Upregulation of ubiquitin carboxy-terminal hydrolase 47 (USP47) in papillary thyroid carcinoma *ex vivo* and reduction of tumor cell malignant behaviors after USP47 knockdown by stabilizing SATB1 expression *in vitro*

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Abstract. Aberrant ubiquitination contributes to cancer development, including thyroid carcinoma. The present study assessed the expression of ubiquitin carboxy-terminal hydrolase 47 (USP47) and underlying molecular events in the development of papillary thyroid carcinoma (PTC). The effects of USP47 on PTC cell invasion and migration were analyzed by Transwell assays, while. the effects of USP47 and SATB1on PTC cell gene expression and changes in tumor cell metabolism were assayed by reverse transcription-quantitative PCR, western bolt, or ELISA, respectively. The expression of USP47 mRNA and protein was upregulated in PTC tissue and associated with the PTC tumor size. Knockdown of USP47 expression in PTC cell lines (TPC-1 and K1), decreased the cell proliferation mobility and invasion capacities, whereas USP47 overexpression in these cell lines showed an inverse effect and promoted cell glycolysis and glutamine metabolism. Moreover, expression of special AT-rich sequence-binding protein-1 (SATB1) was high in PTC tissue and was associated with USP47 expression. SATB1 expression promoted tumor cell glycolysis and glutamine metabolism, while USP47 protein bound to and deubiquitinated SATB1 to increase its intracellular levels, thus promoting glycolysis and glutamine metabolism. USP47 promotion of PTC development may be due to its stabilization of SATB1 protein, suggesting that targeting the USP47/SATB1 signaling axis may serve as a therapeutic intervention for PTC.

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Introduction

According to Global Cancer Statistics, it is estimated that there were more than a half million new thyroid cancer cases and >40,000 cancer-related deaths due to thyroid cancer in 2020 globally; thyroid cancer is the ninth most commonly diagnosed cancer and ranks 25th in terms of cancer deaths globally (1). The worldwide incidence rate is three times higher in females than in males, accounting for 10.1 per 100,000 population in female patients and the incidence in males is 0.33 per 100,000 population (1). The China Cancer Registry data show that the incidence of thyroid cancer is rising annually and threatens human health in Chinese populations (2). However, to date, the precise etiology of thyroid cancer development remains to be defined. Radiation exposure is the only validated thyroid cancer risk factor while other factors, such as being overweight hormonal exposure and certain environmental pollutants may be associated with thyroid cancer development (1,3). Histologically, thyroid cancer is divided into papillary, follicular, medullary, anaplastic or other rare types; of these, papillary thyroid carcinoma (PTC) comprises 75-85% of diagnosed thyroid cancer cases (4,5). To date, the incidence of diagnosed PTC has been increasing because of the use of ultrasonography and other diagnostic imaging equipment (1,6). PTC prognosis is usually good, with a 10-year survival rate of 90% following standardized therapy options, such as surgery alone or combined with inhibition of thyroid-stimulating hormone and radioactive iodine agents (7). Local recurrence and distant metastasis are primary causes of advanced PTC and death (8). Nevertheless, further understanding of the pathogenesis and underlying molecular mechanisms of PTC may help to control PTC progression effectively, as well as to detect PTC earlier.

The Warburg effect describes a phenomenon characterized by tumor cell metabolic reprogramming of predominant energy production by using less efficient aerobic glycolysis in the cytosol instead of the typical mitochondrial citric acid cycle and oxidative phosphorylation in normal cells (9). Such a fermentation process only produces low levels of ATP compared with that of the citric acid cycle and oxidative phosphorylation but it possesses an advantage by allowing proliferating tumor cells to use the resulting nutrients, such as glucose and glutamine, for cell proliferation, leading to fermentation favoring tumor cell proliferation (10,11). Previous studies have shown that an increase in aerobic glycolysis significantly induces myofibroblast activation; meanwhile, neutrophils and 2-deoxyglucose inhibitors have been shown to attenuate unilateral ureter obstruction surgery-associated renal fibrosis, as well as TGF-β1)-induced renal interstitial fibroblasts (12,13). In addition, histone H2B monoubiquitination upregulates expression of multiple human mitochondrial respiratory genes (such as nicotinamide adenine dinucleotide dehydrogenase ubiquinone) 1 alpha subcomplex subunit 9, Nicotinamide adenine dinucleotide phosphate transhydrogenase (NNT), and Nicotinamide adenine dinucleotide-hydrogen dehydrogenase [ubiquinone] 1 beta subcomplex subunit 6 (NDUFB6) proteins in lung cancer cells, which suppresses the Warburg effect and tumorigenesis (14); while isocitrate dehydrogenase 2 has been revealed to stimulate the hypoxia-inducible factor-1α pathway, which promotes the Warburg effect and PTC growth (15).

Glutamine metabolism is a type of metabolic reprogramming in tumor cells (16) and glutamine demand, a non-essential amino acid, is increased in cancer cells (17). For example, glutamine has been reported to be a key amino acid in breast cancer cell growth (18). Moreover, prostate cancer cells can be radio sensitized and treated by targeting glutamine metabolism and autophagy (19), while melanoma cell growth is reduced by interfering with the glutamine transporter (20). Furthermore, deubiquitinases, a type of protease that regulates the activity of the ubiquitin/proteasome system, can regulate cancer development and progression (21). Ubiquitin carboxy-terminal hydrolase 47 (USP47) belongs to the deubiquitination enzyme family of proteins and counteracts the effects of the E3 ubiquitin ligase in lung cancer cell proliferation and differentiation (9). Additionally, USP47 deubiquitination stabilizes YAP-associated protein in colorectal cancer progression (22), while miR-204-5p inhibit ovarian cancer cell proliferation via downregulated USP47 expression (23). By contrast, long non-coding RNA Down syndrome cell adhesion molecule antisense RNA1-upregulated USP47 expression promotes osteosarcoma progression (24), while USP47 has been shown to promote TGF-β2-induced breast cancer cell epithelial-mesenchymal transition (25).

The present study first detected USP47 expression in PTC tissues vs. normal tissue and its association with clinicopathological parameters and follow-up data from patients. In addition the effects of the expression or knockdown of USP47 protein on biological behaviors, such cell proliferation, the Warburg effect and glutamine metabolism, in the PTC cell lines TPC-1 and K1 were investigated. The present study aimed to determine whether targeting USP47 might serve as a novel strategy for control of PTC.

Materials and methods

PTC specimens and cell culture. A total of 30 pairs of PTC and distant non-tumorous tissue (2 cm away from the cancerous lesions; there were no tumor cells confirmed in these tissue specimens) were collected from patients treated at the Department of Thyroid and Breast Surgery, The Second Affiliated Clinical School of Medicine, Fujian Medical University (Quanzhou, China). From October 2020 to October 2022.The patients aged 19-68 years old (median: 43 years old), included 22 females and 8 males andwere pathologically confirmed with PTC and staged according to the 2022 World Health Organization Classification of Thyroid Neoplasms criteria (26); none of the patients had received presurgical treatments, such as chemotherapy or radiation therapy. The present study was approved by the Ethics Review Committees of The Second Affiliated Hospital of Fujian Medical University (2021; approval no. 149) and all participants provided signed informed consent. Fresh tissue specimens were collected from the Surgical Room, quickly frozen in liquid nitrogen and kept at -80˚C. The clinicopathological and follow-up data were obtained from the patients' medical records.

The human PTC cell lines TPC-1 and K1 were purchased from Guangzhou Ryder Liankang Biotechnology Co., Ltd. and the K1 cell line authenticated using short tandem repeat profile analysis. Cells were cultured in RPMI-1640 supplemented with 5% fetal bovine serum (FBS; cat. no. SH30087.01) and 1% penicillin-streptomycin (cat. no. SH30010; both Hyclone; Cytiva) in a humidified incubator with 5% CO_2 and 95% air at 37˚C.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR). The total cellular RNA was isolated from cells with TRIzol (Invitrogen; Thermo Fisher Scientific, Inc.) and reverse-transcribed into cDNA with an RNase-free DNase I kit (Promega Corporation), according to the manufacturer's instructions. cDNA samples were subjected to qPCR using 2X SYBR Green qPCR SuperMix (Invitrogen; Thermo Fisher Scientific, Inc.) in a BioPhotometer plus Ebend nucleic acid protein analyzer machine (Eppendorf). The thermocycling conditions were as follows: 50˚C for 2 min, 95˚C for 2 min, followed by 40 cycles of 95˚C for 15 sec and 60˚C for 32 sec. The GAPDH mRNA was used as a control and the relative USP47 mRNA expression was quantified by the $2^{-\Delta\Delta Cq}$ method (27). The primers sequences were as follows: USP47 forward, 5'-TATGCAGAGTCATGTTTGAT-3' and reverse, 5'-ATCAAGATATGTGTCGATTC-3'; SATB1 forward, 5'-GCTCTGCTGCCCAGGCCAAA-3' and reverse, 5'-CTG TGTAACTGAATTTTCAA-3' and GAPDH forward, 5'-GCT CATTTGCAGGGGGGAG-3' and reverse, 5'-GTTGGTGGT GCAGGAGGCA-3'.

Western blotting. Total cellular protein was extracted with radioimmunoprecipitation assay buffer (cat. no. T1070; Beijing Solarbio Science & Technology Co., Ltd.) and the concentration of the samples was assayed with a bicinchoninic acid protein assay kit (Nanjing KGI Biological Development Co., Ltd.), according to the manufacturer's protocol. The protein samples (20 μg) were separated by 10% SDS-PAGE [Roche Diagnostics (Shanghai) Co., Ltd.] and transferred onto polyvinylidene

fluoride membranes (Millipore Sigma). The membranes were blocked at room temperature for 1 h in 5% non-fat milk and incubated in a solution of phosphate-buffered saline (PBS) containing primary antibody at 4˚C overnight. The primary antibodies were anti-USP47 (cat. no. ab72143; Abcam; 1:2,000) and anti-special AT-rich sequence-binding protein-1 (SATB1; cat. no. ab6688; Abcam). Afterwards, membranes were washed three times with Tris hydrochloride + 0.2%Tween-20 and incubated with a horseradish peroxidase-labeled goat anti-rabbit IgG (H+L) (cat. no. ab6721; Abcam; dilution of 1:20,000) at the room temperature for 1 h. Next, membranes were briefly washed three times with Tris hydrochloride + Tween and the positive protein signaling was visualized with a Western Blotting Detection kit (Human IgG; Beijing Solarbio). ImageJ software, version 1.8.0.345 (National Institutes of Health), was used to quantitate the band density. The GAPDH antibody (1:1,000) (cat. no. ab9485; Abcam) was used as a control. Western blotting was used to detect the protein expression levels of Lactate dehydrogenase (LDH), KH domain 2 (KH2), Amino acid transport vehicle 2 (ASCT2), and Glutaminase 1 (GLS1) in USP47 overexpressing cells and empty vector control cells. LDHA rabbit monoclonal antibody (mAb; cat. no. 3582; Cell Signaling Technology, Inc; 1:1,000); recombinant anti-HK II antibody (cat. no. ab209847; Abcam; 1:1,000); recombinant anti-ASCT2 antibody (cat. no. ab237704; 1:1,000); anti-GLS2 antibody (cat. no. ab150474; Abcam; 1:500).

Plasmids and cell infection. The concentration of nucleic acid was 10 nM and the generation system used was 2nd generation. The ratio of lentivirus and packaging and envelope plasmids was 10:8:6. Overexpression plasmids carrying USP47 and SATB1 cDNA contain the plasmid backbone of the plvx_zsgreen_pgk_puro (LMAI Bio). SATB1 small interfering (si)RNA were from Sigma Biotech. siRNA sequences were as follows: SATB1-siRNA-1, 5'-AUUUCUGAAUGUUCUUUCCCCdTdT-3'; SATB1-siRNA-2, 5'-UAGACAUUUCUGAAUGUUCUUdTdT-3'; SATB1-siRNA-3, 5'-UGUUAGACAUUUCUGAAUGUUdTdT-3' and NC-siRNA, 5'-GAACUGGGGUGCGUGUGAUdTdT-3'. USP47 (si)RNA and NC) targeting cDNA sequences were synthesized by Guangzhou Ribobio Co., Ltd. as follows: USP47 siRNA-1, 5'-UACAACCAUGCAUAGGAUUAdTdT-3'; USP47 siRNA-2, 5'-AACGCUGCUACAAUGAUUUGdTdT-3'; USP47 siRNA-3, 5'-AGCAGUCGACUCCAGAAGACdTdT-3' and USP47 siRNA-NC, 5'-UUCUCCGAACGUGUCACGUUUdTdT-3'. These DNA sequences were cloned into pLKO.1 lentiviral vector (Addgene, Inc.). Lentiviruses were packaged and produced in 293(T) cells (Guangzhou Landliangkang Biotechnology Co., Ltd.) following vector transfection using Lipofectamine 2000 reagent (cat. no. 11668019; Invitrogen; Thermo Fisher Scientific, Inc.) for 24 h at 37˚C and supernatant was collected. In brief, TPC-1 or K1 cells were plated into 24-well cell culture plates ($5x10^4$ cells/well), grown at 37˚C overnight, then infected with viral supernatant or control viral particles at MOI of 10 for 48 h and efficiency of these si-RNAs was analyzed; the most effective siRNA was used for subsequent experiments. Stably transduced cells were selected using puromycin-containing RPMI-1640. The screening concentration was 12 μg/ml and the maintenance concentration was 4 μg/ml).

ELISA. Levels of lactate, ATP, glucose consumption, glutamate, α-ketoglutarate and glutamine following knockdown of USP47 expression or SATB1 expression were assessed using ELISA. kits for lactate (cat. no. WK-SU62; Shanghai Valan Biotechnology Co., Ltd.), ATP content (cat. no. LE-H3380; Lyell Biological Co.), glucose consumption (cat. no. Keshun-0017; Shanghai Keshun Biotechnology Co.), glutamate (cat. no. 140739; Nanjing Senberga Biotechnology Co.), α-ketoglutarate (cat. no. XGE64578; Shanghai Sig Biotechnology Co.) and glutamine (cat. no. KL-Gln-Hu; Kanglang Biotechnology Co.) according to the manufacturer's protocols. Experiments were performed in duplicate and repeated at least twice.

Cell proliferation assay. Cells with stable USP47 siRNA infection were plated into 96-well plates with $1x10^4$ cells/well and grown for 0, 24, 48 and 72 h at 37˚C. The cellTiter96AQ single solution (cat. no. G3582; Promega Corporation) was added to cell culture medium at a ratio of 1:10 and the cells were further cultured at 37˚C for 4 h. The optical density of the cell cultures was measured with a microplate reader (Multiscan MK3; Thermo Fisher Scientific, Inc.) at 490 nm and the data were quantified as the percentage of the control. The experiment was performed in triplicate and repeated three times.

Transwell assay. Transwell chambers were obtained from Corning, Inc. to assess PTC cell mobility. In brief, PTC cells were resuspended in 100 μl serum-free RPMI-1640 and plated into the upper chambers ($1x10^4$ cells per chamber). The filter was precoated with Matrigel (Corning, Inc.) for 48 h at 37˚C for the tumor cell invasion assay or without Matrigel for the migration assay. A total of 100 μl RPMI-1640 containing 20% FBS (Invitrogen; Thermo Fisher Scientific, Inc.) was added to the bottom chambers and the cells were cultured at 37˚C for 12-48 h. Cells remaining on the surface of the filter were removed using a cotton swab, whereas cells that had migrated or invaded to the other side of the filters were fixed in 4% paraformaldehyde at 4˚C for 30 min, washed with distilled water (twice; 2 min), stained with 0.1% crystal violet for 10 min at room temperature and manually counted under an inverted fluorescence microscope (Olympus CKX41 and U-CTR30-2). A total of five microscopic fields (400x magnification) were randomly selected for each chamber. The assay was performed in duplicate and repeated three times.

Statistical analysis. IBM SPSS Statistics 27 software was used for statistical analysis. The paired t test was used to compare paired samples, while the independent sample t test was used to compare the mean difference between the two groups. One-way ANOVA) was used to compare the mean differences between three or more groups. A total of three independent experiments was performed. Data are presented as the mean ± SD. $P<0.05$ was considered to indicate a statistically significant difference.

Results

USP47 is upregulated in PTC tissue. The present study detected the levels of USP47 mRNA and protein in 30 paired PTC and distant normal tissue samples. USP47 mRNA and protein were

Table I. Association of USP47 mRNA expression with clinicopathological characteristics of patients with PTC.

Characteristic	Number	Relative USP47 expression	T-value	P-value
Sex				
Female	22	3.89±0.25	0.215	0.81
Male	8	3.77±0.52		
Age at diagnosis, years				
≤45	18	4.02±0.30	0.901	0.38
>45	12	3.61±0.33		
Tumor size, cm				
<2	28	3.90±0.24	2.149	0.04
≥2	2	3.31±0.12		
N stage (AJCC)				
N0 or Nx	12	3.93±0.37	0.257	0.79
N1	18	3.81±0.29		
Gland outside invasion				
No	23	4.04±0.25	1.507	0.14
Yes	7	3.25±0.46		
Tumor location				
One lobe	24	9.21±1.32	0.928	0.36
More than one lobe	6	9.68±0.82		

AJCC, American Joint Committee on Cancer; Nx, not assessed.

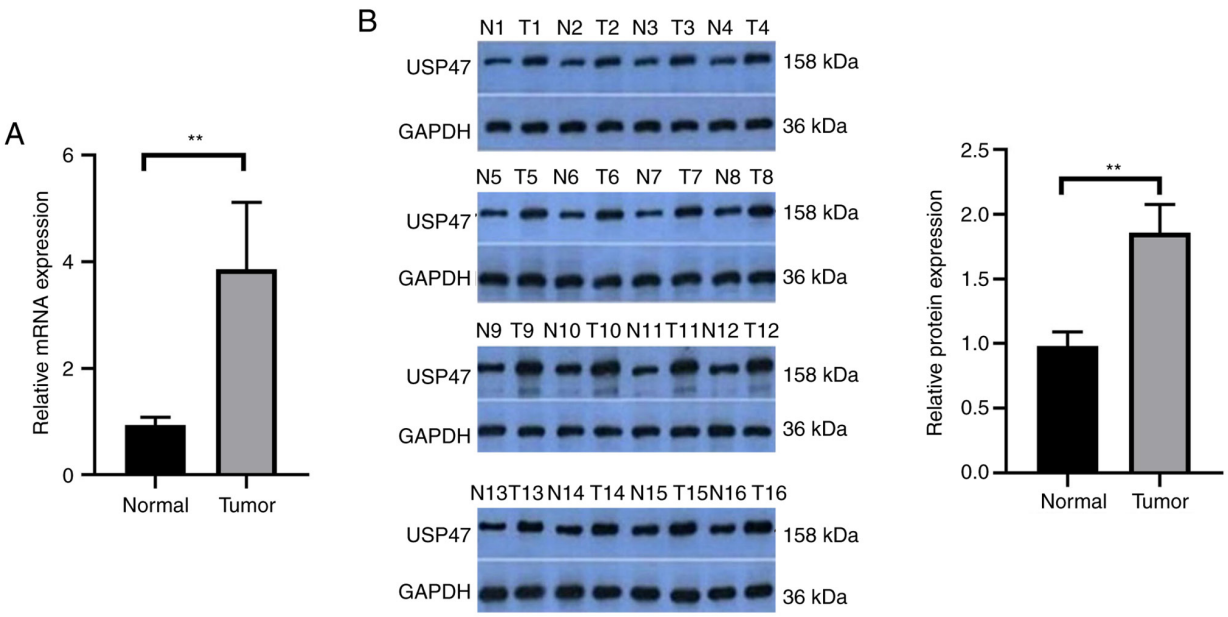

Figure 1. USP47 mRNA and protein are upregulated in PTC and associated with PTC lymphatic metastasis. (A) Paired PTC and adjacent tissue (n=30) was collected from patients and subjected to reverse transcription-quantitative PCR analysis of USP47 mRNA and (B) western blot analysis of USP47 protein. USP47, ubiquitin carboxyl-terminal hydrolase 47; PTC, Papillary thyroid carcinoma; T, Tumor; N, Normal. **P<0.01.

significantly upregulated in PTC tissue (Fig. 1A and B). Analysis of USP47 mRNA and clinicopathological data from the patients demonstrated that the expression of USP47 mRNA was associated with tumor size but not the number of lesions, lymph node metastasis or extrathyroidal extension of PTC (Table I).

PTC cell proliferation, invasion and migration is decreased following after knockdown of USP47 expression. To assess the role of USP47 protein in PTC, USP47 expression was knocked down in PTC cell lines with USP47 siRNA. RT-qPCR data showed that the USP47 mRNA expression was dramatically

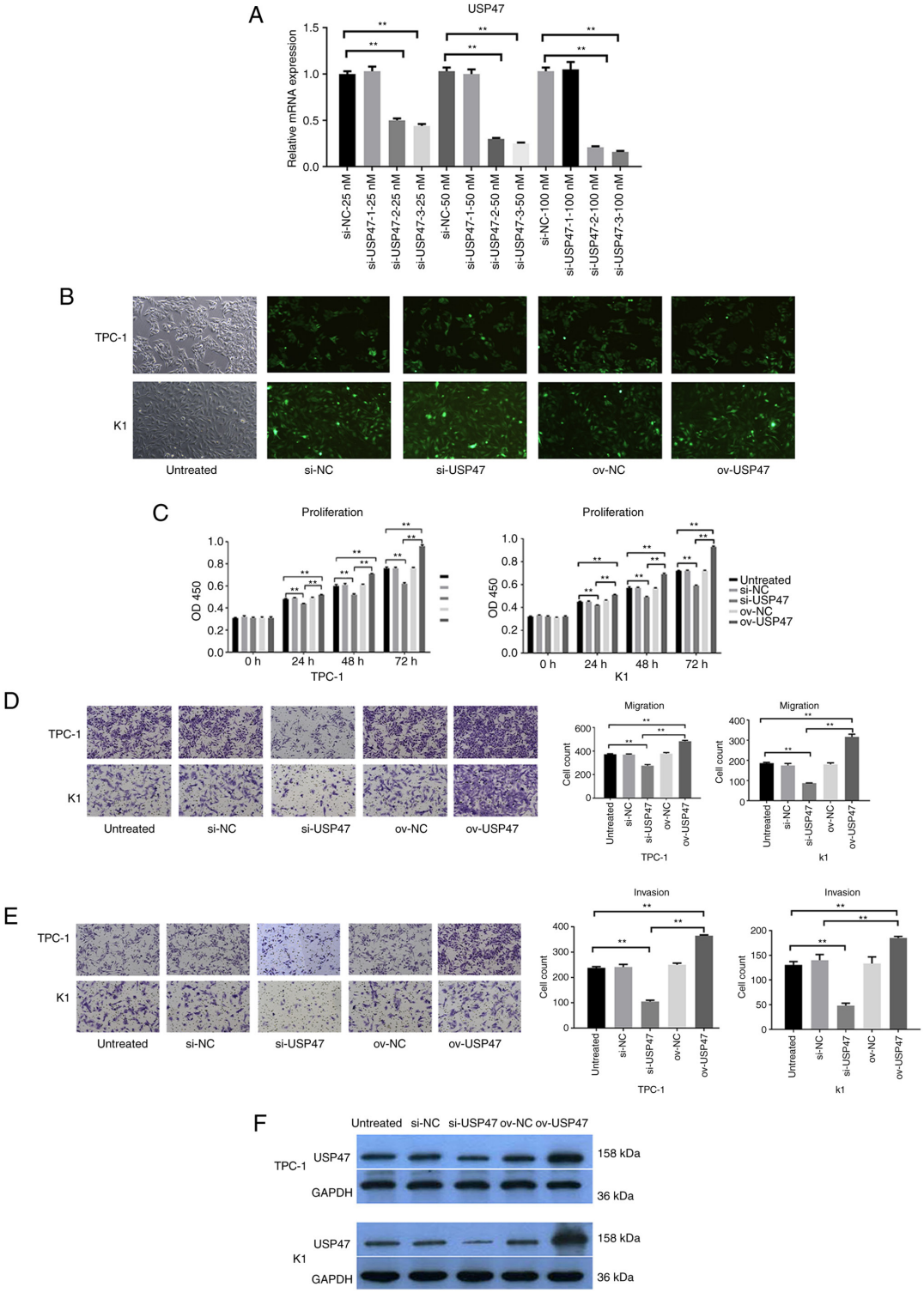

Figure 2. Effect of USP47 on PTC cell proliferation, migration and invasion. (A) TPC-1 and K1 cells were grown and infected with USP47 siRNAs for 48 h and subjected to reverse transcription-quantitative PCR analysis of the USP47 mRNA to test the interference efficiency of USP47 siRNAs. (B) TPC-1 and K1 cells were subjected to light and fluorescence microscopy. (C) TPC-1 and K1 cells with stable USP47 knockdown or ov underwent a cell proliferation assay. (D) Transwell assays were used to detect the effect of USP47 on the migration and (E) invasion of PTC cells. TPC-1 and K1 cells with stable USP47 knockdown or ov were seeded for Transwell assays. (F) TPC-1 and K1 cells with stable USP47 knockdown or ov underwent western blot analysis of USP47 protein expression. USP47, Ubiquitin carboxyl-terminal hydrolase 47; PTC, papillary thyroid carcinoma; si, small interfering; ov, overexpression; NC, negative control; OD, optical density. **P<0.01.

reduced after the introduction of siRNA-3 compared with that of USP47si-NC in both the TPC-1 and K1 cell lines (Fig. 2A) USP47si-3 had the highest interference efficiency, and it was selected for subsequent experiments (Fig. 2B). Cell proliferation assay revealed that knockdown of USP47 expression led to a significant decrease in TPC-1 and K1 cell proliferation (Fig. 2C). Transwell assay revealed that the migration and invasion abilities of both TPC-1 and K1

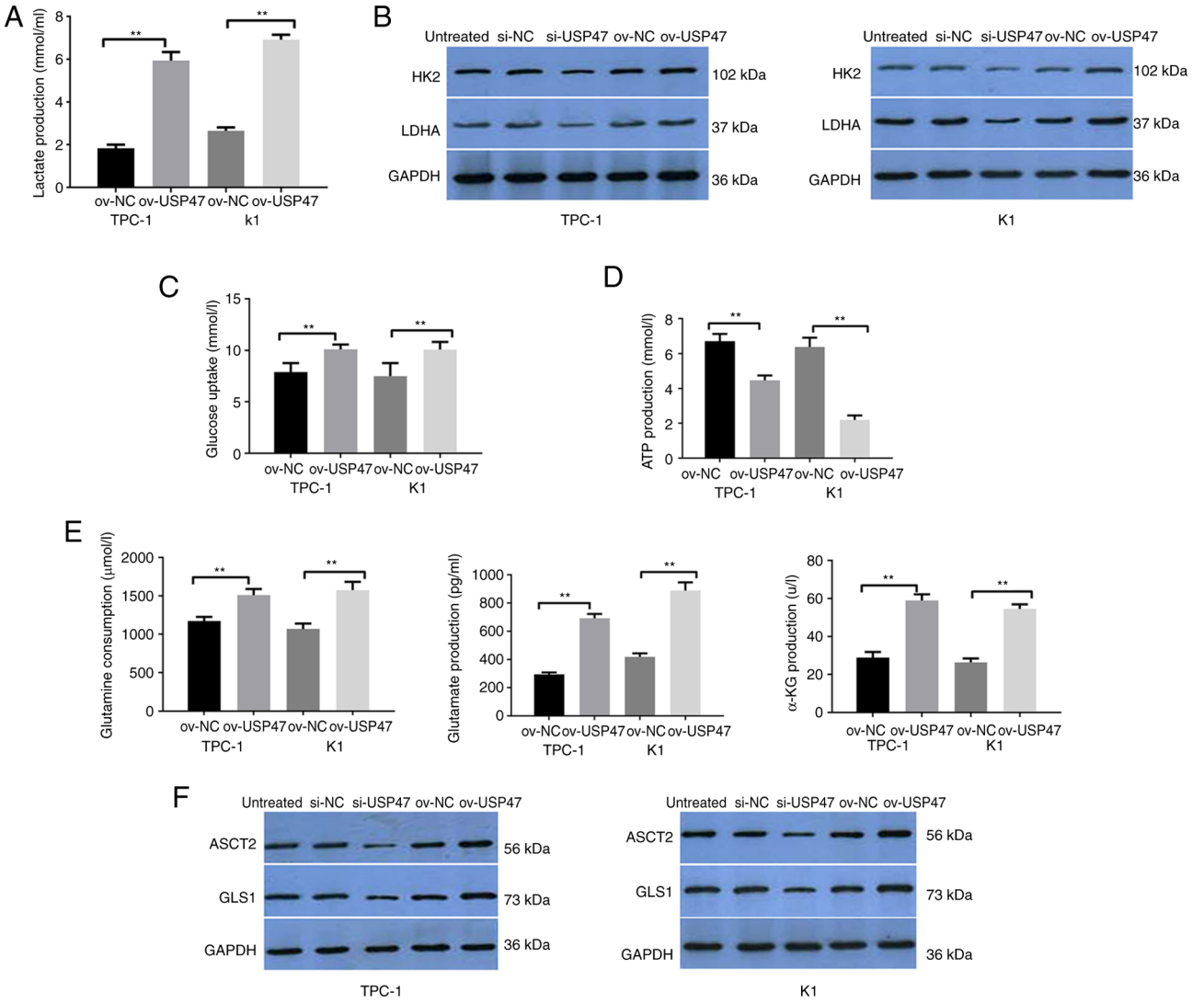

Figure 3. USP47 promotes the Warburg effect and glutamine catabolism in PTC cells *in vitro*. (A) ELISA was performed to assess (A) lactate production, Western blot to assess (B) glucose metabolism key enzymes HK2 and LDHA, ELISA to assess (C) glucose uptake, (D) ATP content and (E) glutamine, glutamate and α-KG consumption. Western blot analysis (F) of key enzymes ASCT2 and GLS1 for glutamine metabolism in TPC-1 and K1 cells with stable USP47 ov. USP47, Ubiquitin carboxyl-terminal hydrolase 47; PTC, Papillary thyroid carcinoma; LDHA, lactate dehydrogenase; HK2, Hexokinase 2; ASCT2, Amino acid transport vehicle 2; α-KG, ketoglutaric acid; GLS1, Glutaminase 1; ov, overexpression; NC, negative control; si, small interfering. **P<0.01.

cells were significantly inhibited following transfection with USP47si-3 (Fig. 2D and E). The aforementioned experiments confirmed that USP47 promoted the proliferation, invasion, and migration of PTC cells.

USP47 knockdown downregulates the Warburg effect in PTC in vitro. To understand the effects of USP47 expression and knockdown in PTC cells, lactate production, lactate dehydrogenase (LDH) and hexokinase 2 (HK2) activity, ATP content and glucose consumption were assessed. USP47-overexpressing TPC-1 and K1 cells demonstrated increased lactate production as well as LDH and HK2 activity compared with vector control-infected cells, whereas knockdown of USP47 expression in PTC-1 and K1 cells decreased lactate production, as well as LDH and HK2 activity (Fig. 3A and B). Furthermore, PTC-1 and K1 cells with USP47 overexpression showed a lower ATP content and glucose consumption than vector control-infected cells, but knockdown of USP47 expression resulted in higher ATP content and glucose consumption (Fig. 3C and D).

USP47 regulates glutamine metabolism. Glutamine catabolism is a characteristic metabolic pattern following cancer cell metabolic reprogramming (28). Thus, the present study assessed glutamine consumption, glutamate and α-ketoglutarate production and the enzymatic activity of ASCT2 and GLS1 in TPC-1 and K1 cells with USP47 overexpression. Compared with the empty vector cells, glutamine consumption as well as glutamate and α-ketoglutarate production in PTC-1 and K1 cells overexpressing USP47 were all increased (Fig. 3E) and levels of ASCT2 and GLS1 protein were also higher in these cells (Fig. 3F).

USP47 interacts with SATB1 in PTC cells in vitro. A previous study has shown that USP47 expression promotes colon cancer progression via STAB1 deubiquitination (29), while another study revealed high SATB1 expression in PTC tissue, which is associated with a poor PTC prognosis (30). Therefore, the present study detected the levels of SATB1 mRNA and protein in TPC-1 and K1 cells following USP47 overexpression and

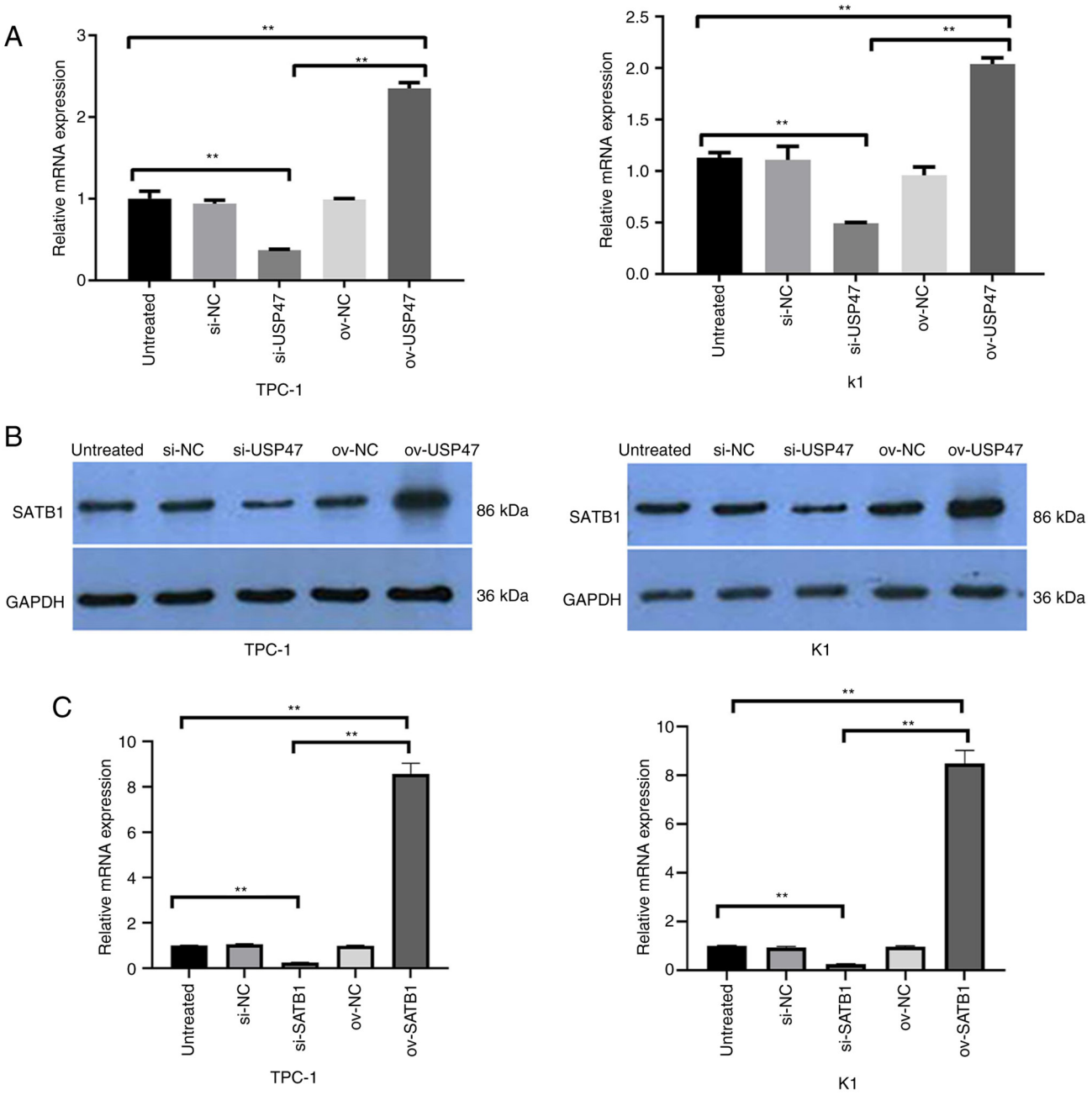

Figure 4. USP47 interacts with and stabilizes SATB1 protein in Papillary thyroid carcinoma cells *in vitro*. TPC-1 and K1 cells with stable USP47 overexpression or knockdown were grown and subjected to (A) RT-qPCR and (B) western blotting. (C) Expression of SATB1 was knocked down in TPC-1 and K1 cell lines and verified by RT-qPCR. **P<0.01. USP47, Ubiquitin carboxyl-terminal hydrolase 47; SATB1, special AT-rich sequence-binding protein-1; RT-qPCR, Reverse transcription-quantitative PCR; si, small interfering; NC, negative control; ov, overexpression.

knockdown. SATB1 mRNA and protein levels were both decreased in USP47 knockdown TPC-1 and K1 cells, whereas USP47 expression increased expression of SATB1 mRNA and protein in PTC cells (Fig. 4A and B). Knockdown of SATB1 was verified by using RT-qPCR in PTC cells (Fig. 4C).

SATB1 promotes glucose and glutamine metabolism in PTC cells in vitro. SATB1 expression was knocked down in PTC cells, resulting in decreased lactate production, glucose consumption and LDH and HK2 activity. ATP content was increased in PTC-1 and K1 cells following knockdown of SATB1 expression, whereas SATB1 overexpression decreased lactate production, glucose consumption and LDH and HK2 activity) (Fig. 5A-D). Furthermore, compared with the control cells, PTC-1 and K1 cells infected with USP47 siRNA showed reduced glutamine consumption, glutamate and α-ketoglutarate production and ASCT2 and GLS1 activities; overexpression of SATB1 in PTC cells increased glutamine depletion, production of glutamate and α-ketoglutaric acid, and ASCT2 and GLS1 activities (Fig. 5E and F).

Discussion

The present study analyzed the expression of USP47 mRNA and protein in 30 paired PTC and normal tissue samples. The expression of USP47 mRNA and protein was upregulated

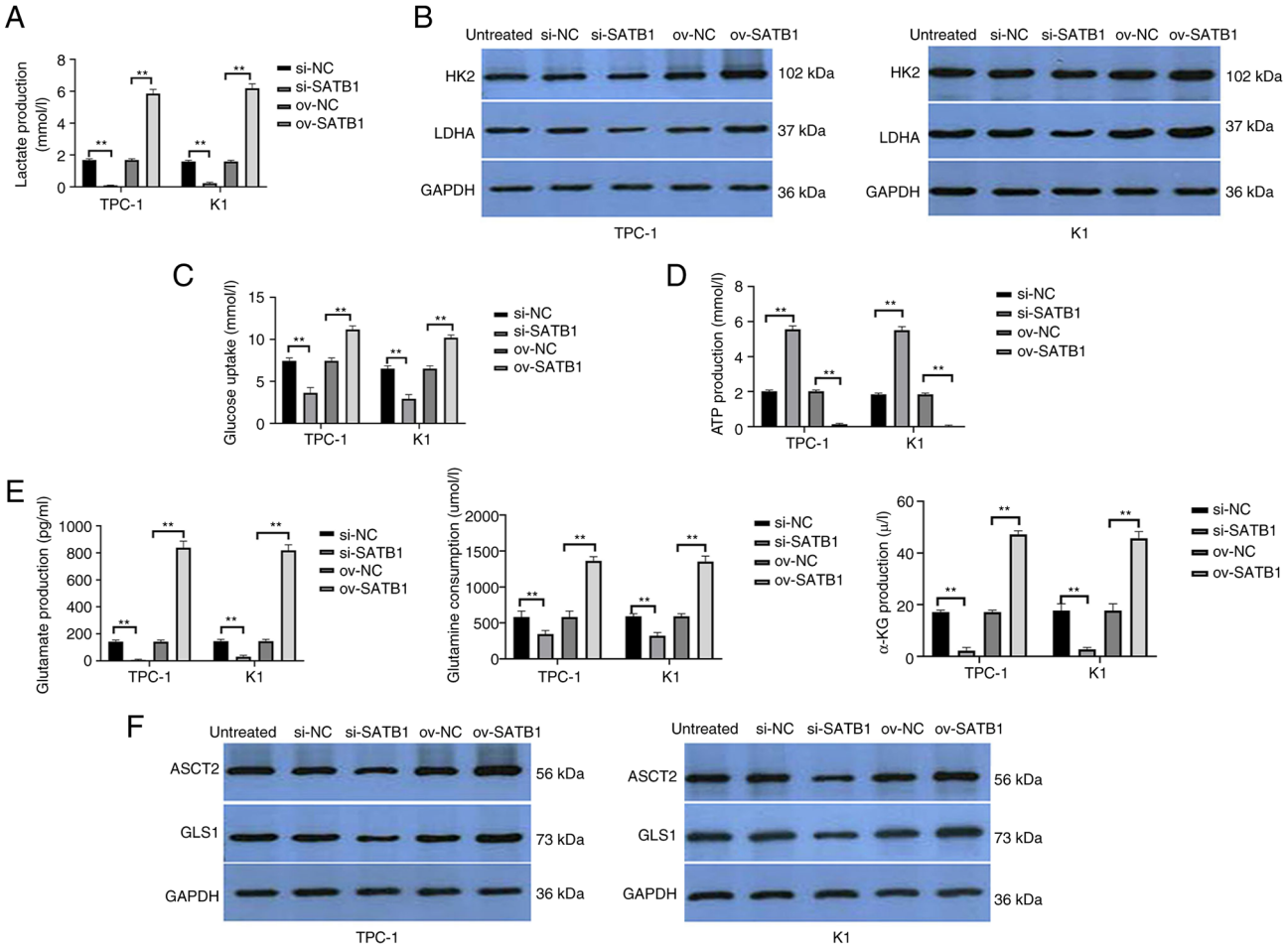

Figure 5. SATB1 regulates the Warburg effect and glutamine catabolism *in vitro* in papillary thyroid carcinoma cells. (A) ELISA was performed with stable SATB1-overexpressing or -knockdown TPC-1 and K1 cells for analysis of (A) lactate, Western blot to assess (B) glucose metabolism key enzymes HK2 and LDHA, ELISA to assess (C) glucose uptake, (D) ATP content and (E) glutamine, glutamate and α-KG consumption. Western blot analysis (F) of key enzymes ASCT2 and GLS1 for glutamine metabolism. SATB1, special AT-rich sequence-binding protein-1; LDH, Lactate dehydrogenase; HK2, Hexokinase 2; ASCT2, amino acid transport vehicle 2; GLS1, glutaminase 1; si, small interfering; NC, negative control; ov, overexpression. **P<0.01.

in PTC compared with normal tissues and that upregulated USP47 mRNA expression was associated with the PTC tumor size. Effects of USP47 and SATB1 overexpression and knockdown on the malignant behaviors of tumor cells were assessed. USP47 expression induced PTC cell proliferation, migration and invasion, whereas knockdown of USP47 expression caused the opposite effects in PTC cells *in vitro*. Compared with vector control-infected cells, USP47-overexpressing TPC-1 and K1 cells showed increased lactate production as well as LDH and HK2 levels, whereas knockdown of USP47 expression demonstrated decreased lactate production as well as LDH and HK2 activity in PTC-1 and K1 cells. However, USP47-overexpressing PTC-1 and K1 cells exhibited lower ATP content and glucose consumption than vector control-infected cells, whereas knockdown of USP47 expression had the opposite effects. In addition, USP47 interacted with and stabilized SATB1 protein in PTC cells *in vitro* and knockdown of SATB1 expression in PTC cells decreased the levels of lactate production and glucose consumption as well as LDH and HK2 activity but increased the ATP content; SATB1 overexpression had the opposite effects. PTC-1 and K1 cells infected with USP47 siRNA showed decreased glutamine consumption, glutamate and α-ketoglutarate production and ASCT2 and GLS1 activities; overexpression of SATB1 in PTC cells had the opposite effects. In summary, the present study demonstrated that USP47 expression induced the Warburg effect and glutamine metabolism in PTC cells by stabilizing SATB1 expression during the progression of PTC. Future studies should investigate knockdown of USP47 and/or SATB1 expression as a therapeutic strategy to control PTC progression clinically.

The recombinant human USP47 protein has been characterized in a previous study (31). USP47 is a monomeric protein ~146 kDa in size. Substitution of Cys109 with Ser significantly abrogates enzymatic activity of USP47 and that N-ethylmaleimide, Zn ion and ubiquitin aldehyde can also dose-dependently inhibit the activity of USP47. In addition, a recent proteomics analysis demonstrated that USP47 plays a role in regulating breast cancer cell epithelial-mesenchymal transition following TGF-β2 treatment (25). Moreover, USP47 has been shown to mediate deubiquitination and stabilization of yes-associated protein during colorectal cancer progression (22). USP47 enhances tumorigenesis via down-regulation of p53 by deubiquitination of ribosomal protein S2 (32). Furthermore, loss of USP47 expression decreases the transcription of SATB1-targeting genes and suppresses

proliferation, migration and tumorigenesis in a mouse colon cancer model (29). USP47 interacts with β-transducin repeat-containing protein, which promotes cell survival (33). Knockdown of USP47 expression has been demonstrated to overcome chronic myelogenous leukemia resistance to a tyrosine kinase inhibitor and to eliminate leukemia stem/progenitor cells (34) and USP47 can also regulate the stemness of colorectal cancer cells (35). The aforementioned studies indicate that USP47 can be an oncogene or exert oncogenic activity in human malignancies. The present study confirmed that USP47 was highly expressed in PTC tissue and that increased USP47 mRNA expression was associated with tumor size in patients with PTC. Moreover, USP47 expression induced PTC cell growth and mobility, whereas knockdown of USP47 expression exerted the opposite effects in PTC cells *in vitro*. Compared with the vector control-infected cells, USP47-overexpressing TPC-1 and K1 cells exhibited an increase in the Warburg effect. The present findings in PTC cells were consistent with those of previous studies of other human malignancy (22,25,29,32,33), although the underlying gene targets may be different.

SATB1 serves as the 'genome organizer' to organize chromatin into spatial loops, which serve as docking sites for transcription factor binding and chromatin-modifying enzymes (29,36,37). A previous study reported that SATB1 expression promotes PTC progression, whereas inhibition of SATB1 expression reverses the malignant biological behaviors of TPC-1 cells (30). SATB1 can reprogram chromatin and tumor cell transcription profiles to enhance tumor cell growth and mobility and inhibit apoptosis resulting in disease progression (36,38). SATB1 expression is upregulated in various human cancers (39-41). For example, knockdown of SATB1 suppresses esophageal cancer cell proliferation and mobility and SATB1 promotes esophageal cancer development by upregulating expression of fibronectin and platelet-derived growth factor receptor β (36). Thus, targeting SATB1 may effectively control human cancers. Our previous study showed that the protein and mRNA expression of SATB1 is upregulated in PTC and associated with USP47 expression in PTC tissue (30). Nevertheless, additional s Studies are required to reveal how USP47 regulates SATB1 expression mechanistically.

Ubiquitylation is an enzymatic modification of translated proteins in cells that is necessary for a variety of cell functions, such as genomic maintenance, cell cycle, antigen processing, DNA repair and cell differentiation and development (42,43); however, deubiquitination is associated with cell functions such as cell cycle distribution, proteasome- and lysosome-dependent protein degradation, DNA repair and microbial pathogenesis (44). Deubiquitination is the reverse process of ubiquitination, the primary role of which is to remove ubiquitin from substrates (45). For example, USP47, a deubiquitinase, maintains the stemness of colorectal cancer cells (35) and promotes the phosphorylation and survival of RelA gastric cancer cells (46). USP47 promotes lung squamous cell carcinoma cell proliferation (47) and stabilizes BTB domain and CNC homolog 1 for induction of the Warburg effect in non-small cell lung cancer (9). By contrast, a recent study demonstrated that long non-coding RNA zinc finger protein 883 mediated USP47 upregulation suppresses NLRP3 ubiquitination to induce epilepsy development (48). The present data revealed that USP47 expression induced PTC cell proliferation and invasion via upregulation of the Warburg effect and glutamine metabolism in PTC cells. However, the present data need to be confirmed *in vivo* and in the clinic. It is also necessary to investigate how USP47 regulates SATB1 and its downstream factors in the control of PTC progression.

Nevertheless, the present study had limitations. For example, the present study demonstrated that USP47 promoted malignant biological behavior and metabolism of PTC but did not determine the mechanism of USP47 action. Second, the present results do not prove that STAB1 is stabilized by USP47; further studies are required to determine the underlying regulatory mechanism.

To the best of our knowledge, the present study is the first to demonstrate upregulated USP47 expression in PTC tissues, which was associated with the PTC tumor size. Moreover, USP47 overexpression induced PTC cell viability and mobility by enhancing the Warburg effect and glutamine metabolism. At the gene level, USP47 interacted with SATB1 protein to stabilize it, thereby enhancing glucose and glutamine metabolism in PTC cells. The present study suggested that targeting the USP47/SATB1 axis may be a potentially novel approach for controlling PTC progression in the clinic.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

GL, WW and LihZ performed experiments. JL, XW and HS analyzed the results. GL wrote and revised the manuscript. LitZ, YY and WQ designed the study. YY and WQ revised the manuscript. JC. HD and XC have made substantial contributions to interpretation of data. All authors have read and approved the final manuscript. GL and WW confirm the authenticity of all the raw data.

Ethics approval and consent to participate

The present study was approved (approval no. 149) by the Ethics Review Committees of the Second Affiliated Hospital, Fujian Medical University Fujian, China and followed the principles of the Declaration of Helsinki. Written informed consent was obtained from all participants.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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