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Downregulated Expression of USP18 Is Associated with a Higher Recurrence Risk of Papillary Thyroid Carcinoma

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As a member of the deubiquitinating protease family, ubiquitin specific peptidase 18 (USP18) is well acknowledged for its roles in stabilizing downstream protein substrates and inhibiting type I interferon signaling. USP18 has been reported to exert distinct roles in different cancer types. However, its expression and function in papillary thyroid carcinoma (PTC) remain unknown. Here we collected 156 PTC patients and retrospectively retrieved their clinicopathological characteristics as well as their survival data. Among them, USP18 was hypoexpressed in 47 PTC samples (30.1%) and significantly correlated with oncogenic characteristics. According to univariate and multivariate analyses, low USP18 can act as an independent prognostic indicator for unfavorable progression-free survival of PTC patients. Ectopic overexpression and knockdown assays indicated that USP18 can negatively regulate the proliferation of PTC cell lines. The anti-tumor effect of USP18 was finally validated by xenografts results from nude mice. Taken together, PTC patients with low level of USP18 have worse survival compared to those possess high USP18 expression. Downregulated USP18 may be involved in the proliferation of PTC, and USP18 expression can serve as an independent survival predictor.

Keywords: papillary thyroid carcinoma; prognosis; proliferation; USP18
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Introduction

Thyroid cancer is the most common endocrine malignancy, of which incidence has tripled in the past decades since the 1990s (Morris et al. 2016). There were 52,890 estimated newly diagnosed thyroid cancer cases in the United States in 2020 (Siegel et al. 2020). Of note, thyroid cancer accounts for up to 11% of cancers in adolescents, therefore more and more attentions have been attracted on both clinical practice and mechanism investigation (Ahn and Welch 2015). Based on histologic type, thyroid cancer can be classified into differentiated (papillary and follicular thyroid carcinoma) and anaplastic thyroid carcinoma. Among them, papillary thyroid carcinoma (PTC) is the predominate type, accounting for approximately 80% of all cases (Cancer Genome Atlas Research Network 2014). Compared to most other malignancies, the overall survival of PTC seems satisfied thanks to achievements in thyroidectomy, radioiodine therapy, and hormone inhibition therapy. However, nearly 60% cases are diagnosed with lymph node metastases and possess a high recurrence risk (Hay et al. 2002; Lundgren et al. 2006). Therefore, it is still essential to explore novel biomarkers for both prognosis prediction and personalized treatment.

Both protein abundance and post-translational modifications are vital for the precise control of protein functions and cellular processes. Many post-translational modifications are reversible, such as phosphorylation, ubiquitination, glycosylation, methylation, and so on. All these modifications are extensively involved in carcinogenesis and cancer progression (Eisenberg-Lerner et al. 2016; Hou et al. 2017; Liu et al. 2019). Among them, ubiquitination and deubiquitination balance is critical on controlling protein function and degradation by attaching or detaching the ubiquitin onto protein substrates. Numerous E3 ubiquitin-protein ligases and deubiquitinases (DUBs) had been reported to participate in cancer progression (Celebi et al. 2020). On one hand, F-box and WD repeat domain-containing 7 (FBW7) can suppress proliferation of hepatocellular carcinoma and colorectal cancer (Lv et al. 2020), while mouse double minute 2 (MDM2) enhances proliferation and invasion of breast cancer. On the other hand, ubiquitin

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specific peptidase 22 (USP22) enhances renal cell carcinoma viability (Lin et al. 2020), while USP33 suppresses colon adenocarcinoma progression (Liu et al. 2016). Therefore, dysfunction of ubiquitin balance plays completely distinct roles in different cancer types.

Similarly, many E3 ubiquitin-protein ligases were dysregulated in thyroid cancer. For example, the E3 ligase $SCF^{\beta\text{-TRCP}}$ stimulates ubiquitination and degradation of vascular endothelial growth factor receptor 2 (VEGFR2), subsequently impairs angiogenesis and metastases of PTC (Shaik et al. 2012). Tripartite motif-containing protein 14 (TRIM14) promotes thyroid cancer progression via ubiquitinating suppressor of cytokine signaling 1 (SOCS1). Similarly, TRIM30 mediates SRY-Box transcription factor 17 (Sox17) ubiquitination thus enhances PTC proliferation. As for DUBs, ubiquitin specific peptidase 7 (USP7) promotes proliferation of papillary thyroid carcinoma cells through T-Box transcription factor 3 (TBX3)-mediated p57 kinesin-like protein 2 (KIP2) repression (Xie et al. 2020). Higher OTU deubiquitinase 1 (OTUD1) also identifies less-aggressive characteristics of thyroid cancer (Carneiro et al. 2014). However, the role of USP18 in PTC remains unknown.

Here we aimed to explore the expression and clinical relevance of USP18 in PTC. The mRNA level of *USP18* was firstly tested in clinical specimens, then its protein expression and intracellular localization were tested via immunohistochemistry. Afterwards, clinical significance of USP18 was assessed by correlation tests and survival analyses. Finally, tumor-related role of USP18 was explored in PTC by both *in vitro* and *in vivo* strategies.

Methods

Patients and specimens

This study contains two retrospective cohorts. The first cohort is comprised of 27 PTC cases whose tumor samples and adjacent thyroid samples were fresh-frozen in liquid nitrogen immediately after thyroidectomy in our hospital. The specimens in this cohort were used for analyzing mRNA levels of USP18.

The second cohort contains 156 PTC patients who underwent radical surgery in our hospital. All cases in this cohort had sufficient formalin-fixed paraffin-embedded (FFPE) specimens for immunohistochemistry (IHC) analyses to test protein levels of USP18. The follow-up information was intact for this cohort thus was also subjected for survival analyses. All diagnoses were validated by pathological tests.

All specimens were tested with prior consents from participants. The study was approved by the Ethic Committee of First Affiliated Hospital of Anhui University of Science and Technology. The tumor grade and staging were made according to American Joint Committee on Cancer (AJCC) 7th edition.

Real time quantitative PCR (RT-qPCR)

Total mRNA was extracted using the Trizol® reagent and quantitated at OD260/280 nm, followed by treating with DNase I and reverse-transcribed into cDNA with Superscript II® retrotranscriptase (Invitrogen, Carlsbad, CA, USA). The obtained cDNAs were then applied for qPCR using SYBR PCR mix on the StepOnePlus Applied Biosystems instrument according to the manufacturer's instructions. The qPCR primers were listed below: USP18 forward, 5′-AACGTGCCCTTGTTTGTCCAA-3′; reverse 5′-GAGTCCTTCACCCGGATCGTA-3′. GAPDH forward, 5′-AGGTCGGTGTGAACGGATTTG-3′; reverse 5′-TGTAGACCATGTAGTTGAGGTCA-3′.

Immunohistochemistry (IHC)

As described above, the 156 FFPE PTC tissue specimens were subjected to IHC analyses. Tissue sections were cut into 4 μm thickness and deparaffinized with xylene and rehydrated through graded ethanol. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide in methanol for 10 min. After heat mediated antigen retrieval, sections were incubated with either primary anti-USP18 antibody (1:300, #LS-C679953, LSBio, Seattle, WA, USA) or control IgG at 4°C overnight. Signal was detected using the HRP secondary antibody and visualized by diaminobenzidine reaction (Liu et al. 2017).

The IHC staining results were scored according to both staining intensity and positive percentage. IHC staining intensity was scored as: 0 (negative), 1 (weak staining), 2 (moderate staining), and 3 (strong staining). The proportion of positive cells was scored as: 1 (0-25%); 2 (25-50%); 3 (50-75%); 4 (75-100%). Finally, the total score of immunoreactivities was calculated by multiplying these two scores above (ranging 1-12).

Cell culture and transfection

The normal thyrocytes-derived cell line Nthy-ori 3-1 (SV-40 immortalized normal human thyroid follicular cells) and four human PTC cell lines (K-1, TPC-1, and IHH-4, BCPAP) were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). Nthy-ori3-1 and K-1 cells were cultured in RPMI 1640 medium. TPC-1 cells were cultured in DMEM medium. IHH-4 cells were cultured in a mixture (1:1) of RPMI 1640 and DMEM medium. BCPAP cells were cultured in a mixture (1:1) of F12 and DMEM medium. All media were supplemented with 10% fetal bovine serum, 100 U/ml penicillin and 100 mg/ml streptomycin. All cells were maintained at 37°C in a humidified atmosphere with 5% CO_2. The overexpression was achieved by transfecting K-1 cells with USP18 plasmids (#22572, Addgene, Watertown, MA, USA) or control vector plasmids. The knockdown was conducted by transducing TPC-1 cells with packaged lentiviruses containing shRNA targeting USP18 (5′-AGCAACATGAAGAGAGAGCA-3′) or scramble shRNA (5′-CATTCCGCAGTGGTGCATT-3′) as control.

Western blotting

Total protein from cultured cells was extracted using NP-40 lysis buffer and quantified using a BCA assay kit. Equal amounts of protein were subjected to 10% SDS-PAGE and then transferred onto polyvinylidene fluoride (PVDF) membranes. After blocked the non-specific antigens, PVDF membranes were incubated at 4°C overnight with anti-USP18 antibody (1:1,000, #LS-C679953, LSBio) and anti-GAPDH antibody (1:1,000, #sc-47724, Santa Cruz Biotechnology, Dallas, TX, USA), followed by incubation with corresponding secondary horseradish peroxidase (HRP)-conjugated antibodies for another 2 h. The protein expression levels were visualized using the Pierce™ Enhanced chemiluminescence (ECL) kit (#32106, Thermo Fisher Scientific, Pittsburgh, PA, USA) and semi-quantified using Image J Software.

Cell proliferation assay

Transfected TPC-1 and K-1 cells were cultured for 24 hours before digested and seeded into 96-well cell-culture plates (3,000 cells/well). After cultured for designated time points, the cell culture medium was discarded and 100 μl 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) solution were added. Then the cells were incubated at 37°C incubator for 4 hours and 200 μl dimethyl sulfoxide (DMSO) reagent was added into each well. Finally, the plates were shaken for 15 min and send to a microplate reader to measure absorbance at 490 nm wavelength (Liu et al. 2021a). The data was obtained from three independent repeats.

Cell invasion assay

Matrigel-transwell assay was conducted to test invasion capacities of TPC-1 and K-1 cells. Briefly, the transwell was pre-coated with Matrigel, then 3×10^4 transfected cells were added into the upper chamber and cultured for 48 hours. Invaded cells below the membrane were fixed, stained, and counted as described by others. The experiments were repeated for three times.

Nude mice experiment

The animal experiments were approved by the Ethics Committee of First Affiliated Hospital of Anhui University of Science and Technology and performed in accordance

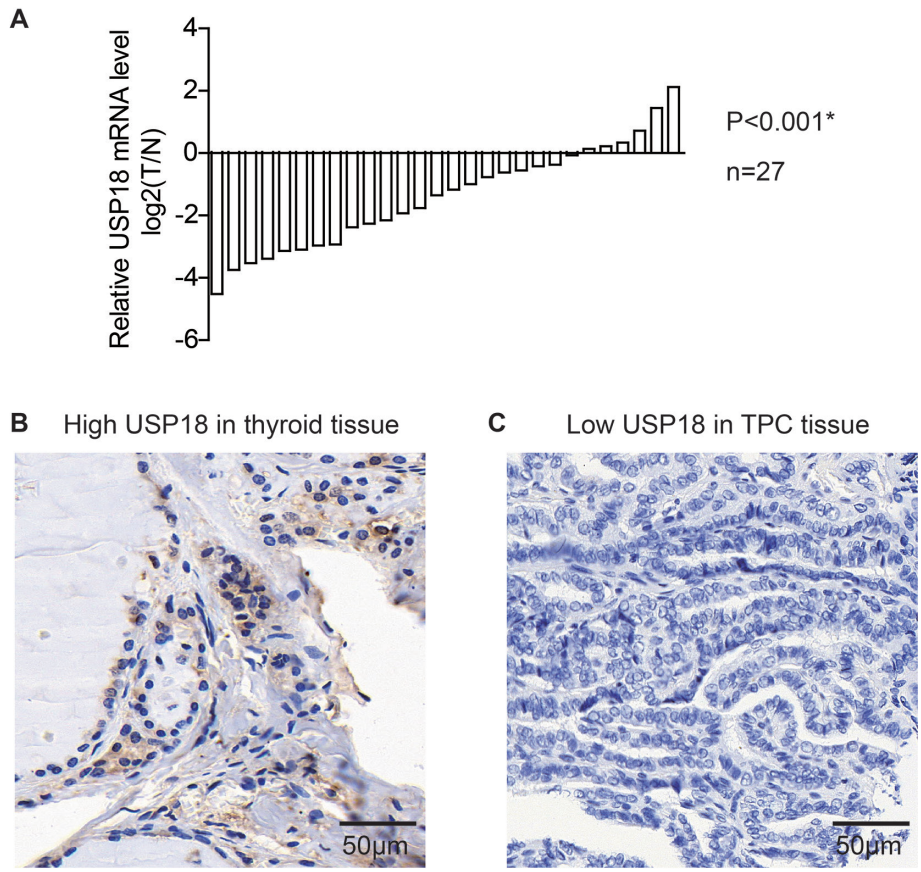

Fig. 1. Expression of USP18 in papillary thyroid carcinoma (PTC) tissues and adjacent thyroid tissues.
(A) RT-qPCR analyses were conducted to test mRNA levels of USP18 in clinical specimens. The data was compared by paired Student's t-test. *indicates $P < 0.05$. (B) Representative high protein level of USP18 in adjacent thyroid tissues, showing a predominant cytoplasm localization. The IHC score is 6. (C) Representative negative protein immunostaining of USP18 in PTC tissues, the IHC score is 0. The samples exhibited in (B) and (C) were obtained from the same patient.

Table 1. Clinicopathologic characteristics of the patients with papillary thyroid carcinoma (PTC) and their correlation with USP18 expression.

Variables	Cases (n = 156)	USP18 expression		P value
		Low (n = 47)	High (n = 109)	
Age (years)				0.139
≤ 45 years	69	25	44	
> 45 years	87	22	65	
Sex				0.378
Female	119	38	81	
Male	37	9	28	
Tumor size				< 0.001*
≤ 2.0 cm	109	22	87	
> 2.0 cm	47	25	22	
ATA risk				0.649
Low	92	29	63	
Middle or high	64	18	46	
TCI				0.325
Negative	111	36	75	
Positive	45	11	34	
ETE				0.293
Negative	83	22	61	
Positive	73	25	48	
LN metastasis				< 0.001*
Negative	114	23	91	
Positive	42	24	18	
TNM stage				0.002*
I-II	129	32	97	
III-IV	27	15	12	

*P < 0.05 was considered as statistically significant by chi-square test.
USP18, ubiquitin specific peptidase 18; ATA, American Thyroid Association; TCI, thyroid capsular invasion; ETE, extrathyroidal extension; LN, lymph node.

with the Guide for the Care and Use of Laboratory Animals (8th edition). Briefly, stably transfected K-1 or TPC-1 cells were subcutaneously injected into female BALB/c nude mice at 4 weeks old. Then the subcutaneous tumor size was measured by a vernier caliper every 5-days, and the tumor volume was calculated by the following formula: (π × length × width^2) / 6. After three weeks, all mice were sacrificed, the xenografts were excised and weighted (Chen et al. 2021).

Statistics

Data analyses were performed using the SPSS 18.0 software and GraphPad Prism 7.0. Chi-square test was used to assess the associations between the protein expression level and clinicopathological factors. Kaplan-Meier curve and log-rank test were used to compare the progression free survival (PFS) of enrolled patients. PFS was defined as the time between the surgical resection and the first diagnosis of disease relapse. Cellular and mice data were obtained from three independent repeats. Cox regression analysis was performed to identify independent prognostic factors. Student's *t*-test was used to compare differences for *in vitro* and *in vivo* experiments. Data were presented as mean ± standard deviation (SD). $P < 0.05$ was considered statistically significant.

Results

Clinicopathological characteristics of enrolled PTC patients

Among the 156 cases, 45 patients were diagnosed at ≤ 45 years old, while the other 87 patients with > 45 years old. Most patients (119/156, 76.3%) were female and here were only 37 male cases. The tumor size was ≤ 2.0 cm in 109 cases and > 2.0 cm in 47 cases. According to the American Thyroid Association (ATA) Risk Stratification System, 92 patients were with low risk, 48 patients with middle risk, and the other 16 patients with high risk. Thyroid capsular invasion (TCI) was observed in 45 cases and negative in the other 111 cases. Extrathyroidal extension (ETE) was diagnosed in 73 cases and negative in the other 83 cases. As for the lymph node (LN) metastasis, 42 cases showed positive LN, while no LN metastasis was

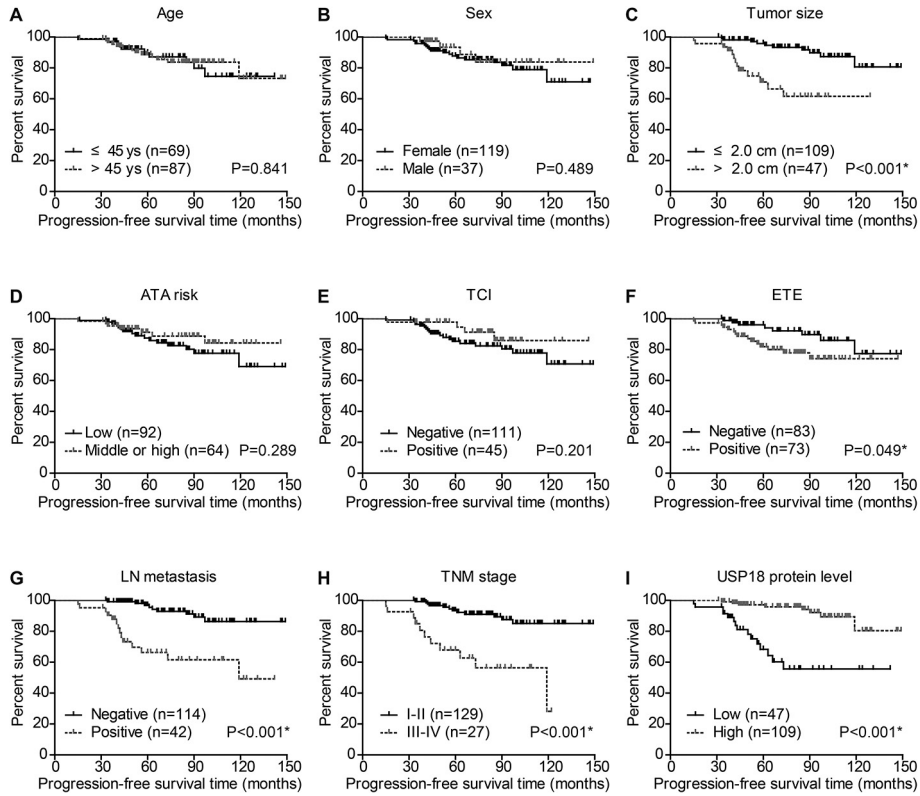

Fig. 2. Overall survival analyses of papillary thyroid carcinoma (PTC) cohort.
Kaplan-Meier method was used to analyze the prognostic significance of all retrieved clinicopathological characteristics, including patients' age (A), sex (B), tumor size (C), the American Thyroid Association (ATA) Risk (D), thyroid capsular invasion (TCI) (E), extrathyroidal extension (ETE) (F), lymph node (LN) metastasis (G), TNM stage (H), and USP18 protein level (I). Data were analyzed by log-rank test. *P < 0.05.

observed in the other 114 cases. Accordingly, 129 cases were classified as TNM stage I-II, and 27 cases with TNM stage III-IV.

Expression of USP18 and its correlation with clinicopathological features

The mRNA levels of *USP18* were firstly tested in fresh-frozen PTC tissues and their paired nontumorous thyroid tissues (Fig. 1A). Among the 27 paired specimens, a lower *USP18*-mRNA level was observed in 22 cases (81.5%), indicating an impaired transcription of *USP18* in PTC. We next conducted IHC experiments to test the protein expression pattern of USP18 in another 156 PTC tissues and adjacent thyroid tissues. As a result, a detective USP18 protein level was detected in most normal thyroid tissues, which mainly localized in cytoplasm (Fig. 1B). In contrast, the TPC tissues showed relative lower USP18 immunoreactivity (Fig. 1C).

To further understand whether USP18 has any correlation with clinicopathological characteristics, we divided the 156 cases into a high expression group (n = 109) and a low expression group (n = 47) according to the receiver operating characteristic curve. As shown in Table 1, chi-square test indicated a negative correlation between USP18 level and tumor size (P < 0.001). Similarly, a low USP18 level was also significantly associated with positive LN metastasis (P < 0.001) and more advanced TNM stages (P = 0.002). However, no significant correlation was observed between USP18 expression and patient age, sex, ATA risk, TCI, or ETE (P > 0.05).

Significance of USP18 on predicting PTC recurrence

The survival status of our retrospective cohort was analyzed by Kaplan-Meier method and compared via log-rank test (Fig. 2). Accordingly, patients with low USP18 protein expression exhibited worse outcomes than those with high USP18 expression (P < 0.001). The mean PFS was 100.8 ± 8.3 months for patients with low-USP18 level, while up to 138.3 ± 3.8 months for patients with high-USP18 level. The 5-year PFS rate of low-USP18 group was 68.3%, while was 95.8% for high-USP18 group. Besides USP18 protein level, larger tumor size, positive ETE, positive LN metastasis, and advanced TNM stages all indicated unfavorable patients' survival (Table 2).

Furthermore, multivariate Cox regression analysis uncovered that USP18 expression [Hazard Ratio (HR) = 0.314, 95% Confidence Interval (CI): 0.124-0.792, P = 0.014; Table 3] was an independent favorable prognostic factor for PTC patients. Meanwhile, the larger tumor size (HR = 3.501, 95% CI: 1.363-8.992, P = 0.009) and positive

Table 2. Univariate analyses.

Variables	Cases (n = 158)	Progression-free survival		P value
		Mean ± SD (Months)	5-year (%)	
Age (years)				0.841
≤ 45 years	69	123.1 ± 5.4	89.7%	
> 45 years	87	129.6 ± 5.1	88.6%	
Sex				0.489
Female	119	126.1 ± 4.4	87.7%	
Male	37	134.4 ± 6.8	93.4%	
Tumor size				< 0.001*
≤ 2.0 cm	109	137.1 ± 3.6	95.8%	
> 2.0 cm	47	97.6 ± 7.2	70.8%	
ATA risk				0.289
Low	92	126.0 ± 5.2	87.4%	
Middle or high	64	132.2 ± 4.9	91.2%	
TCI				0.201
Negative	111	126.4 ± 4.7	85.3%	
Positive	45	134.5 ± 5.4	97.8%	
ETE				0.049*
Negative	83	135.2 ± 4.6	96.0%	
Positive	73	122.5 ± 5.6	81.9%	
LN metastasis				< 0.001*
Negative	114	138.7 ± 3.3	96.8%	
Positive	42	101.1 ± 8.5	66.3%	
TNM stage				< 0.001*
I-II	129	136.9 ± 3.3	93.3%	
III-IV	27	86.8 ± 8.3	67.9%	
USP18 expression				< 0.001*
Low	47	100.8 ± 8.3	68.3%	
High	109	138.3 ± 3.8	95.8%	

*P < 0.05 was considered as statistically significant by log-rank test.
USP18, ubiquitin specific peptidase 18; ATA, American Thyroid Association; TCI, thyroid capsular invasion; ETE, extrathyroidal extension; LN, lymph node.

Table 3. Multivariate analysis.

Variables	Hazard ratio	95% Confidence interval	P value
Tumor size			
(> 2.0 cm vs. ≤ 2.0 cm)	3.501	1.363-8.992	0.009*
ETE			
(Positive vs. negative)	1.312	0.522-3.301	0.564
LN metastasis			
(Positive vs. negative)	2.759	1.045-7.283	0.040*
TNM stage			
(III/IV vs. I/II)	1.768	0.691-4.524	0.234
USP18 expression			
(High vs. low)	0.314	0.124-0.792	0.014*

*P < 0.05 was considered as statistically significant by Cox regression test.
USP18, ubiquitin specific peptidase 18; ETE, extrathyroidal extension; LN, lymph node.

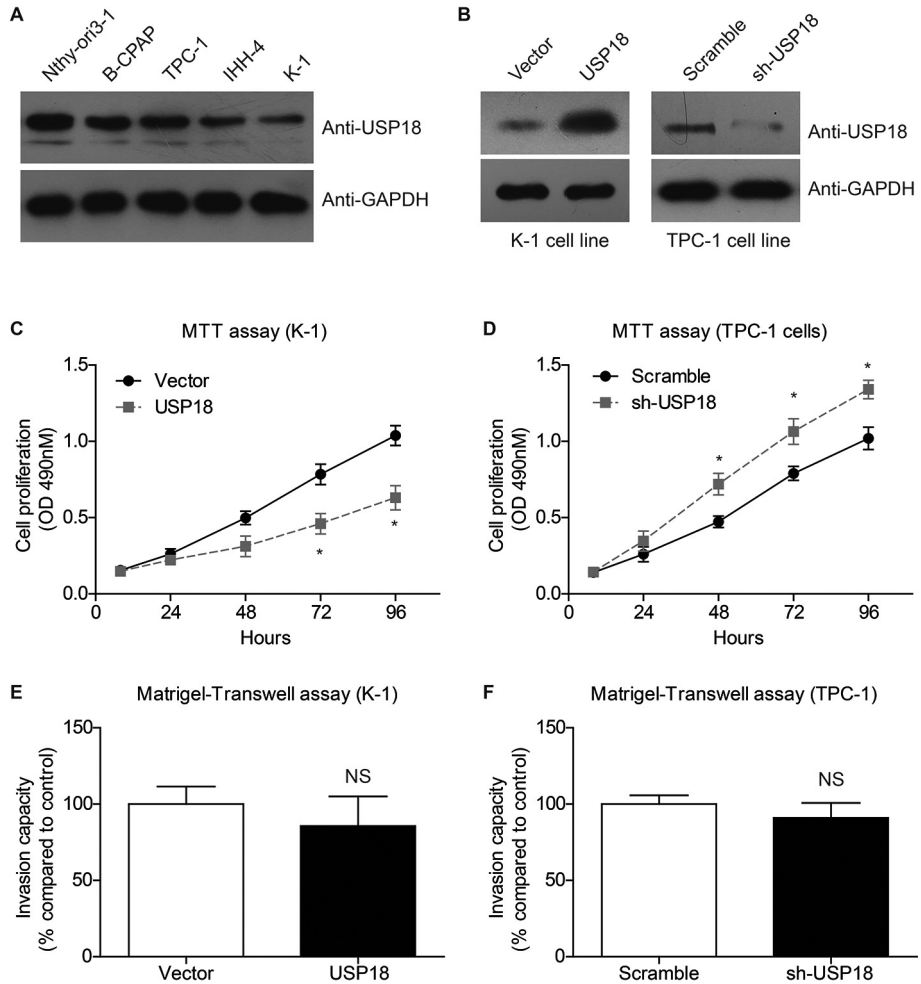

Fig. 3. The effects of USP18 on papillary thyroid carcinoma (PTC) proliferation and invasion.
(A) Protein level of USP18 was tested in different PTC cell lines and normal thyrocytes. (B) Overexpression assay was conducted by transfecting USP18 plasmids into K-1 cell line using blank vector plasmids as control. Knockdown assay was achieved by transducing USP18-specific shRNA (sh-USP18) or scramble shRNA into TPC-1 cells.
(C) MTT assay reflected that overexpressing USP18 can significantly impair the proliferation capacity of K-1 cells. (D) The proliferation process was accelerated in TPC-1 cells that transfected with USP18-shRNA. (E) USP18 overexpression exhibited no significant effect on the invasion capacity of K-1 cells. (F) Silencing USP18 exerted no statistically significant effect on the invasion process of TPC-1 cells. Data was obtained from three independent repeats. *P < 0.05 by Student's t-test. NS, no statistical significance.

LN metastasis (HR = 2.759, 95% CI: 1.045-7.283, P = 0.040) also contributed independently to the PFS of PTC. In all, our clinical data have revealed that a hypoexpression of USP18 in PTC tissues contributes to invasive phenotype and unfavorable prognosis.

USP18 inhibits proliferation of PTC cells without affecting its invasion process

We next tested the expression profile of USP18 in human PTC cell lines via immunoblotting (Fig. 3A). Consistent with the IHC data, all the four PTC cell lines (K-1, TPC-1, and IHH-4, BCPAP) exhibited lower USP18 protein levels than Nthy-ori 3-1 thyrocytes. Therefore, we selected K1 cell line, whose endogenous USP18 level was the lowest, for overexpression assay; and selected TPC-1 cell line, whose endogenous USP18 level was the highest, for knockdown assay (Fig. 3B). After establishing stably transfected cell lines, MTT assays were conducted to evaluate cell viabilities. As a result, overexpressing USP18 led to an impaired proliferation capacity of K-1 cells (Fig. 3C). In contrast, silencing USP18 by specific shRNA resulted in a significantly enhanced cell proliferation process of TPC-1 cells (Fig. 3D). However, the Matrigel-transwell assay demonstrated that USP18 has no statistically significant effect on modulating PTC cell invasion (Fig. 3E, F).

USP18 attenuates PTC growth in vivo

To further validate the tumor-related role of USP18 in PTC, we conducted xenograft experiments by subcutaneously injecting stably transfected PTC cells into nude mice. As reflected by growth curves, the tumor growth rate was significantly slower in USP18-overexpression group than

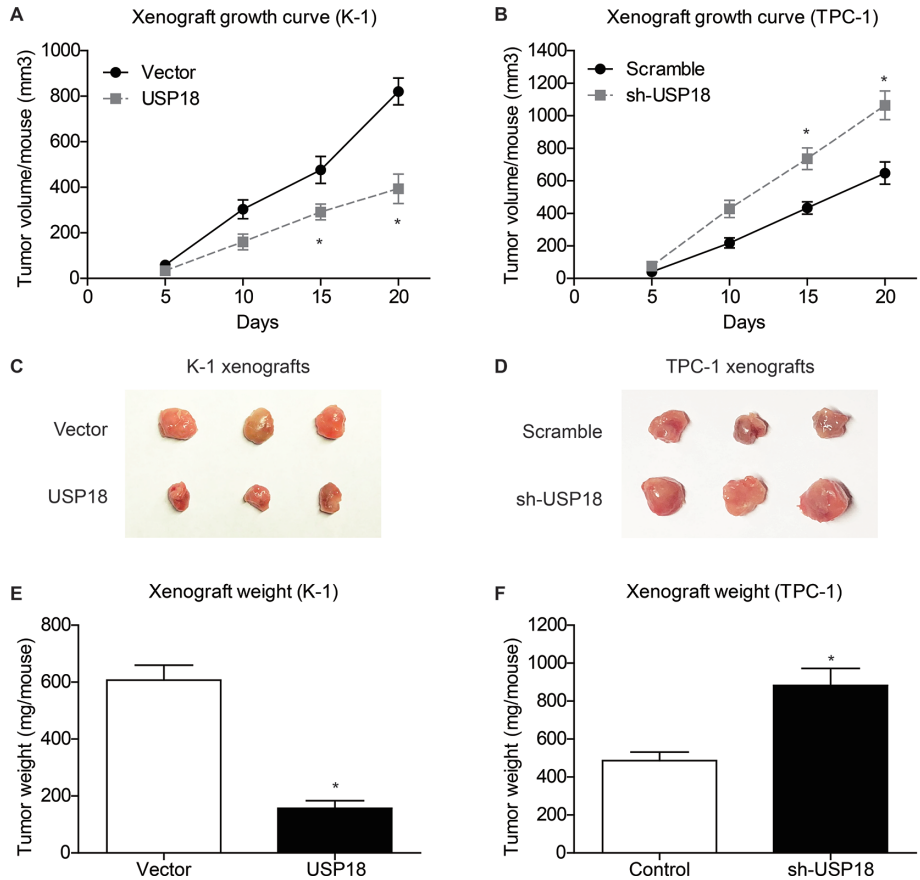

Fig. 4. The effects of USP18 on tumor growth in nude mice.
(A, B) The stably transfected K-1 and TPC-1 cells were subcutaneously injected into nude mice, respectively. Tumor volumes were measured every five days to plot growth curves. (C, D) Three weeks later, xenografts were isolated for photographing and weighting. (E, F) Weight of tumor xenografts. Data was obtained from three independent repeats. *P < 0.05 by Student's t-test.

that of control group (Fig. 4A). In contrast, silencing USP18 accelerated the tumor growth in vivo (Fig. 4B). Consistent with growth curves monitored on live mice, the excised tumors also indicated a tumor-suppressing effect of USP18 on both tumor size (Fig. 4C, D) and tumor weight (Fig. 4E, F). Taken together, USP18 exerts anti-proliferative effects in PTC progression both *in vitro* and *in vivo*.

Discussion

As an endocrine cancer, PTC is characterized by dysregulation of numerous protein functions including ubiquitination balance. Here we tested the tumor-related roles of USP18 in clinical PTC samples, human PTC cell lines, as well as subcutaneous xenografts. USP18 belongs to the ubiquitin specific peptidase subfamily of DUBs, which had been reported to play oncogenic roles in malignancies. For example, USP18 promotes breast cancer growth by upregulating EGFR and activating the AKT/Skp2 pathway (Tan et al. 2018). A similar growth-promoting effect of USP18 was also observed in lung cancer (Liu et al. 2021b). Interestingly, USP18 can also induce apoptotic resistance in glioblastoma (Sgorbissa et al. 2011). Similarly, silencing USP18 led to an increase of apoptosis in breast cancer cells upon chemotherapy or interferon-α (IFN-α) stimulation (Potu et al. 2010). The tumor-promoting role of USP18 was also reported in kidney cancer (Shahidul Makki et al. 2013) and acute promyelocytic leukemia (Guo et al. 2010). Moreover, decreased expression of USP18 was reported to be correlated with longer cancer-specific survival of muscle invasive bladder cancer (Kim et al. 2014).

However, USP18 seems to exert anti-tumor effects in several other cancer types. For example, USP18 exhibits tumor-suppressing effects in melanoma (Hong et al. 2014). A reduced USP18 expression is also associated with the unfavorable survival of leiomyosarcoma (Chinyengetere et al. 2015). Our data provided the first evidence that USP18 is downregulated in PTCs and plays anti-proliferation functions. The multifaced function of USP18 is consistent with complicated tumor-related roles of other DUBs as reviewed by Fraile et al. (2012). Therefore, the detailed role of USP18 in each cancer type deserves precisely determination, which is essential for development and in-depth characterization of USP18 inhibitors (Mustachio et al. 2018).

Our study had some limitations. Firstly, the sample size enrolled in our study is relatively small and is from a single medical center. Secondly, our data only focused on

the disease relapse but not overall survival due to the limited dead patients. Thirdly, this study mainly explored the clinical relevance and significance of USP18 in PTC, therefore we did not fully illustrate its underlying signaling mechanisms. Fourthly, there are cross-talks and complementary effects among different DUBs. Here we only tested the level of USP18 without mapping the expression of other DUBs, and therefore, in the further study, we will identify more other differential expression DUBs and investigate their combination value with USP18 in PTC.

In conclusion, our analysis reveals for the first time that USP18 is an independent prognostic factor in PTC and has a suppressing role on PTC proliferation.

Conflict of Interest

The authors declare no conflict of interest.

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