The p300 inhibitor A-485 exerts antitumor activity in growth hormone pituitary adenoma

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Abstract

**Background:** Growth hormone pituitary adenoma (GHPA) is a major subtype of pituitary adenoma. It can lead to progressive somatic disfigurement, multiple complications and even increased mortality. The efficacy of the current treatments is limited; thus, a novel pharmacological treatment is urgently needed. As a histone acetyltransferase (HAT) coactivator, p300 can regulate the transcription of several genes that are crucial for pituitary adenoma tumorigenesis and progression. However, the role of p300 and its catalytic inhibitor in GHPA is still unclear.

**Methods:** The expression of p300 was detected in GHPA and normal pituitary tissues. Genetic knockdown was performed by siRNA. The efficacy of the p300 inhibitor A-485 in the cell cycle, proliferation, apoptosis and hormone secretion was investigated by flow cytometry, ELISAs, western blotting and qRT-PCR. RNA sequencing, bioinformatic analysis and subsequent validation experiments were performed to reveal the potential biological mechanism of A-485.

**Results:** High expression of p300 was found in GHPA tissues compared with normal pituitary tissues. Knockdown of p300 inhibited cell proliferation and clone formation. Treatment with A-485 suppressed cell growth and inhibited the secretion of GH *in vitro* and *in vivo*. Further mechanistic studies showed that A-485 could downregulate the expression or activity of several oncogenes, such as genes in the Ptg1, c-Myc, cAMP and PI3K/AKT/mTOR signaling pathways, which are crucial for pituitary adenoma tumorigenesis and progression.

**Conclusions:** In this study, our findings demonstrate that inhibition of HAT p300 by its selective inhibitor A-485 is a promising therapy for GHPA.

**Key words:** p300, A-485, growth hormone pituitary adenoma
Introduction

Pituitary adenomas (PAs) are one of the most common intracranial neoplasms, with a prevalence of 10% to 25% \(^1-3\). Based on immunohistochemical staining of the hormone content and primary transcription factors in the tumor, PAs can be classified into several subtypes. Overall, growth hormone pituitary adenomas (GHPAs) constitute approximately 20% of all PAs \(^4-6\). Although the majority of GHPAs are benign, prolonged exposure to excess hormone can lead to progressive somatic disfigurement and a wide range of systemic manifestations, such as gigantism in children and acromegaly in adults. Terminally, the patients will present with cardiovascular, metabolic, respiratory, neoplastic, endocrine, articular and bone complications, which are associated with increased mortality \(^7-10\).

Treatment strategies for GHPA aim to control tumor growth and normalize growth hormone (GH) levels, thereby ameliorating the symptoms and reducing mortality \(^11,12\). As the cornerstone treatment, transsphenoidal adenomectomy is effective in approximately 75% of microadenomas \(^8\). However, remission is not achieved in 50% of invasive macroadenomas \(^13-16\). Pharmacological treatment also has an important role in the management of GHPA. Generally, it contains somatostatin analog (SSA), growth hormone receptor antagonist (GHR antagonist) and dopamine receptor agonist (DA). Nevertheless, all three of these medications have limited efficiency, with remission rates of 10% to 35% \(^17-22\). Radiation is indicated as a third-line therapy after unsuccessful surgery and medical therapy, and biochemical control is only achieved in 50% of GHPA patients \(^23,24\). Thus, it is urgently necessary and practicably feasible to discover new therapeutic targets and medications.

The pathogenesis of GHPA is complex and remains elusive. Current studies indicate that abnormal expression of functional genes, disruption of the cell cycle and dysregulation of signal transduction are associated with the tumorigenesis of GHPA. Emerging evidence has also shown that histone acetylation contributes to the pathogenesis of GHPA \(^25-27\). As a key member of the histone acetyltransferase (HAT) family, p300 can modulate the transcriptional process by acetylating the histone tail or through protein-protein interactions with transcription factor \(^28\). Aberrant expression of
p300 has been involved in various cancers and is closely correlated with poor prognosis and a malignant phenotype \(^{29-32}\). Several studies have shown that p300 can regulate the expression of pituitary tumor transforming gene 1 (PTTG1) and c-MYC and facilitate the transcriptional activity of cAMP \(^{33-35}\). All three of these genes are essential for tumorigenesis and GH secretion \(^{36-38}\). Moreover, a potent, selective and drug-like catalytic p300 inhibitor, A-485, has been discovered and shown to have a more pronounced antitumor effect than previous inhibitors \(^{39}\). It can specifically inhibit p300 catalyzed acetylation of histone H3 lysine 27 (H3K27) and lysine 18 (H3K18) sites \(^{39,40}\). However, the role of p300 and its inhibitor in GHPA remains unclear.

Therefore, the aim of the present study was to identify the expression of p300 in GHPA and in normal pituitary glands. Then, the role of p300 in the GHPA phenotype was assessed by genetic knockdown experiments and treatment with the pharmacological inhibitor A-485 in vivo and in vitro. Our findings suggest that inhibition of p300 with its selective inhibitor A-485 could serve as a potential therapeutic strategy for the treatment of GHPA in the future.

**Material and methods**

**Patients and tissue samples**

GHPA tissues were surgically removed at the Neurosurgery Department, Huashan Hospital, Fudan University. Histological diagnoses were performed independently by two experienced pathologists, according to the 2017 WHO classification. Three normal pituitary tissues were obtained from cadaveric organ donations with no evidence of any endocrine disease (Fudan University). For immunohistochemistry (IHC) study, a total of 39 GHPA samples were collected. For western blotting and qRT-PCR, 5 GHPA samples were included. The clinical characteristics are shown in supplementary Table 1\(^{41}\).
Cell culture

The rat pituitary adenoma GH3 cells (Cat. No. CCL-82.1), MMQ cells (Cat. No. CRL-10609) and mouse pituitary adenoma AtT-20 cells (Cat. No. CCL-89) were purchased from the ATCC and cultured in Ham’s F-12K medium (Shanghai Basalmedia Technologies Co., Ltd.) with 15% horse serum (Gibco), 2.5% fetal bovine serum (FBS, Gibco) and 1% penicillin/streptomycin (Gibco). All the cells were maintained in an incubator at 37°C supplemented with 5% CO₂.

Immunohistochemistry

Paraffin sections of GHPA and normal pituitary tissues were dewaxed by xylene and dehydrated by gradient ethanol. Incubation at room temperature with 3% H₂O₂ for 5-10 minutes and then washed in distilled water. Blocking the sections with 5% goat serum at room temperature in 10 minutes and incubation with primary antibody at 4°C overnight. Next day, HRP-labelled secondary antibody was carried out for incubation at room temperature in 30 minutes after washing in PBS. Then, DAB reagent was used to stain in proper time. Finally, re-staining the nuclei with Harris haematoxylin (blue), washed, dehydrated in gradient ethanol and xylene, and then mounted with permanent mounting medium. The immune-reactivity was measured and quantified with Image-Pro Plus 6.0.

Western blotting analysis

Proteins isolation was performed as previously described, grinded tissues and cell lysates were separated on SDS-polyacrylamide gels, and transferred to Nitrocellulose membrane (Millipore). Membranes were blocked with a Tris/saline solution containing 5% skim milk and 0.1% Tween-20 at room temperature for 1 hour, and incubated with a primary antibody overnight at 4 °C. The next day, membranes were washed with TBST buffer, and then incubated with HRP–conjugated secondary antibody with a 1:10,000 dilution at room temperature for 1 hour. After 3 times washed with TBST buffer, the bands were detected in a ChemiScope 3,400 imaging system using ECL substrate (Cat. No.
The catalog number of antibodies are listed in supplementary Table 2. All experiments were repeated three times.

**RNA extraction and qRT-PCR**

Trizol reagent (Cat. No. R401-01, Vazyme Biotech) was used to isolate total RNA according to manufacturer’s protocol. Reverse transcription was carried out to obtain cDNA using HiScript II qRT SuperMix (Cat. No. R222-01, Vazyme Biotech). Subsequently, qRT-PCR was performed using AceQ qPCR SYBR Green Master Mix (Cat. No. Q141-02, Vazyme Biotech), and amplification was detected with a Quant Studio 6 Flex Real-Time PCR System (ABI). Expression of target genes was normalized with the expression of GAPDH. The fold changes in gene expression were calculated using the equation \( \Delta \Delta Ct = \Delta Ct (\text{GENE-GAPDH})_{\text{normal}} - \Delta Ct (\text{GENE-GAPDH})_{\text{cancer}} \). The primer sequences are listed in Supplementary Table 3.

**RNA interference**

Before transfection, a density of \( 1 \times 10^6 \) GH3 cells/well were seeded into a 6-well plate. Transfection of siRNA was performed with RNAiMAX reagent (Cat. No. 13778075, Invitrogen). After 48 hours of transfection, cells were collected for further experiments. siRNA of p300 and negative control were synthesized by Shanghai GenePharma Co., Ltd.

**Cell viability assay**

For the siRNA-treated GH3 cells, a density of \( 5 \times 10^3 \) cells/well were seeded into a 96-well plate after 24 hours transfection. Cell viability were measured on the 24, 48, 72, 96 hours. For the A-485 treated GH3 cells, a density of \( 5 \times 10^3 \) cells were seeded into a 96-well plate incubated with dimethyl sulfoxide (DMSO; control) or a series of 2-fold-diluted concentrations of A-485 for 4 days. After that, Cell
Titer-Glo luminescent assays (Cat. No. G7572, Promega) were performed to measure cell viability following the manufacturer’s protocol, and the luminescence was monitored with a multifunctional microplate reader (EnVision, Perkin Elmer). Finally, the IC₅₀ values were calculated in GraphPad Prism 7.0.

**Clone formation assay**

For the siRNA-treated GH3 cells, 1×10³ transfected or control cells/well of each group were seeded in a 6-well plate. For the A-485 treated cells, 2×10³ cells/well were seeded in a 6-well plate and incubated with A-485 or DMSO after 24 hours of adhesion. The cell medium containing A-485 was changed every 3 days. After 3 weeks of growth, all the colonies were fixed with 4% paraformaldehyde and then stained with 0.1% crystal violet. The clone formation assay was counted with Image-Pro Plus 6.0.

**Cell cycle and apoptosis assay**

GH3 cells were seeded at a concentration of 1×10⁶ cells/well in a 6-well plate and incubated with different concentration of A-485 for 72 hours. For cell cycle assay, cells were collected and re-suspended in 70% ethanol overnight at 4°C. Then, the samples were washed with PBS and stained with propidium iodide (PI, Cat. No. A211-01, Vazyme Biotech) for 30 minutes at room temperature. For apoptosis assay, cells were incubated with a FITC-labelled annexin V and PI (Cat. No. A211-01, Vazyme Biotech) for 10 minutes. BD FACSCalibur (BD Pharmingen) was applied to analyze the cell cycle phase distribution and apoptosis according to the manufactural protocol as follow:

https://www.bdbiosciences.com/content/dam/bdb/marketing-documents/BD_FACSCalibur_instructions.pdf
ELISA assay

The supernatant of GH3 cells treated with A-485 after 24 hours and the blood of xenograft mice through eyeball method in serum were used to detect the concentrations of growth hormone by Rat/Mouse GH ELISA kit (Cat. No. EZRMGH-45K, RRID: AB_2892711, Merck millipore). All the experiments of ELISA assay were strictly carried out following up kit protocol as follow:

https://www.sigmaaldrich.cn/CN/zh/product/mm/ezrmgh?context=product

Xenograft experiment

Six- to eight-week-old male BALB/c nude mice were selected for the GH3 xenograft model. A density of 2.5x10^6 GH3 cells suspension mixed with Matrigel Matrix (Cat. No. 354262, Corning) were injected into subcutaneous tissue of each mouse. When the tumor formed, all the mice were randomly divided into 3 groups with 8 mice in each group (A-485 100 mg/kg, A-485 50 mg/kg and DMSO control). Tumor volumes was measured every 2 days and calculated as width^2× length × 0.5. A-485 was intraperitoneal injected once daily when the tumor volumes approached about 100 mm^3. All the mice were sacrificed after a 28-day experiment, the tumors were excised, weighted, and frozen at -80°C. The blood were collected from the orbit and frozen at -80°C.

RNA sequencing analysis

A concentration of 1x10^7 GH3 cells were seeded in 10cm dish plates and incubated with 5 μM, 2.5 μM of A-485 or DMSO for 48 hours. After that, cells were harvested and total RNA was isolated for preparation of cDNA library and then sequenced on Illumina Novaseq platform. Differential gene expression analysis was performed using the DESeq2 R package (1.16.1). The resulting P-values were adjusted using the Benjamini and Hochberg’s approach for controlling the false discovery rate. Corrected P-value of 0.05 and absolute foldchange of 2 were set as the threshold for significantly
differential gene expression. Kyoto Encyclopedia of Genes and Genomes (KEGG) database (http://www.genome.jp/kegg/) and Gene Ontology (GO) enrichment analysis were used to further analysis for gene-annotation enrichment.

Statistical analysis

All numerical results were expressed as the mean ± SD. and represented data from a minimum of three independent experiments. A two-tailed unpaired t-test was used to analyze differences between two groups. All analyses were performed using GraphPad Prism 7.0 statistical software (GraphPad Software, Inc., La Jolla, CA, USA). The level of statistical significance was set at P<0.05.

Study approval

This study was approved by the Ethics and Research Committees of Huashan Hospital, Fudan University. Tumor samples and corresponding clinical materials were obtained with the written consent from all patients. All the animal experiments were performed according to the ethical guidelines and approved by the Institute Animal Care and Use Committee (IACUC), Shanghai Institute of Materia Medica (SIMM), Chinese Academy of Sciences.

Results

p300 Expression Profile in GHPA and Normal Pituitary Gland

To assess the expression of p300 in GHPA tissues compared with that in normal pituitary tissues, we detected the protein and mRNA levels of p300 by IHC, western blotting and qRT-PCR. This clearly showed an increase in p300 expression in GHPA tissues compared to normal pituitary tissues (Figure 1, a-d), and p300 expression was relatively higher in the GH3 cell line (secreting GH and PRL) than
in the MMQ cell line (secreting PRL), AtT-20 cell line (secreting ACTH) and normal rat pituitary tissue as a control (Supplementary Figure 1). Hence, we selected the GH3 cell line for subsequent experiments. Analyzing the correlation between the expression of p300 and the clinical data, we found that the expression of p300 was positively correlated with the preoperative GH level and IGF-1 (insulin-like growth factor-1) index (upper limit of normal range for age- and sex-matched IGF-1 level) (Supplementary Figure 2, a and b). As the patients with suppression rate of octreotide suppression test (OST) above 90.51% are considered as SSA sensitive, we divided them into SSA responders and SSA non-responders. Here, the expression of p300 in the SSA non-responders exhibited an increasing trend compared to that in the SSA responders. It indicated that p300 might be involved in SSA resistance, although there was no significant difference (p = 0.08) (Supplementary Figure 2c). Overall, the high expression of p300 and its positive correlation with adverse clinical features established the theoretical foundation for subsequent studies.

**p300 Knockdown Suppresses Cell Proliferation in GH3 Cells**

To elucidate the functional role of p300 in GHPA, p300-specific siRNAs were constructed and transfected into GH3 cells. The knockdown efficiency was validated by western blotting and qRT-PCR (Figure 2, a and b). As the most effective siRNA, siRNA-p300#3 remarkably inhibited the levels of the specific acetylation targets H3K18ac and H3K27ac (Figure 2c); thus, it was chosen for further experiments. Notably, knockdown of p300 significantly inhibited GH3 cell proliferation (p < 0.001) and reduced clone formation (p < 0.001) (Figure 2, d and e). Taken together, our data suggest that p300 exerts a crucial effect on the proliferation of GH3 cells.

**p300 Inhibitor A-485 Reduces Cell Viability and GH Secretion in vitro**

To investigate the potential therapeutic effect of the p300 catalytic inhibitor A-485 on GH3 cells, CellTiter Glo was performed to evaluate cell viability. The IC_{50} was 0.489 μM after 3 days of A-485 treatment (Figure 3a). A-485 significantly induced the dose-dependent and time-dependent inhibition of cell proliferation in GH3 cells (Figure 3b). Gratifyingly, the levels of H3K18ac and H3K27ac were...
also decreased after treatment with A-485, suggesting that the antiproliferative effect of A-485 was associated with enzymatic inhibition of its target p300 in GHPA cells (Figure 3c). After 72 hours of incubation with A-485 at concentrations of 5 μM, 2.5 μM, 1.25 μM and 0 μM, flow cytometry was carried out to detect the cell cycle and apoptosis. Clearly, A-485 treatment increased the percentage of apoptotic cells (Figure 3d) and the protein expression of the apoptosis marker cleaved caspase 3 (Supplementary Figure 3a). In addition, A-485 induced G2/M phase arrest in GH3 cells (Figure 3e) and inhibited the expression of the G2/M phase-related protein cyclin B1 (Supplementary Figure 3b). To evaluate the long-term inhibitory effect of A-485 on GH3 cells, a clone formation assay was performed. Prolonged exposure to A-485 at concentrations of 0.5 μM (p < 0.0001) and 1 μM (p < 0.0001) for 3 weeks significantly inhibited the colonization of GH3 cells (Figure 3f). Furthermore, we tested the mRNA expression of Gh1 and evaluated the secretion of growth hormone after treatment of cells with A-485. It was clear that A-485 inhibited Gh1 mRNA expression and secretion ability in GH3 cells (Figure 3, g and h). In summary, these data suggest that A-485, a highly selective catalytic inhibitor of p300, can inhibit the development of malignant phenotypes and reduce GH expression in GH3 cells.

**p300 Inhibitor A-485 Reduced Tumor Survival and GH Secretion in vivo**

To evaluate the antitumor effects of the p300 selective inhibitor A-485 in vivo, we generated a subcutaneous xenograft model with GH3 cells in BALB/c nude mice. Treatment with A-485 significantly inhibited tumor growth with regard to tumor volume (32.37% inhibition in the 50 mg/kg group and 54.15% inhibition in the 100 mg/kg group) and tumor weight (43.83% inhibition in the 50 mg/kg group and 61.41% inhibition in the 100 mg/kg group) in comparison with the control regimen (Figure 4, a and b, Supplementary Figure 4). In regards to mouse body weight, because of hypersecretion of GH, it was increased in the control group, and A-485 clearly reduced the weight in the treatment group (Figure 4c). Additionally, ELISA was performed to explore GH secretion in...
serum. As expected, serum GH secretion was significantly reduced in the A-485-treated mice (Figure 4d).

**p300 Inhibitor A-485 Effects on Gene Expression in GH3 Cells**

To explore the antitumor molecular mechanism of A-485 in GHPA, RNA sequencing analysis was applied. The differentially expressed genes were revealed in heatmaps and volcano plot maps. Among them, 2,750 upregulated genes and 2,625 downregulated genes or 435 upregulated genes and 392 downregulated genes were observed in cells treated with 5 μM or 2.5 μM A-485, respectively (Figure 5a, Supplementary Figure 5). Venn diagrams show the overlapping genes for treatments with different concentrations. There were 732 genes that showed differential expression at both concentrations (Figure 5b). The fold changes in hub genes are shown in Figure 5c, which were verified by qRT-PCR (Figure 5d). Among the hub genes, Ptg1 and Gh1 were downregulated, which has previously been reported to be related to the occurrence and development of GHPA. Also, Cdk1 and Ccnb1 were downregulated and are known to be cell cycle-related genes. GO enrichment analysis of biological processes and molecular functions showed that most hub genes functioned in chromosome segregation and cell cycle processes (Supplementary Figure 6). KEGG analysis indicated that the PI3K/Akt/mTOR signaling pathway was specifically affected by A-485 (Figure 5e). As crucial molecules in the PI3K/Akt/mTOR pathway, the phosphorylation of Akt, mTOR and S6K was verified by western blotting. A-485 inhibited the phosphorylation level of these proteins (Figure 5f). Moreover, A-485 reduced the expression of c-Myc and inhibited the phosphorylation of Creb, which are crucial in GHPA oncogenicity and essential in GH secretion (Figure 5f).

**Discussion**

GHPA usually possesses two main pathophysiological characteristics, the occupying effect and hypersecretion of GH, which result in severe clinical symptoms and multisystemic comorbidities that shorten the lifespan of patients by 10-20 years. Hence, therapeutic strategies for GHPA aim to
control tumor growth and to achieve biochemical remission, and these strategies are still facing challenges. As mentioned above, transsphenoidal surgery can achieve satisfactory remission rates in microadenoma and intrasellar macroadenoma. However, the effect is much lower in invasive macroadenoma with suprasellar or/and parasellar expansion, and the recurrence rate is high due to active cell proliferation. For medical therapy, drug resistance is still a large obstacle. Therefore, novel therapeutic targets and drugs are urgently needed.

Currently, increasing attention has been given to the function of epigenetic alterations, particularly abnormal levels of histone acetylation in the progression of PA, which would provide novel insights into PA treatment. The levels of acetylation in the histone tail, especially H3 and H4, could be considered active markers that are regulated by HATs and histone deacetylases (HDACs). p300 is a crucial member of the HAT family and can regulate gene expression and facilitate transcriptional activity. p300 is overexpressed in various cancers, such as liver tumors, nasopharyngeal tumors and hemopathy. There is also a strong correlation between high expression of p300 and poor prognosis in these tumors, indicating that p300 promotes tumor growth. More intriguingly, in prostate cancer, p300 is not only involved in the progression of cancer cells but also impacts the secretion of androgen. As a hormone-secreting tumor, we were intensely curious about the role of p300 in GHPA. Overexpression of PTTGI and c-MYC has been shown to be positively correlated with tumor invasiveness, aggressiveness or recurrence in GHPA, and p300 can regulate the expression of these two crucial genes. In addition, p300 can facilitate transcription factor binding to the promoter region during the transcription of cyclic adenosine monophosphate (cAMP), and activation is of cAMP is highly associated with GH secretion and somatotroph proliferation. Thus, we assumed that p300 plays a critical role in the development of GHPA and sought to validate our hypothesis through a series of experiments.

In our study, we demonstrated that the expression of p300 was elevated in GHPA tissues compared with normal pituitary tissues. Subsequent analysis of clinical data showed that the expression of p300 was positively correlated with the preoperative GH level and the IGF-1 index, which was in line with the key characteristic of GHPA. In addition, the expression of p300 in the SSA.
responders group exhibited a higher trend than that in the control group, indicating that p300 might be involved in SSA resistance. There was no significant difference (p = 0.08), but this was probably due to the insufficient number of GHPA patients who received medical therapy before surgery. Consistent with the results observed in other cancers described above, the increased expression of p300 in GHPA and its correlation with clinical features provide the theoretical basis for targeting p300 for GHPA treatment.

Consequently, functional assays involving both genetic knockdown of p300 and treatment with A-485, a highly selective catalytic inhibitor, could lead to significant inhibition of cell proliferation and clone formation of GH3 cells in vitro. Flow cytometry assays and the expression of related markers indicated that the growth inhibition of GH3 cells is due to the induction of apoptosis and G2/M cell cycle arrest by A-485. Strikingly, A-485 significantly inhibited the mRNA expression and secretion of GH in vitro. Subsequently, the pharmacological efficacy of A-485 was evaluated in a subcutaneous xenotransplantation model. In agreement with the efficacy observed in vitro, A-485 effectively inhibited tumor growth and GH secretion. These therapeutic effects of A-485 in vitro and in vivo suggest that p300 inhibitors might serve as potential candidates for GHPA therapy.

To explore the mechanism by which A-485 inhibits GHPA tumorigenesis, transcriptome sequencing analysis was performed. The results of the GO enrichment analysis showed that the most highly differentially expressed genes were enriched in chromosome segregation, the cell cycle and other biological processes. Another bioinformatic analysis of KEGG enrichment revealed that the main pathways influenced by A-485 include the cell cycle pathway and the PI3K/Akt/mTOR pathway. The PI3K/Akt/mTOR pathway is involved in numerous cellular processes, such as cell growth, the cell cycle, and protein synthesis. Its aberrant activation has been confirmed to be associated with the malignant behavior of cancers including glioma, breast cancer, and ovarian cancer. Based on previous studies, aberrant activation of the PI3K/Akt/mTOR pathway has been confirmed to contribute to malignant proliferation and hormone secretion in GHPA. Through the application of the mTOR inhibitor rapamycin or RAD001, clear inhibition of the viability and proliferation of GHPA cells was induced. Similarly, the dual-PI3K/mTOR inhibitor XL765
inhibited GHPA cell growth but showed drug toxicity and produced no significant differences in GH secretion in vivo. Compared with the inhibitors described above, A-485 shows great advantages (a lower IC$_{50}$ and effective inhibition of GH secretion in vivo) in terms of antitumor effects. However, the expression of phosphorylated Akt, S6K and mTOR was also decreased, suggesting that the antitumor effects of A-485 may occur through inhibition of the PI3K/Akt/mTOR pathway. Moreover, A-485 led to the downregulation of c-Myc, Ptgp1 and Gh1, which participate in tumorigenesis and function in GHPA. What’s more, the expression of phosphorylated Creb was also downregulated by A-485. It has been demonstrated that Creb acts as a crucial transcriptional regulator in the cAMP signaling pathway, which is closely related to GH secretion.

In summary, our findings highlight the significance of p300 in the tumorigenesis of GHPA, suggesting that p300 may serve as a novel therapeutic target. The antitumor effect of the p300 inhibitor A-485 in vitro and in vivo demonstrates that A-485 might serve as a potential therapeutic for GHPA.
Disclosure of Potential Conflicts of Interest

The authors declare no potential conflicts of interest.

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Data Availability

All data generated or analyzed during this study are included in this published article.

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**Figure legend**

**Figure 1** p300 expression profile in GHPA and normal pituitary tissue.

(a) IHC image of p300 expression in normal pituitary and GHPA tissues. Each column represents a different sample. Scale bars: 100 μm left, 50 μm right. (b) Logarithmized IOD value of p300 expression according to IHC images of GHPA tissues (n = 39) and normal pituitary tissues (n = 3). (c) The mRNA expression of p300 in specimens (GHPA: n = 5; Normal: n = 3) assessed by qRT-PCR. (d) The protein expression of p300 in specimens (GHPA: n = 5; Normal: n = 3) assessed by western blotting. * p<0.05, ** p<0.01, *** p<0.001. The bar represents ± SD.

**Figure 2** Knockdown of p300 inhibits cell proliferation, clone formation and the acetylation of H3.

(a, b) The protein and mRNA expression of p300 in GH3 cells after transfection with p300-siRNAs, the negative control and the mock control. (c) The protein expression of H3K18ac and H3K27ac in p300-knockdown GH3 cells. (d) Cell proliferation of p300-knockdown GH3 cells. (e) Clone formation by p300-knockdown GH3 cells. * p<0.05, ** p<0.01, *** p<0.001. The bar represents ± SD.

**Figure 3** The p300 inhibitor A-485 exerts antitumor effects in vitro.

(a) The IC₅₀ of A-485 in GH3 cells was detected after 3 days of A-485 incubation. (b) The cell viability was detected at 24h, 48h, 72h and 96h, respectively. (c) The protein expression of H3K18ac and H3K27ac in A-485 treated GH3 cells. (d) Apoptosis analysis of A-485 treated GH3 cells. (e) Cell cycle analysis of A-485 treated GH3 cells. (f) Clone formation of A-485 treated GH3 cells. (g) The mRNA expression of Gh1 in A-485 treated GH3 cells. (h) The secretion of GH in the supernatant of A-485 treated GH3 cells. * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001. The bar represents ± SD.
**Figure 4** The antitumor effect of A-485 in vivo.

(a, b, c) The tumor volume, tumor weight and mouse weight of each group during the experiment are shown. (d) The level of growth hormone in serum was detected by ELISA assays. * p<0.05, ** p<0.01, *** p<0.001. The bar represents ± SD.

**Figure 5** Exploration of the potential mechanism affected by A-485.

(a) Heat maps of differentially expressed genes at different concentrations of A-485. (b) Venn diagrams showing the overlapping genes between each group. (c) The fold changes in hub genes based on the RNA sequencing. (d) The mRNA expression of hub genes was verified by qRT-PCR. (e) KEGG analysis indicated the specific signaling pathways affected by A-485. (f) The protein expression of key molecules affected by A-485 were detected by western blotting. The bar represents ± SD.
Figure 4

(a) Tumor volume (mm³) over time for different groups.
- Control
- A-485 50 mg/kg
- A-485 100 mg/kg

(b) Tumor weight comparison:
- Control
- A-485 50 mg/kg
- A-485 100 mg/kg

(c) Mouse weight over time for different groups.
- Control
- A-485 50 mg/kg
- A-485 100 mg/kg

(d) Growth hormone (ng/ml) comparison:
- Control
- A-485 50 mg/kg
- A-485 100 mg/kg
