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ORIGINAL ARTICLE – CANCER RESEARCH

# TPP1 OB-fold domain protein suppresses cell proliferation and induces cell apoptosis by inhibiting telomerase recruitment to telomeres in human lung cancer cells

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## Abstract

**Purpose** Maintaining telomeres by recruiting telomerase-to-chromosome ends is essential for cancer cell survival. Inhibiting telomerase recruitment to telomeres represents a novel strategy for telomere-based lung cancer therapy. However, approaches for interrupting telomerase recruitment for cancer therapy still need to be explored.

**Methods** The telomere-binding protein TPP1 is responsible for recruiting telomerase to telomeres and synthesizing telomeres through the association between the oligosaccharide/oligonucleotide-binding (OB)-fold domain of TPP1 and telomerase reverse transcriptase. We overexpressed the TPP1 OB domain (TPP1-OB) by lentivirus infection in lung cancer cells. Telomere length was examined by Southern blot analysis of terminal restriction fragments. The effects of TPP1-OB on cell proliferation, the cell cycle, apoptosis, chemosensitivity, and tumor growth were evaluated in vitro and in vivo.

**Result** TPP1-OB inhibited the recruitment of telomerase to telomeres and shortened telomere length by acting as a dominant-negative mutant of TPP1. TPP1-OB resulted in reduced cell proliferation, G1 cell cycle arrest, and increased cell apoptosis in lung cancer cells. Cell apoptosis occurred mainly through the caspase-3-dependent signaling pathway. TPP1-OB also suppressed anchorage-independent growth and tumor growth in vivo. Moreover, we demonstrated that TPP1-OB enhances the sensitivity of lung cancer cells to the chemotherapeutic drug paclitaxel.

**Conclusion** Our results suggest that inhibiting TPP1-mediated telomerase recruitment by expressing the TPP1-OB domain is a potential novel strategy for telomere-targeted lung cancer therapy.

**Keywords** TPP1 · Telomere · Telomerase · Lung cancer

## Abbreviations

| | |
|---|---|
| OB | Oligosaccharide/oligonucleotide-binding |
| TERT | Telomerase reverse transcriptase |
| TEL | TPP1 glutamate (E) and leucine (L)-rich |
| TEN | Telomerase essential N-terminal |
| TIF | Telomere dysfunction-induced foci |
| FBS | Fetal bovine serum |

Jinfang Zhu, Weiran Liu and Chen Chen have contributed equally to this study.

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| | |
|---|---|
| RT-PCR | Reverse transcription-polymerase chain reaction |
| FISH | Fluorescence in situ hybridization |
| PNA | Peptide nucleic acids |

## Introduction

Telomeres are a special structure at the end of linear chromosomes that consist of the repetitive telomere DNA sequence and the six-protein complex called shelterin (Cech 2004; de Lange 2005; Palm and de Lange 2008). Because DNA polymerases cannot fully replicate the extreme ends of chromosomes, telomeres gradually shorten with cell division in normal adult somatic cells. When telomeres reach a critically short length, cellular replicative senescence occurs, and cell division ceases (Blackburn 2000; Shay and Wright 2000, 2005). In cancer cells, a reverse transcription enzyme called telomerase is reactivated. Telomerase synthesizes telomere DNA and maintains a stable telomere length for indefinite cell division, which results in cell immortality (Stewart and Weinberg 2006; Artandi and DePinho 2010; Shay and Wright 2011; Shay 2016). Thus, the recruitment of telomerase-to-telomere ends and the addition of telomere repeats to chromosome ends are essential for cancer cell growth.

The six-protein shelterin complex includes TRF1, TRF2, POT1, TIN2, RAP1, and TPP1, and properly safeguards chromosome ends (de Lange 2005; Palm and de Lange 2008; Lim et al. 2017; Takai et al. 2010). TRF1 and TRF2 bind double-stranded telomere repeats, while POT1 binds single-stranded DNA overhangs. RAP1 is associated with TRF2, and TIN2 connects TRF1 with TRF2. TPP1 bridges POT1 to TIN2. In addition to interacting with the other shelterin components to protect telomere ends, TPP1 interacts with telomerase and recruits telomerase-to-chromosome ends to elongate telomeres (Abreu et al. 2010; Zaug et al. 2010; Chen et al. 2017; Hu et al. 2017). TPP1 has three separate domains that separately interact with TIN2, POT1, and telomerase (Rajavel et al. 2016; Nandakumar et al. 2012). The telomerase-binding domain resides in 87–250 aa, which contains an oligosaccharide/oligonucleotide-binding (OB)-fold domain (referred to as TPP1-OB). TPP1 is a crucial protein in the shelterin complex that regulates the recruitment of telomerase to telomeres and telomerase processivity through the interaction between telomerase and the TPP1 glutamate (E) and leucine (L)-rich (TEL) patch of TPP1-OB (Nandakumar et al. 2012; Zhong et al. 2012). The disruption of binding by a TEL-patch mutation of TPP1 or a telomerase essential N-terminal (TEN) domain mutation of TERT interferes with telomerase localization to telomeres, resulting in telomere shortening (Nakashima et al. 2013; Schmidt et al. 2014). Therefore, inhibiting the recruitment of telomerase represents a novel strategy for telomere-targeted cancer therapy.

Here, we overexpressed the TPP1-OB domain protein to competitively inhibit the binding of endogenous TPP1 to telomerase, blocking the recruitment of telomerase to telomeres. We found that TPP1-OB suppressed cell proliferation and tumor growth through shortening telomeres and inducing cell apoptosis in lung cancer cells in vitro and in vivo. Our study provides a promising strategy for inhibiting the recruitment of telomerase by expressing TPP1-OB in a dominant-negative manner for anticancer therapy.

## Materials and methods

### Cell lines and plasmids

All cell lines obtained from the National Infrastructure of Cell Line Resource (Beijing, China) were maintained at 37 °C in a humidified atmosphere of 5% $CO_2$. Both A549 and H520 cells were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium (Gibco, Thermo Fisher Scientific, MA, USA) supplemented with 10% fetal bovine serum (FBS) (PAN-Biotech, Germany), 100 U/mL penicillin-G, and 100 µg/mL streptomycin. 293T cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FBS.

Flag-TERT was purchased from Addgene (Watertown, MA, USA), and the PCDH vector was purchased from Systems Biosciences. The primers, 2× HA-Forward primer: 5′AATTCTACCCATACGATGTTCCAGATTACGCTTACCCATACGATGTTCCAGATTACGCTTAATAAG3′ and 2× HA-Reverse primer: 5′GATCCTTATTAAGCGTAATCTGGAACATCGTATGGGTAAGCGTAATCTGGAACATCGTATGGGTAG3′, were synthesized, annealed, and inserted into the PCDH vector to produce the PCDH-HA vector. Then, TPP1 cDNA was inserted into the PCDH-HA vector to generate the HA-TPP1 plasmid.

TPP1-OB (259–750 bp) was amplified with primers (Forward: AGCTCTAGAGCCACCATGGCAGGTTCGGGGAGGCT, Reverse: AGCGAATTCTGCATTGGACGAGGTGGACTC) and cloned into the PCDH-puro lentiviral expression vector (Systems Biosciences, Mountain View, CA, USA).

### RT-PCR

The expression of TPP1-OB was determined by reverse transcription-polymerase chain reaction (RT-PCR). Total

RNA was extracted from the stably transfected cells by using TRIzol (Invitrogen, Carlsbad, CA, USA). cDNA was synthesized using the Prime Script II 1st Strand cDNA Synthesis Kit (Takara, Japan) according to the manufacturer's instructions. PCR was performed using the above TPP1-OB primers and GAPDH as a loading control (Forward: 5′GGG AAACTGTGGCGTGAT3′, Reverse: 5′GAGTGGGTGTCG CTGTTGA3′).

### Western blot analysis

Cells were lysed with RIPA lysis buffer, and protein extracts were fractionated on SDS-PAGE gels and transferred onto PVDF membranes. Antibodies against cleaved caspase3, cleaved PARP, and TPP1 were purchased from Cell Signaling Technology (Cell Signaling Technology, MA, USA). Anti-HA, anti-Flag and anti-GAPDH were purchased from Sigma (Sigma-Aldrich, MO, USA).

### Coimmunoprecipitation

PCDH-HA-TPP1, Flag-TERT, and PCDH-puro-TPP1-OB were cotransfected into 293T cells using Lipofectamine 2000 (Thermo Fisher Scientific, MA, USA). After 48 h of transfection, cells were harvested with 1× phosphate-buffered saline (PBS) and lysed with 500 μL of lysis buffer (50 mM Tris–HCl, pH 7.4, 150 mM NaCl, 0.5% NP-40, 1 mM EDTA, and complete protease inhibitor cocktail). Five percent of the cellular lysate was used as the input sample. After sonicating and centrifuging, 30 μL of anti-HA beads (Sigma-Aldrich, MO, USA) or Anti-DDDDK-tag mAb-Magnetic beads (MBL, Nagoya, Japan) were mixed with the supernatants at 4 °C overnight. Then, the beads were washed with 1× lysis buffer three times and boiled in 2× SDS buffer for 10 min, and the proteins were resolved by SDS-PAGE and analyzed by western blotting.

### Cell viability assay

A total of 6000 cells were seeded in triplicate in a 96-well plate. Cells were treated with paclitaxel for 72 h after cell adherence. Then, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution (Sigma-Aldrich, MO, USA) was added and incubated for 4 h at 37 °C. Dimethyl sulfoxide (DMSO) was used to dissolve the precipitant. The 96-well plate was read at 570 nm to determine cell viability using the GloMax® System (Promega, WI, USA). The inhibition of cell growth was calculated as follows: Cell inhibition % = [1 − (treated cells-blank)/(untreated control-blank)] × 100%. Experiments were performed three times.

### Colony formation assay

Approximately 500 cells in complete medium were seeded into each well of 6-well plates and maintained at 37 °C for 12 days. Cells were fixed and stained with Giemsa solution. Colonies containing more than 50 cells were counted.

### Soft agar assay

For the soft agar assay, 0.35% low melting point agar-containing 1000 cells was plated on a 0.6% low melting point agar base in 6-well plates, which were maintained in a 37 °C incubator for 2–3 weeks. Then, colonies were counted in ten random fields with a light microscope (Olympus, Japan).

### Flow cytometry analysis

At least $1 \times 10^6$ cells were harvested and fixed in 70% ice cold ethanol at − 20 °C overnight. Then, the cells were centrifuged, washed with PBS, and resuspended in 1 mL of PBS containing 1 mg/mL of RNase and 50 μg/mL of propidium iodide. The cells were incubated for 15 min in the dark at room temperature and analyzed by a BD FACS Canto II flow cytometer (BD Biosciences, CA, USA). Three independent experiments were performed.

### Caspase-Glo 3/7 assay

Caspase3 activity was detected by the Caspase-Glo 3/7 Assay Kit (Cat. No. G8090, Promega, WI, USA) according to the manufacturer's instructions.

### FISH and immunofluorescence

Telomere fluorescence in situ hybridization (FISH) was performed using the Telomere PNA FISH Kit/Cy3 (Dako Denmark A/S, Glostrup, Denmark) according to the manufacturer's instructions. After hybridization and washing, slides were processed for indirect immunofluorescence. Slides were rinsed in PBS and then incubated with anti-53BP1 primary antibody (Cell Signaling Technology, MA, USA) at 4 °C overnight. Then, Alexa Fluor 488-conjugated goat anti-rabbit IgG was added, and the slides were incubated at 37 °C for 1 h and stained with 4′,6-diamidino-2-phenylindole (DAPI). Slides were mounted with Prolong antifade mounting medium (Molecular Probes Inc., OR, USA) and coverslipped. Images were captured using a Zeiss scanning microscope.

### Telomere length analysis

The TeloTAGGG Telomere Length Assay Kit was used to measure telomere length according to the manufacturer's instructions (Cat. No. 12209136001 Roche, Mannheim, Germany).

### Xenograft mouse model

TPP1-OB-expressing A549 cells ($5 \times 10^6$ cells) and vector control cells were injected subcutaneously into the flanks of female BALB/c nude mice ($n = 12$) that were randomly divided into two groups. After 9 days, the tumors were measured with the same Vernier caliper every 3 or 4 days, and the length and width were recorded to calculate the volume as (length × width$^2$)/2. When the volume of most of the tumors reached approximately 1000 mm$^3$, the tumors were resected from the mice and preserved by snap freezing and paraffin embedding. Tissue slides were used for HE staining and immunohistochemistry (IHC) analysis of Ki-67 (1:200 dilution, Cell Signaling Technology) and cleaved caspase3 (1:50 dilution, Cell Signaling Technology). The animal protocol was approved by the Institutional Animal Care and Use Committee of Tianjin Cancer Hospital.

### Statistical analysis

GraphPad Prism software was used to perform statistical analyses. The data are presented as the mean ± standard deviation (SD). Statistical comparisons were analyzed using a two-tailed Student's *t* test or ANOVA. A *p* value of less than 0.05 was considered significant.

## Results

### TPP1-OB interferes with telomerase binding to TPP1 and telomerase localizing to telomeres

The TPP1-OB domain of TPP1, independent of its POT1-binding domain and TIN2-binding domain, is believed to affect only its interaction with telomerase. We reasoned that an overexpression of TPP1-OB should act as a dominant-negative mutant by displacing endogenous TPP1 from

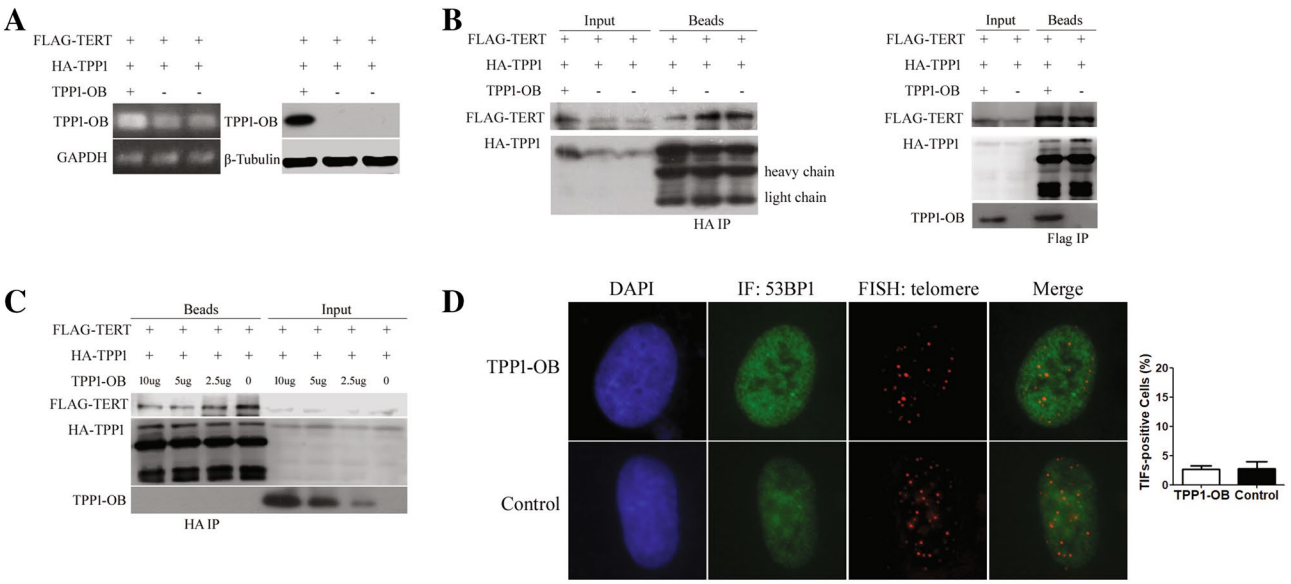



**Fig. 1** TPP1-OB domain protein disrupts the recruitment of telomerase to telomeres. **a** TPP1-OB was cotransfected with HA-TPP1 and Flag-TERT in 293T cells. TPP1-OB is overexpressed by RT-PCR and western blotting. **b** Co-IP assay showing that TPP1-OB overexpression impairs TERT binding to TPP1. Left panel, performing IP with anti-HA conjugated beads followed by immunoblotting (IB) with anti-Flag antibody; Right panel, performing IP with anti-Flag conjugated beads followed by IB with anti-HA antibody. Input indicates cellular lysates before incubation with beads, and 5% of the cellular lysate was used as the input sample. **c** 293T cells were transfected with the indicated doses of TPP1-OB plasmids, followed by IP with anti-HA conjugated beads and IB with anti-Flag antibody. The inhibition of the TERT interaction with TPP1 is dependent on the TPP1-OB dose. **d** After transfection for 48 h, cells were fixed for the immunofluorescence–fluorescence in situ hybridization (IF-FISH) assay. IF detects 53BP1 proteins (green), and FISH detects telomeres (red). DAPI staining detects the nuclei (blue). TIFs are indicated by the colocalization of 53BP1 and telomeres (yellow spots). Cells from ten random fields were counted. The experiment was repeated twice (TPP1-OB group vs Control group, $p > 0.05$. Scale bar = 20 μm)

telomerase, thus inhibiting telomerase access to telomeres without causing telomere dysfunction. To test this hypothesis, we first tested the interaction of TPP1 with telomerase in the presence of TPP1-OB in a coimmunoprecipitation experiment. TPP1-OB was coexpressed with HA-TPP1 and Flag-TERT in 293T cells. As shown in Fig. 1a, b, TPP1-OB inhibited TERT binding to TPP1. Moreover, we transfected different doses of TPP1-OB plasmids with HA-TPP1 and Flag-TERT into 293T cells. With the increase in the amount of TPP1-OB, the coimmunoprecipitation of Flag-TERT and HA-TPP1 progressively decreased (Fig. 1c), confirming that the binding of these two proteins is TPP1-OB dose-dependent. As expected, we did not observe telomere dysfunction-induced focus (TIF) formation in the TPP1-OB transient expressing cells (Fig. 1d and Supplementary Figure 1). Taken together, the results indicate that TPP1-OB inhibits the recruitment of telomerase to telomeres acting in a dominant-negative manner.

## TPP1-OB impedes cell proliferation in lung cancer cells

We then evaluated the effect of TPP1-OB on the long-term growth of lung cancer cells. TPP1-OB was stably transduced into A549 and H520 lung cancer cells by lentivirus infection (Fig. 2a). All cell lines were passaged every 3 days and counted with a hemocytometer. The growth curves showed that cell proliferation slowed down in stable TPP1-OB-overexpressing A549 and H520 cells (Fig. 2b). We observed that the effect of TPP1-OB expression on cell proliferation was not apparent until PD30 in A549 cells and PD20 in H520 cells. Cells in PD30 were seeded into 6-well plates for the colony formation assay to further confirm the effect of TPP1-OB on cell proliferation. The results indicated that the number of clones was significantly lower in stable TPP1-OB-overexpressing A549 and H520 cells than in the control cells (Fig. 2c). Moreover, flow cytometric cell cycle analysis collected from PD30 cells revealed that TPP1-OB led to G1 cell cycle arrest and reduced the percentage of cells in the S phase (Fig. 2d), which might contribute to the decrease in

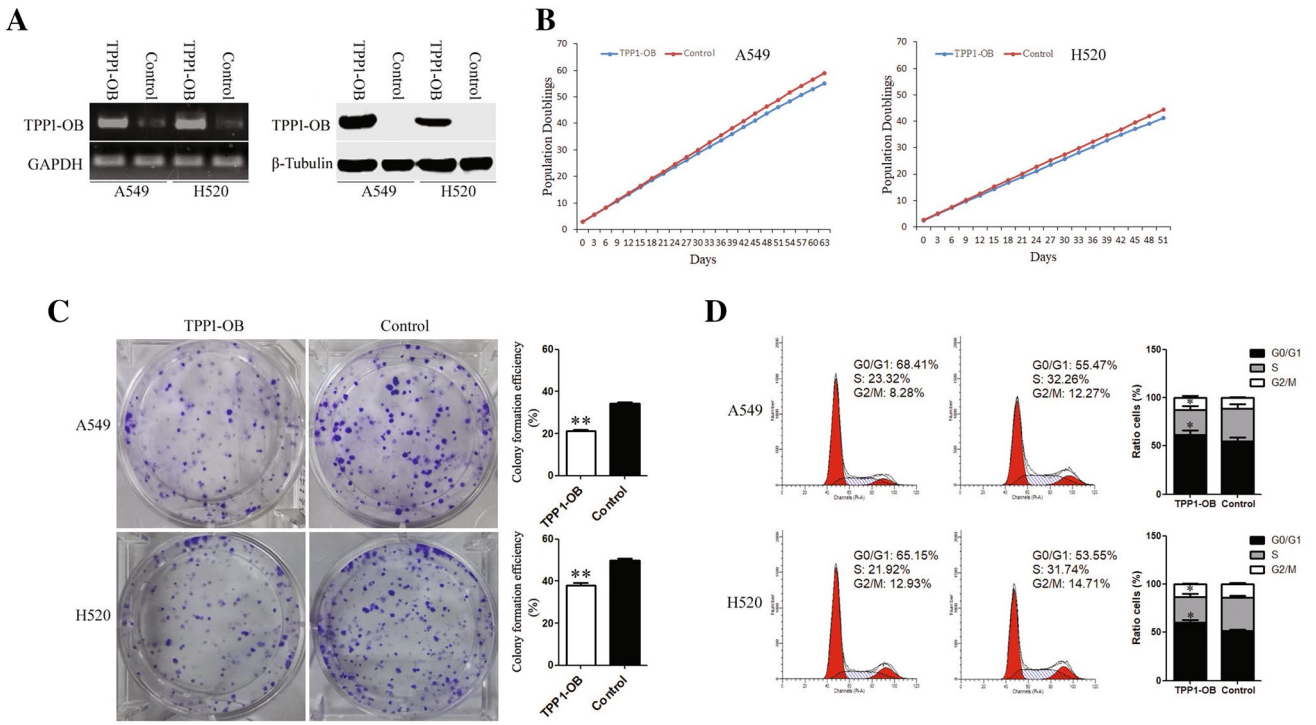



**Fig. 2** TPP1-OB inhibits cell proliferation and clone formation in A549 and H520 lung cancer cells. **a** RT-PCR and western blotting show the expression of TPP1-OB after TPP1-OB was stably transduced into A549 and H520 cells by lentivirus infection. **b** Cell growth curves indicate that cell proliferation decreased in TPP1-OB-overexpressing A549 and H520 lung cancer cells. Data represent population doublings (PDs) in each passage. ANOVA, $p < 0.05$. **c** TPP1-OB reduced clone formation in A549 and H520 lung cancer cells. Colony formation efficiency (%) was calculated as number of colonies formed/number of cells seeded × 100%. **d** Cell cycle analysis shows that the percentage of cells decreased in the S phase and increased in the G1 phase in TPP1-OB-expressing A549 and H520 cells compared with control cells. The cell cycle was analyzed by flow cytometry. Representative FACS plots are shown. The percentages of G0/G1, S, and G2/M phase cells are indicated on the right column graph. Each experiment was repeated in triplicate. Data are presented as the mean ± SD. *$p < 0.05$, **$p < 0.01$

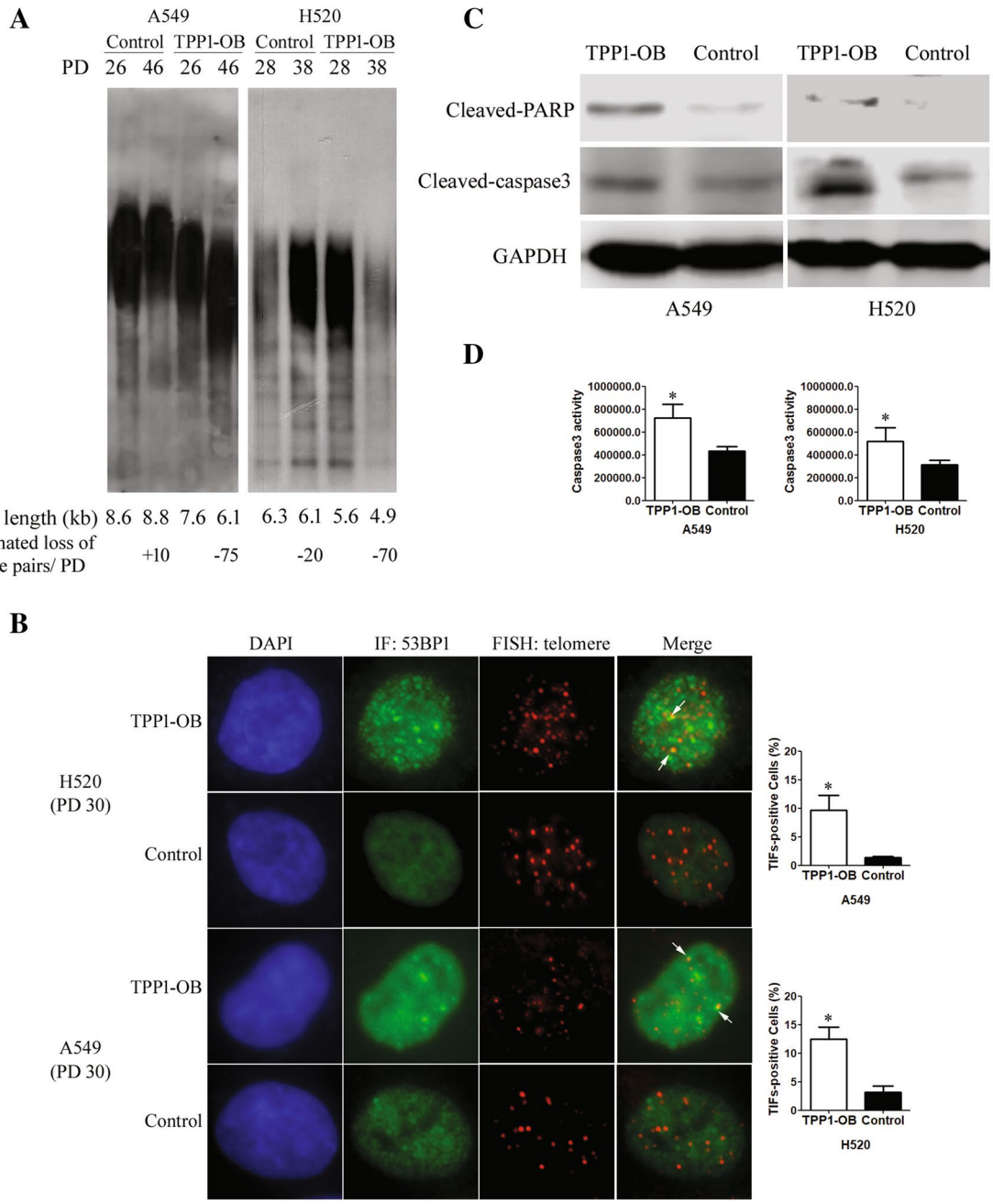



**Fig. 3** TPP1-OB shortens telomere length and induces cell apoptosis. **a** Telomere restriction fragment (TRF) Southern blot analysis from TPP1-OB-expressing A549 and H520 lung cancer cells and control cells at the indicated population doubling (PD) is shown, and mean TRF length was calculated (bottom panel). **b** TIF analysis indicates that there was a higher TIF incidence in TPP1-OB-expressing cells than in control cells at PD30. Representative TIF images are shown. Cells were stained with 53BP1 (green) and a telomere PNA-FISH probe (red). Nuclei were stained with DAPI (blue). Cells with four or more 53BP1 foci colocalizing with telomeres (yellow dots) were scored as TIF-positive. The percentage of TIF-positive cells is shown on the right column graph. Cells from ten random fields were assessed. The experiment was repeated twice. Data are presented as the mean ± SD. Scale bar = 20 μm. **c** TPP1-OB-expressing A549 and H520 cells and control cells from PD30 were lysed. Immunoblot analysis of cleaved caspase-3 and cleaved PARP was performed. GAPDH was used as a loading control. **d** The above-mentioned cells were measured by the Caspase-Glo® 3/7 Assay Kit for the caspase-3 activity assay. Data are presented as the mean ± SD of three independent experiments. A two-tailed Student's t test was used to calculate statistical significance. $*p < 0.05$

cell growth. Collectively, TPP1-OB suppressed proliferation in lung cancer cells.

### TPP1-OB results in telomere shortening and induces apoptosis in lung cancer cells

To determine whether the repression of cell proliferation correlated with the decrease in telomere length, we examined the telomere length of TPP1-OB stably overexpressing A549 and H520 cells by telomere restriction fragment (TRF) Southern blot analysis. With cell passage, the control cells showed an unchanged telomere length. In contrast, the telomere length gradually shortened in both A549 and H520 cells stably overexpressing TPP1-OB (Fig. 3a). The estimated loss of base pairs per population doubling (PD) was 75 bp in A549 cells and 70 bp in H520 cells. We then detected telomere damage by measuring telomere dysfunction-induced foci (TIF) formation. We observed a significant effect of TPP1-OB on TIF formation in the populations of cells with short telomeres (Fig. 3b). A real-time quantitative telomerase activity assay indicated that TPP1-OB did not affect telomerase activity in either cell type (Supplementary Figure 2a).

To clarify the outcomes of telomere shortening, cell senescence and cell apoptosis were further studied. The increased cell apoptosis in A549 and H520 cells stably overexpressing TPP1-OB was evidenced by the upregulation of cleaved caspase-3 and cleaved PARP protein (Fig. 3c). To confirm whether TPP1-OB-induced cell apoptosis involves the activation of caspase-3, we performed a caspase-3 activity assay. We observed a significant increase in caspase-3 activity in A549 and H520 cells stably expressing TPP1-OB (Fig. 3d). The SA-β-gal assay showed that TPP1-OB had no effect on cell senescence (Supplementary Figure 2b). These data demonstrated that TPP1-OB resulted in telomere shortening, which induced cell apoptosis but not cell senescence.

### TPP1-OB suppresses anchorage-independent growth and tumor formation

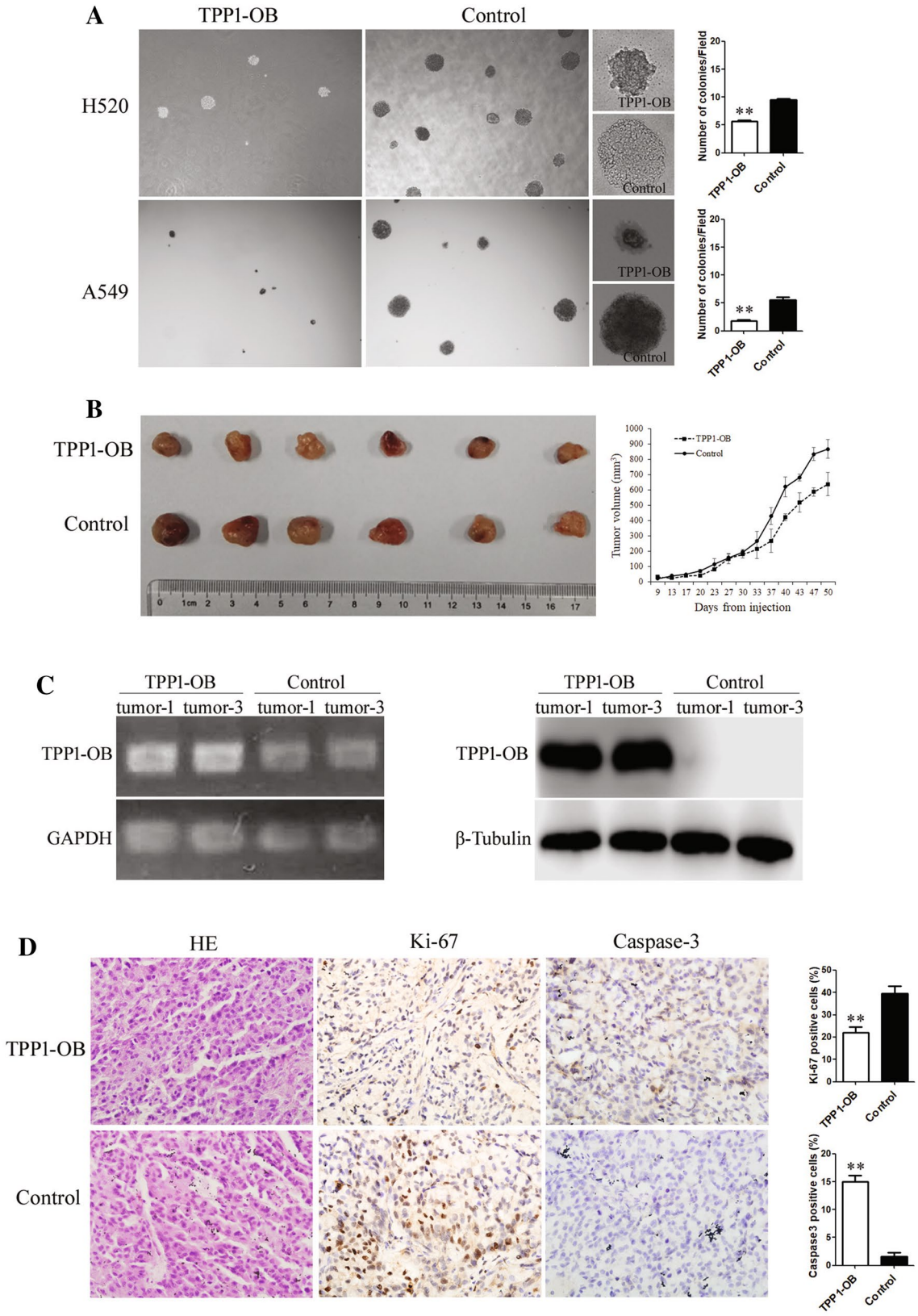
To test whether TPP1-OB could suppress tumor progression, we first performed an anchor-independent growth assay to determine the tumor formation ability of lung cancer cells. As shown in Fig. 4a, the results from the soft agar colony formation experiments indicated that the TPP1-OB-expressing A549 and H520 cells formed fewer and smaller colonies than the control cells. Next, we analyzed the effect of TPP1-OB on tumor growth using A549-xenograft mice. TPP1-OB-expressing A549 cells and the vector control cells were subcutaneously injected into nude mice. Tumor volumes were measured every 3–4 days. The tumor growth curves revealed that TPP1-OB inhibited A549 xenograft growth (Fig. 4b). TPP1-OB overexpression was confirmed by RT-PCR and western blotting of the dissected tumors (Fig. 4c). IHC analysis of the dissected tumors showed that the expression of the cell proliferation marker Ki-67 was lower and the expression of the cell apoptosis marker cleaved caspase-3 was higher in TPP1-OB-expressing A549 xenograft tissues than in the control tissues (Fig. 4d). Altogether, these findings demonstrate that TPP1-OB inhibited tumor growth by decreasing cell proliferation and increasing cell apoptosis.

### TPP1-OB increases the sensitivity of lung cancer cells to paclitaxel

We also investigated the effect of TPP1-OB on chemosensitivity in both A549 and H520 cells. The PD30 TPP1-OB stably expressed A549 and H520 cells were treated with paclitaxel, a common chemotherapeutic agent for non-small cell lung cancer (NSCLC). The viability of the TPP1-OB-expressing cells decreased compared to that of the control cells, and this decrease occurred in a paclitaxel dose-dependent manner (Fig. 5a). In addition, TPP1-OB promoted the degradation of caspase-3 and PARP induced by paclitaxel (Fig. 5b). The results indicated that TPP1-OB enhanced the sensitivity of both A549 and H520 cells to paclitaxel.

## Discussion

The recruitment of telomerase to the ends of telomeres for synthesizing telomere repeats is vital for cancer growth. TPP1, one of the shelterin proteins, has been recently identified as a primary factor in recruiting telomerase to the telomere ends (Zhong et al. 2012; Sexton et al. 2014; Xin et al. 2007; Wang et al. 2007). Blocking the TPP1–telomerase interaction by mutations in the TEL patch of TPP1 inhibits telomerase recruitment to telomeres and telomere synthesis, which suggests that targeting TPP1 could be a potential novel strategy for cancer therapy (Nandakumar et al. 2012; Nakashima et al. 2013). In this study, we used TPP1-OB domain proteins operating in a dominant-negative manner to inhibit TPP1–telomerase interaction. This approach interfered with only telomerase recruitment to telomeres without affecting telomerase activity or other TPP1 functions such as chromosome end protection. TPP1-OB suppressed cell growth in vitro and in vivo by inducing cell apoptosis in lung cancer cells. TPP1 (544 aa in length) has three separate functional domains, including the telomerase-binding domain (TPP1-OB, 87–250 aa), POT1-binding domain (250–334 aa), and TIN2-binding domain (334–544 aa) (Rajavel et al. 2016; Sexton et al. 2014; Grill et al. 2018). In this study, we found that the overexpression of TPP1-OB can inhibit


A
TPP1-OB
Control
H520
A549
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Number of colonies/Field
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B
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C
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tumor-1 tumor-3 tumor-1 tumor-3
TPP1-OB
GAPDH
β-Tubulin
D
HE
Ki-67
Caspase-3
TPP1-OB
Control
Ki-67 positive cells (%)
Caspase3 positive cells (%)

◂**Fig. 4** TPP1-OB suppresses tumor growth. **a** Cells in PD30 were plated. The soft agar colony formation assay showed that TPP1-OB-expressing A549 and H520 lung cancer cells decreased anchor-independent growth capability, as evidenced by the reduced number of colonies. **b** Tumor growth curves in nude mice indicate that TPP1-OB-expressing A549 lung cancer cells grew more slowly than the control cells. The PD15 TPP1-OB-expressing A549 cells and control cells were subcutaneously injected into nude mice. Tumor diameters were measured every 3–4 days, and tumor volume was calculated. Data represent the mean volume ± SD, $n = 6$ mice per group. ANOVA, $p < 0.05$. **c** Parts of resected tumors were used to extract RNA and protein for RT-PCR and western blotting assays to determine TPP1-OB expression. Tumor-1 indicates tumors from one mouse, and tumor-3 indicates tumors from another mouse. **d** Parts of resected tumors were formalin-fixed, paraffin-embedded, and sliced for IHC assay. Representative images of Ki-67 and cleaved caspase-3 staining are shown. Cells from ten random fields were assessed. Scale bar = 20 μm. $^{**}p < 0.01$

the interaction of TPP1 with telomerase by competitively binding to telomerase. Thus, telomerase could not localize to telomeres and synthesize telomeres, resulting in telomere shortening. As a consequence, cell growth was suppressed in vitro and in vivo in TPP1-OB-overexpressing lung cancer cells. The effect of TPP1-OB on cell proliferation is consistent with that of TEL-patch mutations of TPP1.

Telomere shortening can limit cell growth by inducing cellular senescence and apoptosis in cancer cells (Zhang et al. 1999; Riou et al. 2002; Deng et al. 2008; Bernadotte et al. 2016; Wang et al. 2016). In our study, we found that cellular apoptosis, rather than cellular senescence, was the major pathway in repressing cell proliferation in lung cancer cells stably expressing TPP1-OB. This finding is consistent with another study reporting that TEL-patch mutations induced cell apoptosis in HeLa cells (Nakashima et al. 2013), which was likely associated with telomere damage, as TIFs increased in the TEL-patch mutant HeLa cells. We observed elevated levels of TIFs in TPP1-OB stably transfected lung cancer cells upon telomere shortening. It is possible that inhibiting TPP1-dependent telomerase recruitment leads to cell apoptosis via telomere shortening-induced telomere damage.

Many studies have reported that some telomere- or telomerase-targeting drugs can increase the chemosensitivity of cancer cells to chemotherapeutic drugs (Djojosubroto et al. 2005; Biroccio et al. 2003; Ward and Autexier 2005; Saretzki 2003; Cerone et al. 2006; Lipinska et al. 2017). Here, we showed that the overexpression of TPP1-OB shortened telomere length, enhanced the sensitivity of A549 and H520 cells to paclitaxel, and increased apoptotic cell death. In agreement with the other studies, combining telomere or telomerase therapy with chemotherapy could enhance the antitumor effect.

In conclusion, we found that TPP1-OB inhibited telomerase access to telomeres and shortened telomere length in a dominant-negative mutant. As a result, tumor growth was suppressed in vitro and in vivo mainly through the mitochondrial apoptotic pathway and caspase-3 activation. TPP1-OB enhanced the chemosensitivity of lung cancer cells to paclitaxel by inducing apoptosis. Thus, blocking the recruitment of telomerase to telomeres by expressing TPP1-OB represents a new therapeutic strategy for lung cancer. As peptides are promising anticancer agents, especially with the advance of new drug-delivery systems (Wu et al. 2014; Torchilin 2014; Raucher and Ryu 2015; Dissanayake et al. 2017), TPP1-OB merits development as an anticancer peptide drug.

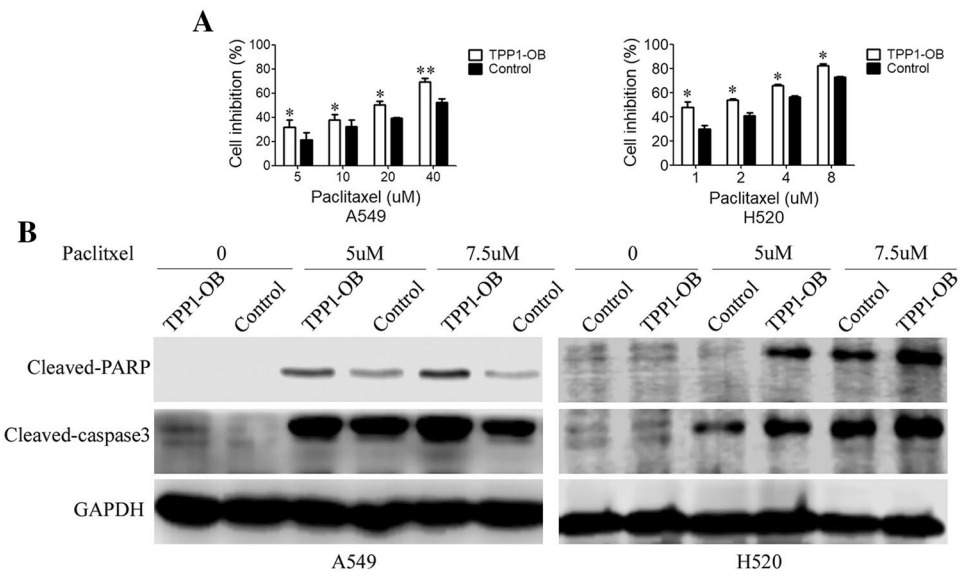


**Fig. 5** TPP1-OB enhances the chemosensitivity of A549 and H520 lung cancer cells to paclitaxel. **a** The MTT cell viability assay showed that TPP1-OB increased the chemosensitivity of A549 and H520 cells to paclitaxel. **b** Cells in PD30 were treated with the indicated concentration paclitaxel for 48 h. The levels of cleaved caspase-3 and cleaved PARP were detected by immunoblot analysis. $^{*}p < 0.05$, $^{**}p < 0.01$

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## Compliance with ethical standards

**Conflict of interest** The authors declare no conflicts of interest.

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