

## Original Article

# Exploration of the mechanism by which H3K27ac activates APOC1 to promote PTC proliferation

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**Abstract:** Early studies have suggested that apolipoprotein C1 (APOC1) functions as an oncogenic factor in papillary thyroid carcinoma (PTC), where it promotes tumor cell proliferation. Here, we focus on the regulatory mechanisms underlying APOC1-mediated PTC cell growth, with particular attention to histone modification-driven changes in APOC1 expression and disease progression. Our bioinformatics analysis revealed that APOC1 was differentially expressed in PTC and might be involved in tumor-related biological processes. The influence of APOC1 on PTC cell growth and glycolysis was studied. The impact of APOC1-induced glycolytic reprogramming on PTC cell proliferation was assessed. Cells were treated with the selective p300/CBP histone acetyltransferase inhibitor (C646) and the histone deacetylase inhibitor sodium butyrate (NaB) to modulate histone acetylation levels. Subsequently, chromatin immunoprecipitation (ChIP) assays were carried out to assess the role of histone modifications in APOC1 expression regulation. Finally, nude mouse xenograft models were used to confirm the oncogenic role of APOC1 in PTC development. Bioinformatics and experimental analyses confirmed that APOC1 expression showed a strong correlation with glycolysis-related genes and PTC cell proliferation was influenced by changes in glycolysis. Meanwhile, APOC1 expression was activated by H3K27ac. Xenograft models were subsequently used to further validate these findings in vivo. H3K27ac-mediated activation of APOC1 may promote glycolytic reprogramming in PTC cells, thereby driving their proliferation.

**Keywords:** Apolipoprotein C1 (APOC1), glycolysis, histone H3 lysine 27 acetylation (H3K27ac), papillary thyroid carcinoma (PTC), histone modification

## Introduction

Thyroid carcinoma (TC) is a common head and neck malignancy with a steadily increasing incidence [1]. In 2020, China reported 586,202 new TC cases and 43,646 deaths. TC is the seventh most frequently diagnosed malignancy in men and ranks third among women [2]. Previous studies have shown that apolipoprotein C1 (APOC1) promotes papillary thyroid cancer (PTC) proliferation [3]. APOC1, a small protein primarily found in the liver, is key in lipid metabolism and the structure of high- and very low-density lipoproteins [4-6]. Recent studies indicate that APOC1 levels are markedly elevated in several malignancies such as lung cancer [7], hepatocellular carcinoma [8], gastric carcinoma [9], renal cell carcinoma [10], and ovari-

an cancer [11], and contributes to tumor cell proliferation, migration, and invasion. In gastric carcinoma, elevated APOC1 expression is significantly linked to clinical stage and lymph node metastasis [12]. Moreover, patients with breast cancer show significantly higher serum APOC1 levels than those who are healthy, suggesting its usefulness as a serum biomarker for diagnosing breast cancer [13].

Metabolic reprogramming supports cancer progression by promoting rapid proliferation and metastasis [14, 15]. Cancer cells are characterized by uncontrolled growth. They depend on aerobic glycolysis. Under normal oxygen conditions, glucose is mainly converted into lactate rather than being fully oxidized through the tricarboxylic acid cycle. Glycolytic phenotype is

validated by glucose transporters (GLUTs) and multiple core rate-limiting enzymes maintain the glycolytic phenotype. The related enzymes are Hexokinase 2 (HK2), Lactate dehydrogenase A (LDHA), 6-phosphofructo-2-kinase (PFKFB3) and Pyruvate Kinase M2 (PKM2). Breast cancer patients with higher expression of these genes tend to have poorer prognoses. They may serve as indicators for prognosis evaluation [16]. Previous studies by Xu et al. showed that APOC1 promotes tumor glycolysis. In addition, histone modification has been reported to regulate APOC1 expression in gastric cancer [9].

Histone acetylation is an important form of post-translational regulation. Increasing evidence suggests that abnormal acetylation plays a critical role. It contributes to cancer initiation and progression [17]. Histone H3 Lysine 27 acetylation (H3K27ac) is a common epigenetic marker of active regulatory elements and closely related to genes that control cell proliferation and differentiation [18]. Prior studies suggested that APOC1 enhances the proliferation of PTC cells [3]. Data from the UCSC database showed clear H3K27ac enrichment in the APOC1 promoter region. This finding suggests that acetylation may contribute to the regulation of APOC1. In this study, we explored how acetylation influences APOC1 expression and determine whether APOC1 promotes PTC cell proliferation through glycolytic reprogramming. These findings may provide further insight into the epigenetic and metabolic mechanisms of PTC.

### Materials and methods

#### *Differential expression and statistical analysis of APOC1*

APOC1 transcriptome data were retrieved from the TCGA-THCA and GTEx cohorts. After integrating the datasets, we divided samples into two groups based on the median APOC1 expression level. We then examined transcriptional differences associated with varying APOC1 expression. Differential expression analysis was carried out using DESeq2, applying a threshold of  $|\log_2FC| > 1$  and adjusted  $P$  value  $< 0.05$  to define significance. A volcano plot was generated to display the results.

#### *Correlation analysis between APOC1 expression and glycolysis-related genes*

Using RNA-seq data from the TCGA-THCA cohort, we investigated the potential association between APOC1 and glycolysis-related genes. Glycolysis-related gene sets were retrieved from the Molecular Signatures Database, and their relationships with APOC1 were assessed using Pearson correlation and co-expression analyses. Heatmaps were generated to display both expression profiles and correlation patterns, with red and blue indicating high and low expression levels, respectively. All analyses and visualizations were conducted in R 4.3.0 using *corrplot* and *ggplot2*.

#### *Functional enrichment analysis*

We first filtered genes with  $|\log_2FC| > 1$  and adjusted  $P < 0.05$ . Based on these genes, Gene Set Enrichment Analysis (GSEA) was conducted to explore pathways potentially linked to APOC1 in thyroid cancer.

#### *Cell cultivation and gene transfer*

PTC cell lines (BCPAP, K1, and TPC-1) and Nthy-ori 3-1 cells were purchased from the Shanghai Institute for Biological Sciences, Chinese Academy of Sciences (ATCC). The cells were maintained in RPMI-1640 medium (Bio-channel, China) at 37°C under 5% CO<sub>2</sub>. Following Lipofectamine 2000-mediated transfection (Thermo Fisher Scientific, USA), APOC1 was successfully upregulated in BCPAP cells and downregulated in TPC-1 cells. The experimental design therefore comprised OENC, OE, shRNAC, and three shRNA groups (#1, #2, and #3). Additional treatment groups were TPC-1 + a selective p300/CBP histone acetyltransferase inhibitor (C646), BCPAP + sodium butyrate (NaB), OE + Alkannin, and shRNA#2 + DASA-58.

#### *Western blot*

Stably transfected PTC cells were lysed to extract total protein using a commercial kit (Applygen, USA). Using a BCA assay kit (Abbkine, China), protein concentration was subsequently determined. Subsequently, samples were separated by SDS-PAGE and transferred onto PVDF membranes (Merck, Germany). After bl-

ocking with 5% skim milk in TBST, membranes were treated with primary antibodies against APOC1 (Affinity, 1:1000), HK2 (Affinity, 1:1000), GLUT1 (Affinity, 1:1000), LDHA (Affinity, 1:1000), Ki-67 (Affinity, 1:1000), and c-Myc (Affinity, 1:1000), PKM2 (Affinity, 1:1000); cyclin D1 (ZENBIO, 1:1000), cyclin A2 (ZENBIO, 1:1000), H3K27ac (ZENBIO, 1:1000), H3 (ZENBIO, 1:1000), and  $\beta$ -actin (Servicebio, 1:5000), followed by HRP-conjugated secondary antibodies (Servicebio, 1:5000). Protein bands were evaluated through ECL (Lablied, China) and quantified with ImageJ.

### *Patient specimens*

The papillary thyroid carcinoma (PTC) tissue microarray (Batch No. ThyP120C-S01) was purchased from Shanghai Superchip Biotechnology Co., Ltd. The microarray comprises 58 thyroid carcinoma specimens along with their corresponding adjacent non-tumorous tissues. In addition, this study collected specimens from The First Affiliated Hospital of Hebei North University, including samples from 10 PTC patients admitted between January 2021 and December 2023. This study was approved by the Ethics Committee of The First Affiliated Hospital of Hebei North University. All participants provided informed consent and signed written informed consent forms. The inclusion criteria were as follows: (1) first-time presentation to the Department of Otolaryngology-Head and Neck Surgery at The First Affiliated Hospital of Hebei North University; (2) a histopathological diagnosis of papillary thyroid carcinoma (PTC); (3) consent to undergo thyroidectomy (total or partial) combined with lymph node dissection; and (4) availability of complete preoperative laboratory data, electronic medical records, and postoperative pathological reports.

### *RT-qPCR*

Total RNA was obtained from both PTC cells and thyroid cancer tissues, and RNA concentration was determined using a NanoDrop 2000 spectrophotometer (Thermo Scientific, USA). cDNA synthesis was performed from 1  $\mu$ g RNA using the FastKing Kit (Tiangen, China). MORT-qPCR reactions (10  $\mu$ L) were prepared with 2 $\times$  SYBR Green Master Mix (LABLEAD, China), gene-specific primers, and cDNA template. Amplification was conducted on an ABI

7500 system (TaKaRa, Japan) under standard cycling conditions (95°C for 30 s; 40 cycles of 95°C for 5 s and 60°C for 34 s). Relative expression was calculated using the  $2^{-\Delta\Delta Ct}$  method with  $\beta$ -actin as an internal control. Primer sequences are listed in [Supplementary Table 1](#).

### *Colony formation*

For the colony formation assay, stably transfected PTC cells were plated into six-well plates (500 cells per well) and allowed to grow for one week. Colonies were subsequently fixed with 4% paraformaldehyde and stained using 0.1% Giemsa (Solarbio, China). Only colonies with more than 50 cells were counted, and analysis was performed using ImageJ.

### *Growth curve analysis of cell proliferation*

To evaluate cell proliferation,  $1 \times 10^4$  cells were seeded into 96-well plates in triplicate for each condition. Cell growth was monitored in real time using the IncuCyte ZOOM system, with images acquired every 3 hours. The observation was stopped once cells reached about 80% confluence, and the resulting data were used to plot growth curves.

### *Cell cycle analysis*

Cell-cycle distribution was evaluated in  $1 \times 10^6$  stably transfected cells from each group. After overnight fixation in 75% ethanol at -20°C, cells were collected by centrifugation and subjected to RNase A digestion (100  $\mu$ L, 37°C, 30 min). Samples were then incubated with propidium iodide (400  $\mu$ L) in the dark at 4°C for 30 min prior to DNA content analysis using an ACEA NovoCyteR flow cytometer with 488 nm excitation.

### *Immunohistochemistry experiment (IHC)*

Tissue sections embedded in paraffin (4  $\mu$ m) underwent deparaffinization and rehydration. This was followed by antigen retrieval in citrate buffer. Using 3% hydrogen peroxide, endogenous peroxidase activity was quenched to reduce background staining. Sections were incubated with primary antibodies - APOC1 (Affinity, 1:150), HK2 (Affinity, 1:100), PKM2 (Affinity, 1:100), LDHA (Affinity, 1:100), Ki-67 (Affinity, 1:100), c-Myc (Affinity, 1:100), Cyclin D1 (ZENBIO, 1:100), and Cyclin A2 (ZENBIO, 1:100)

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- followed by an HRP-conjugated secondary antibody. After DAB staining finished initially, hematoxylin was utilized to re-dye these tissue slices. A series of steps followed one after another: differentiation, bluing, dehydration, transparency and sealing.

### *Immunofluorescence (IF)*

Frozen sections were first immersed in 4% paraformaldehyde for fixation. After that, they were washed three times with PBST, with each rinse lasting 15 min, to eliminate residual fixative while maintaining tissue integrity. The sections were then blocked with 5% goat serum for 1 h, incubated overnight with the LDHA (Affinity, 1:500) primary antibody, and subsequently treated with a fluorescent secondary antibody for 1 h. Finally, the stained sections were mounted with DAPI-containing medium and observed under a fluorescence microscope.

### *Assessment of lactate production*

Lactate levels were assessed in samples from  $1 \times 10^6$  cells or 0.1 g of tissue using a Solarbio lactate assay kit (China). After the addition of the appropriate reagents, the samples were incubated at 37°C, and the subsequent procedures were carried out according to the manufacturer's instructions.

### *Determination of adenosine triphosphate (ATP) content*

ATP levels were assessed in samples from  $5 \times 10^6$  cells or 0.1 g of tissue using a Solarbio ATP assay kit (China). After the addition of extraction solution and chloroform, the samples were vortexed and placed on ice. The subsequent procedures were performed according to the kit instructions. ATP content was derived from the absorbance values obtained at 450 nm after incubation at 37°C. To enable comparison between samples, the results were adjusted based on cell number or tissue mass.

### *Determination of glucose uptake capacity*

To assess glucose uptake,  $1 \times 10^6$  cells were processed with a Beyotime glucose assay kit (China). Following cell lysis and centrifugation, supernatants were collected. Samples were

incubated at 95°C for 8 min and subsequently cooled to 4°C on ice. Next, 180 µL of each preparation was loaded into 96-well plates for absorbance measurement at 630 nm. Results were normalized against total cell content.

### *Chromatin immunoprecipitation (ChIP) assay*

A chromatin immunoprecipitation assay was performed using a commercial kit from Boxin Biotechnology (China). Cell detachment was performed with trypsin. In parallel, tissue samples were dissected into small fragments and fixed in formaldehyde. Glycine was then added to terminate cross-linking. After lysis, the samples were subjected to sonication and centrifugation, and the supernatants were incubated with magnetic beads and an H3K27ac-specific antibody (ZENBIO). Following washing, both Input and IP samples were treated with Proteinase K and incubated at 65°C to reverse cross-linking. The precipitated DNA was collected and quantified by PCR.

### *Subcutaneous xenograft model*

All animal experiments were conducted in accordance with the regulations of the IACUC of Beijing Biotechnology Co., Ltd. Female BALB/c nude mice were used for xenograft experiments, and all procedures met the relevant ethical requirements. BCPAP ( $1 \times 10^6$ , OENC or OE) and TPC-1 cells ( $1 \times 10^6$ , shRNAC, shRNA#1, or shRNA#2) were resuspended in 100 µL PBS and inoculated into the right axillary dorsal region of nude mice. Tumor volumes were measured three times weekly with a vernier caliper. At 28 days, the acquisition of tumor-related data was achieved by imaging the mice and collecting tumors after CO<sub>2</sub> euthanasia, which allowed further photographic recording and analytical assessment. Mice were placed in a 9.6 L polyurethane chamber connected to a CO<sub>2</sub> gas cylinder. CO<sub>2</sub> was delivered at two flow rates: a low-flow condition of 30% chamber volume displacement per minute and a high-flow condition of 100% chamber volume displacement per minute. Animals were exposed until cessation of respiration, after which CO<sub>2</sub> administration was maintained for an additional 1 min. Mice were then removed and subjected to cervical dislocation as a secondary method of euthanasia. The chamber was subsequently disinfected with 70% ethanol.



## Statistical analysis

GraphPad Prism version 10 was applied for statistical analysis and data plotting. Data are expressed as mean  $\pm$  standard deviation (SD) or mean  $\pm$  standard error of the mean (SEM), as appropriate. The independent-samples t-test was used for comparisons between two groups. One-way ANOVA, two-way ANOVA, and repeated-measures ANOVA were used for multiple-group and repeated-measures comparisons. Nonparametric rank-sum tests were applied when data did not meet normality or homogeneity of variance assumptions. *P* value <0.05 was considered statistically significant.

## Results

### Bioinformatics analysis of APOC1 expression

TCGA-based analysis revealed significantly elevated APOC1 mRNA levels were significantly higher in THCA tissues than in neighboring normal tissues (**Figure 1A, 1B**). Moreover, the high- and low-APOC1 expression groups exhibited distinct transcriptional profiles. Differential expression analysis revealed 8,410 genes, among which 4,655 were upregulated and 3,755 were downregulated (**Figure 1C**). APOC1 mRNA was correlated with glycolysis-related genes (**Figure 1D, 1E**). GSEA revealed that high APOC1 expression was associated with the enrichment of fibroblast growth factor signaling and immune regulation pathways (**Figure 1F**).

### APOC1 expression is increased in PTC tissues

Compared with Nthy-ori 3-1 cells and adjacent normal tissues, APOC1 expression was markedly increased in PTC cell lines and tissues (**Figure 2A, 2B, 2D, 2E**). IHC staining further demonstrated that APOC1 was mainly detected in the cytoplasm and was highly expressed in PTC tissues (**Figure 2C**). To investigate its role, APOC1 was overexpressed in BCPAP cells (low endogenous levels) and silenced in TPC-1 cells (high endogenous levels). Western blot analysis confirmed efficient overexpression in the OE group (**Figure 2F, 2I**) and effective knockdown by shRNA#1 and shRNA#2 (**Figure 2G, 2H**); therefore, these cells were used in subsequent experiments. Furthermore, in our previous study, we investigated the association between

APOC1 expression and clinicopathological characteristics [3]. High APOC1 expression was significantly associated with advanced clinical stage (Stage III-IV) and lymph node metastasis (**Supplementary Table 2**).

### APOC1 promotes PTC cell proliferation

Colony formation, growth curve, and cell cycle assays were performed to assess the effect of APOC1 in PTC cell proliferation. APOC1 overexpression promoted colony formation (**Figure 3A, 3B**) and accelerated cell growth (**Figure 3C, 3D**). It also increased the proportion of cells in S/G2 phase (**Figure 3E, 3F**). In contrast, APOC1 knockdown produced the opposite effects (**Figure 4A-F**). Taken together, our data support a pro-proliferative role for APOC1 in PTC cells.

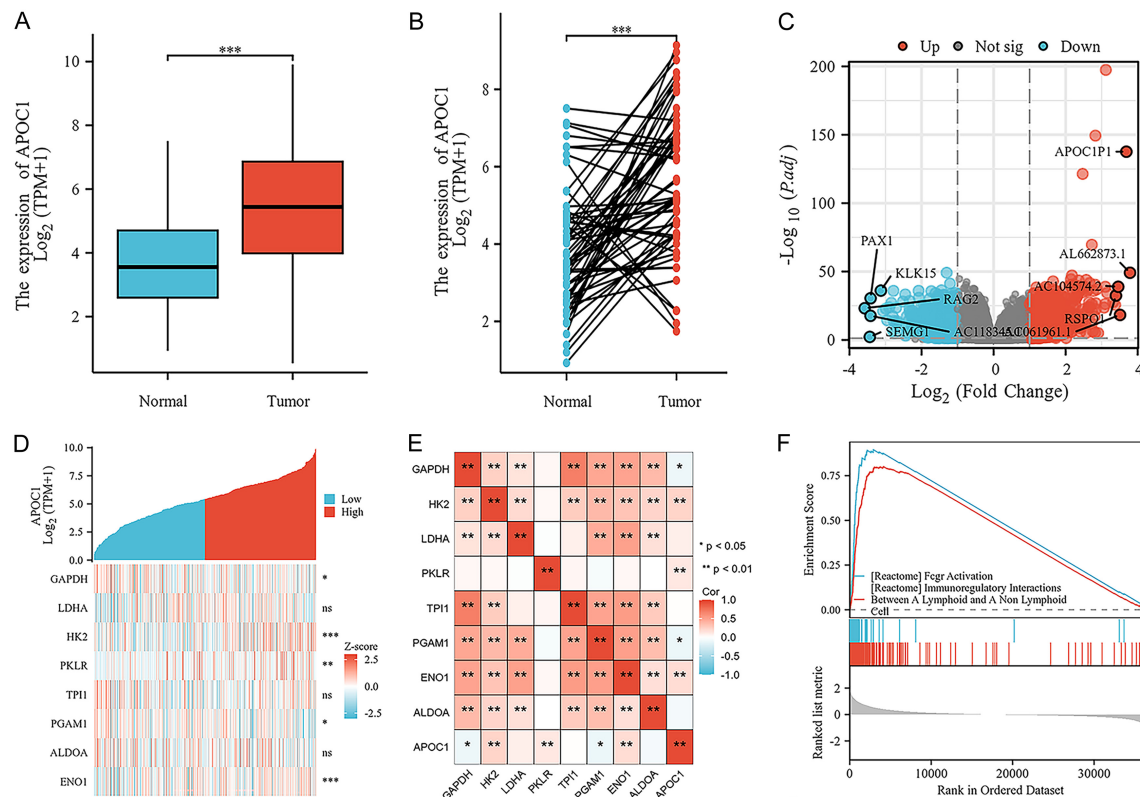
### APOC1 promotes glycolysis in PTC cells

Overexpression of APOC1 enhanced glycolytic activity in PTC cells, reflected by higher lactate secretion, ATP level, and glucose uptake. By contrast, APOC1 depletion reduced these metabolic indicators, underscoring its positive association with glycolysis (**Figure 5A-F**). Western blot and RT-qPCR analyses showed elevated expression of glycolytic proteins (GLUT1, PKM2, HK2, and LDHA) in the OE group and reduced levels in knockdown cells (**Figure 5G-L**). These findings indicate that APOC1 promotes glycolysis in PTC cells.

### APOC1 facilitates PTC cell proliferation by promoting glycolysis

To explore the link between APOC1-induced proliferation and glycolysis, OE cells were treated with the PKM2 inhibitor Alkannin. Western blot showed reduced PKM2 and proliferation proteins (Ki-67, c-Myc, cyclin D1 and cyclin A2) in the OE + Alkannin group (**Figure 6A, 6E**). Growth curve and cell cycle analyses confirmed that Alkannin treatment suppressed APOC1-driven proliferation (**Figure 6C, 6D, 6H, 6I**). Conversely, treatment with the PKM2 activator DASA-58 in the shRNA#2 group restored PKM2 expression and increased proliferation-related proteins (**Figure 6B, 6F**). Growth curve and cell cycle analyses further confirmed that DASA-58 enhanced cell proliferation and promoted S/G2-phase progression compared with the

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**Figure 1.** Differential expression and correlation of APOC1. A. Boxplot depicting the differences in Apolipoprotein C1 (APOC1) expression levels between Papillary thyroid cancer (PTC) tissues and their adjacent normal tissues. B. Paired analysis of APOC1 expression in matched PTC tissues and adjacent normal tissues. C. Volcano plot of differential gene expression. D. Co-expression heatmap of APOC1 and glycolysis-related genes. E. Correlation heatmap of glycolysis-related genes with APOC1. F. Representative GSEA plot of APOC1-related genes. \* $P < 0.05$ , \*\* $P < 0.01$ .

shRNA#2 group (Figure 6G, 6J-L). Taken together, these findings indicate that APOC1 promotes PTC cell proliferation through a glycolysis-dependent mechanism.

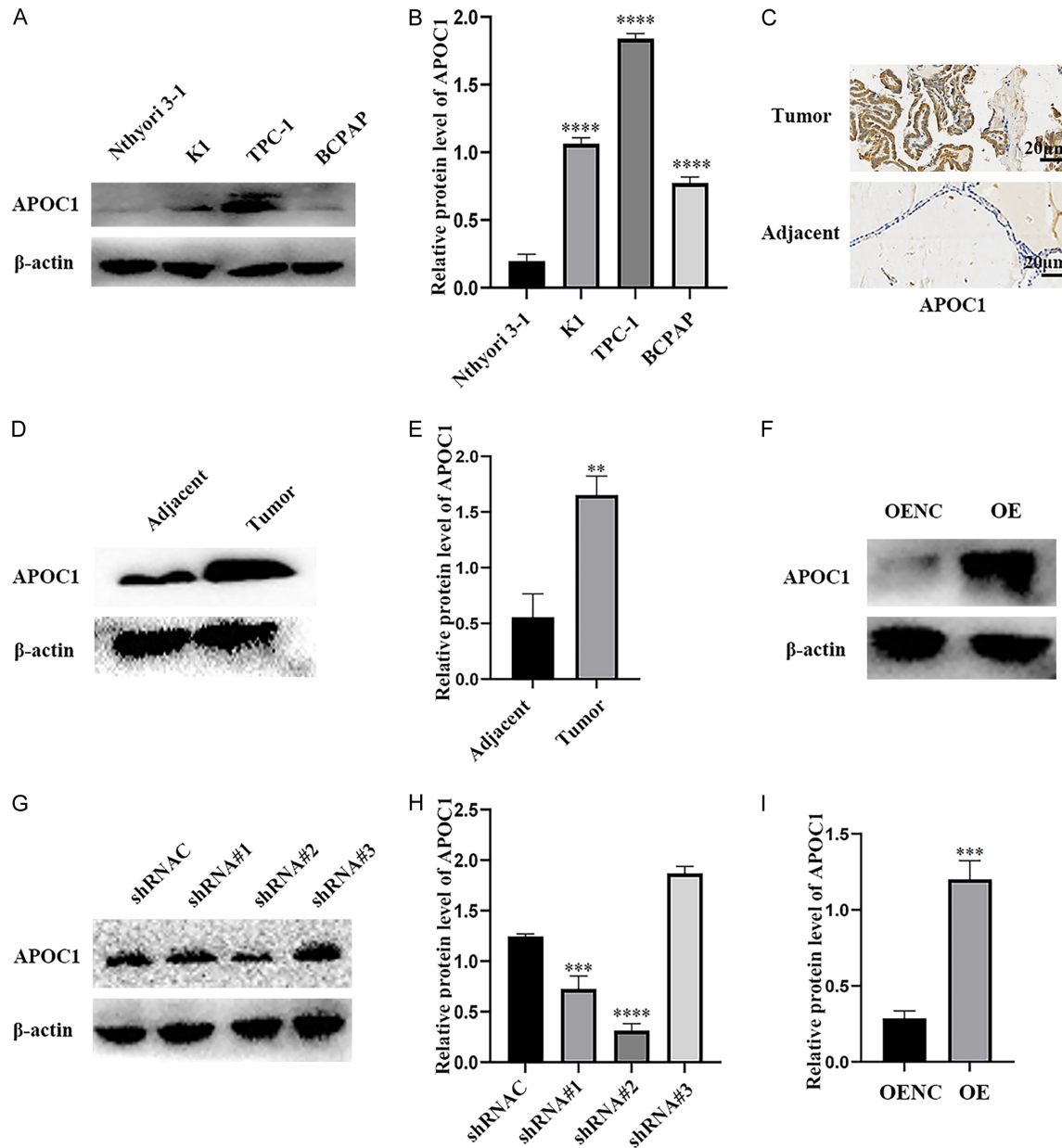
### *APOC1 is regulated by H3K27ac in PTC*

Western blot analysis revealed significantly higher H3K27ac levels in PTC tissues relative to adjacent non-tumorous tissue (Figure 7A, 7B). Both H3K27ac and APOC1 were upregulated, suggesting a potential association. UCSC Genome Browser data showed an H3 acetylation peak within the APOC1 locus (Figure 7C). ChIP-qPCR further confirmed increased H3K27ac enrichment at the APOC1 promoter in PTC tissues relative to normal tissues (Figure 7D), indicating that APOC1 may be regulated by H3K27ac. Consistently, PTC cell lines exhibited higher H3K27ac and APOC1 levels than normal thyroid cells (Figure 7E, 7F), with ChIP-qPCR showing greater H3K27ac enrichment at the

APOC1 promoter in TPC-1 and BCPAP cells (Figure 7G).

### *H3K27 acetylation activates APOC1 expression in PTC cells*

Western blot analysis indicated significantly elevated levels of H3K27ac and APOC1 in the BCPAP + NaB group compared to the BCPAP and BCPAP + DMSO groups (Figure 7H, 7I). Western blot analysis indicated that H3K27ac and APOC1 expression were significantly reduced in the TPC-1 + C646 group (Figure 7J, 7K). This decrease was evident when compared with both the TPC-1 and TPC-1 + DMSO groups. The activation of APOC1 expression in PTC cells appeared to be associated with H3K27ac enrichment, suggesting a potential regulatory contribution of this histone modification. These data suggest that H3K27ac may indicate a potential regulatory role in activating APOC1 expression in PTC cells.



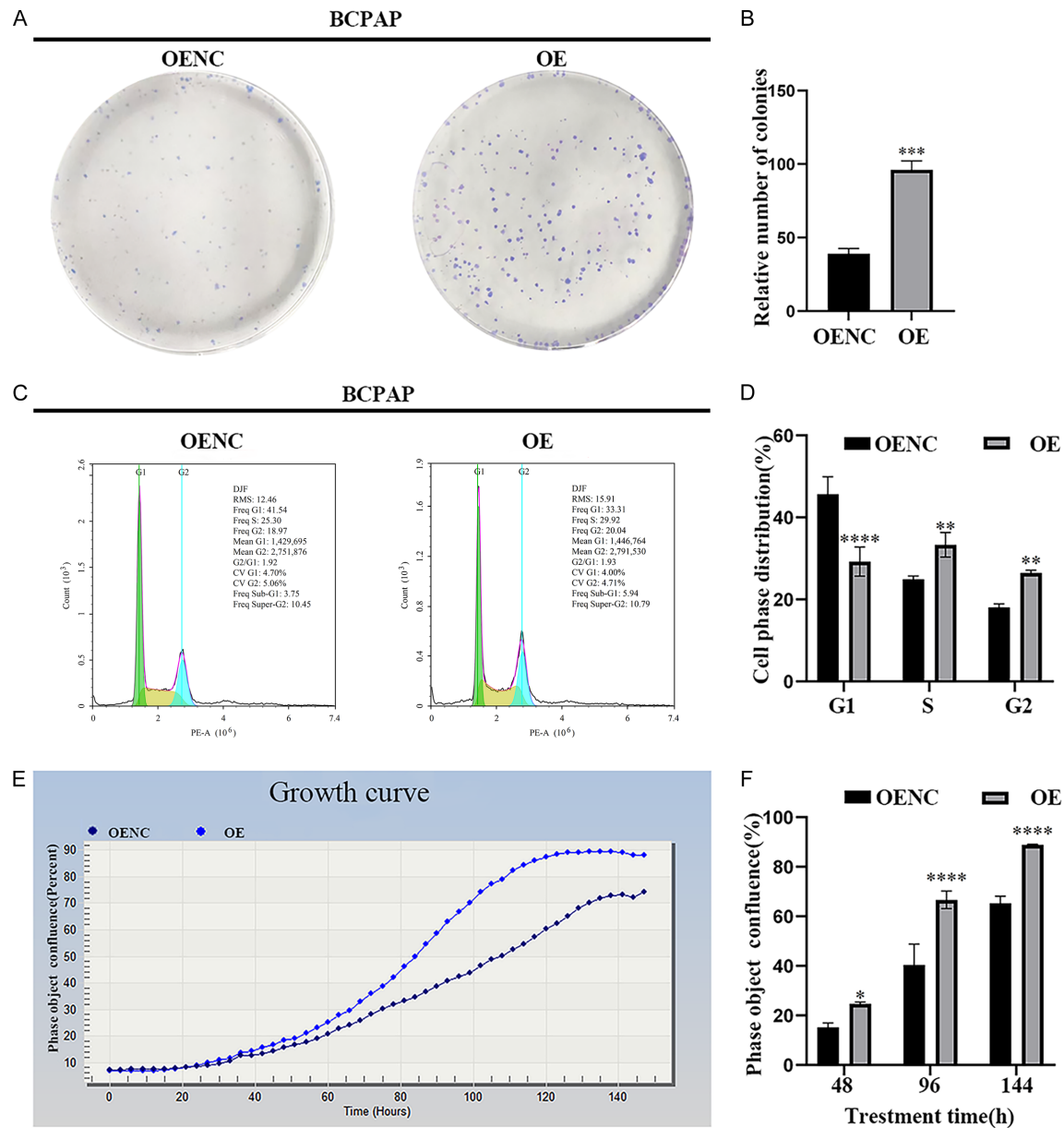
**Figure 2.** Upregulation of APOC1 expression in PTC. A, B. Differences in APOC1 expression between PTC cells and normal thyroid cells were assessed by Western blot. C. Immunohistochemistry (IHC) staining (400×) showed the expression pattern of APOC1 in PTC tissues and paired adjacent noncancerous tissues. D, E. APOC1 protein expression in PTC tissues and corresponding adjacent noncancerous tissues was further determined by western blot. F, I. APOC1 expression level following APOC1 overexpression in BCPAP cells. G, H. Expression level of APOC1 after APOC1 knockdown in TPC-1 cells. Unpaired Student's *t*-test and one-way analysis of variance (ANOVA) were used for statistical evaluation in the experiments. \**P*<0.05, \*\**P*<0.01, \*\*\**P*<0.001, \*\*\*\**P*<0.0001.

#### *APOC1 promotes PTC tumor growth in vivo*

A xenograft-based tumor model established to investigate the contribution of APOC1 to PTC growth in vivo. Tumors in the OE group grew markedly faster than those in the OENC group

(Figure 8A, 8E) whereas growth was inhibited in the shRNA#1 and shRNA#2 groups (Figure 8B, 8F). Representative tumor images from independent in vivo experiments are shown in Figure 8 to confirm reproducibility. Western blot and IHC analyses showed elevated prolifera-

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**Figure 3.** Impact of APOC1 overexpression on the proliferation of PTC cells. A. Colony formation analysis of cells with APOC1 overexpression. B. Quantitative assessment of cell colonies in APOC1-overexpressing cells. C. Cell cycle distribution of APOC1-overexpressing cells. D. Percentage analysis of cell cycle phases in APOC1-overexpressing cells. E. Cell growth profile following APOC1 overexpression. F. Analysis of cell growth following APOC1 overexpression. Data were analyzed using unpaired t-tests, two-way ANOVA and repeated-measures ANOVA, as appropriate, followed by post hoc tests where applicable. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , \*\*\*\* $P < 0.0001$ .

tion-related protein levels in APOC1-overexpressing tumors (Figure 8C, 8G) and reduced levels in knockdown groups (Figure 8D, 8H, 8I). These results demonstrate that APOC1 accelerates PTC tumor growth and proliferation.

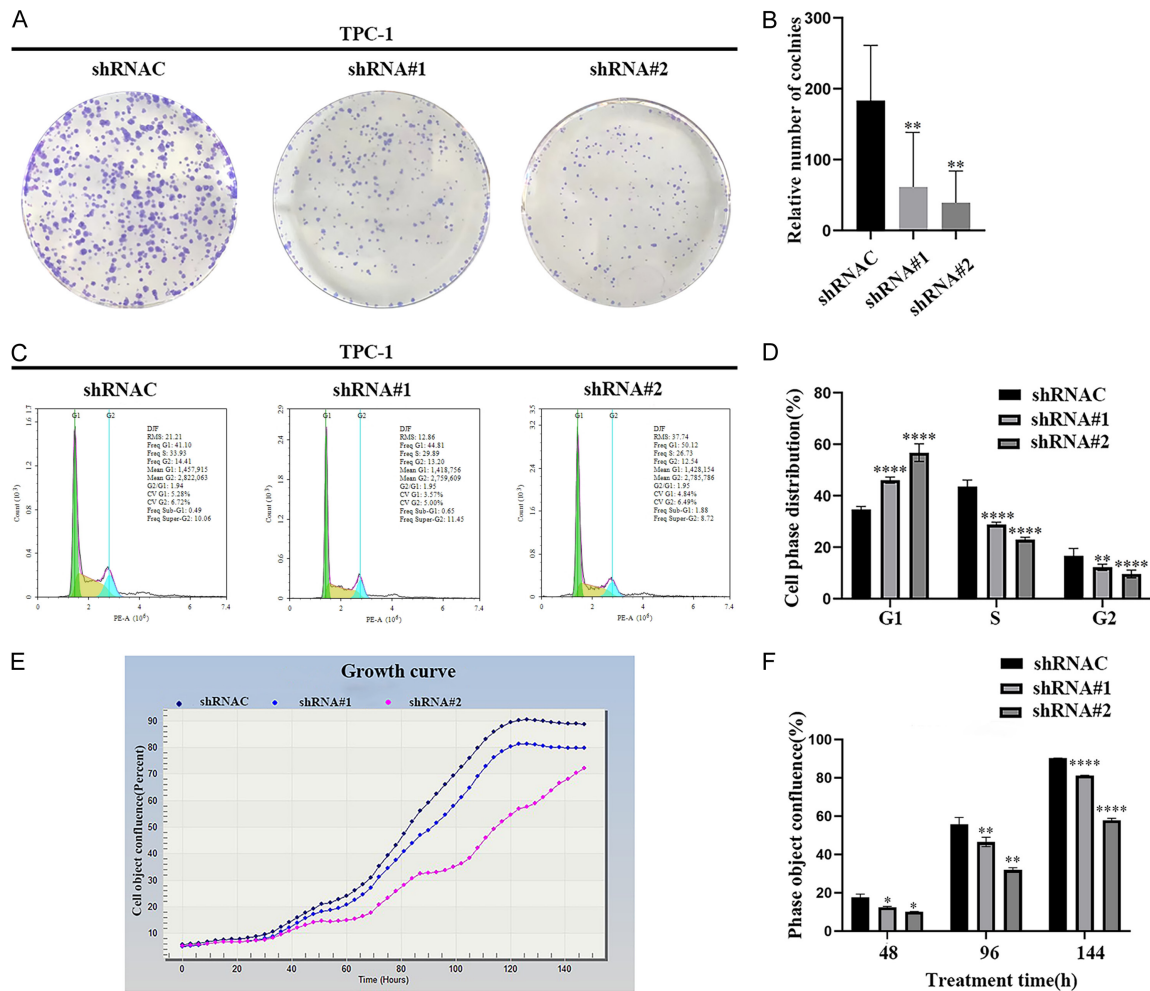
### *APOC1 enhances glycolysis in PTC tumors in vivo*

To evaluate the effect of APOC1 on glycolysis in PTC tumors in vivo, glycolysis-related proteins

were analyzed by Western blot and IHC. A higher expression level was observed in the OE group, while lower levels were detected in the shRNA#1 and shRNA#2 groups compared with controls (Figure 9A-E). Tumors from the OE group showed increased lactate and ATP levels, whereas these metabolites were decreased in knockdown groups (Figure 9F-I). IF analysis of LDHA expression further confirmed these results (Figure 9J). Collectively, these findings



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**Figure 4.** Effect of APOC1 silencing on PTC cell proliferation. A. Assessment of colony formation in APOC1-silenced cells. B. Quantification of colonies in cells with APOC1 silenced. C. Cell cycle distribution diagram of APOC1-silenced cells. D. Percentage analysis of cell cycle phases in APOC1-silenced cells. E. Growth curve of APOC1-silenced cells. F. Cell confluence analysis of APOC1-silenced cells. Data were statistically evaluated using one-way and two-way analysis of variance (ANOVA), as well as repeated-measures ANOVA, with post hoc testing performed where appropriate. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , \*\*\*\* $P < 0.0001$ .

indicate that APOC1 enhances glycolysis in PTC tumors in vivo.

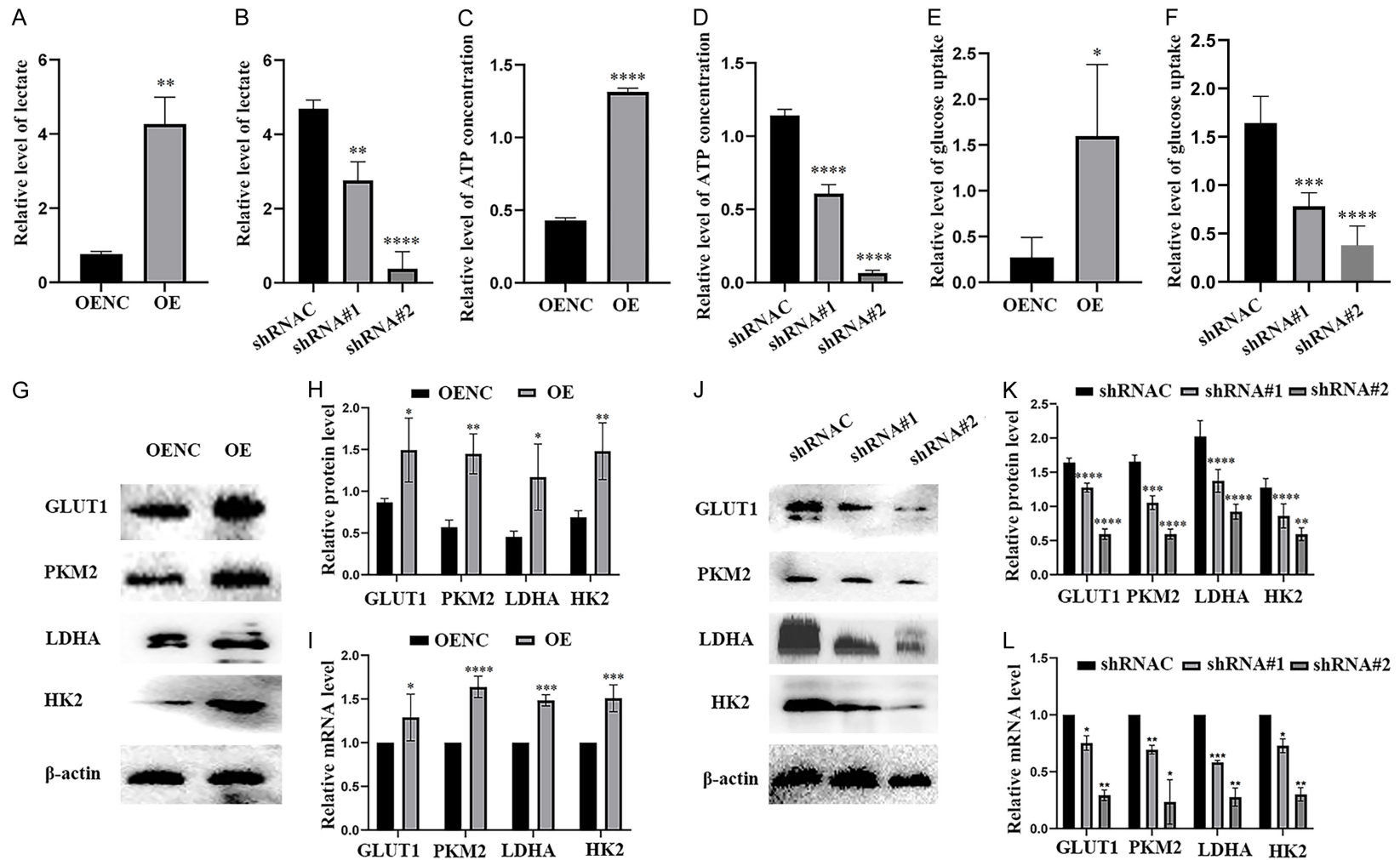
### Discussion

Over the past decades, thyroid cancer (TC) has shown a substantial increase in incidence, reaching a 313% rise, which is thought to be driven largely by improved diagnostic capability and heightened public awareness. Accounting for approximately 84% of cases, PTC is the predominant pathological type. The observed rise in thyroid cancer cases is likely linked to better diagnostic tools and increased routine health screenings [19]. While most patients experience favorable outcomes, the risk of overdiag-

nosis and overtreatment has drawn concern. This highlights the importance of identifying new molecular targets to guide more precise and individualized treatment strategies [20].

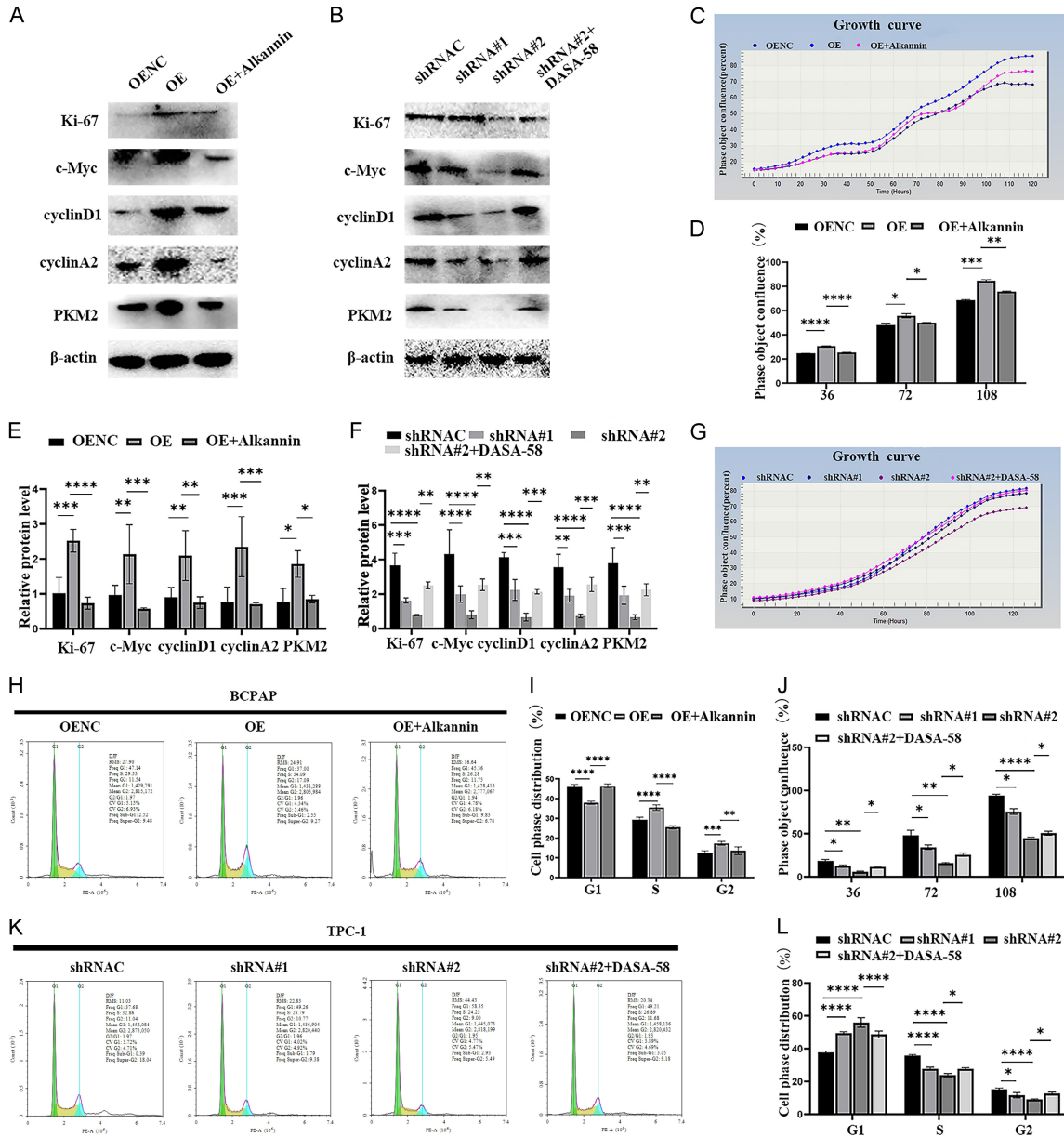
Examination of the THCA cohort showed higher APOC1 levels in PTC tissues than in matched adjacent non-tumor samples. APOC1 expression was also positively associated with multiple glycolytic genes, implying that it may contribute to metabolic reprogramming in PTC [21]. For tumor cells, metabolic reprogramming serves as a crucial mechanism for adaptation, supporting both survival and fast growth [22]. In this context, a typical metabolic feature is the reliance on aerobic glycolysis rather than

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**Figure 5.** Effect of APOC1 on glycolysis in PTC cells. A. Lactate production in cells with APOC1 overexpression. B. Lactate production in cells with silenced APOC1 expression. C. ATP levels in cells with APOC1 overexpression. D. ATP levels in APOC1-silenced cells. E. Glucose uptake in APOC1-overexpressing cells. F. Glucose uptake in APOC1-silenced cells. G-I. Glycolysis-related protein expression in PTC cells overexpressing APOC1 was analyzed using Western blot and RT-qPCR. J-L. Glycolysis-related protein expression in APOC1-silenced PTC cells was measured using Western blot and RT-qPCR. Between-group comparisons were carried out using unpaired Student's *t*-test, one-way analysis of variance (ANOVA), or two-way ANOVA, depending on the study design. \**P*<0.05, \*\**P*<0.01, \*\*\**P*<0.001, \*\*\*\**P*<0.0001.

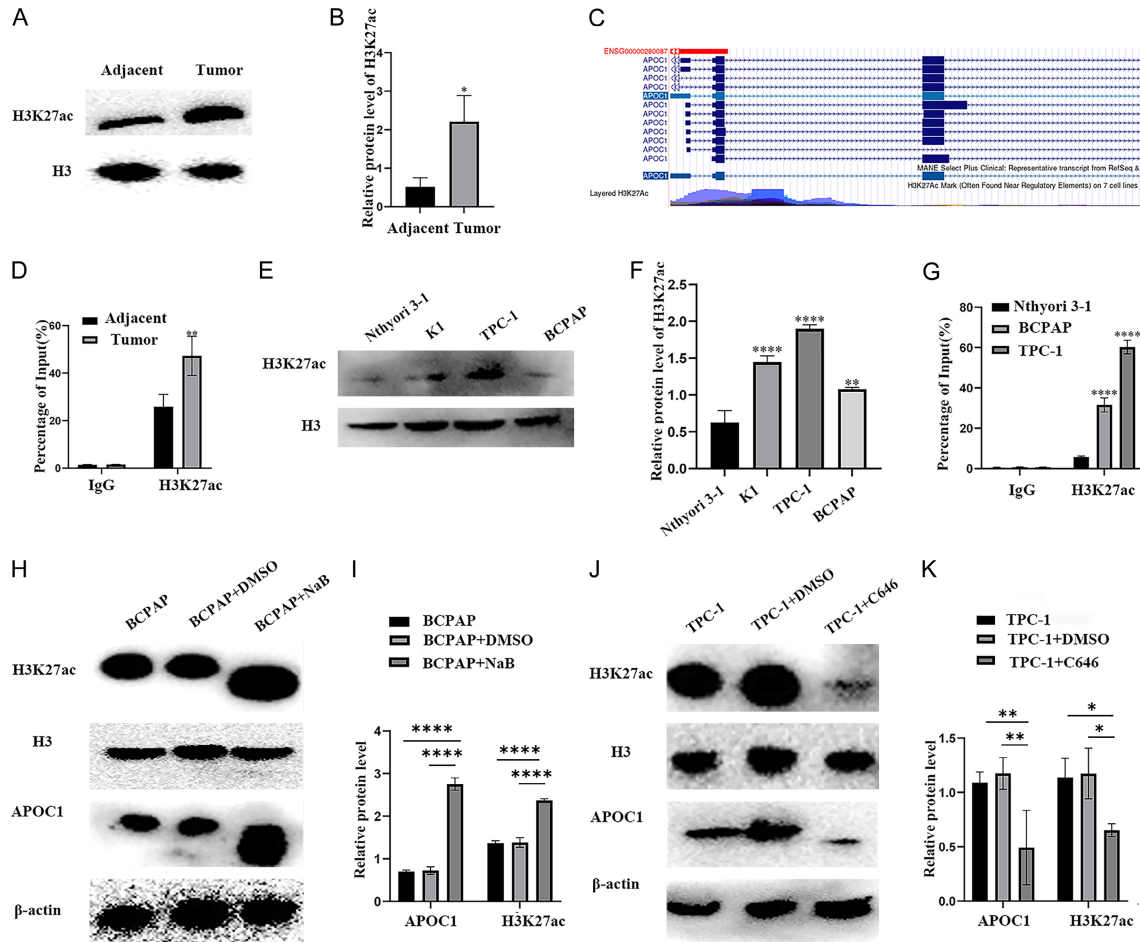
## Mechanisms of PTC proliferation



**Figure 6.** APOC1 promotes PTC cell proliferation through glycolysis. A, E. Expression of Pyruvate Kinase M2 (PKM2) and proliferation-related proteins after treatment with a PKM2 inhibitor in APOC1-overexpressing cells. B, F. Expression of PKM2 and proliferation-related proteins after treatment with a PKM2 agonist in APOC1-silenced cells. C. Growth curve of APOC1-overexpressing cells after treatment with a PKM2 inhibitor. D. Cell confluency analysis of APOC1-overexpressing cells after treatment with a PKM2 inhibitor. G. Growth curve of APOC1-silenced cells after treatment with a PKM2 agonist. H. Cell-cycle distribution of APOC1-overexpressing cells after treatment with a PKM2 inhibitor. I. Quantitative analysis of the percentage of cells in each cell-cycle phase in APOC1-overexpressing cells after treatment with a PKM2 inhibitor. J. Cell confluency analysis of APOC1-silenced cells after treatment with a PKM2 agonist. K. Cell cycle distribution diagram of APOC1-silenced cells after treatment with a PKM2 agonist. L. PKM2 agonist treatment altered the distribution of APOC1-silenced cells across different cell-cycle phases, as reflected by the quantitative proportion of cells in each phase. Statistical analyses were conducted using one-way and two-way ANOVA, and repeated-measures ANOVA, followed by post hoc comparisons when applicable. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , \*\*\*\* $P < 0.0001$ .

oxidative phosphorylation, even under normal oxygen availability [23]. A single glucose mole-

cule yields more ATP through oxidative phosphorylation. Even so, aerobic glycolysis can pro-

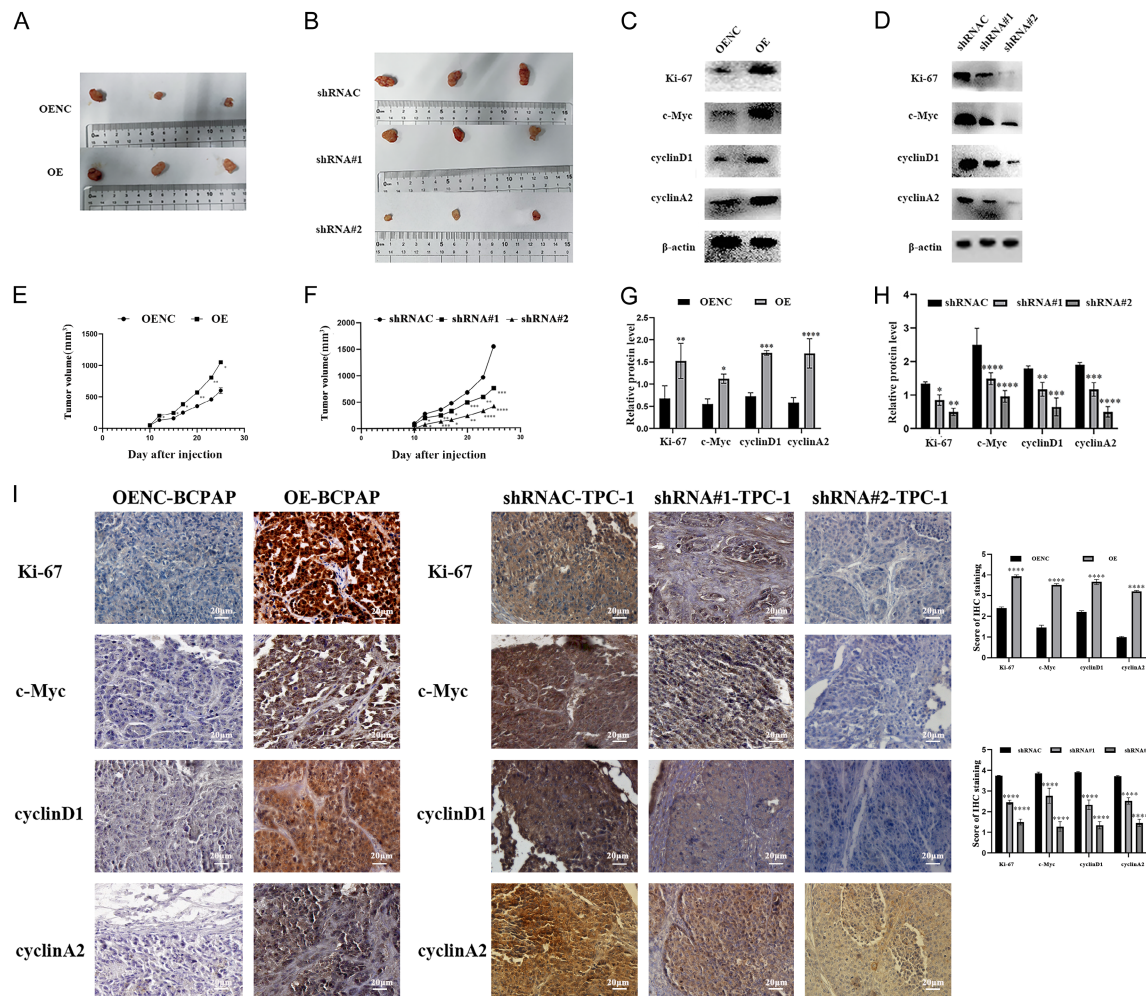


vide energy more rapidly to meet cellular demands. It also contributes important intermediate metabolites that are required for biosynthesis during cell proliferation [24]. Glucose metabolism undergoes reprogramming. Many regulatory factors exert control over this process. Among them, GLUT1 is a major glucose transporter in cancer cells and plays a key role in maintaining glucose uptake [25]. FOXD1 has been reported to promote pancreatic cancer progression by enhancing GLUT1-mediated aerobic glycolysis. This effect relies on boosted GLUT1-associated aerobic glycolysis [26]. HK2

catalyzes the first step of glycolysis. This enzyme converts glucose into glucose-6-phosphate through phosphorylation [27], and this molecule participates in tumor immune escape. In particular, its action depends on Ikbα phosphorylation [28]. LDHA is responsible for the last step of glycolysis, where it converts pyruvate into lactate and helps sustain the glycolytic process [29, 30]. Glucose undergoes phosphorylation to form glucose-6-phosphate. HK2 executes this reaction in the early stage of glycolysis [31]. Altogether, a potential association exists. The data confirms such relation



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**Figure 8.** Impact of APOC1 on tumor progression in a nude mouse xenograft model. A, B. Representative tumor images from an independent in vivo experiment are shown to confirm reproducibility. C, G. Expression of proliferation-associated proteins in tumor tissues from APOC1-overexpressing nude mice. D, H. Proliferation-related protein expression in tumor tissues of APOC1-silenced nude mice. E, F. Tumor volume changes during experiments in nude mice. I. IHC analysis evaluated the levels of proliferation-associated proteins in tumor tissues from nude mice (400 $\times$ ). Data analysis was performed using unpaired *t*-tests, one-way and two-way ANOVA, and repeated-measures ANOVA, with subsequent post hoc tests as appropriate. \**P*<0.05, \*\**P*<0.01, \*\*\**P*<0.001, \*\*\*\**P*<0.0001.

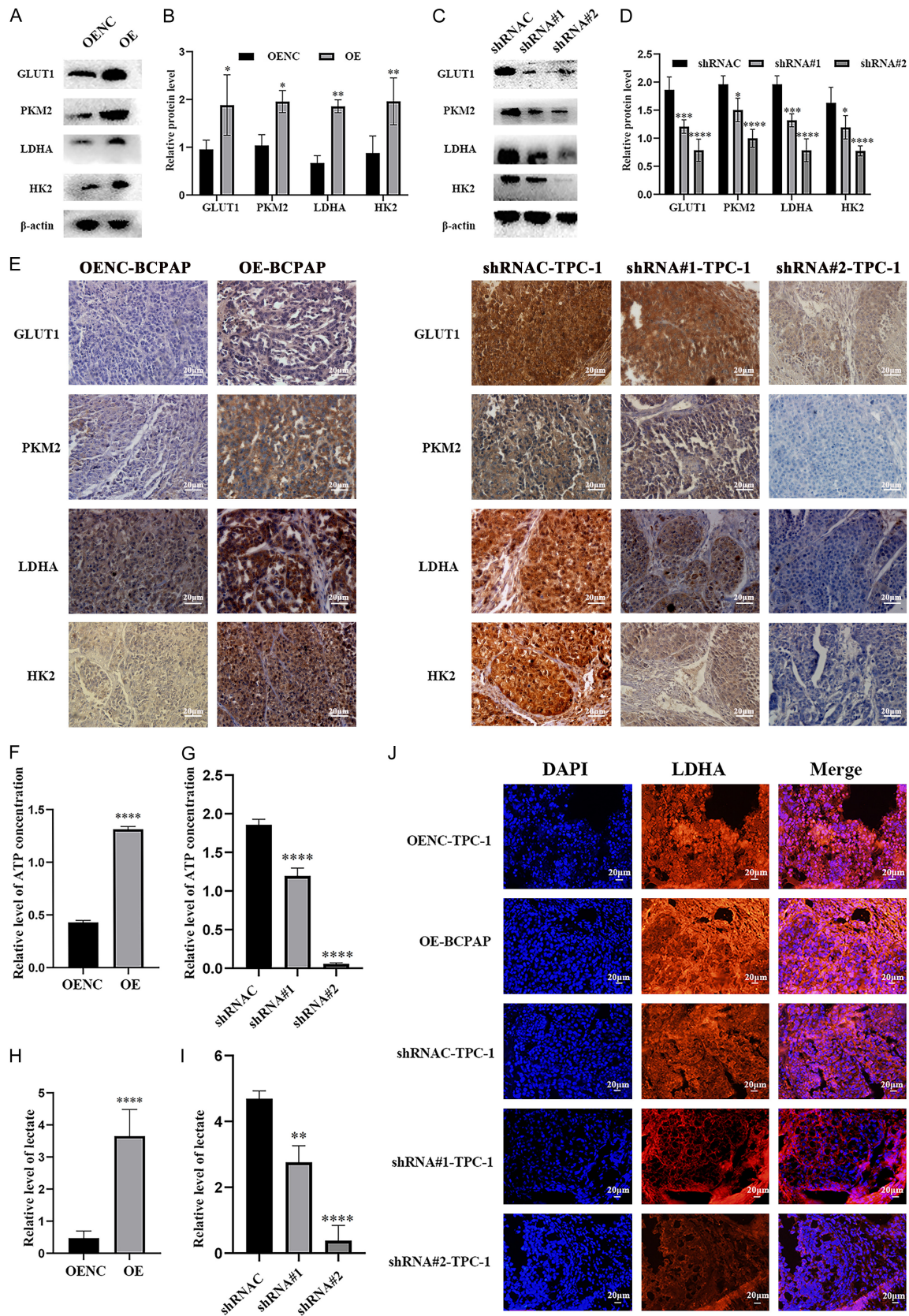
between APOC1 and glycolytic reprogramming [9]. To investigate whether APOC1 influences glycolysis in PTC cells, we established two cell models with APOC1 overexpression or knock-down. The expression of glycolysis-related genes such as GLUT1, HK2, PKM2, and LDHA changed in parallel with APOC1 levels. Overall, both glycolytic products (lactate and ATP) and glucose uptake capacity showed a positive correlation with APOC1 expression levels. These data imply that APOC1 may play a role in enhancing glycolysis in PTC cells.

Given the role of glycolysis-related genes in tumor growth, we examined the role of APOC1 on PTC cell proliferation. Growth curve analysis

showed that cell growth rate correlated positively with APOC1 expression, and clonogenic capacity increased accordingly. Proper cell-cycle progression is essential for cell division [32]. Dysregulation of the G1, S, G2, and M phases of the cell cycle is closely associated with uncontrolled cellular proliferation, thereby contributing to a fundamental hallmark of cancer [33-35]. Flow cytometry showed that APOC1 overexpression promoted S/G2-phase progression, while APOC1 knockdown induced G0/G1 arrest. Taken together, data from growth curve analysis, colony formation assays, and cell-cycle profiling consistently indicate that APOC1 promotes the proliferative capacity of PTC cells.



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**Figure 9.** Effect of APOC1 on tumor glycolysis in the nude mouse xenograft model. A, B. Western blot analysis of glycolysis-related protein expression in tumor tissues from APOC1-overexpressing nude mice. C, D. Western blot

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analysis of glycolysis-related protein expression in tumor tissues from APOC1-silenced nude mice. E. IHC assessment of glycolysis-related proteins (400×). F. ATP levels in tumor tissues from APOC1-overexpressing nude mice. G. ATP levels in tumor tissues from APOC1-silenced nude mice. H. Lactate levels in tumor tissues from APOC1-overexpressing nude mice. I. Lactate levels in tumor tissues from nude mice with silenced APOC1. J. Immunofluorescence (IF) analysis was performed to examine LDHA expression in tissues obtained from nude mice (400×). Appropriate statistical tests, including unpaired Student's *t*-test, one-way ANOVA, and two-way ANOVA, were used to compare groups based on experimental conditions. \**P*<0.05, \*\**P*<0.01, \*\*\**P*<0.001, \*\*\*\**P*<0.0001.

Whether APOC1 promotes PTC cell proliferation by enhancing glycolysis remains unclear. To test this, APOC1-overexpressing cells were treated with the PKM2 inhibitor Alkannin, which effectively reduced PKM2 levels. Cyclin D1, a key regulator of the G1-S phase transition, was utilized as a proliferation marker [36]. Ki-67 is highly expressed during the G2 and mitotic phases and is considered an important pathological marker of cellular proliferation [37]. c-Myc, a nuclear transcription factor, regulates cell growth, metabolism, and proliferation. Its dysregulation contributes to genomic instability, uncontrolled proliferation, and tumor progression [38]. Frequently overexpressed in cancers, cyclin A2 regulates cell-cycle progression and proliferation [39]. Therefore, cyclin D1, cyclin A2, Ki-67, and c-Myc were selected as proliferation-related markers.

Following PKM2 inhibition, APOC1-overexpressing cells showed markedly reduced levels of proliferation-related proteins by Western blot analysis. Recovery of PKM2 expression and a concomitant elevation in proliferation-related proteins were observed in APOC1-silenced cells (shRNA#2) following DASA-58 treatment, suggesting a partial reversal of the suppressive effect induced by APOC1 depletion. To elucidate the regulatory mechanism of APOC1 in PTC, analysis of the UCSC database revealed a strong H3K27ac enrichment peak at the APOC1 locus, suggesting that APOC1 may be transcriptionally regulated by H3K27ac. APOC1 and H3K27ac showed consistent expression patterns in PTC cells and tissues relative to their normal counterparts. Treatment with the histone acetyltransferase inhibitor C646 reduced APOC1 expression and H3K27ac levels in TPC-1 cells, whereas sodium butyrate increased both in BCPAP cells. ChIP assays further confirmed the enrichment of H3K27ac at the APOC1 promoter. Xenograft experiments further verified that APOC1 promotes glycolysis and tumor growth in PTC *in vivo*. Thus, H3K27ac-mediated activation of APOC1 may promote gly-

colytic reprogramming in PTC cells, thereby driving their proliferation.

It should be noted that in the present study, metabolic parameters including lactate, ATP, and glucose uptake were assessed using indirect methods, and oxidative phosphorylation-related function (e.g., ROS) was not directly evaluated. Thus, these measures may not fully capture the dynamic regulation of glycolysis and mitochondrial respiration. Future studies will employ Seahorse analysis to directly assess cellular energy metabolism flux and further validate and extend these findings.

### Conclusion

In summary, H3K27ac activates APOC1, which promotes PTC proliferation through glycolysis.

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### Disclosure of conflict of interest

None.

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# Mechanisms of PTC proliferation

**Supplementary Table 1.** Information for primers

| Name of primer | Sequences               |
|----------------|-------------------------|
| GLUT1-F        | TCTGGCATCAACGCTGTCTTC   |
| GLUT1-R        | CGATACCGGAGCCAATGGT     |
| PKM2-F         | ATGTCGAAGCCCATAGTGAA    |
| PKM2-R         | TGGGTGGTGAATCAATGTCCA   |
| HK2-F          | GAGCCACCACTCACCCTACT    |
| HK2-R          | CCAGGCATTTCGGCAATGTG    |
| LDHA-F         | ATGGCAACTCTAAAGGATCAGC  |
| LDHA-R         | CCAACCCCAACAACTGTAATCT  |
| APOC1-F        | CTGGTGGTGGTTCTGTCTCGTAT |
| APOC1-R        | TCACTCTGTTTGATGCGGCT    |
| β-actin-F      | CCTGGGCATGGAGTCCTGTG    |
| β-actin-R      | AGGGGCCGGAAGTCGTCATAC   |
| GAPDH-F        | GGAGCGAGATCCCTCCAAAAT   |
| GAPDH-G        | GGCTGTTGTCATACTTCTCATGG |

**Supplementary Table 2.** APOC1 expression and clinicopathological characteristics of 58 specimens

| Characteristics      | Total | APOC1 expression |      | P value | $\chi^2$ value | Correlation<br>r value |
|----------------------|-------|------------------|------|---------|----------------|------------------------|
|                      |       | Low              | High |         |                |                        |
| Age (years)          |       |                  |      | 0.185   |                |                        |
| <45                  | 25    | 10               | 15   |         |                |                        |
| ≥45                  | 33    | 20               | 13   |         |                |                        |
| Gender               |       |                  |      | 0.98    |                |                        |
| Female               | 35    | 18               | 17   |         |                |                        |
| Male                 | 23    | 12               | 11   |         |                |                        |
| Tumor size           |       |                  |      | 0.001   | 10.749         | 0.66                   |
| <2 cm                | 23    | 18               | 5    |         |                |                        |
| ≥2.0                 | 35    | 12               | 23   |         |                |                        |
| Clinical stage       |       |                  |      | 0.001   | 21.28          | 0.77                   |
| 1-2                  | 38    | 28               | 10   |         |                |                        |
| 3-4                  | 20    | 2                | 18   |         |                |                        |
| Lymphatic metastasis |       |                  |      | 0.02    | 5.78           | 0.56                   |
| No                   | 26    | 18               | 8    |         |                |                        |
| Yes                  | 32    | 12               | 20   |         |                |                        |