

A Potential Impact of SARS-CoV-2 on Pituitary Glands and Pituitary Neuroendocrine Tumors

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Research Article

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Abstract

Introduction: Angiotensin-converting enzyme 2 (ACE2) is the receptor of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The effects of SARS-CoV-2 on normal pituitary glands function or pituitary neuroendocrine tumors (PitNETs) have not yet been elucidated. Thus, the present study aimed to investigate the potential risks of SARS-CoV-2 infection on the impairment of pituitary glands and the development of PitNETs.

Methods: PitNETs tissues were obtained from 114 patients, and normal pituitary gland tissues were obtained from the autopsy. The mRNA levels of ACE2 and angiotensin II receptor type 1 (AGTR1) were examined by quantitative real-time PCR. Immunohistochemical staining was performed for ACE2 in 69 PitNETs and 3 normal pituitary glands. The primary tumor cells and pituitary cell lines (MMQ, GH3 and AtT-20/D16v-F2) were treated with diminazene aceturate (DIZE), an ACE2 agonist, with various dose regimens. The pituitary hormones between 43 patients with SARS-CoV-2 infection were compared with 45 healthy controls.

Results: Pituitary glands and the majority of PitNET tissues showed low/negative ACE2 expression at both the mRNA and protein levels, while AGTR1 showed high expression in normal pituitary and corticotroph adenomas. ACE2 agonist increased the secretion of ACTH in AtT-20/D16v-F2 cells through downregulating AGTR1. The level of serum adrenocorticotrophic hormone (ACTH) was significantly increased in COVID-19 patients as compared to normal controls ($p < 0.001$), but was dramatically decreased in critical cases as compared to non-critical patients ($p = 0.003$).

Conclusion: This study revealed a potential impact of SARS-CoV-2 infection on corticotroph cells and adenomas.

Introduction

Coronavirus disease 2019 (COVID-19) is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). It has led to > 112,000,000 infections and > 2,400,000 deaths all over the world [1]. Presently, specific therapies preventing COVID-19 infection are lacking, which is essential to control the outbreak [2].

Angiotensin-converting enzyme 2 (ACE2) is regarded as the receptor of SARS virus and SARS-CoV-2 [3,4]. In renin-angiotensin systems (RAS), ACE2 plays a key role. It antagonizes the activation of the classical RAS system and exerts a protective effect against inflammation [5]. The expression and distribution of ACE2 is indicative of the risk and severity of COVID-19 [6]. Reportedly, ACE2 is expressed in the lung, heart, kidneys, and the central nervous system [7]. In the ACE2 transgenic mice model, the brain served as a target organ for SARS infection [8],[9]. However, whether ACE2 could be valuable for the prevention of SARS-CoV-2 infection is yet controversial [10].

The pituitary gland is the center of the endocrine system. Pituitary neuroendocrine tumors (PitNETs) are benign and originated from the anterior pituitary, accounting for approximately 10–25% of all intracranial tumors. Although the overexpression of ACE2 has been observed in several malignant tumors [11], none of the studies have yet focused on the role of ACE2 on normal pituitary or PitNETs.

In this article, we study the potential risks of SARS-CoV-2 infection on the pituitary glands and PitNETs. First, we explored the expression the expression level of ACE2 in the normal pituitary glands and PitNETs, second, we explore the effect of ACE2 agonsit over cellular proliferation and hormonal secretion of pituitary cell lines and third, we elucidate the changes in the pituitary function as a response to 2019-nCoV infection. The current study revealed that SARS-CoV-2 has a potential impact on corticotrophs.

Materials And Methods

Patients and tissue samples

We performed a retrospective study involving 43 patients with COVID-19 as the study group, who were hospitalized in Renmin Hospital of Wuhan University from February 5 to Apr 5, 2020. All cases were laboratory-confirmed as SARS-CoV-2 positive using quantitative RT-PCR (qRT-PCR) on nasal and pharyngeal swab specimens. The diagnosis of COVID-19 and the severity was determined according to the New Coronavirus Pneumonia Prevention and Control Program (7th edition) published by the National Health Commission of China. Briefly, patients with accompanying respiratory failure (mechanical ventilation needed), shock or multiple organ dysfunctions were diagnosed as critical type. The control group came from the population who previously received pituitary function evaluation and were classified as normal in the same center. 45 age – and gender-matched healthy controls were randomly selected and the data of their pituitary hormones were collected. Patients with a history of pituitary disease and/or renal insufficiency were excluded from the study. All the patients who underwent a complete pituitary hormone assessment and their matched-controls did not have a pituitary MRI.

A total of 114 PitNETs from a single expert center were collected. The cohort comprised of 4 normal pituitary tissues obtained from autopsy. This consecutive series encompassed PitNET types, including 24 (20.9%) lactotrophs, 16 (13.9%) somatotrophs, 13 (11.3%) corticotrophs, 31 (27.0%) gonadotrophs, 23 (20.0%) null-cells, 4 (3.5%) plurihormonal PIT1-positive PitNETs, 3 (2.6%) mixed growth hormone/prolactin (GH-PRL), and 1 (0.9%) thyrotroph. The study was approved by the Ethical Review Board in Renmin Hospital of Wuhan University and Ruijin Hospital of Shanghai Jiao Tong University School of Medicine.

Pituitary hormone assessment

In the study group, prolactin (PRL), somatotropin (GH), adrenocorticotrophic hormone (ACTH), cortisol (8AM), thyroid stimulating hormone (TSH), free triiodothyronine (FT3), free thyroxine (FT4), luteinizing hormone (LH) and follicle stimulating hormone (FSH) were detected by electrochemiluminescent immunoassays according to the instructions from the manufacturer. In the control group, the data of

serum PRL, GH, ACTH, cortisol, TSH, FT3, FT4, LH and FSH levels were retrieved from the dataset already kept in the same medical center. All the cases in the two groups had not received corticosteroid therapy or medications that could suppress TSH, ACTH and PRL within 5 days before pituitary hormone assessment.

Public datasets acquisition and analysis

RNA-seq data of normal tissues and PitNETs were downloaded from the Gene Expression Omnibus (GEO; <https://www.ncbi.nlm.nih.gov/geo>). 46 normal tissues, which included pituitary glands (5 tissues), distributed in different organs and 5 lactotroph adenomas were obtained for this study.

Quantitative real-time PCR (qPCR)

Total RNA of tumor tissues was extracted using TRIzol (Invitrogen, Carlsbad, CA, USA), following the manufacturer's instructions. 5µg RNA was reversely transcribed into cDNA using the cDNA Synthesis Kit (TaKaRa, Shiga, Japan). The mRNA expression level was quantified using qPCR with the SYBR Green real-time PCR Master Mix kit (TaKaRa Bio). PCR primers used were as follows: human ACE2 forward, 5'-CATTGGAGCAAGTGTGGATCTT-3'; reverse: 5'-GAGCTAATGCATGCCATTCTCA-3'; human angiotensin II receptor type 1 (AGTR1) forward, 5'-GATGATTGTCCCAAAGCTGG-3'; reverse, 5'-TAGGTAATTGCCAAAGGGCC-3'; human proto-oncogene receptor (MAS) forward, 5'-TTG TTG AGG AAC CCA CGA AC-3'; reverse, 5'-CCA CTG GGG AGA TGC TCA TA-3'; human β-actin forward, 5'-GGATGCAGAAGGAGATCACTG-3'; reverse: 5'-CGATCCACACGGAGTACTTG-3' [12-15]. The amplification reaction was carried out on an ABI PRISM 7500 Sequence Detection System (Applied Biosystems, Warrington, UK). The mRNA expression of the target genes was normalized to that of the endogenous reference gene β-actin and expressed as the fold-difference ($2^{-\Delta\Delta Ct}$). Low and high measurable levels were defined by ratio of specific/beta-actin transcripts <1 and ≥ 1 , respectively [16].

Immunohistochemistry (IHC) and scoring

Tissue samples were fixed in 4% formalin, embedded in paraffin, and sliced into 4-mm-thick sections. IHC was conducted using the Vectastain ABC Kit (Vector Laboratories, Burlingame, CA, USA). Rabbit primary antibody for ACE2 (1:200, ab108252) was purchased from Abcam (Cambridge, MA, USA). Finally, the slides were developed with DAB and counterstained with hematoxylin.

Slices were scanned by Panoramic 1000 slide digitalization system (3DHISTECH, Budapest, Hungary). Images were captured at ×20 magnification using CaseViewer 2.3(3DHISTECH, Budapest, Hungary). The field was selected with a good contrast of DAB chromogen and hematoxylin which is considered region of interest. Before capturing the images, the color density and white balance were standardized for all images. All the acquired images were saved as JPEG format. After IHC images acquisition, ImageJ 1.48 version (NIH, Bethesda, Maryland) analysis was performed and ACE2 immunostaining of pituitary glands and tumor tissues was scored as negative (0+), low positive (1+), positive (2+) and high positive (3+), as described in the previous study [17].

Cell culture and reagents

Pituitary cell lines MMQ (CRL-10609™), GH3 (CCL-82.1™), and AtT-20/D16v-F2 (AtT-20, CRL-1795™) were purchased from the American Type Culture Collection (Manassas, USA). The MMQ and GH3 cell lines were cultured in Dulbecco's modified Eagle medium and F12 medium (Gibco, Grand Island, NY, USA), supplemented with 15% horse serum (Gibco) and 2.5% fetal bovine serum (FBS; Gibco). The AtT-20 cell line was cultured in RPMI1640 (Sangon Biotech, Shanghai, China) supplemented with 10% FBS (Gibco).

Primary human tumor cells were obtained from patients who underwent endoscopy surgery for PitNETs between March 2020 and August 2020 at the Department of Neurosurgery, Ruijin Hospital of Jiaotong University, Shanghai, China. As described in the previous study [18], the tumor cells were cultured in DMEM with 10% FBS and 100U/mL penicillin/streptomycin (Gibco). Cells were seeded onto 10cm dishes at a density of 10^6 cells per well and were cultured for 48 hours before treatment.

All cell lines and primary tumor cells were maintained in a humidified atmosphere with 5% CO₂ at 37 °C. Diminazene aceturate (DIZE) was purchased from MedChemExpress (Shanghai, China).

Establishment of stably transfected cells

The recombinant plasmids (pCDH-puro-AGTR1) were constructed, and sequenced by Tongyong Biotechnologies (Anhui, China). AtT-20 cell lines stably expressing DEPTOR or empty vectors, were constructed using the lentiviral technique. After 48 h transduction with lentiviral supernatant, cells were selected with 2 µg/mL puromycin for 1–2 weeks for stable transfectants.

Western blotting

Total proteins were extracted using the Total Protein Extraction Kit (Millipore Co., Billerica, MA, USA). The cultured tumor cells were lysed with RIPA lysis buffer (Beyotime, Shanghai, China). An equivalent of 30 mg protein/sample was resolved by SDS-PAGE (Sangon Biotech, Shanghai, China) and transferred to polyvinylidene difluoride membranes (PVDF; Millipore). The membranes were blocked with 5% nonfat milk in Tris-buffered saline with 1% Tween-20 (TBST) buffer for 1 h and probed overnight with primary antibodies at 4 °C. The antibodies used were as follows: ACE2 (ab108252, Abcam), and POMC (ab210605, Abcam), Tubulin (ab7291, Abcam) and AGTR1 (25343-1-AP, Proteintech). The immunoreactive bands were detected using ECL detection reagent (Millipore) according to the manufacturer's instructions. Kidney paracancer tissues from two renal cell carcinoma patients were obtained for use as a positive control. Protein expression was quantified using ImageJ software (Wayne Rasband, National Institute of Mental Health, Bethesda, MD, USA).

Cell proliferation assay

Cell proliferation was measured using a WST-8 Cell Counting Kit-8 (CCK8) (Bimake, Houston, TX, USA) according to the manufacturer's instructions. MMQ, GH3, and AtT-20 and primary tumor cells were plated

in 96-well plates at a density of 1×10^4 cells/well, respectively. All cells were treated with different concentrations of DIZE and assessed after 24h, 48h and 72h.

Enzyme-linked immunosorbent assay (ELISA)

The levels of the hormone in the cell culture supernatants were measured using ELISA kits (Signalway Antibody LLC, Maryland, MD, USA) at the indicated time points, with the appropriate treatment according to the manufacturer's recommendations.

Statistical analysis

All data were analyzed using GraphPad Prism, version 5 (GraphPad Software, La Jolla, CA, USA). The differences among categorical variables were analyzed using independent-sample Student's t-test or one-way analysis of variance (ANOVA). The immunoreactive scores of ACE2 were analyzed using the non-parametric Kruskal–Wallis H test. $P < 0.05$ indicated statistical significance.

Results

Expression of ACE2 in human PitNETs and pituitary gland

To identify whether the pituitary is a target organ of SARS-CoV-2, we detected the expression of ACE2 in human normal pituitary glands and PitNETs tissues. First, we obtained RNA-seq profile data of 42 normal tissues from GEO (GDS3834). Strikingly, ACE2 showed lower expression in normal pituitary as compared to the other normal tissues. Then, western blotting of 8 tumor sections showed that ACE2 protein expression was lower in primary PitNET specimens (Suppl. Table 1) than that in renal tissue (Fig. 1a). To further confirm these clinical results, immunohistochemical staining of 69 tumor sections from 114 PitNETs and 3 normal pituitary glands was conducted (Fig. 1b and 1c). A low expression of ACE2 was detected in all normal pituitary glands. Moreover, among 69 samples of PitNET tissues, only 10 (14.5%) samples showed positive ACE2 expression, while 59 (85.5%) showed low positive/negative expression (Suppl. Table 2). No statistically significant differences ($p = 0.589$) were observed in ACE2 in pituitary glands or different PitNET subtypes. Additionally, we detected the expression of ACE2 in 4 human normal pituitary glands and 114 PitNETs tissues by real-time PCR (Fig. 1d and Suppl. Table 3). We found that the overall expression of ACE2 was low, albeit with a relative high expression in gonadotropic adenomas as compared to normal pituitary and other subtypes of pituitary tumors ($p = 0.016$).

These data suggested that ACE2 showed a low expression in pituitary glands and PitNETs at the protein and mRNA levels.

Expression of RAS components in PitNETs

The classical RAS ACE-Ang II-AGTR1 regulatory axis and the ACE2-Ang1-7-Mas counter-regulatory axis play an essential role in maintaining homeostasis in humans (Fig. 1e). The entry of SARS-CoV-2 into cells is facilitated by the interaction between viral S-protein with the extracellular domains of the

transmembrane ACE2 proteins, followed by subsequent downregulation of ACE2 expression and markedly elevated levels of circulating Ang II [3,19-22]. Angiotensin II is converted to angiotensin (1–7) by ACE2. Angiotensin II binds to AGTR1, and angiotensin (1–7) binds to Mas. The expression of AGTR1 was increased in normal pituitary tissues as compared to PitNETs (0.004312 ± 0.000748 in normal pituitary glands and 0.001351 ± 0.000136 in PitNETs, $p < 0.001$). We also analyzed the correlation between the expression of AGTR1 and PitNET types and found that the expression of AGTR1 was increased in corticotroph adenomas as compared to somatotroph adenomas ($p = 0.016$), gonadotroph adenomas ($p = 0.014$), and null-cell adenomas ($p = 0.033$). The expression of MAS was increased in normal pituitary tissues as compared to PitNETs (0.000889 ± 0.000034 in normal pituitary glands and 0.0005968 ± 0.000020 in PitNETs, $p = 0.008$). However, no significant difference was observed among the PitNETs subtypes (Suppl. Table 4).

Additionally, we calculated the ratio of AGTR1 to MAS (AGTR1/MAS) in pituitary glands and PitNETs tissues. The AGTR1/MAS was increased in corticotroph adenomas and normal pituitary tissues as compared to other subtypes PitNETs ($p < 0.001$). There is no significant difference between corticotroph adenomas and normal pituitary tissues. These results suggested corticotroph adenomas and normal pituitary tissues had higher mRNA levels for genes of the ACE-Ang II-AGTR1 regulatory axis compared to other subtypes PitNETs.

Function of ACE2 on the growth and hormone secretion of pituitary cells in vitro

To clarify the role of ACE2 on different pituitary cells, we manipulated its function in pituitary MMQ, GH3, and AtT-20 cell lines. First, the expression of ACE2 was demonstrated in MMQ, GH3, and AtT-20 cells by immunoblotting analysis (Fig. 2a). Next, we used DIZE to activate ACE2 in pituitary cells. First, we conducted CCK-8 assays to investigate the function of ACE2 on the proliferation of pituitary cell lines and primary tumor cells. The growth curve analysis revealed that DIZE does not inhibit the proliferation of pituitary cells at a dose of $100 \mu\text{M}$ (Fig. 2b-2j, Suppl. Fig.1 and Suppl. Table 5). Next, ELISA was performed to examine the effect of ACE2 on the hormone secretion in GH3, MMQ and AtT-20 cells (Fig. 2k-2m). The activation of ACE2 by DIZE could increase the ACTH secretion in AtT-20 cells; however, it did not show an obvious influence on the secretion of PRL in MMQ or GH3 cells, respectively. The effect of ACE2 on ACTH secretion was further explored by Western blot (Fig. 2n). Proopiomelanocortin (POMC) is the precursor for ACTH, which acts on the adrenal glands to induce synthesis and secretion of adrenal steroids. The activation of ACE2 could downregulate AGTR1 and upregulate POMC (Fig. 2n). To test whether agtr1 decreased POMC expression in corticotroph, AtT-20 cells cells were transfection with AGTR1 overexpression plasmids, and POMC were examined by western blotting. We found that activation of ACE2 increased POMC and decreased AGTR1 expression, while AGTR1 overexpression had the opposite effects on POMC expression (Fig. 2o).

Together, these data indicated that the activation of ACE2 by DIZE increases the ACTH secretion, but no difference was detected in PRL secretion and cell viability.

Pituitary function in COVID-19 patients

To investigate the role of COVID-19 on pituitary function, ELISA was performed to detect the level of hormone secretion. Compared to the control group (Table 1), COVID-19 patients had significantly higher PRL (15.46 ± 1.016 ng/mL vs. 11.34 ± 1.184 ng/mL, $p=0.010$) and ACTH secretion (46.8 ± 3.383 pg/mL vs. 28.34 ± 1.765 pg/mL, $p<0.0001$). However, no statistically significant difference was detected in serum GH, cortisol, TSH, FT3, FT4, LH, or FSH between the two groups. Interestingly, 6 (14.0%) patients with COVID-19 diagnosed as “critical cases” had significantly lower serum ACTH as compared to the those non-critical COVID-19 patients (23.00 ± 4.737 pg/mL vs. 50.66 ± 3.47 pg/mL, $p= 0.003$). Also, no significant difference was detected in the serum PRL, GH, cortisol, TSH, FT3, FT4, LH, or FSH between critical and non-critical patients (Table 2).

Taken together, these results suggested that ACTH was inhibited in critical COVID-19 patients and SARS-CoV-2 might have potential impact on corticotroph via the ACE2 and AGTR1 proteins.

Discussion

Hitherto, few studies have referred to the expression of ACE2 in pituitary and PitNETs. The present study conducted real-time PCR, Western blot, and IHC and demonstrated that low expression of ACE2 in pituitary glands and PitNETs. These findings indicated that the pituitary glands and PitNETs have a low susceptibility to SARS-CoV-2 infection.

Low ACE2 might reduce the entry of SARS-CoV-2 or local viral load, or deteriorate the function of the organ under stress or fail to play an effective protective role. We compared the pituitary hormone profiles of COVID-19 patients and age - and gender-matched healthy controls in this study. Compare to healthy control, a significant increase was observed in serum PRL and ACTH; however, in critical cases, the ACTH level were dramatically decreased as compared to that in non-critical patients. The secretion of PRL and ACTH could be influenced by multiple factors, such as diet, stress, and drugs, etc. Based on similar PRL and cortisol levels in non-critical COVID-19 patients and critical COVID-19 patients, respectively, we inferred that the decreased ACTH level in critical COVID-19 patients was more likely to be caused by dysfunction, such as the possible damage of corticotrophs, in stead of stress responses or feedback regulation of the adrenal axis. Thus, the current results provided evidence that the hypothalamic–pituitary–adrenal (HPA) axis is activated in non-critical COVID-19 patients but suppressed in critical patients. Furthermore, we found that DIZE increases ACTH secretion in vitro, which could have the potential for the therapy of critical COVID-19 patients [23],[24], [25].

The normal HPA axis response to SARS-CoV-2 remains unknown. Both the HPA axis and the RAS are activated in response to stress in humans that maintain the organ function by modulating a multitude of homeostatic processes, including immune defense mechanisms, inflammation, and cellular metabolism [26][27]. Although presented as independent systems, the HPA and RAS are highly interactive, sharing several endocrine factors, including ACTH, vasopressin, angiotensin-II (Ang II), and aldosterone. ACE2 antagonizes the role of Ang II [28] that regulates the homeostasis system of the RAS [29] [30]. The ACE-Ang II-AGTR1 axis has proinflammatory effects that may lead to acute lung injury or myocarditis,

whereas the ACE2-Ang1-7-MAS axis has anti-inflammatory effects[31,32]. We found that corticotroph adenomas and normal pituitary tissues had higher mRNA levels for genes of the ACE-Ang II-AGTR1 axis compared to other subtypes PitNETs. Although the current data showed that ACE2 was highly expressed in the gonadotroph, high expression of AGTR1 in the pituitary gland and corticotroph adenomas was also detected. We further found that activation of ACE2 increased ACTH secretion by upregulating POMC and downregulating AGTR1 expression, while AGTR1 overexpression had the opposite effects on POMC expression in AtT-20 cells. Since RAS could act at both local and systemic levels [33,34], AGTR1 might be another key protein in COVID-19 infection patients.

According to our current results, the impact of SARS-CoV-2 on HPA with/without pituitary disease should be considered. Since previous studies have indicated that glucocorticoid therapy was not beneficial to patients affected with SARS and MERS, and the guidelines of the World Health Organization (WHO) did not prescribe glucocorticoids [35,36]. The physiological stress doses of hydrocortisone could be administered [37]. Moreover, some of the patients with PitNets presented hypopituitarism, requiring extra stress dose of glucocorticoid supplementation [37].

Nevertheless, the present study has some limitations, such as lack of direct evidence from the tissue and blood samples of COVID-19 patients and viral infection of cell line experiments. In conclusion, the current study revealed a potential impact of SARS-CoV-2 on pituitary glands and corticotrophs. Thus, the results of the current study might provide some evidence for the prevention strategy of the disease in clinical practice.

Declarations

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Conflict of Interest

The authors declare that they have no conflict of interest.

Informed consent

Informed consent was obtained from all individual participants included in the study.

Data Availability Statement

Some or all data, models, or code generated or used during the study are available from the corresponding author by request (List items).

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Tables

Table. 1. Hormone Profiles in COVID-19 group and the control group			
Feature	Patients with COVID-19 n=43	Healthy Control n=45	p
Age (Y):	58.14 ± 1.96	53.2 ± 1.85	0.070
Sex:			0.355
Female	28(65.12%)	25(55.96%)	
Male	15(34.88%)	20(44.44%)	
PRL (ng/mL)	15.46 ± 1.02	11.34 ± 1.18	0.010
GH (ng/mL)	0.76 ± 0.24	0.50 ± 0.13	0.342
ACTH (pg/mL)	46.80 ± 3.38	28.34 ± 1.77	<0.001
Serum Cortisol (µg/dL)	17.60 ± 1.39	14.66 ± 0.71	0.059
FSH (mIU/mL)	33.12 ± 4.92	27.06 ± 3.94	0.336
LH (mIU/ml)	16.47 ± 2.372	12.12 ± 1.58	0.127
TSH (µIU/mL)	2.13 ± 0.22	2.21 ± 0.21	0.787
FT3 (pg/mL)	3.11 ± 0.11	2.98 ± 0.06	0.269
FT4 (ng/dL)	1.16 ± 0.03	1.20 ± 0.02	0.392
Abbreviations: PRL: prolactin, GH: somatotropin, ACTH: adrenocorticotrophic hormone, cortisol (8AM), TSH: thyroid stimulating hormone, FT3: free triiodothyronine, FT4: free thyroxine , LH: luteinizing hormone, FSH: follicle stimulating hormone			

Table 2. Hormone Profiles in Critical cases and Non-Critical cases			
Feature	Critical cases n=6	Non-Critical cases n=37	P
Age (Y):	65.33 ± 2.85	56.97 ± 2.18	0.141
Sex(%):			0.413
Female	3(50.00%)	25(67.57%)	
Male	3(50.00%)	12(32.43%)	
PRL (ng/mL)	18.27 ± 3.60	15.01 ± 1.03	0.271
GH (ng/mL)	0.56 ± 0.30	0.79 ± 0.28	0.746
ACTH (pg/mL)	23.00 ± 4.74	50.66 ± 3.47	0.003
Serum Cortisol (µg/dL)	22.49 ± 7.63	16.80 ± 1.08	0.158
FSH (mIU/mL)	19.44 ± 9.13	35.34 ± 5.47	0.267
LH (mIU/ml)	11.44 ± 4.90	17.29 ± 2.64	0.399
TSH (µIU/mL)	3.13 ± 1.08	1.97 ± 0.18	0.064
FT3 (pg/mL)	2.60 ± 0.41	3.20 ± 0.11	0.061
FT4 (ng/dL)	1.18 ± 0.11	1.16 ± 0.03	0.827
Abbreviations: PRL: prolactin, GH: somatotropin, ACTH: adrenocorticotrophic hormone, cortisol (8AM), TSH: thyroid stimulating hormone, FT3: free triiodothyronine, FT4: free thyroxine , LH: luteinizing hormone, FSH: follicle stimulating hormone			

Figures

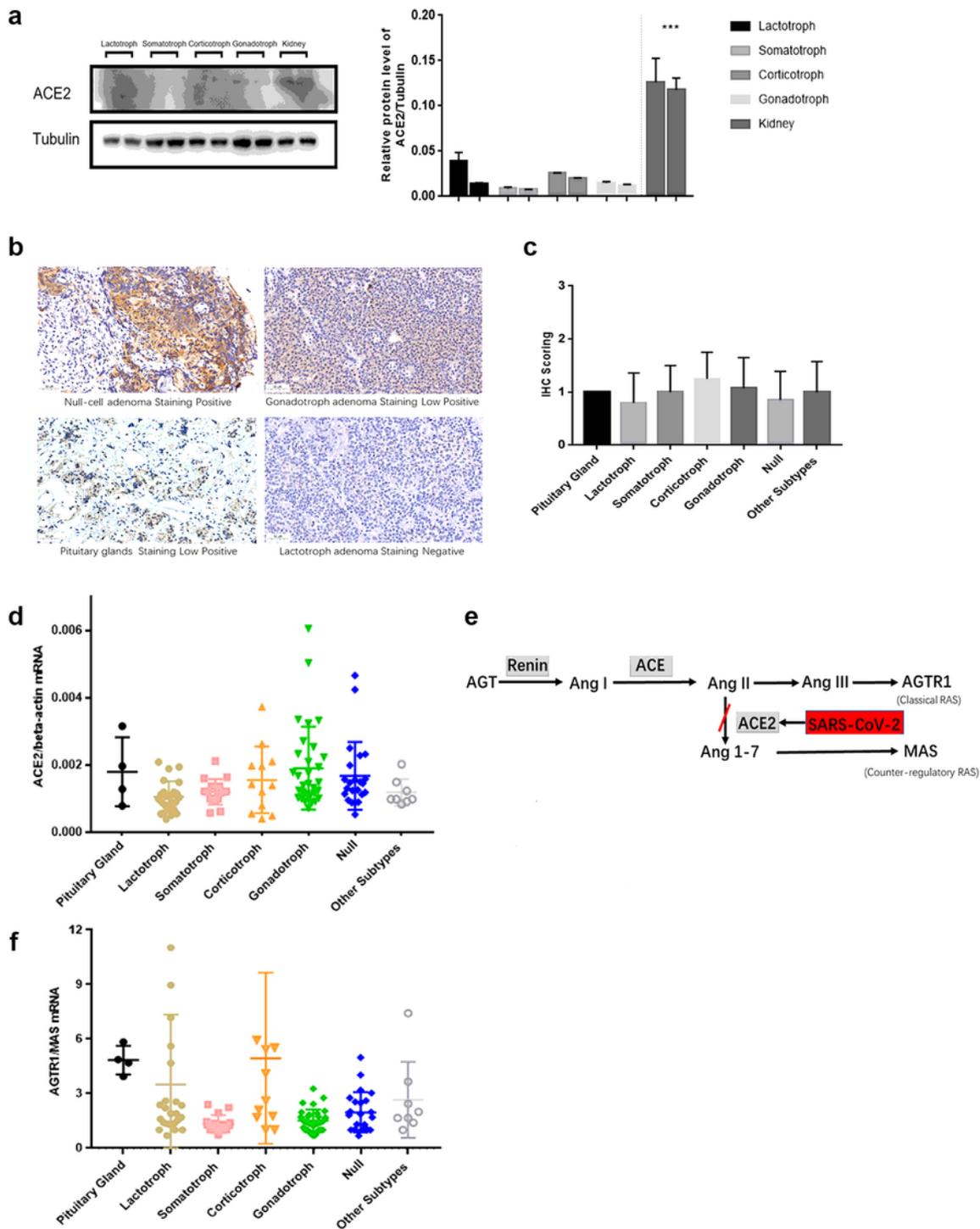


Figure 1

The expression of ACE2 and RAS components in PitNETs and pituitary gland. (a) Western blot showed that ACE2 was low expressed in PitNETs compare to kidney tissues. (b) Representative immunostaining scores of ACE2 expressed in normal pituitary glands and PitNETs. Pituitary glands and PitNETs tissues was scored as negative(0+), low positive (1+), positive (2+), and high positive (3+), Scale bar, 50 μm . (c) Among 69 samples of PitNET tissues and 2 normal pituitary tissues, most samples showed low positive

expression. No statistically significant differences ($p = 0.589$) were observed in ACE2 in pituitary glands or different PitNET subtypes. (d) Pituitary gland and PitNETs showed relatively low expression of ACE2 mRNA, which was otherwise relatively higher in gonadotroph adenomas ($P = 0.016$). Other Subtypes includes 4 plurihormonal PIT1-positive PitNETs, 3 mixed growth hormone/prolactin (GH-PRL), and 1 thyrotroph. (e) Schematic illustration of RAS. (f) AGTR1/MAS in pituitary gland and PitNETs. (g) AGTR1 expression in PitNETs and pituitary gland. AGTR1/MAS was otherwise higher in pituitary gland and corticotroph adenomas [$p < 0.001$]. Abbreviation: RAS, renin-angiotensin system; AGT, angiotensinogen; ACE, angiotensin-converting enzyme; ACE2, angiotensin-converting enzyme 2; Ang I, angiotensin I; Ang II, angiotensin II; Ang III, angiotensin III; Ang 1-7, angiotensin 1-7; Mas, MAS proto-oncogene receptor; AGTR1, angiotensin II receptor type 1. * $P < 0.05$; ** $P < 0.01$. * $P < 0.001$.

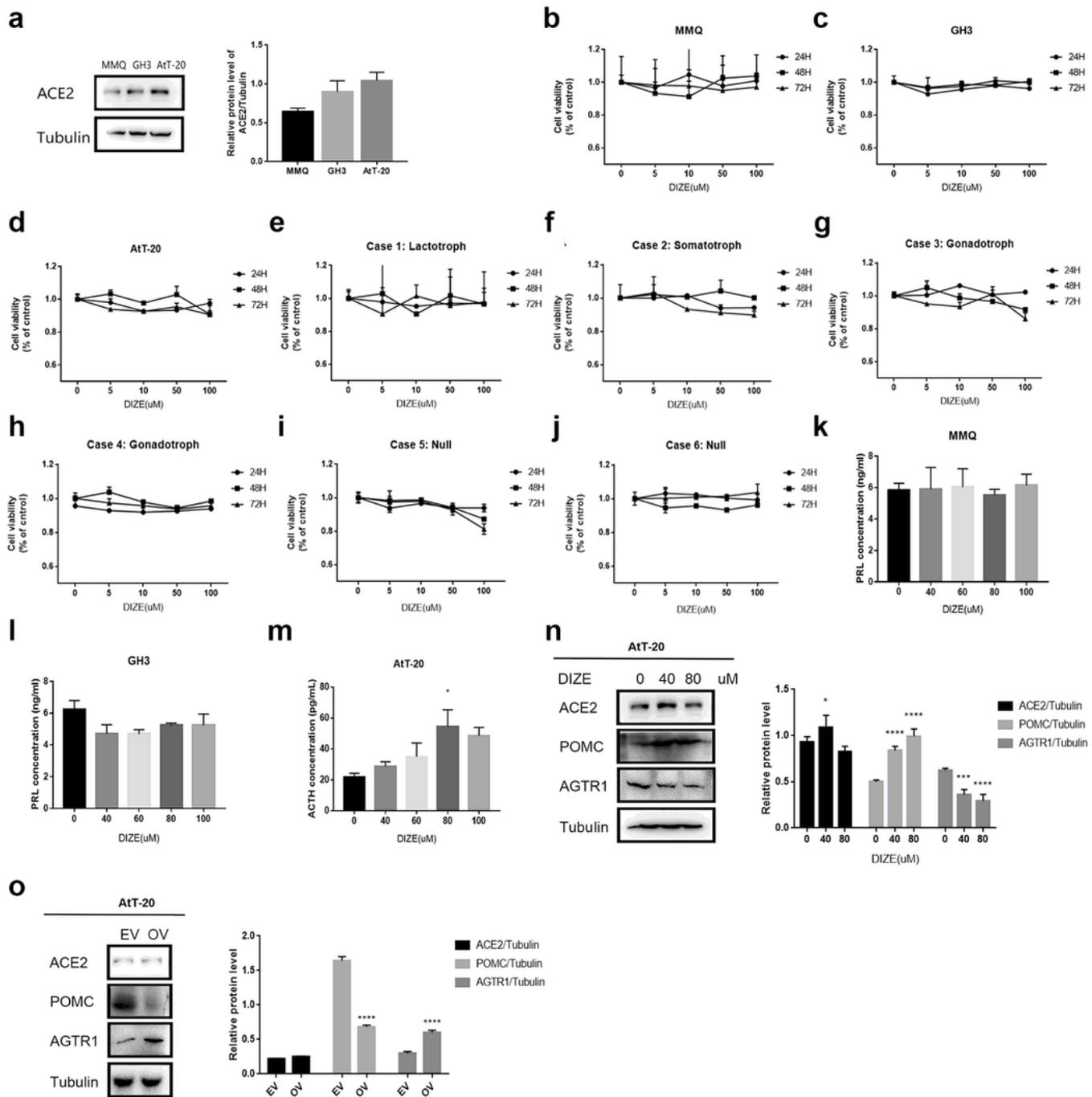


Figure 2

Function of ACE2 on the hormone secretion of pituitary cells in vitro. (a) The expression of ACE2 in MMQ, GH3, and AtT-20/D16v-F2(AtT-20) cells by western blot. (b-j) The function of ACE2 on the growth of pituitary cells in vitro. Pituitary cell lines (b-d) and primary pituitary tumor cells (e-j) were incubated with different concentrations of DIZE for 24, 48, and 72 h, and cell proliferation was measured by CCK-8 assay. (k-m) MMQ cells, GH cells and AtT-20 cells were incubated with different concentrations of DIZE for 48h

and hormones secretion was detected by ELISA. (n) The effect of ACE2 was confirmed by western blot. (o) AGTR1 overexpression had the opposite effects on ACTH secretion . Abbreviation: PRL, prolactin; ACTH: adrenocorticotrophic hormone; RAS, renin-angiotensin system; DIZE, diminazene aceturate; AGT, angiotensinogen; ACE, angiotensin-converting enzyme; ACE2, angiotensin-converting enzyme 2; Ang I, angiotensin I; Ang II, angiotensin II; Ang III, angiotensin III; Ang 1-7, angiotensin 1-7; Mas, MAS proto-oncogene receptor; AGTR1, angiotensin II receptor type 1; POMC, proopiomelanocortin. *P < 0.05; **P < 0.01. *P < 0.001.

Supplementary Files

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