type 1-Associated Thyrotropin-Producing Pituitary Carcinoma: Report of a Probable De Novo Example. *Hum Pathol* (2009) 40(2):270–8. doi: 10.1016/j.humpath.2008.06.013
33. Koyama J, Ikeda K, Shose Y, Kimura M, Obora Y, Kohmura E. Long-Term Survival With non-Functioning Pituitary Carcinoma - Case Report. *Neurol Med Chir (Tokyo)* (2007) 47(10):475–8. doi: 10.2176/nmc.47.475
34. Horvath E, Kovacs K, Killinger DW, Smyth HS, Platts ME, Singer W. Silent Corticotropic Adenomas of the Human Pituitary Gland: A Histologic, Immunocytologic, and Ultrastructural Study. *Am J Pathol* (1980) 98 (3):617–38.
35. Raverot G, Wierinckx A, Jouanneau E, Auger C, Borson-Chazot F, Lachuer J, et al. Clinical, Hormonal and Molecular Characterization of Pituitary ACTH Adenomas Without (Silent Corticotroph Adenomas) and With Cushing's Disease. *Eur J Endocrinol* (2010) 163(1):35–43. doi: 10.1530/EJE-10-0076
36. Cooper O. Silent Corticotroph Adenomas. *Pituitary* (2015) 18(2):225–31. doi: 10.1007/s11102-014-0624-3
37. Cho HY, Cho SW, Kim SW, Shin CS, Park KS, Kim SY. Silent Corticotroph Adenomas Have Unique Recurrence Characteristics Compared With Other Nonfunctioning Pituitary Adenomas. *Clin Endocrinol (Oxf)* (2010) 72 (5):648–53. doi: 10.1111/j.1365-2265.2009.03673.x
38. Cheres AF, ElAsmar N, Rajpal A, Selman WR, Arafah BM. Perioperative Hypothalamic Pituitary Adrenal Function in Patients With Silent Corticotroph Adenomas. *Pituitary* (2017) 20(4):471–6. doi: 10.1007/s11102-017-0809-7
39. Yamada S, Ohyama K, Taguchi M, Takeshita A, Morita K, Takano K, et al. A Study of the Correlation Between Morphological Findings and Biological Activities in Clinically Nonfunctioning Pituitary Adenomas. *Neurosurgery* (2007) 61(3):580–4; discussion 4-5. doi: 10.1227/01.NEU.0000290906.53685.79
40. Oystese KA, Casar-Borota O, Normann KR, Zucknick M, Berg JP, Bollerslev J. Estrogen Receptor Alpha, a Sex-Dependent Predictor of Aggressiveness in Nonfunctioning Pituitary Adenomas: SSTR and Sex Hormone Receptor Distribution in NFPA. *J Clin Endocrinol Metab* (2017) 102(9):3581–90. doi: 10.1210/jc.2017-00792
41. Nishioka H, Inoshita N, Mete O, Asa SL, Hayashi K, Takeshita A, et al. The Complementary Role of Transcription Factors in the Accurate Diagnosis of Clinically Nonfunctioning Pituitary Adenomas. *Endocr Pathol* (2015) 26 (4):349–55. doi: 10.1007/s12022-015-9398-z
42. Cazabat L, Dupuy M, Boulin A, Bernier M, Baussart B, Foubert L, et al. Silent, But Not Unseen: Multimicrocystic Aspect on T2-weighted MRI in Silent Corticotroph Adenomas. *Clin Endocrinol (Oxf)* (2014) 81(4):566–72. doi: 10.1111/cen.12443
43. Drummond J, Roncaroli F, Grossman AB, Korbonits M. Clinical and Pathological Aspects of Silent Pituitary Adenomas. *J Clin Endocrinol Metab* (2019) 104(7):2473–89. doi: 10.1210/jc.2018-00688
44. Thodou E, Argyrakos T, Kontogeorgos G. Galectin-3 as a Marker Distinguishing Functioning From Silent Corticotroph Adenomas. *Horm (Athens)* (2007) 6(3):227–32.
45. Mete O, Hayhurst C, Alahmadi H, Monsalves E, Gucer H, Gentili F, et al. The Role of Mediators of Cell Invasiveness, Motility, and Migration in the Pathogenesis of Silent Corticotroph Adenomas. *Endocr Pathol* (2013) 24 (4):191–8. doi: 10.1007/s12022-013-9270-y
46. Horvath E, Kovacs K, Lloyd R. Pars Intermedia of the Human Pituitary Revisited: Morphologic Aspects and Frequency of Hyperplasia of POMC-peptide Immunoreactive Cells. *Endocr Pathol* (1999) 10(1):55–64. doi: 10.1007/BF02738816
47. Langlois F, Lim DST, Varlamov E, Yedinak CG, Cetas JS, McCartney S, et al. Clinical Profile of Silent Growth Hormone Pituitary Adenomas; Higher Recurrence Rate Compared to Silent Gonadotroph Pituitary Tumors, a Large Single Center Experience. *Endocrine* (2017) 58(3):528–34. doi: 10.1007/s12020-017-1447-6
48. Matsuno A, Okazaki R, Oki Y, Nagashima T. Secretion of High-Molecular-Weight Adrenocorticotropic Hormone From a Pituitary Adenoma in a Patient Without Cushing Stigmata. Case Report. *J Neurosurg* (2004) 101 (5):874–7. doi: 10.3171/jns.2004.101.5.0874

49. Kovacs K, Horvath E, Bayley TA, Hassaram ST, Ezrin C. Silent Corticotroph Cell Adenoma With Lysosomal Accumulation and Crinophagy. A Distinct Clinicopathologic Entity. *Am J Med* (1978) 64(3):492–9. doi: 10.1016/0002-9343(78)90236-X
50. Artenstein AW, Opal SM. Proprotein Convertases in Health and Disease. *N Engl J Med* (2011) 365(26):2507–18. doi: 10.1056/NEJMra1106700
51. Tateno T, Izumiyama H, Doi M, Yoshimoto T, Shichiri M, Inoshita N, et al. Differential Gene Expression in ACTH -Secreting and non-Functioning Pituitary Tumors. *Eur J Endocrinol* (2007) 157(6):717–24. doi: 10.1530/EJE-07-0428
52. Righi A, Faustini-Fustini M, Morandi L, Monti V, Asioli S, Mazzatenta D, et al. The Changing Faces of Corticotroph Cell Adenomas: The Role of Prohormone Convertase 1/3. *Endocrine* (2017) 56(2):286–97. doi: 10.1007/s12020-016-1028-0
53. Reincke M, Sbiera S, Hayakawa A, Theodoropoulou M, Osswald A, Beuschlein F, et al. Mutations in the Deubiquitinase Gene USP8 Cause Cushing's Disease. *Nat Genet* (2015) 47(1):31–8. doi: 10.1038/ng.3166
54. Ma ZY, Song ZJ, Chen JH, Wang YF, Li SQ, Zhou LF, et al. Recurrent Gain-of-Function USP8 Mutations in Cushing's Disease. *Cell Res* (2015) 25 (3):306–17. doi: 10.1038/cr.2015.20
55. Fukuoka H, Cooper O, Ben-Shlomo A, Mamelak A, Ren SG, Bruyette D, et al. EGFR as a Therapeutic Target for Human, Canine, and Mouse ACTH-secreting Pituitary Adenomas. *J Clin Invest* (2011) 121(12):4712–21. doi: 10.1172/JCI60417
56. Araki T, Liu X, Kameda H, Tone Y, Fukuoka H, Tone M, et al. Egfr Induces E2f1-Mediated Corticotroph Tumorigenesis. *J Endocr Soc* (2017) 1(2):127–43. doi: 10.1210/js.2016-1053
57. Chen J, Jian X, Deng S, Ma Z, Shou X, Shen Y, et al. Identification of Recurrent USP48 and BRAF Mutations in Cushing's Disease. *Nat Commun* (2018) 9(1):3171. doi: 10.1038/s41467-018-05275-5
58. Sbiera S, Perez-Rivas LG, Taranets L, Weigand I, Flitsch J, Graf E, et al. Driver Mutations in USP8 Wild-Type Cushing's Disease. *Neuro Oncol* (2019) 21(10):1273–83. doi: 10.1093/neuonc/noz109
59. Tanizaki Y, Jin L, Scheithauer BW, Kovacs K, Roncaroli F, Lloyd RV. P53 Gene Mutations in Pituitary Carcinomas. *Endocr Pathol* (2007) 18(4):217–22. doi: 10.1007/s12022-007-9006-y
60. Uzilov AV, Taik P, Cheesman KC, Javanmard P, Ying K, Roehnelt A, et al. USP8 and TP53 Drivers are Associated With CNV in a Corticotroph Adenoma Cohort Enriched for Aggressive Tumors. *J Clin Endocrinol Metab* (2020) 106:826–42. doi: 10.1210/clinem/dgaa853
61. Roussel-Gervais A, Couture C, Langlais D, Takayasu S, Balsalobre A, Rueda BR, et al. The Cables1 Gene in Glucocorticoid Regulation of Pituitary Corticotrope Growth and Cushing Disease. *J Clin Endocrinol Metab* (2016) 101(2):513–22. doi: 10.1210/jc.2015-3324
62. Casar-Borota O, Boldt HB, Engstrom BE, Andersen MS, Baussart B, Bengtsson D, et al. Corticotroph Aggressive Pituitary Tumours and Carcinomas Frequently Harbour ATRX Mutations. *J Clin Endocrinol Metab* (2020) 106:1183–94. doi: 10.1210/clinem/dgaa749
63. Hernandez-Ramirez LC, Gam R, Valdes N, Lodish MB, Pankratz N, Balsalobre A, et al. Loss-of-Function Mutations in the CABLES1 Gene are a Novel Cause of Cushing's Disease. *Endocr Relat Cancer* (2017) 24(8):379–92. doi: 10.1530/ERC-17-0131
64. Chasseloup F, Pankratz N, Lane J, Faucz FR, Keil MF, Chittiboina P, et al. Germline CDKN1B Loss-of-Function Variants Cause Pediatric Cushing's Disease With or Without an MEN4 Phenotype. *J Clin Endocrinol Metab* (2020) 105(6):1983–2005. doi: 10.1210/clinem/dgaa160
65. Martinez de LaPiscina I, Hernandez-Ramirez LC, Portillo N, Gomez-Gila AL, Urrutia I, Martinez-Salazar R, et al. Rare Germline Dicer1 Variants in Pediatric Patients With Cushing's Disease: What Is Their Role? *Front Endocrinol (Lausanne)* (2020) 11:433. doi: 10.3389/fendo.2020.00433
66. Sahakitrungruang T, Srichomthong C, Pornkunwilai S, Amornfa J, Shuangshoti S, Kulawonganunchai S, et al. Germline and Somatic DICER1 Mutations in a Pituitary Blastoma Causing Infantile-Onset Cushing's Disease. *J Clin Endocrinol Metab* (2014) 99(8):E1487–92. doi: 10.1210/jc.2014-1016
67. de Kock L, Sabbaghian N, Plourde F, Srivastava A, Weber E, Bouron-Dal Soglio D, et al. Pituitary Blastoma: A Pathognomonic Feature of Germ-Line DICER1 Mutations. *Acta Neuropathol* (2014) 128(1):111–22. doi: 10.1007/s00401-014-1285-z
68. Coli A, Asa SL, Fadda G, Scannone D, Chiloiro S, De Marinis L, et al. Minichromosome Maintenance Protein 7 as Prognostic Marker of Tumor Aggressiveness in Pituitary Adenoma Patients. *Eur J Endocrinol* (2016) 174 (3):307–14. doi: 10.1530/EJE-15-0586
69. Garbicz F, Mehlich D, Rak B, Sajjad E, Maksymowicz M, Paskal W, et al. Increased Expression of the microRNA 106b~25 Cluster and its Host Gene MCM7 in Corticotroph Pituitary Adenomas is Associated With Tumor Invasion and Crooke's Cell Morphology. *Pituitary* (2017) 20(4):450–63. doi: 10.1007/s11102-017-0805-y
70. Gicquel C, Le Bouc Y, Luton JP, Girard F, Bertagna X. Monoclonality of Corticotroph Macroadenomas in Cushing's Disease. *J Clin Endocrinol Metab* (1992) 75(2):472–5. doi: 10.1210/jcem.75.2.1322426
71. Herman V, Fagin J, Gonsky R, Kovacs K, Melmed S. Clonal Origin of Pituitary Adenomas. *J Clin Endocrinol Metab* (1990) 71(6):1427–33. doi: 10.1210/jcem-71-6-1427
72. Dahia PL, Grossman AB. The Molecular Pathogenesis of Corticotroph Tumors. *Endocr Rev* (1999) 20(2):136–55. doi: 10.1210/edrv.20.2.0358
73. Raffin-Sanson ML, de Keyzer Y, Bertagna X. Proopiomelanocortin, a Polypeptide Precursor With Multiple Functions: From Physiology to Pathological Conditions. *Eur J Endocrinol* (2003) 149(2):79–90. doi: 10.1530/eje.0.1490079
74. de Keyzer Y, Bertagna X, Lenne F, Girard F, Luton JP, Kahn A. Altered Proopiomelanocortin Gene Expression in Adrenocorticotropin-Producing Nonpituitary Tumors. Comparative Studies With Corticotropic Adenomas and Normal Pituitaries. *J Clin Invest* (1985) 76(5):1892–8. doi: 10.1172/JCI112184
75. de Keyzer Y, Rene P, Lenne F, Auzan C, Clauser E, Bertagna X. V3 Vasopressin Receptor and Corticotropic Phenotype in Pituitary and Nonpituitary Tumors. *Horm Res* (1997) 47(4-6):259–62. doi: 10.1159/000185473
76. Huizenga NA, de Lange P, Koper JW, Clayton RN, Farrell WE, van der Lely AJ, et al. Human Adrenocorticotropin-Secreting Pituitary Adenomas Show Frequent Loss of Heterozygosity at the Glucocorticoid Receptor Gene Locus. *J Clin Endocrinol Metab* (1998) 83(3):917–21. doi: 10.1210/jc.83.3.917
77. Dahia PL, Honegger J, Reincke M, Jacobs RA, Mirtella A, Fahlbusch R, et al. Expression of Glucocorticoid Receptor Gene Isoforms in Corticotropin-Secreting Tumors. *J Clin Endocrinol Metab* (1997) 82(4):1088–93. doi: 10.1210/jcem.82.4.3861
78. Pinto EM, Siqueira SA, Cukier P, Fragoso MC, Lin CJ, de Mendonca BB. Possible Role of a Radiation-Induced p53 Mutation in a Nelson's Syndrome Patient With a Fatal Outcome. *Pituitary* (2011) 14(4):400–4. doi: 10.1007/s11102-009-0194-y
79. Fountas A, Lim ES, Drake WM, Powlson AS, Gurnell M, Martin NM, et al. Outcomes of Patients With Nelson's Syndrome After Primary Treatment: A Multicenter Study From 13 UK Pituitary Centers. *J Clin Endocrinol Metab* (2020) 105(5):dgz200. doi: 10.1210/clinem/dgz200
80. Bengtsson D, Joost P, Aravidis C, Askmalm Stenmark M, Backman AS, Melin B, et al. Corticotroph Pituitary Carcinoma in a Patient With Lynch Syndrome (LS) and Pituitary Tumors in a Nationwide Ls Cohort. *J Clin Endocrinol Metab* (2017) 102(11):3928–32. doi: 10.1210/jc.2017-01401
81. Hosoyama T, Nishijo K, Garcia MM, Schaffer BS, Ohshima-Hosoyama S, Prajapati SI, et al. A Postnatal Pax7 Progenitor Gives Rise to Pituitary Adenomas. *Genes Cancer* (2010) 1(4):388–402. doi: 10.1177/1947601910370979
82. Castellnou S, Vasiljevic A, Lapras V, Raverot V, Alix E, Borson-Chazot F, et al. SST5 Expression and USP8 Mutation in Functioning and Silent Corticotroph Pituitary Tumors. *Endocr Connect* (2020) 9:243–53. doi: 10.1530/endoabs.70.AEP617
83. Tani Y, Inoshita N, Sugiyama T, Kato M, Yamada S, Shichiri M, et al. Upregulation of CDKN2A and Suppression of Cyclin D1 Gene Expressions in ACTH-secreting Pituitary Adenomas. *Eur J Endocrinol* (2010) 163 (4):523–9. doi: 10.1530/EJE-10-0245
84. Eieland AK, Normann KR, Sundaram AYM, Nyman TA, Oystese KAB, Lekva T, et al. Distinct Pattern of Endoplasmic Reticulum Protein Processing and Extracellular Matrix Proteins in Functioning and Silent Corticotroph Pituitary Adenomas. *Cancers (Basel)* (2020) 12(10):2980. doi: 10.3390/cancers12102980
85. Hammer GD, Tyrrell JB, Lamborn KR, Applebury CB, Hannegan ET, Bell S, et al. Transsphenoidal Microsurgery for Cushing's Disease: Initial Outcome

and Long-Term Results. *J Clin Endocrinol Metab* (2004) 89(12):6348–57. doi: 10.1210/jc.2003-032180
86. Sheehan JP, Niranjan A, Sheehan JM, Jane JAJr., Laws ER, Kondziolka D, et al. Stereotactic Radiosurgery for Pituitary Adenomas: An Intermediate Review of its Safety, Efficacy, and Role in the Neurosurgical Treatment Armamentarium. *J Neurosurg* (2005) 102(4):678–91. doi: 10.3171/jns.2005.102.4.0678
87. Cordeiro D, Xu Z, Nasser M, Lopes B, Vance ML, Sheehan J. The Role of Crooke's Changes in Recurrence and Remission After Gamma Knife Radiosurgery. *J Neurooncol* (2019) 142(1):171–81. doi: 10.1007/s11060-018-03078-8
88. Raverot G, Burman P, McCormack A, Heaney A, Petersenn S, Popovic V, et al. European Society of Endocrinology Clinical Practice Guidelines for the Management of Aggressive Pituitary Tumours and Carcinomas. *Eur J Endocrinol* (2018) 178(1):G1–G24. doi: 10.1530/EJE-17-0796
89. Nishioka H, Yamada S. Cushing's Disease. *J Clin Med* (2019) 8(11):1951. doi: 10.3390/jcm8111951
90. Valassi E, Franz H, Brue T, Feelders RA, Netea-Maier R, Tsagarakis S, et al. Preoperative Medical Treatment in Cushing's Syndrome: Frequency of Use and its Impact on Postoperative Assessment: Data From ERCUSYN. *Eur J Endocrinol* (2018) 178(4):399–409. doi: 10.1530/EJE-17-0997
91. Pivonello R, De Leo M, Cozzolino A, Colao A. The Treatment of Cushing's Disease. *Endocr Rev* (2015) 36(4):385–486. doi: 10.1210/er.2013-1048
92. Yoshida K, Fukuoka H, Odake Y, Nakajima S, Tachibana M, Ito J, et al. Multiple Salivary Cortisol Measurements are a Useful Tool to Optimize Metyrapone Treatment in Patients With Cushing's Syndromes Treatment: Case Presentations. *Front Endocrinol (Lausanne)* (2017) 8:375. doi: 10.3389/fendo.2017.00375
93. Fukuoka H, Takeuchi T, Matsumoto R, Bando H, Suda K, Nishizawa H, et al. D-Dimer as a Significant Marker of Deep Vein Thrombosis in Patients With Subclinical or Overt Cushing's Syndrome. *Endocr J* (2014) 61(10):1003–10. doi: 10.1507/endocrj.EJ14-0102
94. Stroud A, Dhaliwal P, Alvarado R, Winder MJ, Jonker BP, Grayson JW, et al. Outcomes of Pituitary Surgery for Cushing's Disease: A Systematic Review and Meta-Analysis. *Pituitary* (2020) 23(5):595–609. doi: 10.1007/s11102-020-01066-8
95. Zhuang Z, Liu X, Bao X, Pan B, Deng K, Yao Y, et al. Invasive ACTH-secreting Pituitary Macroadenoma in Remission After Transsphenoidal Resection: A Case Report and Literature Review. *Med (Baltimore)* (2018) 97(46):e13148. doi: 10.1097/MD.0000000000013148
96. Barber TM, Adams E, Ansorge O, Byrne JV, Karavitaki N, Wass JA. Nelson's Syndrome. *Eur J Endocrinol* (2010) 163(4):495–507. doi: 10.1530/EJE-10-0466
97. Tritos NA, Biller BM. Update on Radiation Therapy in Patients With Cushing's Disease. *Pituitary* (2015) 18(2):263–8. doi: 10.1007/s11102-014-0615-4
98. Mehta GU, Ding D, Patibandla MR, Kano H, Sisterson N, Su YH, et al. Stereotactic Radiosurgery for Cushing Disease: Results of an International, Multicenter Study. *J Clin Endocrinol Metab* (2017) 102(11):4284–91. doi: 10.1210/jc.2017-01385
99. Jagannathan J, Sheehan JP, Pouratian N, Laws ER, Steiner L, Vance ML. Gamma Knife Surgery for Cushing's Disease. *J Neurosurg* (2007) 106 (6):980–7. doi: 10.3171/jns.2007.106.6.980
100. Sheehan JP, Xu Z, Salvetti DJ, Schmitt PJ, Vance ML. Results of Gamma Knife Surgery for Cushing's Disease. *J Neurosurg* (2013) 119(6):1486–92. doi: 10.3171/2013.7.JNS13217
101. Kara M, Guduk M, Samanci Y, Yilmaz M, Sengoz M, Peker S. Gamma Knife Radiosurgery in Patients With Cushing's Disease: Comparison of Aggressive Pituitary Corticotroph Tumor Versus Corticotroph Adenoma. *Clin Neurol Neurosurg* (2020) 197:106151. doi: 10.1016/j.clineuro.2020.106151
102. Gheorghiu ML, Fleseriu M. Stereotactic Radiation Therapy in Pituitary Adenomas, Is it Better Than Conventional Radiation Therapy? *Acta Endocrinol (Buchar)* (2017) 13(4):476–90. doi: 10.4183/aeb.2017.476
103. Lacroix A, Gu F, Gallardo W, Pivonello R, Yu Y, Witek P, et al. Efficacy and Safety of Once-Monthly Pasireotide in Cushing's Disease: A 12 Month Clinical Trial. *Lancet Diabetes Endocrinol* (2018) 6(1):17–26. doi: 10.1016/S2213-8587(17)30326-1
104. Colao A, Petersenn S, Newell-Price J, Findling JW, Gu F, Maldonado M, et al. A 12-Month Phase 3 Study of Pasireotide in Cushing's Disease. *N Engl J Med* (2012) 366(10):914–24. doi: 10.1056/NEJMoa1105743
105. Broersen LHA, Jha M, Biermasz NR, Pereira AM, Dekkers OM. Effectiveness of Medical Treatment for Cushing's Syndrome: A Systematic Review and Meta-Analysis. *Pituitary* (2018) 21(6):631–41. doi: 10.1007/s11102-018-0897-z
106. Shimon I, Rot L, Inbar E. Pituitary-Directed Medical Therapy With Pasireotide for a Corticotroph Macroadenoma: Pituitary Volume Reduction and Literature Review. *Pituitary* (2012) 15(4):608–13. doi: 10.1007/s11102-012-0427-3
107. Cannavo S, Messina E, Albani A, Ferrau F, Barresi V, Priola S, et al. Clinical Management of Critically Ill Patients With Cushing's Disease Due to ACTH-secreting Pituitary Macroadenomas: Effectiveness of Presurgical Treatment With Pasireotide. *Endocrine* (2016) 52(3):481–7. doi: 10.1007/s12020-015-0601-2
108. Greenman Y, Stern N. Paradoxical and Atypical Responses to Pasireotide in Aggressive ACTH-secreting Pituitary Tumors. *Pituitary* (2016) 19(6):605–11. doi: 10.1007/s11102-016-0755-9
109. Katznelson L. Sustained Improvements in Plasma ACTH and Clinical Status in a Patient With Nelson's Syndrome Treated With Pasireotide LAR, a Multireceptor Somatostatin Analog. *J Clin Endocrinol Metab* (2013) 98 (5):1803–7. doi: 10.1210/jc.2013-1497
110. Daniel E, Debono M, Caunt S, Girio-Fragkoulakis C, Walters SJ, Akker SA, et al. A Prospective Longitudinal Study of Pasireotide in Nelson's Syndrome. *Pituitary* (2018) 21(3):247–55. doi: 10.1007/s11102-017-0853-3
111. Hanson PL, Aylwin SJ, Monson JP, Burrin JM. FSH Secretion Predominates In Vivo and In Vitro in Patients With non-Functioning Pituitary Adenomas. *Eur J Endocrinol* (2005) 152(3):363–70. doi: 10.1530/eje.1.01854
112. Ferriere A, Cortet C, Chanson P, Delemer B, Caron P, Chabre O, et al. Cabergoline for Cushing's Disease: A Large Retrospective Multicenter Study. *Eur J Endocrinol* (2017) 176(3):305–14. doi: 10.1530/EJE-16-0662
113. Godbout A, Manavela M, Danilowicz K, Beauregard H, Bruno OD, Lacroix A. Cabergoline Monotherapy in the Long-Term Treatment of Cushing's Disease. *Eur J Endocrinol* (2010) 163(5):709–16. doi: 10.1530/EJE-10-0382
114. Pivonello R, De Martino MC, Cappabianca P, De Leo M, Faggiano A, Lombardi G, et al. The Medical Treatment of Cushing's Disease: Effectiveness of Chronic Treatment With the Dopamine Agonist Cabergoline in Patients Unsuccessfully Treated by Surgery. *J Clin Endocrinol Metab* (2009) 94(1):223–30. doi: 10.1210/jc.2008-1533
115. Casulari LA, Naves LA, Mello PA, Pereira Neto A, Papadia C. Nelson's Syndrome: Complete Remission With Cabergoline But Not With Bromocriptine or Cyproheptadine Treatment. *Horm Res* (2004) 62(6):300–5. doi: 10.1159/000082235
116. Shraga-Slutzky I, Shimon I, Weinshtein R. Clinical and Biochemical Stabilization of Nelson's Syndrome With Long-Term Low-Dose Cabergoline Treatment. *Pituitary* (2006) 9(2):151–4. doi: 10.1007/s11102-006-9290-4
117. Petrossians P, Ronci N, Valdes Socin H, Kalife A, Stevenaert A, Bloch B, et al. ACTH Silent Adenoma Shrinking Under Cabergoline. *Eur J Endocrinol* (2001) 144(1):51–7. doi: 10.1530/eje.0.1440051
118. Tateno T, Kato M, Tani Y, Oyama K, Yamada S, Hirata Y. Differential Expression of Somatostatin and Dopamine Receptor Subtype Genes in Adrenocorticotropin (ACTH)-Secreting Pituitary Tumors and Silent Corticotroph Adenomas. *Endocr J* (2009) 56(4):579–84. doi: 10.1507/endocrj.K08E-186
119. Hegi ME, Diserens AC, Gorlia T, Hamou MF, de Tribolet N, Weller M, et al. MGMT Gene Silencing and Benefit From Temozolomide in Glioblastoma. *N Engl J Med* (2005) 352(10):997–1003. doi: 10.1056/NEJMoa043331
120. Fadul CE, Kominsky AL, Meyer LP, Kingman LS, Kinlaw WB, Rhodes CH, et al. Long-Term Response of Pituitary Carcinoma to Temozolomide. Report of Two Cases. *J Neurosurg* (2006) 105(4):621–6. doi: 10.3171/jns.2006.105.4.621
121. Lasolle H, Cortet C, Castinetti F, Cloix L, Caron P, Delemer B, et al. Temozolomide Treatment can Improve Overall Survival in Aggressive Pituitary Tumors and Pituitary Carcinomas. *Eur J Endocrinol* (2017) 176 (6):769–77. doi: 10.1530/EJE-16-0979
122. Elbelt U, Schlaffer SM, Buchfelder M, Knappe UJ, Vila G, Micko A, et al. Efficacy of Temozolomide Therapy in Patients With Aggressive Pituitary Adenomas and Carcinomas-A German Survey. *J Clin Endocrinol Metab* (2020) 105(3):dgz211. doi: 10.1210/clinem/dgz211

123. Tanaka S, Yamamoto M, Morita M, Takeno A, Kanazawa I, Yamaguchi T, et al. Successful Reduction of ACTH Secretion in a Case of Intractable Cushing's Disease With Pituitary Crooke's Cell Adenoma by Combined Modality Therapy Including Temozolomide. *Endocr J* (2019) 66(8):701–8. doi: 10.1507/endocrj.EJ18-0547
124. Scheithauer BW, Horvath E, Abel TW, Robital Y, Park SH, Osamura RY, et al. Pituitary Blastoma: A Unique Embryonal Tumor. *Pituitary* (2012) 15 (3):365–73. doi: 10.1007/s11102-011-0328-x
125. Tatsi C, Stratakis CA. Aggressive Pituitary Tumors in the Young and Elderly. *Rev Endocr Metab Disord* (2020) 21(2):213–23. doi: 10.1007/s11154-019-09534-8
126. Salehi F, Scheithauer BW, Kovacs K, Horvath E, Syro LV, Sharma S, et al. O-6-methylguanine-DNA Methyltransferase (MGMT) Immunohistochemical Expression in Pituitary Corticotroph Adenomas. *Neurosurgery* (2012) 70 (2):491–6; discussion 6. doi: 10.1227/NEU.0b013e318230ac63
127. Cohen-Inbar O, Xu Z, Lee CC, Wu CC, Chytka T, Silva D, et al. Prognostic Significance of Corticotroph Staining in Radiosurgery for non-Functioning Pituitary Adenomas: A Multicenter Study. *J Neurooncol* (2017) 135(1):67–74. doi: 10.1007/s11060-017-2520-y
128. Ceccato F, Lombardi G, Manara R, Emanuelli E, Denaro L, Milanese L, et al. Temozolomide and Pasireotide Treatment for Aggressive Pituitary Adenoma: Expertise at a Tertiary Care Center. *J Neurooncol* (2015) 122 (1):189–96. doi: 10.1007/s11060-014-1702-0
129. Fountas A, Lavrentaki A, Subramanian A, Toulis KA, Nirantharakumar K, Karavitaki N. Recurrence in Silent Corticotroph Adenomas After Primary Treatment: A Systematic Review and Meta-Analysis. *J Clin Endocrinol Metab* (2018) 104:1039–48. doi: 10.1210/jc.2018-01956
130. Whitelaw BC. How and When to Use Temozolomide to Treat Aggressive Pituitary Tumours. *Endocr Relat Cancer* (2019) 26(9):R545–R52. doi: 10.1530/ERC-19-0083
131. Trouillas J, Roy P, Sturm N, Dantony E, Cortet-Rudelli C, Viennet G, et al. A New Prognostic Clinicopathological Classification of Pituitary Adenomas: A Multicentric Case-Control Study of 410 Patients With 8 Years Post-Operative Follow-Up. *Acta Neuropathol* (2013) 126(1):123–35. doi: 10.1007/s00401-013-1084-y
132. Syro LV, Rotondo F, Camargo M, Ortiz LD, Serna CA, Kovacs K. Temozolomide and Pituitary Tumors: Current Understanding, Unresolved Issues, and Future Directions. *Front Endocrinol (Lausanne)* (2018) 9:318. doi: 10.3389/fendo.2018.00318
133. Hirohata T, Asano K, Ogawa Y, Takano S, Amano K, Isozaki O, et al. DNA Mismatch Repair Protein (MSH6) Correlated With the Responses of Atypical Pituitary Adenomas and Pituitary Carcinomas to Temozolomide: The National Cooperative Study by the Japan Society for Hypothalamic and Pituitary Tumors. *J Clin Endocrinol Metab* (2013) 98(3):1130–6. doi: 10.1210/jc.2012-2924
134. Zacharia BE, Gulati AP, Bruce JN, Carminucci AS, Wardlaw SL, Siegelin M, et al. High Response Rates and Prolonged Survival in Patients With Corticotroph Pituitary Tumors and Refractory Cushing Disease From Capecitabine and Temozolomide (CAPTEM): A Case Series. *Neurosurgery* (2014) 74(4):E447–55; discussion E55. doi: 10.1227/NEU.0000000000000251
135. Heaney AP. Clinical Review: Pituitary Carcinoma: Difficult Diagnosis and Treatment. *J Clin Endocrinol Metab* (2011) 96(12):3649–60. doi: 10.1210/jc.2011-2031
136. Lin AL, Donoghue MTA, Wardlaw SL, Yang TJ, Bodei L, Tabar V, et al. Approach to the Treatment of a Patient With an Aggressive Pituitary Tumor. *J Clin Endocrinol Metab* (2020) 105(12):3807–20. doi: 10.1210/clinem/dgaa649
137. Lin AL, Jonsson P, Tabar V, Yang TJ, Cuaron J, Beal K, et al. Marked Response of a Hypermutated Acth-Secreting Pituitary Carcinoma to Ipilimumab and Nivolumab. *J Clin Endocrinol Metab* (2018) 103 (10):3925–30. doi: 10.1210/jc.2018-01347
138. Sol B, de Filette JMK, Awada G, Raeymaeckers S, Aspeslagh S, Andreescu CE, et al. Immune Checkpoint Inhibitor Therapy for ACTH-secreting Pituitary Carcinoma: A New Emerging Treatment? *Eur J Endocrinol* (2021) 184(1): K1–5. doi: 10.1530/EJE-20-0151
139. Bedard PL, Hyman DM, Davids MS, Siu LL. Small Molecules, Big Impact: 20 Years of Targeted Therapy in Oncology. *Lancet* (2020) 395(10229):1078–88. doi: 10.1016/S0140-6736(20)30164-1
140. Cohen M, Persky R, Stegemann R, Hernandez-Ramirez LC, Zeltser D, Lodish MB, et al. Germline USP8 Mutation Associated With Pediatric Cushing Disease and Other Clinical Features: A New Syndrome. *J Clin Endocrinol Metab* (2019) 104(10):4676–82. doi: 10.1210/jc.2019-00697
141. Liu X, Feng M, Dai C, Bao X, Deng K, Yao Y, et al. Expression of EGFR in Pituitary Corticotroph Adenomas and Its Relationship With Tumor Behavior. *Front Endocrinol (Lausanne)* (2019) 10:785. doi: 10.3389/fendo.2019.00785
142. McCormack A, Dekkers OM, Petersenn S, Popovic V, Trouillas J, Raverot G, et al. Treatment of Aggressive Pituitary Tumours and Carcinomas: Results of a European Society of Endocrinology (ESE) Survey 2016. *Eur J Endocrinol* (2018) 178(3):265–76. doi: 10.1530/EJE-17-0933
143. Cooper O, Bonert VS, Rudnick J, Pressman BD, Lo J, Salvatori R, et al. Egfr/ErbB2 Targeting Lapatinib Therapy for Aggressive Prolactinomas. *J Clin Endocrinol Metab* (2020) 106:e917–25. doi: 10.1210/clinem/dgaa805
144. Ortiz LD, Syro LV, Scheithauer BW, Ersen A, Uribe H, Fadul CE, et al. Anti-VEGF Therapy in Pituitary Carcinoma. *Pituitary* (2012) 15(3):445–9. doi: 10.1007/s11102-011-0346-8
145. Rotman LE, Vaughan TB, Hackney JR, Riley KO. Long-Term Survival After Transformation of an Adrenocorticotropic Hormone-Secreting Pituitary Macroadenoma to a Silent Corticotroph Pituitary Carcinoma. *World Neurosurg* (2019) 122:417–23. doi: 10.1016/j.wneu.2018.11.011
146. Lamb LS, Sim HW, McCormack AI. Exploring the Role of Novel Medical Therapies for Aggressive Pituitary Tumors: A Review of the Literature- "Are We There Yet?" *Cancers (Basel)* (2020) 12(2):e917–25. doi: 10.3390/cancers12020308
147. Yang J, Yan J, Liu B. Targeting VEGF/VEGFR to Modulate Antitumor Immunity. *Front Immunol* (2018) 9:978. doi: 10.3389/fimmu.2018.00978
148. Monsalves E, Juraschka K, Tateno T, Agnihotri S, Asa SL, Ezzat S, et al. The PI3K/AKT/mTOR Pathway in the Pathophysiology and Treatment of Pituitary Adenomas. *Endocr Relat Cancer* (2014) 21(4):R331–44. doi: 10.1530/ERC-14-0188
149. Jia W, Sanders AJ, Jia G, Liu X, Lu R, Jiang WG. Expression of the mTOR Pathway Regulators in Human Pituitary Adenomas Indicates the Clinical Course. *Anticancer Res* (2013) 33(8):3123–31.
150. Yao JC, Fazio N, Singh S, Buzzoni R, Carnaghi C, Wolin E, et al. Everolimus for the Treatment of Advanced, non-Functional Neuroendocrine Tumours of the Lung or Gastrointestinal Tract (RADIANT-4): A Randomised, Placebo-Controlled, Phase 3 Study. *Lancet* (2016) 387(10022):968–77. doi: 10.1016/S0140-6736(15)00817-X
151. Donovan LE, Arnal AV, Wang SH, Odia Y. Widely Metastatic Atypical Pituitary Adenoma With mTOR Pathway STK11(F298L) Mutation Treated With Everolimus Therapy. *CNS Oncol* (2016) 5(4):203–9. doi: 10.2217/cns-2016-0011
152. Jouanneau E, Wierinckx A, Ducray F, Favrel V, Borson-Chazot F, Honnorat J, et al. New Targeted Therapies in Pituitary Carcinoma Resistant to Temozolomide. *Pituitary* (2012) 15(1):37–43. doi: 10.1007/s11102-011-0341-0
153. Liu NA, Jiang H, Ben-Shlomo A, Wawrowsky K, Fan XM, Lin S, et al. Targeting Zebrafish and Murine Pituitary Corticotroph Tumors With a Cyclin-Dependent Kinase (CDK) Inhibitor. *Proc Natl Acad Sci USA* (2011) 108(20):8414–9. doi: 10.1073/pnas.1018091108
154. Liu NA, Araki T, Cuevas-Ramos D, Hong J, Ben-Shlomo A, Tone Y, et al. Cyclin E-Mediated Human Proopiomelanocortin Regulation as a Therapeutic Target for Cushing Disease. *J Clin Endocrinol Metab* (2015) 100(7):2557–64. doi: 10.1210/jc.2015-1606
155. Daniel E, Newell-Price JD. Therapy of Endocrine Disease: Steroidogenesis Enzyme Inhibitors in Cushing's Syndrome. *Eur J Endocrinol* (2015) 172(6): R263–80. doi: 10.1530/EJE-14-1014
156. Pivonello R, Ferrigno R, De Martino MC, Simeoli C, Di Paola N, Pivonello C, et al. Medical Treatment of Cushing's Disease: An Overview of the Current and Recent Clinical Trials. *Front Endocrinol (Lausanne)* (2020) 11:648. doi: 10.3389/fendo.2020.00648
157. Guelho D, Grossman AB. Emerging Drugs for Cushing's Disease. *Expert Opin Emerg Drugs* (2015) 20(3):463–78. doi: 10.1517/14728214.2015.1047762
158. Fleseriu M, Pivonello R, Elenkova A, Salvatori R, Auchus RJ, Feelders RA, et al. Efficacy and Safety of Levoketoconazole in the Treatment of Endogenous Cushing's Syndrome (SONICS): A Phase 3, Multicentre,

Open-Label, Single-Arm Trial. *Lancet Diabetes Endocrinol* (2019) 7(11):855–65. doi: 10.1016/S2213-8587(19)30313-4
159. Fleseriu M, Pivonello R, Young J, Hamrahian AH, Molitch ME, Shimizu C, et al. Osilodrostat, a Potent Oral 11beta-Hydroxylase Inhibitor: 22-Week, Prospective, Phase II Study in Cushing's Disease. *Pituitary* (2016) 19(2):138–48. doi: 10.1007/s11102-015-0692-z
160. Cuevas-Ramos D, Lim DST, Fleseriu M. Update on Medical Treatment for Cushing's Disease. *Clin Diabetes Endocrinol* (2016) 2:16. doi: 10.1186/s40842-016-0033-9
161. Castinetti F, Fassnacht M, Johanssen S, Terzolo M, Bouchard P, Chanson P, et al. Merits and Pitfalls of Mifepristone in Cushing's Syndrome. *Eur J Endocrinol* (2009) 160(6):1003–10. doi: 10.1530/EJE-09-0098
162. Fein HG, Vaughan TB3rd, Kushner H, Cram D, Nguyen D. Sustained Weight Loss in Patients Treated With Mifepristone for Cushing's Syndrome: A Follow-Up Analysis of the SEISMIC Study and Long-Term Extension. *BMC Endocr Disord* (2015) 15:63. doi: 10.1186/s12902-015-0059-5

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Endocrinology

ORIGINAL RESEARCH
published: 21 October 2021
doi: 10.3389/fendo.2021.726448

The Upregulation of Molecules Related to Tumor Immune Escape in Human Pituitary Adenomas

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Human pituitary adenomas are one of the most common intracranial neoplasms. Although most of these tumors are benign and can be treated medically or by transsphenoidal surgery, a subset of these tumors are fast-growing, aggressive, recur, and remain a therapeutic dilemma. Because antibodies against immune checkpoint receptors PD-1 and CLTA-4 are now routinely used for cancer treatment, we quantified the expression of mRNA coding for PD-1, CLTA-4, and their ligands, PD-L1, PD-L2, CD80, and CD86 in human pituitary adenomas and normal pituitary glands, with the ultimate goal of exploiting immune checkpoint therapy in aggressive pituitary adenomas. Aggressive pituitary adenomas demonstrated an increased expression of PD-L2, CD80, and CD86 in compared to that of normal human pituitary glands. Furthermore, aggressive pituitary tumors demonstrated significantly higher levels of CD80 and CD86 compared to non-aggressive tumors. Our results establish a rationale for studying a potential role for immune checkpoint inhibition therapy in the treatment of pituitary adenomas.

Keywords: pituitary adenoma, immune checkpoint blockade, immunotherapy, immune escape, aggressive pituitary adenoma

OPEN ACCESS

Edited by:
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Peking Union Medical College Hospital (CAMS), China

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Specialty section:
This article was submitted to Pituitary Endocrinology, a section of the journal Frontiers in Endocrinology

Received: *16 June 2021*
Accepted: *01 September 2021*
Published: *21 October 2021*

Citation:
Xi Z, Jones PS, Mikamoto M, Jiang X, Faje AT, Nie C, Labelle KE, Zhou Y, Miller KK, Soberman RJ and Zhang X (2021) The Upregulation of Molecules Related to Tumor Immune Escape in Human Pituitary Adenomas. Front. Endocrinol. 12:726448. doi: 10.3389/fendo.2021.726448

INTRODUCTION

Pituitary adenomas are the second most common primary intracranial tumor (1–3). While the majority of these benign tumors can be managed effectively medically or with transsphenoidal surgery, there is a subset that remains resistant and/or recurs (4, 5). While there are no reliable predictors of aggressive behavior, elevated Ki67 index >3%, tumor invasiveness, large size, functioning adenomas, silent adenomas, and the rare event of distant metastasis have all been correlated with increased tumor aggressiveness (6–8). For tumors that grow despite radiation therapy, the alkylating agent temozolomide is commonly used. Only 50% of patients respond, and the vast majority of these tumors escape control (9). Therefore, finding additional therapeutic modalities is critical to their management.

The host response to tumors involves the recruitment and activation of T cell subsets and macrophages. Tumors escape this response by expressing immune checkpoint molecules on their

surface. These, in turn, interact with their ligand/receptor on the surface of immune cells down-regulating their anti-tumor function (10). Among the immune checkpoint pathways, the most well studied molecules are programmed cell death protein 1 (PD-1), cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) and their ligands (11–15). PD-1 is mostly located on the surface of activated T cells, B cells, monocytes, natural killer (NK) cells, and dendritic cells. It has two ligands: programmed death-ligand 1 (PD-L1) and programmed death-ligand 2 (PD-L2) (16, 17). Unlike PD-1, which inhibits immune activity after T cells are activated, CTLA-4, which is mainly expressed on regulatory T cells, regulates the activation of T cells (14, 15). The CTLA-4 ligands are CD80 and CD86, which are found on antigen presenting cells. Monoclonal antibodies that block PD-1, PD-L1, and CTLA-4 have each been found to demonstrate persuasive anti-tumor effects (18–21). Immune checkpoint therapy has been effective in treating selective tumors, as exemplified by melanomas and renal cell carcinoma (22, 23).

The large majority of immune checkpoint blockade applications have been in the treatment of malignant tumors (e.g. melanomas), and there are few data on their efficacy in the treatment of aggressive benign tumors. Some reports showed increased expression of PD-L1 in functioning pituitary adenomas (24, 25), and recently, Lin et al. (26) reported a case of refractory Cushing's disease (CD) in which a dramatic response to ipilimumab (anti-CLTA-4 antibody) and nivolumab (anti-PD-1 antibody) was observed. This suggests that the use of immune checkpoint blockade agents may be effective in a subset of refractory cases. We therefore analyzed mRNA expression of immune checkpoint molecules including PD-1, PD-L1, PD-L2, CLTA-4, CD80, and CD86, in clinically aggressive pituitary adenomas, adenomas that had not exhibited aggressive behavior and normal pituitary tissue to explore whether there is a scientific rationale for investigating whether immunotherapeutic agents are effective against aggressive pituitary tumors.

MATERIALS AND METHODS

Sample Collection

Fresh human pituitary samples were provided by the Massachusetts General Hospital Neurosurgery service. A total of 60 tumor samples were collected; among them, 43 samples were aggressive tumors (28 clinically non-functioning, 7 GH-secreting, 4 ACTH-secreting, 3 PRL-secreting adenomas, as well as 1 silent TSH-expressing) and 17 samples were typical benign adenomas (11 clinically non-functioning, 4 GH-secreting, 1 ACTH-secreting, and 1 PRL-secreting adenomas) that had exhibited no signs of aggressive behavior. Aggressive tumors were defined as adenomas that were recurrent despite surgery and radiation, giant adenomas (≥4cm) with invasiveness, adenomas requiring multiple surgeries and radiation therapy, macroadenomas that recurred unusually rapidly (typically within 2-3 years) requiring radiation, and invasive macroadenomas. One control group was macroadenomas that were non-invasive and not recurrent following surgery. In addition to the tumor controls, 12 normal pituitary tissue samples were obtained from Harvard Brain Bank. These data are locate in **Supplementary Table 1**. This research was approved by the Mass General Brigham institutional review board.

Total RNA Extraction

Briefly, tissues were cut into pieces and homogenized in TRIZOL reagent (Invitrogen, Waltham, MA, USA; 1 mL TRIZOL reagent per 50~100 mg tissue). The samples were kept at room temperature for a few minutes, then centrifuged. The supernatant was transferred to a new tube, and 0.2 ml chloroform was added followed by vigorous vertexing. After one additional centrifugation, the upper aqueous phase was collected, and an equal volume of isopropyl alcohol was added. The mixture was incubated at room temperature for 10 minutes and centrifuged to precipitate the RNA. Finally, the RNA was washed and dissolved, then diluted for spectrophotometric analysis to determine the concentration.

RT-qPCR

First strand cDNA was synthesized using iScript cDNA Synthesis Kits (Bio-Rad, Hercules, CA, USA), starting with 1 μg of mRNA and finishing with 50 μL in volume. Quantificational PCR was performed with PowerUp™ SYBR™ Green Master Mix (Applied Biosystems, Foster City, CA, USA) following the manufacturer's instructions. Each PCR mix contained 1 μL of cDNA and 20 nmol of each primer; and each reaction started with 50 C for 2 min, followed by 95 C for 10 min and 40 cycles of 95 C for 15 sec followed by 60 C for 1 min. The primer sequences used were as follows:

PD-1: 5'- CCAGGATGGTTCTTAGACTCCC -3'(forward),
5'- TTTAGCACGAAGCTCTCCGAT -3' (reverse);
PD-L1: 5'- TGGCATTTGCTGAACGCATTT -3'(forward),
5'- TGCAGCCAGGTCTAATTGTTTT -3'(reverse);
PD-L2: 5'- ATTGCAGCTTCACCAGATAGC -3'(forward),
5'- AAAGTTGCATTCCAGGGTCAC -3'(reverse);
CTLA-4: 5'- GCCCTGCACTCTCCTGTTTTT -3'(forward),
5'- GGTTGCCGCACAGACTTCA -3' (reverse);
CD80: 5'- AAACTCGCATCTACTGGCAAA -3'(forward),
5'- GGTTCTTGTACTCGGGCCATA -3'(reverse);
CD86: 5'- CTGCTCATCTATACACGGTTACC -3'(forward),
5'- GGAAACGTCGTACAGTTCTGTG -3'(reverse);
GADPH: 5'- GGAGCGAGATCCCTCCAAAAT -3' (forward),
5'- GGCTGTTGTCATACTTCTCATGG -3' (reverse).

Statistical Analysis

Data were analyzed using JMP Pro 15 (SAS, Cary, NC, USA). All data are expressed as median (interquartile range). Statistical comparisons were performed using Wilcoxon rank sum test. A two-tailed $p < 0.05$ indicates a statistically significant difference.

RESULTS

Adenoma *vs.* Normal Pituitary Samples

To examine the differences between aggressive behaving tumors (N=43) and normal pituitary samples (N=12), we compared the mRNA levels of the immune checkpoint molecules PD-1, PD-L1, PD-L2, CTLA-4, CD80, and CD86 by qRT-PCR. As shown in **Figure 1A**, there were significantly higher mRNA levels of PD-L2 ($p < 0.0001$), CD-80 ($p = 0.0035$), and CD-86 ($p = 0.004$) in aggressive pituitary adenomas. However, there was no significant difference in PD-1, PD-L1, and CTLA-4 mRNA levels between aggressive pituitary adenomas and normal pituitary samples.

When we compared the relative mRNA levels of the immune checkpoint molecules between non-aggressive tumors (N=17) and normal pituitary samples, we found significantly higher mRNA expression of ligand PD-L1 (p=0.02), PD-L2 ($p < 0.0001$) (**Figure 1B**). There was no significant difference in PD-1, CTLA-4, CD80, and CD86 mRNA expression between non-aggressive pituitary adenomas and normal pituitary samples.

Aggressive *vs.* Non-Aggressive Pituitary Adenomas

When we compared the levels of mRNA representing immune checkpoint molecules between these 43 aggressive tumors and 17 non-aggressive tumors (**Table 1**), only levels of CD86 were significantly higher in aggressive tumors ($p = 0.035$) (**Figure 2A**). No statistically significant differences were found between PD-1, PD-L1, PD-L2, CLTA-4, and CD80 mRNA levels in these groups.

We also compared a subset of aggressive tumors that were recurrent through radiation or were giant, invasive adenomas on presentation (N=15) to non-aggressive clinically non-functioning (N=11) pituitary adenomas. The mRNA levels of CD80 and CD86 were significantly higher in the aggressive tumors ($p = 0.03$, $p = 0.002$, respectively) (**Figure 2B**). No statistically significant difference was found for PD-1, PD-L1, PD-L2, and CLTA-4 mRNA levels between groups.

TABLE 1 | Clinical features of pituitary adenoma patients.

	All patients	Aggressive adenomas	Non-aggressive adenomas
N	**60**	**43**	**17**
Age	51.8	50.1	56.2
Gender (male, %)	38 (63.3%)	28 (65.1)	10 (58.8)
Classification			
NFA	39	28	11
GH	12	8	4
ACTH	5	4	1
Prolactin	4	3	1
Tumor size (mean, cm)	2.7	3.1	1.7
Ki67 (n = 59, %)			
<3	45	28	17
≥3	14	14	0

NFA, nonfunctioning adenoma; GH, growth hormone-secreting adenoma; ACTH, adrenocorticotropic hormone-secreting adenomas.

Using the well-established marker of Ki67 as an indicator of tumor aggressiveness, we compared mRNA levels of immune checkpoint molecules between tumors with Ki67 < 3.0% and those with Ki67 ≥ 3.0%. We found that the mRNA levels of PD-L1 were significantly elevated in tumors with higher Ki67 ($p < 0.0001$) (**Figure 2C**). No statistically significant difference was found for PD-1, PD-L2, CLTA-4, CD80, and CD86 mRNA levels between groups.

Finally, we compared the non-functioning aggressive (N=28) to the non-aggressive (N=11) pituitary adenomas. mRNA levels of CD86 were significantly higher in aggressive tumors ($p = 0.02$) (**Figure 2D**). No statistically significant difference was found for PD-1, PD-L1, PD-L2, CLTA-4 and CD80 mRNA levels between aggressive and non-aggressive NFTs.

Functioning *vs.* Non-Functioning Adenoma Samples

Given reports suggesting higher immune checkpoint molecules in functioning tumors, we next explored the differences in our

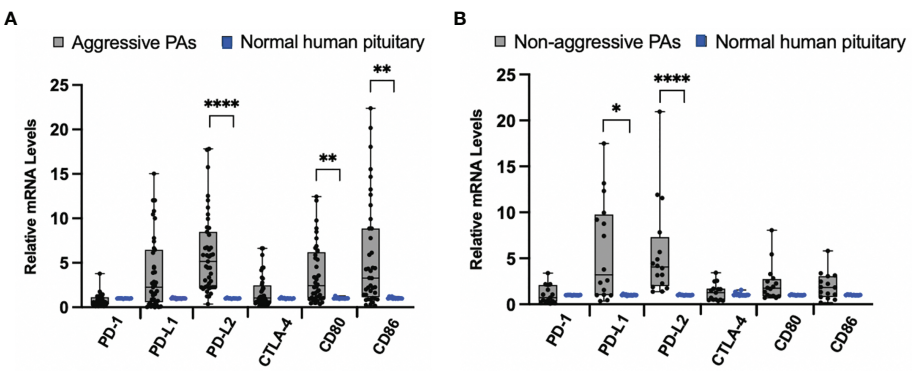

FIGURE 1 | Immune checkpoint molecule mRNA expression in pituitary adenomas (PAs) compared to normal human pituitary tissue. **(A)** Comparison of relative mRNA levels of immune checkpoint molecules between aggressive PAs (gray bar, black dots) and normal human pituitary tissue (blue dots). **(B)** Comparison of relative mRNA levels of immune checkpoint molecules in non-aggressive PAs (gray bar, black dots) and normal human pituitary tissue samples (blue dots). *$p < 0.05$; **$p < 0.01$; ****$p < 0.0001$.

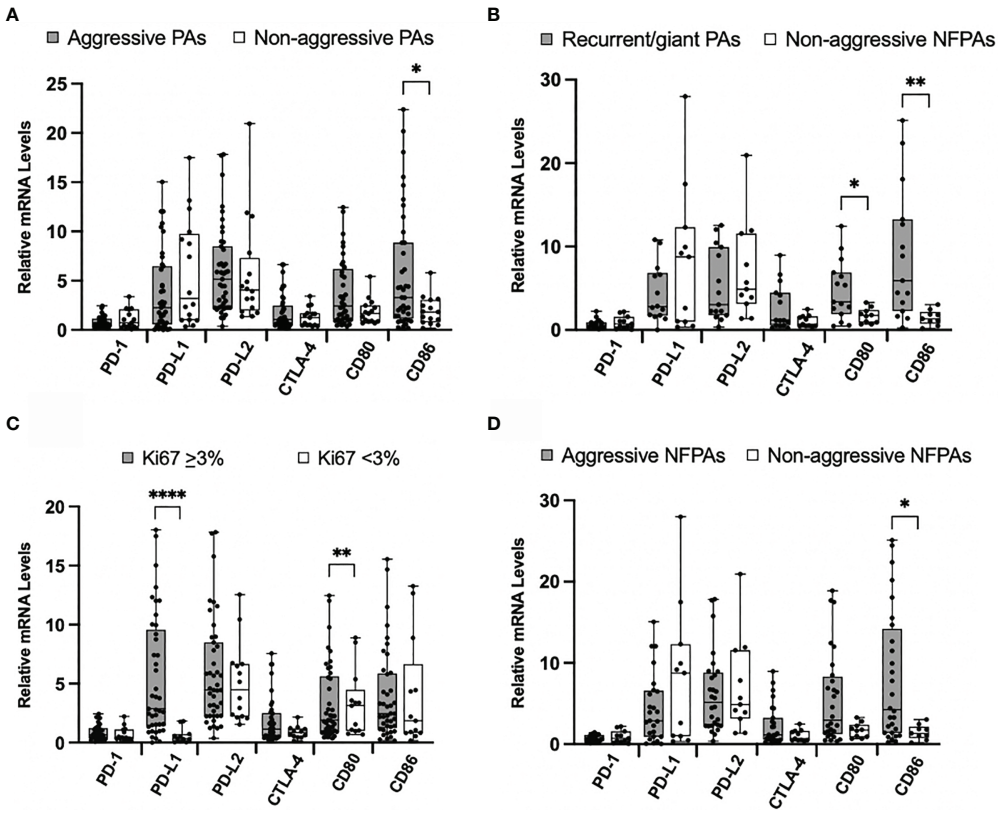

FIGURE 2 | Immune checkpoint molecule mRNA expression in aggressive PAs compared to non-aggressive PAs. **(A)** Comparison of relative mRNA levels of immune checkpoint molecules between all aggressive PAs (gray) and all non-aggressive PAs (white). **(B)** Comparison of relative mRNA levels of immune checkpoint molecules between PAs recurrent through radiation or giant, invasive on presentation (gray) compared to non-functioning non-aggressive PAs (white). **(C)** Comparison of relative mRNA levels of immune checkpoint molecules between PAs with Ki67 ≥ 3% (gray) and PAs with Ki67 < 3% (white). **(D)** Comparison of relative mRNA levels of immune checkpoint molecules between non-functioning aggressive PAs (gray) and non-functioning non-aggressive PAs (white). *$p < 0.05$; **$p < 0.01$; ****$p < 0.0001$.

cohorts of functioning (N=21) and nonfunctioning non-aggressive tumors (N=11). We found no significant difference in relative mRNA levels of PD-1, CTLA-4, CD80, and CD86 (**Figure 3A**). We then compared ACTH-staining tumors (N=5) to nonfunctioning non-aggressive pituitary adenomas. The mRNA levels of CD80 and CD86 were significantly elevated in ACTH tumors ($p = 0.02$, $p = 0.04$, respectively) (**Figure 3B**). We also analyzed GH-staining tumors (N=12) compared to nonfunctioning non-aggressive pituitary adenomas. There were no statistically significant differences in mRNA levels between the groups (**Figure 3C**).

DISCUSSION

The last decade has seen a rapid growth in the use of immunotherapy, and specifically checkpoint blockade, to successfully treat a variety of solid tumors. While pituitary adenomas remain a largely benign disease, the challenging nature of invasive, recurrent, and/or hormonally functioning tumors leaves a gap in current management. In this study, we have found that the mRNA expression levels of the ligands for immune checkpoint receptors PD-1 and CTLA-4—namely, PD-L1 and PD-L2; CD80 and CD86, respectively— were all significantly higher in pituitary adenomas than in the normal human pituitary.

Furthermore, the mRNA levels of CD80 and CD86 were significantly higher in the most aggressive subset of PAs compared to non-aggressive pituitary adenomas. Tumors with Ki67 elevated to ≥3%, levels correlated with an increased risk of recurrence, had significantly elevated PD-L1 and, although we found no differences in immune checkpoint molecules among the functioning adenomas compared to non-functioning adenomas at large, a small subset of 4 ACTH-secreting tumors had significantly elevated CD80 and CD86.

The tumor cells and their surrounding components, including fibroblasts, immune cells, vascular networks, extracellular matrix, etc., comprise the tumor microenvironment (TME) (27). Physiologically, these stromal components maintain homeostasis, immune regulation and anti-tumorigenesis (28, 29). We found higher expression of PD-L2, CD80, and CD86 in aggressive pituitary adenomas when compared to normal pituitary tissues, suggesting the accumulation of peripheral immune cells like regulatory T cells, NK cells and dendritic cells in the pituitary

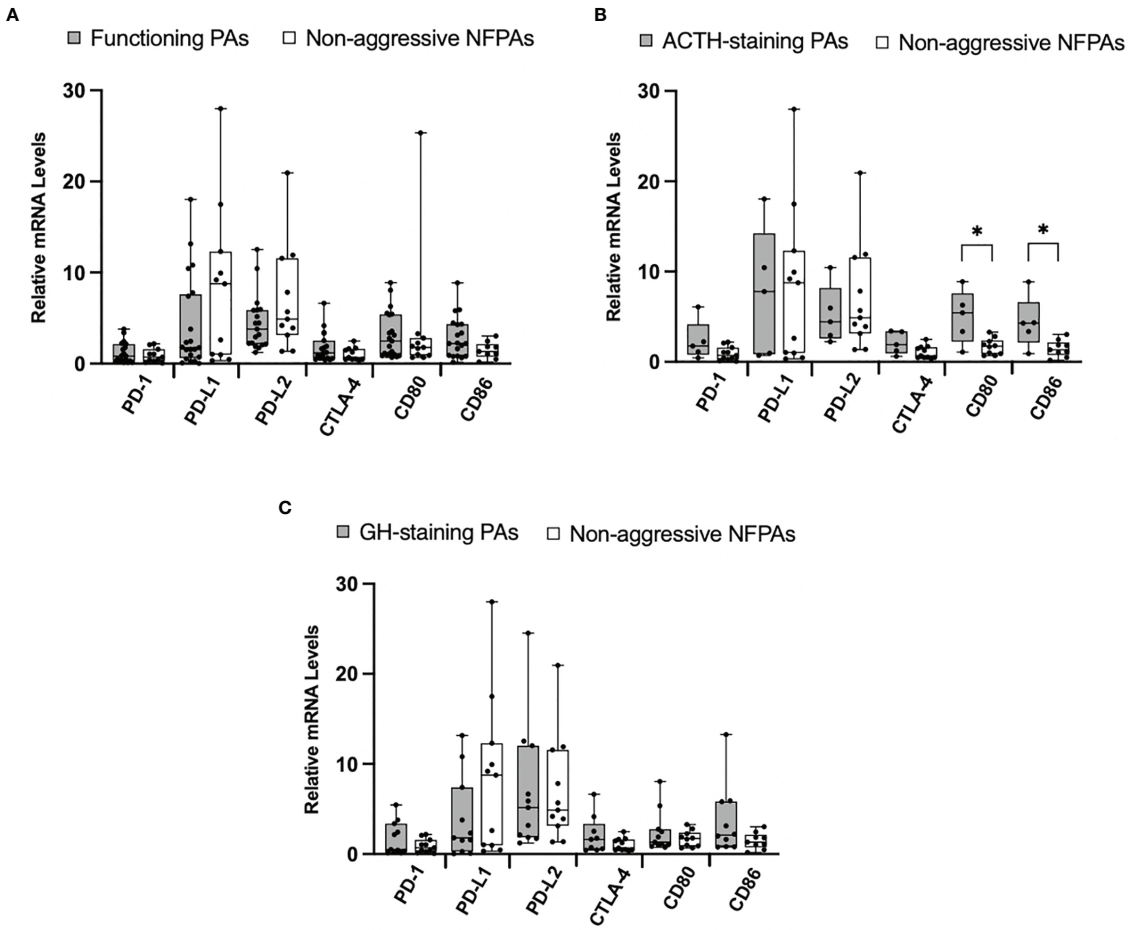

FIGURE 3 | Immune checkpoint molecule mRNA expression in functioning PAs compared to non-functioning PAs. **(A)** Comparison of relative mRNA levels of immune checkpoint molecules between functioning PAs (gray) and non-functioning non-aggressive PAs (white). **(B)** Comparison of relative mRNA levels of immune checkpoint molecules between ACTH-staining PAs (gray) compared to non-functioning non-aggressive PAs (white). **(C)** Comparison of relative mRNA levels of immune checkpoint molecules between GH-staining PAs (gray) compared to non-functioning non-aggressive PAs (white). *$p < 0.05$.

tumor immune microenvironment (TIME). In non-aggressive pituitary adenomas, we found higher expression of PD-L1 and PD-L2 when compared to normal pituitary tissues. This indicates not only that increased immune infiltrate is not unique to aggressive tumors, but also that non-aggressive tumors infiltrates may be linked closer to PD-1 mediated pathways and aggressive tumors to CTLA-4 pathways. PD-1 mRNA, which is expressed by activated T cells, was found at the same level in tumor and normal tissues. These findings suggest that PD-1 and CTLA-4 immune checkpoint pathways may be useful therapeutic targets for pituitary adenomas.

Our transcriptional data complements recent findings of proteomic work by other groups by showing that, despite their benign nature, pituitary adenomas are not immunologically inert environments. In 2016, Mei et al. first reported that PD-L1 RNA and protein expression were significantly increased in functioning tumors compared to non-functioning adenomas (25). While this group and others have studied and found increased immune markers in functioning tumors, we focused on tumors with a clinically aggressive course, which included both functioning and non-functioning pituitary adenomas. In the most aggressive tumors, those that had recurred following surgery and radiation and those that were giant and invasive at presentation, we found significantly elevated CD80 and CD86. Furthermore, in tumors with elevated Ki67 index ≥3%, we found significantly elevated levels of PD-L1 mRNA levels. This is consistent with data from Wang et al., who reported that PD-L1 immunostaining occurred more frequently in tumors with Ki-67 index ≥3%, as well as increased PD-L1 immunostaining in GH and prolactin-secreting tumors (24). Our data demonstrated higher levels of PD-L1 mRNA in pituitary adenomas as compared to normal pituitary gland, but we did not find differences in immune markers when comparing all functioning pituitary adenomas to non-functioning pituitary adenomas. We did find that a subset of five ACTH-staining tumors had significantly elevated CD80 and CD86 mRNA levels compared to our nonfunctioning controls. Kemeny et al. found elevated PD-L1 immunostaining in human ACTH-secreting

tumors and, in a murine model of Cushing's disease, found that treatment with anti-PD-L1 led to restricted tumor growth and lower ACTH production (30). These findings are consistent with a potential role for checkpoint blockade in the management of pituitary adenomas with an aggressive clinical course, particularly refractory Cushing's disease.

The significance of increased CD80 and CD86 expression in pituitary adenomas has not been previously reported, but we hypothesize it predicts an increased reliance on CTLA-4 mediated pathway of tumor suppression. Both CD80 and CD86 serve as co-stimulatory molecules in the immune environment, serving to activate T cells when they bind to CD28 or deactivate T cells when they bind to CTLA-4. These proteins are the most ubiquitous members of the B7 ligand family but are not currently targets for immune checkpoint blockade. B7-H3, another member of the B7 ligand family, is a target currently under active investigation for antibody-based immunotherapy, as it has been found to be expressed in many different cancer types but has a limited expression in normal tissues (31). Next steps may be to further study this ligand expression in pituitary adenomas. Furthermore, our data suggest that it is possible that CD80, CD86, and the CTLA-4-dependent tumor immune escape is involved in the development of tumor aggressiveness, a hypothesis that also merits further study.

Despite the success of immune checkpoint blockade, there remain significant challenges in predicting which patients will respond to therapy. Correlating treatment response with immune checkpoint expression remains an active area of investigation and some studies have shown that anti-PD-1/PD-L1 drugs lead to improved outcomes in patients harboring tumors with high PD-1/PD-L1 expression (32, 33). In addition, Van Allen et al. showed that patients who achieved clinical benefit from the anti-CTLA-4 drug ipilimumab for metastatic melanoma had higher levels of CTLA-4 and PD-L2 expression (34). Our results demonstrating increased expression of PD-L2, CD80, and CD86 in aggressive pituitary adenoma samples provide a rationale for studying whether immune checkpoint blockade is effective for tumor control. Lin et al. attributed the response of a corticotroph pituitary carcinoma to ipilimumab and nivolumab in part to the hypermutated status of the tumor (26). This group is leading a multi-center clinical trial (NCT04042753) to further investigate the efficacy of this combined immunotherapy.

There are several limitations of our work. First, the findings of our study are limited by these known challenges in correlating levels of immune checkpoint molecules with response. Furthermore, our data included small sample sizes, particularly in sub-groups of functioning adenomas. Given promising results in the role of immune checkpoint therapy for corticotroph adenomas, a larger analysis of ACTH-secreting tumors would be valuable.

Overall, our findings indicate that there is a significant immunologic profile difference between pituitary adenomas and normal pituitary as well as between clinically aggressive pituitary adenomas and non-aggressive pituitary adenomas. Our results suggest a possible role of immune checkpoint pathways in pituitary adenoma tumorigenesis and growth and also support a potential role for immune checkpoint blockade in pituitary adenomas that prove difficult to control with standard therapies. The increased expression of the ligands for PD-1 and CLTA-4 in human pituitary tumors suggests that immunotherapeutic antibodies such as ipilimumab and nivolumab may be able to directly target clinically aggressive pituitary tumors resistant to therapy. In addition, although we had small numbers of tumor samples on which to base any firm conclusions, our data suggest that corticotroph tumors may be particularly targetable by these agents.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Mass General Brigham IRB Mass General Brigham 399 Revolution Drive, Suite 710 Somerville, MA 02145 Tel: 857-282-1900 Fax: 857-282-5693. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

ZX: performing experiments, data analysis, and manuscript preparation. PJ: project designed and discussion, data analysis, and manuscript preparation. MM: data analysis and discussion. XJ: performing experiments, and data analysis. AF: project designed and discussion. CN: performing experiments, and data analysis. KL: performing experiments. YZ: project designed and discussion. KM: project designed and discussion, and data analysis. RS: project designed and discussion. XZ: project designed and discussion, performing experiments, data analysis, and manuscript preparation. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported in part by the National Institutes of Health (R01 CA193520), the Jarislowsky Foundation, and a gift from Tom and Siobhan Quinn.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2021.726448/full#supplementary-material

REFERENCES

1. Raappana A, Koivukangas J, Ebeling T, Pirilä T. Incidence of Pituitary Adenomas in Northern Finland in 1992–2007. *J Clin Endocrinol Metab* (2010) 95:4268–75. doi: 10.1210/jc.2010-0537
2. Fernandez A, Karavitaki N, Wass JAH. Prevalence of Pituitary Adenomas: A Community-Based, Cross-Sectional Study in Banbury (Oxfordshire, Uk). *Clin Endocrinol* (2010) 72:377–82. doi: 10.1111/j.1365-2265.2009.03667.x
3. Hauser BM, Lau A, Gupta S, Bi WL, Dunn IF. The Epigenomics of Pituitary Adenoma. *Front Endocrinol* (2019) 10:290. doi: 10.3389/fendo.2019.00290
4. Casanueva FF, Molitch ME, Schlechte JA, Abs R, Bonert V, Bronstein MD, et al. Guidelines of the Pituitary Society for the Diagnosis and Management of Prolactinomas. *Clin Endocrinol* (2006) 65:265–73. doi: 10.1111/j.1365-2265.2006.02562.x
5. Molitch ME. Diagnosis and Treatment of Pituitary Adenomas: A Review. *JAMA* (2017) 317:516–24. doi: 10.1001/jama.2016.19699
6. Raverot G, Burman P, McCormack A, Heaney A, Petersenn S, Popovic V, et al. European Society of Endocrinology Clinical Practice Guidelines for the Management of Aggressive Pituitary Tumours and Carcinomas. *Eur J Endocrinol* (2018) 178:G1–G24. doi: 10.1530/eje-17-0796
7. Gejman R, Swearingen B, Hedley-Whyte ET. Role of Ki-67 Proliferation Index and P53 Expression in Predicting Progression of Pituitary Adenomas. *Hum Pathol* (2008) 39:758–66. doi: 10.1016/j.humpath.2007.10.004
8. Drummond J, Roncaroli F, Grossman AB, Korbonits M. Clinical and Pathological Aspects of Silent Pituitary Adenomas. *The Journal of Clinical Endocrinology & Metabolism* (2019) 104:2473–89. doi: 10.1210/jc.2018-00688
9. Lin AL, Donoghue MTA, Wardlaw SL, Yang TJ, Bodei L, Tabar V, et al. Approach to the Treatment of a Patient With an Aggressive Pituitary Tumor. *J Clin Endocrinol Metab* (2020) 105:3807–20. doi: 10.1210/clinem/dgaa649
10. Friedrich M, Jasinski-Bergner S, Lazaridou M-F, Subbarayan K, Massa C, Tretbar S, et al. Tumor-Induced Escape Mechanisms and Their Association With Resistance to Checkpoint Inhibitor Therapy. *Cancer Immunol Immunother* (2019) 68:1689–700. doi: 10.1007/s00262-019-02373-1
11. Nishimura H, Okazaki T, Tanaka Y, Nakatani K, Hara M, Matsumori A, et al. Autoimmune Dilated Cardiomyopathy in PD-1 Receptor-Deficient Mice. *Science* (2001) 291:319–22. doi: 10.1126/science.291.5502.319
12. Topalian SL, Drake CG, Pardoll DM. Targeting the PD-1/B7-H1(PD-L1) Pathway to Activate Anti-Tumor Immunity. *Curr Opin Immunol* (2012) 24:207–12. doi: 10.1016/j.coi.2011.12.009
13. Taube JM, Klein A, Brahmer JR, Xu H, Pan X, Kim JH, et al. Association of PD-1, PD-1 Ligands, and Other Features of the Tumor Immune Microenvironment With Response to Anti–PD-1 Therapy. *Clin Cancer Res* (2014) 20:5064–74. doi: 10.1158/1078-0432.ccr-13-3271
14. Rowshanravan B, Halliday N, Sansom DM. CTLA-4: A Moving Target in Immunotherapy. *Blood* (2018) 131:58–67. doi: 10.1182/blood-2017-06-741033
15. Zou W, Chen L. Inhibitory B7-Family Molecules in the Tumour Microenvironment. *Nat Rev Immunol* (2008) 8:467–77. doi: 10.1038/nri2326
16. Cha J-H, Chan L-C, Li C-W, Hsu JL, Hung M-C. Mechanisms Controlling PD-L1 Expression in Cancer. *Mol Cell* (2019) 76:359–70. doi: 10.1016/j.molcel.2019.09.030
17. Zak KM, Grudnik P, Magiera K, Dömling A, Dubin G, Holak TA. Structural Biology of the Immune Checkpoint Receptor PD-1 and Its Ligands PD-L1/PD-L2. *Structure* (2017) 25:1163–74. doi: 10.1016/j.str.2017.06.011
18. Schadendorf D, Ascierto PA, Haanen J, Espinosa E, Demidov L, Garbe C, et al. Safety and Efficacy of Nivolumab in Challenging Subgroups With Advanced Melanoma Who Progressed on or After Ipilimumab Treatment: A Single-Arm, Open-Label, Phase II Study (CheckMate 172). *Eur J Cancer* (2019) 121:144–53. doi: 10.1016/j.ejca.2019.08.014
19. Möhn N, Beutel G, Gutzmer R, Ivanyi P, Satzger I, Skripuletz T. Neurological Immune Related Adverse Events Associated With Nivolumab, Ipilimumab, and Pembrolizumab Therapy-Review of the Literature and Future Outlook. *J Clin Med Res* (2019) 8(11):1777. doi: 10.3390/jcm8111777
20. Naumann RW, Wendel Naumann R, Hollebecque A, Meyer T, Devlin M-J, Oaknin A, et al. Safety and Efficacy of Nivolumab Monotherapy in Recurrent or Metastatic Cervical, Vaginal, or Vulvar Carcinoma: Results From the Phase I/II CheckMate 358 Trial. *J Clin Oncol* (2019) 37:2825–34. doi: 10.1200/jco.19.00739
21. Kato K, Cho BC, Takahashi M, Okada M, Lin C-Y, Chin K, et al. Nivolumab *Versus* Chemotherapy in Patients With Advanced Oesophageal Squamous Cell Carcinoma Refractory or Intolerant to Previous Chemotherapy (ATTRACTION-3): A Multicentre, Randomised, Open-Label, Phase 3 Trial. *Lancet Oncol* (2019) 20:1506–17. doi: 10.1016/S1470-2045(19)30626-6
22. Rappold PM, Silagy AW, Kotecha RR, Hakimi AA. Immune Checkpoint Blockade in Renal Cell Carcinoma. *J Surg Oncol* (2021) 123:739–50. doi: 10.1002/jso.26339
23. Rausch MP, Hastings KT. Immune Checkpoint Inhibitors in the Treatment of Melanoma: From Basic Science to Clinical Application. In: WH Ward, JM Farma, editors. *Cutaneous Melanoma: Etiology and Therapy*. Brisbane (AU): Codon Publications (2017).
24. Wang P-F, Wang T-J, Yang Y-K, Yao K, Li Z, Li YM, et al. The Expression Profile of PD-L1 and CD8 Lymphocyte in Pituitary Adenomas Indicating for Immunotherapy. *J Neurooncol* (2018) 139:89–95. doi: 10.1007/s11060-018-2844-2
25. Mei Y, Bi WL, Greenwald NF, Du Z, Agar NYR, Kaiser UB, et al. Increased Expression of Programmed Death Ligand 1 (PD-L1) in Human Pituitary Tumors. *Oncotarget* (2016) 7:76565–76. doi: 10.18632/oncotarget.12088
26. Lin AL, Jonsson P, Tabar V, Yang TJ, Cuaron J, Beal K, et al. Marked Response of a Hypermutated ACTH-Secreting Pituitary Carcinoma to Ipilimumab and Nivolumab. *J Clin Endocrinol Metab* (2018) 103:3925–30. doi: 10.1210/jc.2018-01347
27. Baghban R, Roshangar L, Jahanban-Esfahlan R, et al. Tumor Microenvironment Complexity and Therapeutic Implications at a Glance *Cell Commun Signal* (2020) 18:59. doi: 10.1186/s12964-020-0530-4
28. Chen F, Zhuang X, Lin L, Yu P, Wang Y, Shi Y, et al. New Horizons in Tumor Microenvironment Biology: Challenges and Opportunities. *BMC Med* (2015) 13:45. doi: 10.1186/s12916-015-0278-7
29. Whiteside TL. The Tumor Microenvironment and Its Role in Promoting Tumor Growth. *Oncogene* (2008) 27:5904–12. doi: 10.1038/onc.2008.271
30. Kemeny HR, Elsamadicy AA, Harrison Farber S, Champion CD, Lorrey SJ, Chongsathidkiet P, et al. Targeting PD-L1 Initiates Effective Antitumor Immunity in a Murine Model of Cushing Disease. *Clin Cancer Res* (2020) 26:1141–51. doi: 10.1158/1078-0432.ccr-18-3486
31. Kontos F, Michelakos T, Kurokawa T, Sadagopan A, Schwab JH, Ferrone CR, et al. B7-H3: An Attractive Target for Antibody-Based Immunotherapy. *Clin Cancer Res* (2021) 27:1227–35. doi: 10.1158/1078-0432.CCR-20-2584
32. Rosenberg JE, Hoffman-Censits J, Powles T, van der Heijden MS, Balar AV, Necchi A, et al. Atezolizumab in Patients With Locally Advanced and Metastatic Urothelial Carcinoma Who Have Progressed Following Treatment With Platinum-Based Chemotherapy: A Single-Arm, Multicentre, Phase 2 Trial. *Lancet* (2016) 387:1909–20. doi: 10.1016/s0140-6736(16)00561-4
33. Herbst RS, Soria J-C, Kowanetz M, Fine GD, Hamid O, Gordon MS, et al. Predictive Correlates of Response to the Anti-PD-L1 Antibody MPDL3280A in Cancer Patients. *Nature* (2014) 515:563–7. doi: 10.1038/nature14011
34. Van Allen EM, Miao D, Schilling B, Shukla SA, Blank C, Zimmer L, et al. Genomic Correlates of Response to CTLA-4 Blockade in Metastatic Melanoma. *Science* (2015) 350:207–11. doi: 10.1126/science.aad0095

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Endocrinology

REVIEW
published: 15 November 2021
doi: 10.3389/fendo.2021.725014

Treatment of Aggressive Pituitary Adenomas: A Case-Based Narrative Review

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Management of aggressive pituitary adenomas is challenging due to a paucity of rigorous evidence supporting available treatment approaches. Recent guidelines emphasize the need to maximize standard therapies as well as the use of temozolomide and radiation therapy to treat disease recurrence. However, often these adenomas continue to progress over time, necessitating the use of additional targeted therapies which also impact quality of life and long-term outcomes. In this review, we present 9 cases of aggressive pituitary adenomas to illustrate the importance of a multidisciplinary, individualized approach. The timing and rationale for surgery, radiation therapy, temozolomide, somatostatin receptor ligands, and EGFR, VEGF, and mTOR inhibitors in each case are discussed within the context of evidence-based guidelines and clarify strategies for implementing an individualized approach in the management of these difficult-to-treat-adenomas.

Keywords: pituitary adenomas, surgery, radiation therapy, temozolomide, targeted therapy, aggressive adenomas

OPEN ACCESS

Edited by:
Monica Livia Gheorghiu, Carol Davila University of Medicine and Pharmacy, Romania

Reviewed by:
Greisa Vila, Medical University of Vienna, Austria
Jacqueline Trouillas, Université Claude Bernard Lyon 1, France

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Specialty section:
This article was submitted to Pituitary Endocrinology, a section of the journal Frontiers in Endocrinology

Received: *14 June 2021*
Accepted: *28 October 2021*
Published: *15 November 2021*

Citation:
Cooper O, Bonert V, Liu NA and Mamelak AN (2021) Treatment of Aggressive Pituitary Adenomas: A Case-Based Narrative Review. Front. Endocrinol. 12:725014. doi: 10.3389/fendo.2021.725014

INTRODUCTION

Pituitary adenomas are benign, slow-growing tumors that typically respond to standard surgical and medical therapies, and only ~0.2% become malignant (1, 2). However, there is an intermediate stage, in which benign pituitary adenomas follow a more aggressive clinical course. Although they do not metastasize, these adenomas may be large and may be invasive, showing Knosp scores of 3-4 and invasion of the sphenoid sinus and other vital structures. They also may recur at early timepoints and do not respond to multiple therapies, with >20% exhibiting persistent growth despite optimal medical, surgical, and radiotherapy (3). In these patients, escalated treatment strategies beyond standard therapies are required to control continued tumor growth and prevent tumor-associated local and/or systemic morbidities.

Overall, patients harboring aggressive adenomas require multidisciplinary focused expert care by a team dedicated to pituitary disorders (4). In this review, we present 9 cases of aggressive pituitary adenomas treated at the Cedars-Sinai Pituitary Center, a tertiary referral center with a multidisciplinary care team in line with the recommended structure for a Pituitary Tumor Center of Excellence (4). We defined aggressive adenomas as radiologically invasive adenomas with unusually rapid growth rate or clinically relevant growth despite optimal standard therapies (3). We aim to illustrate the importance of a multidisciplinary, individualized treatment approach. We discuss these cases within the context of current evidence-based guidelines, highlighting critical

issues in determining the appropriate time to intervene and in weighing patient- and disease-specific factors that influence treatment selection.

SUMMARY OF CASES

The 9 patients with aggressive adenomas included 7 males and 2 females, ranging in age from 20 to 56 years, diagnosed with 3 silent corticotroph adenomas (SCAs) that later converted to Cushing disease (CD); with one becoming a carcinoma, 1 CD; 1 null cell carcinoma; and 4 prolactinomas, of which one progressed to a pituitary carcinoma. There were no cases of aggressive growth hormone (GH)-secreting adenomas;. Initial adenoma size ranged from 19 to 90 mm (**Table 1**). Pathologic features are described in **Table 2**. Of note, SF-1 immunostaining was not available (**Table 1**).

The 4 patients with prolactinomas were treated initially with the dopamine agonist (DA) cabergoline, and the remaining adenomas were treated initially with surgery. Recurrences were defined as growth of the residual adenoma after subtotal resection or *de novo* recurrence after gross total resection. One patient developed a *de novo* recurrence while the remainder experienced residual adenoma growth. Eight of the 9 patients had more than one recurrence: 2 had 2 recurrences, 3 had 3 recurrences, 1 had 5 recurrences, and 2 had 6 recurrences. Time to first recurrence ranged from 1 to 9 years, with 4 patients exhibiting recurrence at 1 year, 2 patients at 2 years, and 1 patient each recurring at 5 years and 9 years. Duration of disease ranged from 8 to 21 years; 2 patients are deceased.

MAXIMIZING STANDARD MEDICAL THERAPY FOR FUNCTIONING ADENOMAS

For patients with apparently aggressive functioning adenomas, standard medical therapies should be optimized to ensure that continued adenoma growth is not due to under-dosing (3). For prolactinomas, cabergoline should be administered at a dose of least 3.5 mg a week, and PRL normalization has been reported with doses as high as 11 mg a week (5, 6). For CD, pituitary-directed therapy with the SRL pasireotide can be attempted to decrease ACTH and control adenoma growth (7). When maximal doses have been unsuccessful in controlling disease, further treatment options should be considered.

In the cases presented here, the 4 patients with prolactinomas received maximally tolerated doses of cabergoline ranging from 10.5 to 14 mg a week, although the adenomas continued to progress and PRL levels remained elevated, necessitating additional therapeutic interventions. In one case of an SCA that converted to florid CD, the patient received several medications, including ketoconazole, metyrapone, and mitotane, but hypercortisolism remained uncontrolled and she ultimately underwent bilateral adrenalectomy. The other CD patients underwent surgical resection of the recurrent adenoma without receiving prior medical therapy.

SURGICAL RESECTION

MRI evidence of total adenoma removal after initial surgery correlates with long-term disease-free survival in more than 90% of patients among patients with non-functioning adenomas (NFAs) (8–10). Even when residual tumor tissue is visible on postoperative MRI, approximately 60% to 70% of adenomas will show little evidence of regrowth over a period of approximately 10 years (11, 12); in these cases, regrowth occurs at a rate of approximately 1 mm per year. For functioning adenomas, recurrence of hormonal hypersecretion can occur in up to 25% of patients with CD and up to 40% of patients with prolactinomas at 5 years (13–15), for which re-operation or medical therapy is often effective.

By contrast, for patients with refractory adenomas, surgery plays a lesser role (16–19). These adenomas may invade the cavernous sinus, skull base, or intracranial structures beyond the sella and parasellar region, reducing the opportunity for gross total resection, and there is high likelihood of further growth even with extensive resection. On initial presentation, adenomas are more likely to have a soft texture and a pseudo-capsule, which is a thin, non-fibrotic band of tissue that surrounds and encapsulates the tissue, separating the tumor from the normal gland and other structures, allowing for "extracapsular" removal of the entire mass. However, recurrent adenomas are far more fibrotic and poorly encapsulated (20, 21), which, when combined with previous surgical scarring, makes surgical debulking far more challenging. Nonetheless, particularly for rapidly growing adenomas, reoperation can be considered to reduce mass effect, i.e., to decompress critical structures such as the pituitary gland or optic apparatus, in anticipation of subsequent radiation therapy (RT) or chemotherapy (22).

In general, there is no absolute limit to the number of times transsphenoidal surgery or craniotomy can be attempted, although potential benefits are typically diminished after 3-4 surgeries due to progression of tumor into areas in which surgical resection is difficult or tumor texture renders meaningful debulking impossible.

Among the 9 cases of aggressive adenoma, 4 patients underwent 3 surgeries, 3 had 4 surgeries, 1 patient had 2 surgeries, and 1 underwent surgery after progression on temozolomide (TMZ). While surgery was successful in debulking the adenoma in all cases, gross total resection was not achieved, and the residual adenoma continued to grow. This pattern of subtotal resection and growth of postoperative residual adenoma is typical of aggressive adenomas.

RADIATION THERAPY

RT has proven reliable when surgical resection is deemed not feasible or when medical therapy is no longer efficacious or

TABLE 1 | Summary of cases.

Case	Year of Dx	Age at Dx	Sex	Type	Features*	Recurrences	Initial therapy	Surgery	RT	Medical therapy	Follow-up	Disease duration
1	1997	45	M	SCA→CD	25 mm, right cavernous sinus invasion	6	TSS	1997 TSS 2012 TSS 2015 TSS	1998 GK SRS 2010 CK SRS 2015 FRT 2016 Proton SRS	2013: TMZ x 7 cycles: PR 2013: Pasireotide x 1yr: SD 2017: Bevacizumab x 3 mo: PD	2017: Transformed to carcinoma with cerebellar and cervical spine metastases 2018: Deceased	21 yrs
2	2010	45	F	Null cell	19 mm, left cavernous sinus invasion	2	TSS	2010 TSS 2011 TSS 2012 TSS	2011 IMRT 2012 IMRT	2011: TMZ x 9 cycles: PD 2012: Lapatinib x 2 yrs: no recurrence	2012: Developed *de novo* orbital metastasis, consistent with transformation to carcinoma 2020: No tumor	10 yrs
3	2012	29	M	PRLoma	90 mm; left cavernous invasion, mass effect on brainstem and 4th ventricle.	5	Cab with maximum dose of 10.5 mg/week	2014 CRX 2016 TSS 2017 TSS 2019 TSS	2019 FRT	2019: TMZ x 6 cycles: SD	2020: Stable residual tumor with no evidence of metastases	8 yrs
4	2001	24	F	SCA→CD	59 mm; invasion of right cavernous sinus, Meckel's cave and prepontine cistern	6	CRX	2001 CRX 2002 TSS 2007 CRX 2008 CRX 2014 BLA	2002 GK SRS 2015 FRT/ TMZ	2014: TMZ x 4 cycles: SD 2014: Pasireotide x 2 mo: PD Ketoconazole: PD Metyrapone: PD Mitotane: PD 2016: Cabergoline: SD Pasireotide: SD	2020: Decreased tumor size, ACTH 134 with no evidence of metastases	19 yrs
5	2004	40	M	SCA→CD	24 mm; right cavernous sinus invasion, encasing carotid	2	TSS	2004 TSS 2013 TSS 2015 CRX	2013 RT 2015 RT/ TMZ	2015: TMZ x 6 cycles: PR 2016: Bevacizumab x 6 mo	2019: No visible tumor and no evidence of metastases	15 yrs
6	1996	56	M	CD	28 mm; clival invasion	3	TSS	1997 TSS 1998 TSS 2000 TSS 2013 TSS	2000 RT		2015: No sellar tumor New dural masses consistent with meningiomas	18 yrs
7	1994	54	M	PRLoma	40 mm; in anterior foramen, right infratemporal fossa, occipital condyle	3	Cab with maximum dose of 14 mg/week	1996 TSS 2005 TSS	1996 GK SRS	2006: TMZ x 2 cycles: PD	2006: Developed metastatic disease to the skull base, neck, and lymph node, consistent with carcinoma Deceased	12 yrs
8	2009	20	M	PRLoma	40 mm; invasion of bilateral cavernous sinuses, Meckel's cave	1	Cab with maximum dose of 10.5 mg/week	2021 TSS		2017: TMZ x 6 cycles: PR	2021: Tumor growth (pre-TSS) with no sign of metastatic disease	12 yrs
9	2008	36	M	PRLoma	40 mm; bilateral cavernous sinuses	3	Cab with maximum dose of 12 mg/week	2013 TSS 2014 TSS 2019 CRX	2015 RT	2020: Octreotide 2020: TMZ x 7 cycles: PR	2021: Decreased tumor size with no sign of metastatic disease	13 yrs

**Features of recurrent adenomas are listed if data were not available from initial presentation.*

ACTH, adrenocorticotropin; BLA, bilateral adrenalectomy; Cab, cabergoline; CD, Cushing disease; CK, CyberKnife; CRX, craniotomy; Dx, diagnosis; FRT, fractionated radiation therapy; GK, GammaKnife; IMRT, intensity-modulated radiation therapy; PD, progressive disease; PRL, prolactin; PRLoma, prolactinoma; PR, partial response; RT, radiation therapy; SCA, silent corticotroph adenoma; SD, stable disease; SRS, stereotactic radiosurgery; TMZ, temozolomide; TSS, transsphenoidal surgery; Yrs, years.

TABLE 2 | Pathologic data.

Case	Pathologic adenoma type	Ki-67	Mitotic count	p53
1	Corticotroph	5%	Not increased	Weakly positive
2	Null cell	10%	Many mitotic figures	positive
3	Densely granulated lactotroph	0.5%	Not increased	60% positive (1-2+)
4	Null cell (initially)	Moderately high	Not increased	N/A
5	Densely granulated corticotroph	20%	rare	80% positive
6	Densely granulated corticotroph	1%	Not increased	< 0.5%
7	Lactotroph	N/A	N/A	N/A
8*	N/A	N/A	N/A	N/A
9	Densely granulated lactotroph	9%	Not increased	< 0.1% (1+)

Patient underwent TSS for drainage of cyst. No viable tumor was visualized or resected; pathologic analysis therefore is not available (N/A).

tolerated (23–26). Treatment doses in the range of 12-15 Gy delivered in a single fraction or in multiple fractions can prevent further adenoma growth in more than 95% of adenomas. However, rates of radiation-induced hypopituitarism are quite significant, with at least 25% and up to 90% of patients developing a degree of anterior pituitary hormone deficiency within 10 years (25, 27, 28). In addition, other factors such as patient age, symptoms, adenoma aggressivity, and adenoma type must be taken into consideration when weighing treatment benefit and timing. RT also can be used to reduce hormone secretion in nonresectable functioning adenomas that have failed to respond to medical therapy or that demonstrate feedback-induced adenoma growth (e.g., Nelson's syndrome in patients with progressive CD) (29–31).

A role for repeat irradiation of adenomas that progress or recur despite previous RT is far more limited due to substantial risk of radiation necrosis to adjacent brain structures or breakdown of the carotid artery wall leading to catastrophic bleeding or death (32, 33). The overlap between new and old radiation treatment fields as well as the time between radiation treatments should be considered when determining the potential risk-benefit ratio of additional RT. Improved planning software, more accurate treatment devices, and better understanding of adenoma physiology has allowed for lower radiation dose, which, in turn has resulted in reduced long-term secondary risk such as cognitive deterioration or carotid injury. Nonetheless, the absolute dose tolerance of brain and surrounding structures typically limits use to one or two sessions.

Eight of our 9 patients had RT immediately after initial surgery or after multiple surgeries: 4 patients had a single course, 3 had 2 courses, and 1 had 4 courses. Five patients initially responded to RT, demonstrating either stable disease or some decrease in size, while the remaining 3 patients had continued progressive disease despite RT. All patients showed disease recurrence/progression over time.

TEMOZOLOMIDE

TMZ demonstrates clinical efficacy in up to one-third of aggressive adenomas and its use in these cases is widely reported (34). TMZ is recommended for aggressive pituitary adenomas that grow despite surgery, RT, and optimized medical therapy (3). TMZ may also be considered in frail patients who may not tolerate repeat surgery or have relative contraindications to RT (35). However, the timing of treatment initiation, the duration of therapy, and the potential additive benefits of combining TMZ with RT or another agent all remain unknown.

The standard treatment schedule for TMZ monotherapy is 150-200 mg/m^2/day for 5 days every 4 weeks. Regimens that administer lower TMZ doses for longer periods in a cycle, such as 50 mg/m^2 daily for 21 days (36), have been associated with increased myelotoxicity (37). In patients where the combination of TMZ with RT is considered, such as in those with rapidly growing adenomas with elevated Ki-67 >10%, extensive p53 expression, and/or high mitotic count (3), TMZ can be initiated at lower doses during and then escalated after RT. For example, treatment may be administered as 6 weeks of continuous TMZ therapy at 75 mg/m^2 daily with RT, followed by 6-12 months of standard TMZ monotherapy at 150 mg/m^2 daily for 5 days every 28 days and increasing as tolerated to 200 mg/m^2 (38). Among 166 patients with aggressive pituitary adenomas treated by 67 surveyed members of the European Endocrine Society (ESE), 14 patients received concomitant TMZ and RT with complete or partial response observed in 71% compared to 34% in those on TMZ monotherapy (39), suggesting the efficacy of combination therapy.

Treatment efficacy with TMZ is typically evident by 3 months (40), although many patients may not see maximal adenoma response until 6 months (39). Of note, biochemical response often parallels response on MRI, and may even improve further once adenoma growth stabilizes (39, 41). In the ESE survey, complete radiologic response, i.e., no visible adenoma, was observed in 6% and complete biochemical response in 19% of cases, while partial radiologic response with >30% reduction in size was seen in 31% and partial biochemical response with >20% hormone reduction in 24% of cases (39). A systematic review of TMZ in aggressive pituitary adenomas and carcinomas showed similar response rates, with 6.5% of 31 aggressive adenomas achieving complete response, 45% partial response, 29% stable disease, and 19% progressive disease, while 8.7% of 23 pituitary carcinomas showed complete response, 61% partial response, 13% stable disease, and 17% progressive disease (42).

TMZ therapy is usually administered for 6 months (3), although case reports describe up to 12 months of treatment. Median treatment duration of 9 months in the ESE survey was

associated with treatment response (39). A study of 30 adenomas treated with at least 6 cycles of TMZ showed that 27% had adenoma regression, 47% stable disease, and 27% adenoma progression. After a medial follow-up of 34 months, 40% maintained stable disease and 60% had progression (41).

Longer duration of TMZ treatment has been shown to be beneficial in maintaining disease-free progression up to 120 months (42). However, it can be difficult to quantify because reports describe widely varying treatment durations. In a multicenter study, patients treated for more than 12 months with TMZ had a median relapse-free survival of 57 months versus 18 months for those treated for 12 months or less (43), suggesting the benefit with "longer-term" therapy is seen after 12 months. By contrast, the systematic review of 31 aggressive adenomas and 23 carcinomas defined "short-term" TMZ treatment as a median of 8 months (range, 1-12 cycles) and "long-term" as a median of 26 months (range, 14-45) cycles. Among patients who responded, 36% were receiving long term TMZ; 5-year progression-free survival rate was numerically but not significantly different at 61.3% for long-term TMZ compared to 16.3% for short-term TMZ, but overall survival rate was significantly improved with long-term therapy (91.7% vs 54.1%; p=0.08). Of note, 95% of patients had received prior RT and 75% were previously treated with other standard therapies (42). In a report of 8 patients with aggressive adenomas treated with TMZ for more than 12 months, 4 patients had partial response and the other 4 had stable disease; response was maintained while on TMZ. Of the 3 patients with ACTH-secreting adenomas, one showed complete remission of hypercortisolism, one with Nelson's syndrome showed >90% reduction in ACTH level, and the third showed ~40% reduction in ACTH and urinary free cortisol (44).

Based on available reports, it is reasonable to consider continued TMZ therapy beyond 6 months in patients who are responding to treatment.

The combination of TMZ with the oral chemotherapy capecitabine in the CAPTEM protocol has shown some efficacy in aggressive CD. In a report of 4 cases, 2 had complete adenoma resolution, 1 stable disease, and 1 a 75% decrease in adenoma size concomitant with ACTH reduction (45); another reported case of CAPTEM in an aggressive CD patient showed radiologic stable disease and biochemical response (39). However, use of CAPTEM in 2 corticotroph carcinomas and one lactotroph carcinoma were not successful (39, 40).

MGMT status has been proposed as a predictor of response to TMZ therapy. MGMT (O6-methyl guanine DNA methyl transferase) is a DNA repair enzyme that negates the effect of TMZ (34) and thus tumoral expression of MGMT is inversely related to TMZ efficacy (40, 46–48). However, patients with high MGMT expression may still respond to TMZ (36, 43, 49); in the ESE survey, 76% of those with low MGMT expression responded, while 46% with high MGMT expression did not (39). Therefore, TMZ should still be considered, regardless of MGMT status (3). Functioning aggressive adenomas have been shown to respond better to TMZ compared to NFAs (45% vs 17%), although the rationale is unknown (39).

A second course of TMZ is usually less effective than the initial course and response has not been consistently demonstrated (39, 43, 50). However, it may be reasonable to attempt a 3-month rechallenge in patients with late relapse and low MGMT, as a report of 9 adenomas treated with a second course of TMZ showed that late relapse after the first course was associated with improved response (34).

Eight of the 9 cases presented here were treated with TMZ. Three patients with SCA that progressed to CD were treated with 4-6 cycles of TMZ, but response varied widely, likely due to both MGMT status and prior/concurrent treatments. One patient with 95% MGMT expression showed radiologic progression after 2 cycles of TMZ. However, the addition of pasireotide enabled biochemical and radiologic response, and he completed 7 cycles of combination treatment before showing signs of relapse. The second patient underwent RT followed by 6 cycles of TMZ and experienced both biochemical and radiologic improvement. MGMT in this patient was negative. The third patient, with an unknown MGMT status, showed both biochemical and radiologic response after 2 cycles but progressed again after 4 cycles. Of 4 patients with prolactinoma, one with an unknown MGMT status showed disease progression after concurrent administration of TMZ and RT followed by TMZ monotherapy for 1 cycle. However, the other 3 patients showed sustained biochemical and radiologic response to 6 cycles of TMZ; MGMT was 1% positive in 2 cases and unknown in the other. Finally, 1 patient with a null cell adenoma underwent RT and 9 cycles of TMZ before the disease progressed. MGMT at the initial surgery and upon removal of an orbital metastasis was 98% positive.

Thus, of 8 patients treated, 2 had initial response but progressed after 4-6 cycles of TMZ, 1 did not respond at all, and 4 had partial response to TMZ.

SRL THERAPY

Clinical response of aggressive pituitary adenomas to SRLs is variable. Somatostatin receptor subtype (SST) expression and differences in SRL affinity for each SST may provide some insight into the likelihood of response. Corticotroph adenomas primarily express SST5, and octreotide and lanreotide, which have a strong affinity for SST2, are largely ineffective (51). Pasireotide, which has a stronger affinity for SST5, has been shown to normalize cortisol levels in 20-40% of CD patients (51). However, reports of aggressive CD treated with pasireotide show minimal effect (39). Of the reported 22 aggressive adenomas treated with pasireotide, only 4 (18%) adenomas showed adenoma response and 5 (23%) showed biochemical response (39, 50, 52). In a report of 3 patients with aggressive ACTH-secreting macroadenoma or carcinoma, 2 showed paradoxical increase in ACTH and urinary free cortisol with pasireotide treatment (53), while another report of 3 patients with recurrent CD after cessation of TMZ showed no effect of pasireotide (50). However, one case of an SCA showed a 6% adenoma volume decrease with TMZ and a 21% decrease after adding pasireotide,

and a CD patient showed biochemical response to pasireotide 12 months after partial response to TMZ (52). Some reports show pasireotide reduced ACTH secretion and adenoma size in some patients with Nelson's syndrome (54), but others show no response (55) or mixed response, with 5 patients in one series showing minimal impact on adenoma volume with pasireotide even though ACTH levels decreased (56).

Pasireotide was administered in 2 SCA cases that progressed to CD. In 1 patient it was added to TMZ and enabled adenoma stabilization for a year. In the second patient, it was added after bilateral adrenalectomy and subsequent RT, and has maintained stabilization of both adenoma volume and ACTH levels for 4 years.

NFAs primarily express SST3 (57). Although octreotide and lanreotide may inhibit cell proliferation in cultured NFAs (58), clinical efficacy is limited. In 39 patients with residual adenoma tissue, 81% of those with positive octreotide uptake on scintigraphy demonstrated stable adenoma volumes with octreotide LAR treatment, but none had adenoma reduction (59). In a review of 100 NFAs in 11 published studies treated with octreotide for an average of 6 months, 12% showed adenoma reduction, 83% stable residual, and 5% increased volume (60). Although pasireotide has a higher affinity for SST3 and has been shown to inhibit NFA cell viability *in vitro* (61), no clinical studies have confirmed control of adenoma growth. Of note, there was no clear association between SST expression and clinical features of aggressiveness in 113 NFAs, of which 46% were invasive (62).

Prolactinomas show poor clinical response to SRLs and treatment of cultured DA-resistant adenomas did not show reduced prolactin (PRL) levels (63). However, clinical improvement has been demonstrated in some patients with confirmed SST5 expression. In 5 patients with DA-resistant prolactinomas, treatment with octreotide LAR and cabergoline reduced PRL levels in 2 patients, with a 93% reduction in adenoma size; SST5 expression was noted in 1 responder (64). Others reported PRL and/or adenoma response in prolactinomas treated with combination cabergoline and pasireotide, including in an aggressive prolactinoma strongly positive for SST5 that demonstrated PRL normalization after 2 months that was maintained for 31 months, with >50% reduction in adenoma size (65), as well as in a cabergoline-resistant giant silent prolactinoma showing high expression of SST5 that demonstrated adenoma shrinkage with pasireotide treatment (66). However, among 47 macroprolactinomas, SST2 and SST5 were expressed in only 3/23 and 3/21 adenomas, respectively, and 1 adenoma with both subtypes (66).

Of the 4 prolactinomas in the set, 1 showed continued adenoma growth and rising PRL levels despite cabergoline dose up to 7 mg/day, multiple surgeries, and RT. Although SST5 expression was negative, he was started on octreotide LAR 30 mg monthly in an attempt to stabilize PRL levels, but PRL remained elevated and adenoma size increased.

Peptide receptor radionuclide therapy (PRRT), an SRL-based therapy in which radiolabeled SST-binding molecules target SST2 and SST5, has been evaluated in aggressive pituitary adenomas, demonstrating octreotide uptake on PET/CT. Although 50% of NFAs, 38% of prolactinomas, and 50% of CD adenomas demonstrated positive uptake with ^{111}In-octreotide scintigraphy (67), only 3 of 20 aggressive pituitary adenomas treated with PRRT, showed a partial response and 3 stable disease (reviewed in [68)].

EGFR-, VEGF-, AND MTOR-TARGETING THERAPY

Another target for aggressive pituitary adenomas is EGFR, which belongs to the ErbB family of membrane receptor kinases (69). To date, 3 EGFR tyrosine kinase inhibitors (TKIs), gefitinib, lapatinib, and canertinib, have been evaluated in the treatment of pituitary adenomas. Gefitinib selectively inhibits EGFR/ErbB1 activity, lapatinib prevents activation of EGFR/ErbB2, and canertinib is an experimental pan-ErbB receptor TKI (70).

EGFR is expressed in 75% of human corticotroph adenomas (69, 71), and gefitinib treatment of human primary pituitary corticotroph tumor cultures reduced POMC mRNA and reversed features of hypercortisolemia (71). Lapatinib treatment of corticotroph tumor cells decreased POMC mRNA and ACTH levels. Canertinib, which targets multiple ErbB receptors, suppressed POMC mRNA and ACTH secretion in cultured human corticotroph adenomas, with a 70% reduction seen in those with higher ErbB expression (72).

Gain-of-function mutations in *USP8* have been implicated in aberrant EGFR signaling in corticotrophs (73–75) and studies show *USP8* mutations in 21-62% of corticotroph adenomas (76). However, it remains unclear whether *USP8* mutations and EGFR overexpression are associated with more aggressive adenomas (73, 75, 77–79). Whole exome sequencing of corticotroph adenomas enriched with aggressive adenomas identified *USP8* mutations in only 5 of 22 adenomas (80), but another study found EGFR expression in the cytoplasm of 29 of 52 CD adenomas, and protein expression associated with recurrence (81). There are as yet no reports of EGFR-targeting therapy in patients with aggressive CD.

The strongest evidence supporting use of these agents is for aggressive prolactinomas. EGFR, ErbB2, and ErbB3 are all endogenously expressed in rat lacto-somatotroph tumor cells, and *in vitro* treatment with EGFR TKIs was shown to prevent PRL secretion (69, 82, 83). GH3 cell lines transfected with a constitutively active form of ErbB2 showed a 250-fold induction of PRL, while treatment of these cells with lapatinib led to 40% suppression of PRL secretion (82). In Wistar-Furth rats inoculated with these same transfectants, treatment with lapatinib led to a 40% suppression of adenoma growth and 50% decrease in PRL levels (82), and Fischer rats implanted with estrogen pellets to recapitulate an endogenous prolactinoma model developed pituitary adenomas with hyperprolactinemia that were suppressed by 35% with lapatinib treatment (82). More than 50% of prolactinomas express EGFR and 25% express ErbB2 protein (reviewed in (69), with 40% of invasive adenomas staining positive for ErbB2, compared to 1.2% of

noninvasive adenomas (84). However, rates of ErbB receptor expression were even higher in 28 human prolactinoma specimens analyzed (85), with approximately 80% expressing ErbB receptors, including EGFR (82%), ErbB2 (92%), ErbB3 (25%), and ErbB4 (71%). Higher ErbB3 receptor expression correlated with higher rates of optic chiasm compression, suprasellar extension, and encasement of the carotids, while higher ErbB4 was observed in adenomas with sphenoid sinus invasion (85).

In a 6-month proof-of-concept clinical trial, patients with DA-resistant prolactinomas were treated with lapatinib. One subject demonstrated near normalization of PRL and a 22% reduction in adenoma volume after showing continued adenoma growth and PRL elevation on cabergoline 7 mg/week and surgical adenoma resection; a second subject with persistent hyperprolactinemia and adenoma growth on cabergoline 7 mg/week and surgical resection achieved a 42% reduction in PRL and tumor size stabilization (85).

In a follow-up phase IIa trial, 3 of 4 patients with aggressive prolactinomas treated with 6 months of lapatinib showed stable radiologic disease, with 1 subject showing up to 17% decrease in diameter and a 42% decrease in PRL (86). EGFR expression did not correlate with response to lapatinib therapy.

The 1 patient with a null cell adenoma was treated for 2 years with lapatinib following surgery and RT for an orbital metastasis to help prevent recurrence. After 8 years, she continues to show no signs of recurrence. Her adenoma was positive for EGFR and ErbB2 on immunohistochemistry.

VEGF, expressed in pituitary adenomas, stimulates angiogenesis and regulates the tumor microenvironment to contribute to pituitary tumorigenesis (43, 87, 88). However, treatment of aggressive pituitary adenomas with bevacizumab, a monoclonal antibody binding VEGFR, has yielded mixed results. The ESE survey reported adenoma stabilization in 1 of 3 patients on bevacizumab monotherapy, with another showing partial adenoma regression after combination therapy with TMZ, while a third showed adenoma progression (39). Treatment with bevacizumab after multiple prior therapies including temozolomide in 2 patients with corticotroph adenomas showed decreased ACTH after the first cycle but no longer-term benefit, while 1 patient with NFA showed disease stabilization for 18 months (89).

VEGF-targeting agents in combination with RT and TMZ demonstrated stabilization of a silent corticotroph carcinoma (90), as well as prolonged recurrence-free survival after treatment with RT, TMZ, and bevacizumab (91); progression-free survival for 8 years was observed after RT, TMZ, and bevacizumab in a corticotroph carcinoma with CNS metastases (92). Treatment of 17 pituitary carcinoma patients who recurred after TMZ use also showed some response to bevacizumab monotherapy or combination therapy, including 1 patient with a null cell carcinoma who remains on bevacizumab (93).

Bevacizumab was attempted in 2 patients in our series, both of whom have SCAs that progressed to CD. One had failed multiple courses of RT and showed spinal and dural metastases when he was started on bevacizumab. After 3 months on therapy, he developed cognitive decline, transitioned to hospice, and died. The other patient was started on bevacizumab after undergoing 3 surgeries and 2 courses of RT as well as TMZ. Treatment was given for 6 cycles and he remains recurrence-free 15 years after his initial diagnosis. VEGF expression status was unknown.

The mTOR inhibitor everolimus, approved for the treatment of neuroendocrine tumors, exhibits antiproliferative and proapoptotic activity in pituitary adenomas (94). Clinical response to everolimus is variable, with reports describing partial response in a prolactinoma and stable disease in a corticotroph carcinoma, but progressive disease in 4 other cases of aggressive adenomas (39, 95–97) (**Table 3**).

SUMMARY OF TREATMENT OPTIONS

Treatment of aggressive pituitary adenomas is challenging. The rarity of the disorder, heterogeneity of aggressive adenomas, the lack of consistent response to treatment options, and limited experience on when and how to initiate each treatment modality all underscore the need to individualize management. The cases presented here illustrate the importance of applying an individualized, multidisciplinary approach within the context of current evidence-based guidelines (**Table 4**).

Surgical resection and repeat adenoma debulking are recommended where feasible, especially when mass effects are evident. However, rapid re-growth of residual adenoma tissue requires use of additional therapy. RT, although not curative, may stabilize adenoma growth for months to years, with eventual progression. TMZ is effective in most patients, but responses may be partial and not sustained. Repeat treatment with TMZ is often less effective. Other medical therapies, including SRLs and EGFR-, VEGF-, and mTOR-targeting agents, may stabilize adenoma size and maintain biochemical control in patients with functioning adenomas, but evidence for this is limited. It

TABLE 3 | Summary of responses to targeted medical therapies.

Therapy	N	Tumor response	Biochemical response
EGFR-targeting gefitinib, lapatinib, canertinib	8	25% (n=2) decrease 50% (n=4) stable	38% (n=3)
VEGF-targeting bevacizumab	15	26% (n=4) stable	25% (n =2)
mTOR-targeting everolimus	7	14% (n=1) partial; 14% (n=1) stable	

Data from McCormack et al, 2018 (39), Cooper et al, 2021 (86), Osterhage et al, 2021 (89), Ortiz et al, 2012 (90), Touma et al, 2017 (91), Donovan et al, 2016 (96), and Zhang et al, 2019 (97).

TABLE 4 | Summary of treatment options.

Treatment option	Clinical Considerations
Surgery	• Total gross resection and/or adenoma debulking preferred where feasible • No upper limit on number of surgeries, although scarring and adenoma location and texture may limit benefits after 3-4
RT	• Typically highly effective in controlling nonresectable adenoma growth • For repeat irradiation, careful planning is required to avoid injury to surrounding structures
TMZ	• Recommended for adenoma that grow despite surgery, RT, and optimized medical therapy • Most common regimens: standard dose for 6 cycles if monotherapy, lower dose if given with RT • Rechallenge after progression is typically less effective • Unclear whether MGMT status is useful in predicting response • Longer duration of therapy may sustain response
SRL	• Octreotide and lanreotide typically not effective • Pasireotide has limited effect in aggressive CD, may be effective in prolactinoma • Unclear whether SST5 expression is useful in predicting response
EGFR-targeting	• Best evidence for efficacy is with lapatinib in prolactinoma • Optimal timing of initiation and treatment duration are unclear
VEGF-targeting	• Bevacizumab shows mixed results • Combination with RT and/or TMZ may be effective for pituitary carcinomas or some aggressive CD converted from SCA

EGFR, epidermal growth factor receptor; CD, Cushing disease; MGMT, O6-methylguanine-DNA methyltransferase; PRLoma, prolactinoma; RT, radiation therapy; SRL, somatostatin receptor ligand; SST, somatostatin receptor; TMZ, temozolomide; VEGF, vascular endothelial growth factor.

is unclear whether initiation of these therapies before adenoma growth is evident may help delay progression, allowing TMZ therapy to be 'reserved' when deemed clinically necessary. Identification of actionable molecular targets in aggressive adenomas may further help determine whether other kinase inhibitors and checkpoint inhibitors can be of additional benefit (80, 98). Importantly, regardless of whether an adenoma is defined as aggressive, some subtypes may show aggressive behavior and may warrant use of aggressive treatment strategies. These include SCA that evolve to clinically apparent CD, and lactotroph tumors in males that show resistance to dopamine agonist therapy. Continued study of aggressive pituitary adenomas is essential to establishing a treatment paradigm that maintains adenoma control while optimizing patient quality of life.

AUTHOR CONTRIBUTIONS

OC and AM conceptualized the project, performed the literature search and data analysis, and drafted the article. OC, VB, and NAL contributed case discussions. All authors contributed to the article and approved the submitted version.

REFERENCES

1. Lopes MBS. The 2017 World Health Organization Classification of Tumors of the Pituitary Gland: A Summary. *Acta Neuropathol* (2017) 134(4):521–35. doi: 10.1007/s00401-017-1769-8
2. Melmed S. Pituitary-Tumor Endocrinopathies. *N Engl J Med* (2020) 382 (10):937–50. doi: 10.1056/NEJMra1810772
3. Raverot G, Burman P, McCormack A, Heaney A, Petersenn S, Popovic V, et al. European Society of Endocrinology Clinical Practice Guidelines for the Management of Aggressive Pituitary Tumours and Carcinomas. *Eur J Endocrinol* (2018) 178(1):G1–G24. doi: 10.1530/EJE-17-0796
4. Casanueva FF, Barkan AL, Buchfelder M, Klibanski A, Laws ER, Loeffler JS, et al. Criteria for the Definition of Pituitary Tumor Centers of Excellence (PTCOE): A Pituitary Society Statement. *Pituitary* (2017) 20(5):489–98. doi: 10.1007/s11102-017-0838-2
5. Melmed S, Casanueva FF, Hoffman AR, Kleinberg DL, Montori VM, Schlechte JA, et al. Diagnosis and Treatment of Hyperprolactinemia: An Endocrine Society Clinical Practice Guideline. *J Clin Endocrinol Metab* (2011) 96(2):273–88. doi: 10.1210/jc.2010-1692
6. Ono M, Miki N, Kawamata T, Makino R, Amano K, Seki T, et al. Prospective Study of High-Dose Cabergoline Treatment of Prolactinomas in 150 Patients. *J Clin Endocrinol Metab* (2008) 93(12):4721–7. doi: 10.1210/jc.2007-2758
7. Nieman LK, Biller BM, Findling JW, Murad MH, Newell-Price J, Savage MO, et al. Treatment of Cushing's Syndrome: An Endocrine Society Clinical Practice Guideline. *J Clin Endocrinol Metab* (2015) 100(8):2807–31. doi: 10.1210/jc.2015-1818
8. Gondim JA, Schops M, de Almeida JP, de Albuquerque LA, Gomes E, Ferraz T, et al. Endoscopic Endonasal Transsphenoidal Surgery: Surgical Results of 228 Pituitary Adenomas Treated in a Pituitary Center. *Pituitary* (2010) 13 (1):68–77. doi: 10.1007/s11102-009-0195-x
9. Ludecke DK, Abe T. Transsphenoidal Microsurgery for Newly Diagnosed Acromegaly: A Personal View After More Than 1,000 Operations. *Neuroendocrinology* (2006) 83(3-4):230–9. doi: 10.1159/000095533
10. Mamelak AN, Carmichael J, Bonert VH, Cooper O, Melmed S. Single-Surgeon Fully Endoscopic Endonasal Transsphenoidal Surgery: Outcomes in Three-Hundred Consecutive Cases. *Pituitary* (2013) 16(3):393–401. doi: 10.1007/s11102-012-0437-1
11. Dekkers OM, Hammer S, de Keizer RJ, Roelfsema F, Schutte PJ, Smit JW, et al. The Natural Course of Non-Functioning Pituitary Macroadenomas. *Eur J Endocrinol* (2007) 156(2):217–24. doi: 10.1530/eje.1.02334
12. Dekkers OM, Pereira AM, Roelfsema F, Voormolen JH, Neelis KJ, Schroijen MA, et al. Observation Alone After Transsphenoidal Surgery for Nonfunctioning Pituitary Macroadenoma. *J Clin Endocrinol Metab* (2006) 91(5):1796–801. doi: 10.1210/jc.2005-2552
13. Burke WT, Penn DL, Castlen JP, Donoho DA, Repetti CS, Iuliano S, et al. Prolactinomas and Nonfunctioning Adenomas: Preoperative Diagnosis of Tumor Type Using Serum Prolactin and Tumor Size. *J Neurosurg* (2020) 133 (2)321–328. doi: 10.3171/2019.3JNS19121
14. Rees DA, Hanna FW, Davies JS, Mills RG, Vafidis J, Scanlon MF. Long-Term Follow-Up Results of Transsphenoidal Surgery for Cushing's Disease in a

Single Centre Using Strict Criteria for Remission. *Clin Endocrinol (Oxf)* (2002) 56(4):541–51. doi: 10.1046/j.1365-2265.2002.01511.x
15. Roelfsema F, Biermasz NR, Pereira AM. Clinical Factors Involved in the Recurrence of Pituitary Adenomas After Surgical Remission: A Structured Review and Meta-Analysis. *Pituitary* (2012) 15(1):71–83. doi: 10.1007/s11102-011-0347-7
16. Benveniste RJ, King WA, Walsh J, Lee JS, Delman BN, Post KD. Repeated Transsphenoidal Surgery to Treat Recurrent or Residual Pituitary Adenoma. *J Neurosurg* (2005) 102(6):1004–12. doi: 10.3171/jns.2005.102.6.1004
17. Kurosaki M, Luedecke DK, Abe T. Effectiveness of Secondary Transnasal Surgery in GH-Secreting Pituitary Macroadenomas. *Endocr J* (2003) 50 (5):635–42. doi: 10.1507/endocrj.50.635
18. Almeida JP, Ruiz-Trevino AS, Liang B, Omay SB, Shetty SR, Chen YN, et al. Reoperation for Growth Hormone-Secreting Pituitary Adenomas: Report on an Endonasal Endoscopic Series With a Systematic Review and Meta-Analysis of the Literature. *J Neurosurg* (2018) 129(2):404–16. doi: 10.3171/2017.2.JNS162673
19. Delgado-Lopez PD, Pi-Barrio J, Duenas-Polo MT, Pascual-Llorente M, Gordon-Bolanos MC. Recurrent Non-Functioning Pituitary Adenomas: A Review on the New Pathological Classification, Management Guidelines and Treatment Options. *Clin Transl Oncol* (2018) 20(10):1233–45. doi: 10.1007/s12094-018-1868-6
20. Galm BP, Martinez-Salazar EL, Swearingen B, Torriani M, Klibanski A, Bredella MA, et al. MRI Texture Analysis as a Predictor of Tumor Recurrence or Progression in Patients With Clinically Non-Functioning Pituitary Adenomas. *Eur J Endocrinol* (2018) 179(3):191–8. doi: 10.1530/EJE-18-0291
21. Wei L, Lin SA, Fan K, Xiao D, Hong J, Wang S. Relationship Between Pituitary Adenoma Texture and Collagen Content Revealed by Comparative Study of MRI and Pathology Analysis. *Int J Clin Exp Med* (2015) 8(8):12898–905.
22. Forster N, Warnick R, Takiar V, Pater L, Breneman J. Debulking Surgery of Pituitary Adenoma as a Strategy to Facilitate Definitive Stereotactic Radiosurgery. *J Neurooncol* (2018) 138(2):335–40. doi: 10.1007/s11060-018-2801-0
23. Minniti G, Flickinger J. The Risk/Benefit Ratio of Radiotherapy in Pituitary Tumors. *Best Pract Res Clin Endocrinol Metab* (2019) 33(2):101269. doi: 10.1016/j.beem.2019.04.003
24. Heringer LC, Machado de Lima M, Rotta JM, Botelho RV. Effect of Stereotactic Radiosurgery on Residual or Relapsed Pituitary Adenoma: A Systematic Review and Meta-Analysis. *World Neurosurg* (2020) 136:374–81.e4. doi: 10.1016/j.wneu.2019.11.041
25. Sheehan JP, Niranjan A, Sheehan JM, Jane JAJr., Laws ER, Kondziolka D, et al. Stereotactic Radiosurgery for Pituitary Adenomas: An Intermediate Review of Its Safety, Efficacy, and Role in the Neurosurgical Treatment Armamentarium. *J Neurosurg* (2005) 102(4):678–91. doi: 10.3171/jns.2005.102.4.0678
26. Swearingen B, Barker FG2nd, Katznelson L, Biller BM, Grinspoon S, Klibanski A, et al. Long-Term Mortality After Transsphenoidal Surgery and Adjunctive Therapy for Acromegaly. *J Clin Endocrinol Metab* (1998) 83(10):3419–26. doi: 10.1210/jc.83.10.3419
27. Pollack IF, Claassen D, al-Shboul Q, Janosky JE, Deutsch M. Low-Grade Gliomas of the Cerebral Hemispheres in Children: An Analysis of 71 Cases. *J Neurosurg* (1995) 82(4):536–47. doi: 10.3171/jns.1995.82.4.0536
28. Ding D, Starke RM, Sheehan JP. Treatment Paradigms for Pituitary Adenomas: Defining the Roles of Radiosurgery and Radiation Therapy. *J Neurooncol* (2014) 117(3):445–57. doi: 10.1007/s11060-013-1262-8
29. Mehta GU, Ding D, Patibandla MR, Kano H, Sisterson N, Su YH, et al. Stereotactic Radiosurgery for Cushing Disease: Results of an International, Multicenter Study. *J Clin Endocrinol Metab* (2017) 102(11):4284–91. doi: 10.1210/jc.2017-01385
30. Tritos NA, Biller BM, Swearingen B. Management of Cushing Disease. *Nat Rev Endocrinol* (2011) 7(5):279–89. doi: 10.1038/nrendo.2011.12
31. Vladyka V, Liscak R, Novotny JJr., Marek J, Jezkova J. Radiation Tolerance of Functioning Pituitary Tissue in Gamma Knife Surgery for Pituitary Adenomas. *Neurosurgery* (2003) 52(2):309–16; discussion 16-7. doi: 10.1227/01.NEU.0000043709.53906.31
32. Alonso CE, Bunevicius A, Trifiletti DM, Larner J, Lee CC, Pai FY, et al. Safety and Efficacy of Repeat Radiosurgery for Acromegaly: An International Multi-Institutional Study. *J Neurooncol* (2019) 145(2):301–7. doi: 10.1007/s11060-019-03296-8
33. Schoenthaler R, Albright NW, Wara WM, Phillips TL, Wilson CB, Larson DA. Re-Irradiation of Pituitary Adenoma. *Int J Radiat Oncol Biol Phys* (1992) 24(2):307–14. doi: 10.1016/0360-3016(92)90686-C
34. Burman P, Lamb L, McCormack A. Temozolomide Therapy for Aggressive Pituitary Tumours - Current Understanding and Future Perspectives. *Rev Endocr Metab Disord* (2020) 21(2):263–76. doi: 10.1007/s11154-020-09551-y
35. Whitelaw BC. How and When to Use Temozolomide to Treat Aggressive Pituitary Tumours. *Endocr Relat Cancer* (2019) 26(9):R545–R52. doi: 10.1530/ERC-19-0083
36. Bush ZM, Longtine JA, Cunningham T, Schiff D, Jane JAJr., Vance ML, et al. Temozolomide Treatment for Aggressive Pituitary Tumors: Correlation of Clinical Outcome With O(6)-Methylguanine Methyltransferase (MGMT) Promoter Methylation and Expression. *J Clin Endocrinol Metab* (2010) 95 (11):E280–90. doi: 10.1210/jc.2010-0441
37. Gilbert MR, Wang M, Aldape KD, Stupp R, Hegi ME, Jaeckle KA, et al. Dose-Dense Temozolomide for Newly Diagnosed Glioblastoma: A Randomized Phase III Clinical Trial. *J Clin Oncol* (2013) 31(32):4085–91. doi: 10.1200/JCO.2013.49.6968
38. Stupp R, Mason WP, van den Bent MJ, Weller M, Fisher B, Taphoorn MJ, et al. Radiotherapy Plus Concomitant and Adjuvant Temozolomide for Glioblastoma. *N Engl J Med* (2005) 352(10):987–96. doi: 10.1056/NEJMoa043330
39. McCormack A, Dekkers OM, Petersenn S, Popovic V, Trouillas J, Raverot G, et al. Treatment of Aggressive Pituitary Tumours and Carcinomas: Results of a European Society of Endocrinology (ESE) Survey 2016. *Eur J Endocrinol* (2018) 178(3):265–76. doi: 10.1530/EJE-17-0933
40. Bengtsson D, Schroder HD, Andersen M, Maiter D, Berinder K, Feldt Rasmussen U, et al. Long-Term Outcome and MGMT as a Predictive Marker in 24 Patients With Atypical Pituitary Adenomas and Pituitary Carcinomas Given Treatment With Temozolomide. *J Clin Endocrinol Metab* (2015) 100(4):1689–98. doi: 10.1210/jc.2014-4350
41. Elbelt U, Schlaffer SM, Buchfelder M, Knappe UJ, Vila G, Micko A, et al. Efficacy of Temozolomide Therapy in Patients With Aggressive Pituitary Adenomas and Carcinomas-A German Survey. *J Clin Endocrinol Metab* (2020) 105(3):e660–e775. doi: 10.1210/clinem/dgz211
42. Ji Y, Vogel RI, Lou E. Temozolomide Treatment of Pituitary Carcinomas and Atypical Adenomas: Systematic Review of Case Reports. *Neurooncol Pract* (2016) 3(3):188–95. doi: 10.1093/nop/npv059
43. Lasolle H, Cortet C, Castinetti F, Cloix L, Caron P, Delemer B, et al. Temozolomide Treatment Can Improve Overall Survival in Aggressive Pituitary Tumors and Pituitary Carcinomas. *Eur J Endocrinol* (2017) 176 (6):769–77. doi: 10.1530/EJE-16-0979
44. Lizzul L, Lombardi G, Barbot M, Ceccato F, Gardiman MP, Regazzo D, et al. Long-Course Temozolomide in Aggressive Pituitary Adenoma: Real-Life Experience in Two Tertiary Care Centers and Review of the Literature. *Pituitary* (2020) 23(4):359–66. doi: 10.1007/s11102-020-01040-4
45. Zacharia BE, Gulati AP, Bruce JN, Carminucci AS, Wardlaw SL, Siegelin M, et al. High Response Rates and Prolonged Survival in Patients With Corticotroph Pituitary Tumors and Refractory Cushing Disease From Capecitabine and Temozolomide (CAPTEM): A Case Series. *Neurosurgery* (2014) 74(4):E447–55; discussion E55. doi: 10.1227/NEU.0000000000000251
46. Liu G, Yuan X, Zeng Z, Tunici P, Ng H, Abdulkadir IR, et al. Analysis of Gene Expression and Chemoresistance of CD133+ Cancer Stem Cells in Glioblastoma. *Mol Cancer* (2006) 5:67. doi: 10.1186/1476-4598-5-67
47. McCormack AI, Wass JA, Grossman AB. Aggressive Pituitary Tumours: The Role of Temozolomide and the Assessment of MGMT Status. *Eur J Clin Invest* (2011) 41(10):1133–48. doi: 10.1111/j.1365-2362.2011.02520.x
48. Hagen C, Schroeder HD, Hansen S, Andersen M. Temozolomide Treatment of a Pituitary Carcinoma and Two Pituitary Macroadenomas Resistant to Conventional Therapy. *Eur J Endocrinol* (2009) 161(4):631–7. doi: 10.1530/EJE-09-0389
49. Annamalai AK, Dean AF, Kandasamy N, Kovacs K, Burton H, Halsall DJ, et al. Temozolomide Responsiveness in Aggressive Corticotroph Tumours: A Case Report and Review of the Literature. *Pituitary* (2012) 15(3):276–87. doi: 10.1007/s11102-011-0363-7

50. Losa M, Bogazzi F, Cannavo S, Ceccato F, Curto L, De Marinis L, et al. Temozolomide Therapy in Patients With Aggressive Pituitary Adenomas or Carcinomas. *J Neurooncol* (2016) 126(3):519–25. doi: 10.1007/s11060-015-1991-y
51. Pivonello R, De Leo M, Cozzolino A, Colao A. The Treatment of Cushing's Disease. *Endocr Rev* (2015) 36(4):385–486. doi: 10.1210/er.2013-1048
52. Ceccato F, Lombardi G, Manara R, Emanuelli E, Denaro L, Milanese L, et al. Temozolomide and Pasireotide Treatment for Aggressive Pituitary Adenoma: Expertise at a Tertiary Care Center. *J Neurooncol* (2015) 122(1):189–96. doi: 10.1007/s11060-014-1702-0
53. Greenman Y, Stern N. Paradoxical and Atypical Responses to Pasireotide in Aggressive ACTH-Secreting Pituitary Tumors. *Pituitary* (2016) 19(6):605–11. doi: 10.1007/s11102-016-0755-9
54. Katznelson L. Sustained Improvements in Plasma ACTH and Clinical Status in a Patient With Nelson's Syndrome Treated With Pasireotide LAR, a Multireceptor Somatostatin Analog. *J Clin Endocrinol Metab* (2013) 98 (5):1803–7. doi: 10.1210/jc.2013-1497
55. Fountas A, Lim ES, Drake WM, Powlson AS, Gurnell M, Martin NM, et al. Outcomes of Patients With Nelson's Syndrome After Primary Treatment: A Multicenter Study From 13 UK Pituitary Centers. *J Clin Endocrinol Metab* (2020) 105(5):1527–37. doi: 10.1210/clinem/dgz200
56. Daniel E, Debono M, Caunt S, Girio-Fragkoulakis C, Walters SJ, Akker SA, et al. A Prospective Longitudinal Study of Pasireotide in Nelson's Syndrome. *Pituitary* (2018) 21(3):247–55. doi: 10.1007/s11102-017-0853-3
57. Gatto F, Arvigo M, Ferone D. Somatostatin Receptor Expression and Patients' Response to Targeted Medical Treatment in Pituitary Tumors: Evidences and Controversies. *J Endocrinol Invest* (2020) 43(11):1543–53. doi: 10.1007/s40618-020-01335-0
58. Florio T, Thellung S, Arena S, Corsaro A, Spaziante R, Gussoni G, et al. Somatostatin and Its Analog Lanreotide Inhibit the Proliferation of Dispersed Human Non-Functioning Pituitary Adenoma Cells *In Vitro*. *Eur J Endocrinol* (1999) 141(4):396–408. doi: 10.1530/eje.0.1410396
59. Fusco A, Giampietro A, Bianchi A, Cimino V, Lugli F, Piacentini S, et al. Treatment With Octreotide LAR in Clinically Non-Functioning Pituitary Adenoma: Results From a Case-Control Study. *Pituitary* (2012) 15(4):571–8. doi: 10.1007/s11102-011-0370-8
60. Colao A, Di Somma C, Pivonello R, Faggiano A, Lombardi G, Savastano S. Medical Therapy for Clinically Non-Functioning Pituitary Adenomas. *Endocr Relat Cancer* (2008) 15(4):905–15. doi: 10.1677/ERC-08-0181
61. Zatelli MC, Piccin D, Vignali C, Tagliati F, Ambrosio MR, Bondanelli M, et al. Pasireotide, a Multiple Somatostatin Receptor Subtypes Ligand, Reduces Cell Viability in Non-Functioning Pituitary Adenomas by Inhibiting Vascular Endothelial Growth Factor Secretion. *Endocr Relat Cancer* (2007) 14(1):91–102. doi: 10.1677/ERC-06-0026
62. Flores-Martinez A, Venegas-Moreno E, Dios E, Remon-Ruiz P, Gros-Herguido N, Vazquez-Borrego MC, et al. Quantitative Analysis of Somatostatin and Dopamine Receptors Gene Expression Levels in Non-Functioning Pituitary Tumors and Association With Clinical and Molecular Aggressiveness Features. *J Clin Med* (2020) 9(9):3052. doi: 10.3390/jcm9093052
63. Fusco A, Gunz G, Jaquet P, Dufour H, Germanetti AL, Culler MD, et al. Somatostatinergic Ligands in Dopamine-Sensitive and -Resistant Prolactinomas. *Eur J Endocrinol* (2008) 158(5):595–603. doi: 10.1530/EJE-07-0806
64. Sosa-Eroza E, Espinosa E, Ramirez-Renteria C, Mendoza V, Arreola R, Mercado M. Treatment of Multiresistant Prolactinomas With a Combination of Cabergoline and Octreotide LAR. *Endocrine* (2018) 61 (2):343–8. doi: 10.1007/s12020-018-1638-9
65. Coopmans EC, van Meyel SWF, Pieterman KJ, van Ipenburg JA, Hofland LJ, Donga E, et al. Excellent Response to Pasireotide Therapy in an Aggressive and Dopamine-Resistant Prolactinoma. *Eur J Endocrinol* (2019) 181(2):K21–K7. doi: 10.1530/EJE-19-0279
66. Raverot G, Vasiljevic A, Jouanneau E, Lasolle H. Confirmation of a New Therapeutic Option for Aggressive or Dopamine Agonist-Resistant Prolactin Pituitary Neuroendocrine Tumors. *Eur J Endocrinol* (2019) 181(2):C1–3. doi: 10.1530/EJE-19-0359
67. Petersenn S. Management of Aggressive Pituitary Tumors - A 2019 Update. *Horm Metab Res* (2019) 51(12):755–64. doi: 10.1055/a-1060-1883
68. Ilie MD, Lasolle H, Raverot G. Emerging and Novel Treatments for Pituitary Tumors. *J Clin Med* (2019) 8(8):1107. doi: 10.3390/jcm8081107
69. Cooper O, Vlotides G, Fukuoka H, Greene MI, Melmed S. Expression and Function of ErbB Receptors and Ligands in the Pituitary. *Endocr Relat Cancer* (2011) 18(6):R197–211. doi: 10.1530/ERC-11-0066
70. Singh B, Carpenter G, Coffey RJ. EGF Receptor Ligands: Recent Advances. *F1000Res* (2016) 5:2270. doi: 10.12688/f1000research.9025.1
71. Fukuoka H, Cooper O, Ben-Shlomo A, Mamelak A, Ren SG, Bruyette D, et al. EGFR as a Therapeutic Target for Human, Canine, and Mouse ACTH-Secreting Pituitary Adenomas. *J Clin Invest* (2011) 121(12):4712–21. doi: 10.1172/JCI60417
72. Fukuoka H. New Potential Targets for Treatment of Cushing's Disease: Epithelial Growth Factor Receptor and Cyclin-Dependent Kinases. *Pituitary* (2015) 18(2):274–8. doi: 10.1007/s11102-015-0637-6
73. Ma ZY, Song ZJ, Chen JH, Wang YF, Li SQ, Zhou LF, et al. Recurrent Gain-of-Function USP8 Mutations in Cushing's Disease. *Cell Res* (2015) 25(3):306–17. doi: 10.1038/cr.2015.20
74. Reincke M, Sbiera S, Hayakawa A, Theodoropoulou M, Osswald A, Beuschlein F, et al. Mutations in the Deubiquitinase Gene USP8 Cause Cushing's Disease. *Nat Genet* (2015) 47(1):31–8. doi: 10.1038/ng.3166
75. Perez-Rivas LG, Theodoropoulou M, Ferrau F, Nusser C, Kawaguchi K, Stratakis CA, et al. The Gene of the Ubiquitin-Specific Protease 8 Is Frequently Mutated in Adenomas Causing Cushing's Disease. *J Clin Endocrinol Metab* (2015) 100(7):E997–1004. doi: 10.1210/jc.2015-1453
76. Sbiera S, Perez-Rivas LG, Taranets L, Weigand I, Flitsch J, Graf E, et al. Driver Mutations in USP8 Wild-Type Cushing's Disease. *Neuro Oncol* (2019) 21 (10):1273–83. doi: 10.1093/neuonc/noz109
77. Hayashi K, Inoshita N, Kawaguchi K, Ibrahim Ardisasmita A, Suzuki H, Fukuhara N, et al. The USP8 Mutational Status may Predict Drug Susceptibility in Corticotroph Adenomas of Cushing's Disease. *Eur J Endocrinol* (2016) 174(2):213–26. doi: 10.1530/EJE-15-0689
78. Faucz FR, Tirosh A, Tatsi C, Berthon A, Hernandez-Ramirez LC, Settas N, et al. Somatic USP8 Gene Mutations Are a Common Cause of Pediatric Cushing Disease. *J Clin Endocrinol Metab* (2017) 102(8):2836–43. doi: 10.1210/jc.2017-00161
79. Albani A, Perez-Rivas LG, Dimopoulou C, Zopp S, Colon-Bolea P, Roeber S, et al. The USP8 Mutational Status may Predict Long-Term Remission in Patients With Cushing's Disease. *Clin Endocrinol (Oxf)* (2018). 89:454–8. doi: 10.1111/cen.13802
80. Uzilov AV, Taik P, Cheesman KC, Javanmard P, Ying K, Roehnelt A, et al. USP8 and TP53 Drivers Are Associated With CNV in a Corticotroph Adenoma Cohort Enriched for Aggressive Tumors. *J Clin Endocrinol Metab* (2021) 106(3):826–42. doi: 10.1210/clinem/dgaa853
81. Liu X, Feng M, Dai C, Bao X, Deng K, Yao Y, et al. Expression of EGFR in Pituitary Corticotroph Adenomas and Its Relationship With Tumor Behavior. *Front Endocrinol (Lausanne)* (2019) 10:785. doi: 10.3389/fendo.2019.00785
82. Fukuoka H, Cooper O, Mizutani J, Tong Y, Ren SG, Bannykh S, et al. HER2/ErbB2 Receptor Signaling in Rat and Human Prolactinoma Cells: Strategy for Targeted Prolactinoma Therapy. *Mol Endocrinol* (2011) 25(1):92–103. doi: 10.1210/me.2010-0353
83. Vlotides G, Cooper O, Chen YH, Ren SG, Greenman Y, Melmed S. Heregulin Regulates Prolactinoma Gene Expression. *Cancer Res* (2009) 69(10):4209–16. doi: 10.1158/0008-5472.CAN-08-4934
84. Nose-Alberti V, Mesquita MI, Martin LC, Kayath MJ. Adrenocorticotropin-Producing Pituitary Carcinoma With Expression of c-erbB-2 and High PCNA Index: A Comparative Study With Pituitary Adenomas and Normal Pituitary Tissues. *Endocr Pathol* (1998) 9(1):53–62. doi: 10.1007/BF02739952
85. Cooper O, Mamelak A, Bannykh S, Carmichael J, Bonert V, Lim S, et al. Prolactinoma ErbB Receptor Expression and Targeted Therapy for Aggressive Tumors. *Endocrine* (2014) 46(2):318–27. doi: 10.1007/s12020-013-0093-x
86. Cooper O, Bonert VS, Rudnick J, Pressman BD, Lo J, Salvatori R, et al. EGFR/ErbB2-Targeting Lapatinib Therapy for Aggressive Prolactinomas. *J Clin Endocrinol Metab* (2021) 106(2):e917–e25. doi: 10.1210/clinem/dgaa805
87. Chauvet N, Romano N, Lafont C, Guillou A, Galibert E, Bonnefont X, et al. Complementary Actions of Dopamine D2 Receptor Agonist and Anti-Vegf Therapy on Tumoral Vessel Normalization in a Transgenic Mouse Model. *Int J Cancer* (2017) 140(9):2150–61. doi: 10.1002/ijc.30628
88. Cristina C, Luque GM, Demarchi G, Lopez Vicchi F, Zubeldia-Brenner L, Perez Millan MI, et al. Angiogenesis in Pituitary Adenomas: Human Studies

and New Mutant Mouse Models. *Int J Endocrinol* (2014) 2014:608497. doi: 10.1155/2014/608497
89. Osterhage K, Rotermund R, Droste M, Dierlamm J, Saeger W, Petersenn S, et al. Bevacizumab in Aggressive Pituitary Adenomas - Experience With 3 Patients. *Exp Clin Endocrinol Diabetes* (2021) 129(3):178–85. doi: 10.1055/a-1260-3975
90. Ortiz LD, Syro LV, Scheithauer BW, Ersen A, Uribe H, Fadul CE, et al. Anti-VEGF Therapy in Pituitary Carcinoma. *Pituitary* (2012) 15(3):445–9. doi: 10.1007/s11102-011-0346-8
91. Touma W, Hoostal S, Peterson RA, Wiernik A, SantaCruz KS, Lou E. Successful Treatment of Pituitary Carcinoma With Concurrent Radiation, Temozolomide, and Bevacizumab After Resection. *J Clin Neurosci* (2017) 41:75–7. doi: 10.1016/j.jocn.2017.02.052
92. Rotman LE, Vaughan TB, Hackney JR, Riley KO. Long-Term Survival After Transformation of an Adrenocorticotropic Hormone-Secreting Pituitary Macroadenoma to a Silent Corticotroph Pituitary Carcinoma. *World Neurosurg* (2019) 122:417–23. doi: 10.1016/j.wneu.2018.11.011
93. Santos-Pinheiro F, Penas-Prado M, Kamiya-Matsuoka C, Waguespack SG, Mahajan A, Brown PD, et al. Treatment and Long-Term Outcomes in Pituitary Carcinoma: A Cohort Study. *Eur J Endocrinol* (2019) 181(4):397–407. doi: 10.1530/EJE-18-0795
94. Zatelli MC, Minoia M, Filieri C, Tagliati F, Buratto M, Ambrosio MR, et al. Effect of Everolimus on Cell Viability in Nonfunctioning Pituitary Adenomas. *J Clin Endocrinol Metab* (2010) 95(2):968–76. doi: 10.1210/jc.2009-1641
95. Alshaikh OM, Asa SL, Mete O, Ezzat S. An Institutional Experience of Tumor Progression to Pituitary Carcinoma in a 15-Year Cohort of 1055 Consecutive Pituitary Neuroendocrine Tumors. *Endocr Pathol* (2019) 30(2):118–27. doi: 10.1007/s12022-019-9568-5
96. Donovan LE, Arnal AV, Wang SH, Odia Y. Widely Metastatic Atypical Pituitary Adenoma With mTOR Pathway STK11(F298L) Mutation Treated With Everolimus Therapy. *CNS Oncol* (2016) 5(4):203–9. doi: 10.2217/cns-2016-0011
97. Zhang D, Way JS, Zhang X, Sergey M, Bergsneider M, Wang MB, et al. Effect of Everolimus in Treatment of Aggressive Prolactin-Secreting Pituitary Adenomas. *J Clin Endocrinol Metab* (2019) 104(6):1929–36. doi: 10.1210/jc.2018-02461
98. Tatsi C, Stratakis CA. The Genetics of Pituitary Adenomas. *J Clin Med* (2019) 9(1):30. doi: 10.3390/jcm9010030

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