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## ABOUT COVER

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ORIGINAL ARTICLE

Basic Study

# Tankyrase 2 promotes lung cancer cell malignancy

Ying Wang, Yong-Jun Zhang

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## Abstract

***BACKGROUND***

Tankyrase 2 (TNKS2) is a potential candidate molecular target for the prognosis and treatment of non-small cell lung cancer (NSCLC), but its biological functions are unclear.

***AIM***

To investigate the biological functions of TNKS2 in NSCLC.

***METHODS***

Using a lentiviral vector, we generated H647 model cells with TNKS2 knockdown by RNA interference and A549 model cells with TNKS2 overexpression by transfection with a TNKS2 overexpressing plasmid. Increased and decreased expression levels of TNKS2 in the two cell lines were verified using real-time reverse transcriptase-polymerase chain reaction and Western blot analyses. Cell apoptosis, proliferation, and migration were determined using flow cytometry, carboxyfluorescein succinimidyl ester staining, and scratch assay, respectively. Immunofluorescence staining was conducted to examine TNKS2 and β-catenin expression levels in the two transfected cell lines and the non-transfected cells.

***RESULTS***

TNKS2 mRNA and protein expression was significantly higher in the highly malignant NCI-H647 cells, while it remained at a low level in the less malignant A549 cells. Lentivirus-mediated overexpression of TNKS2 in A549 cells resulted in a 3-fold increase in gene expression and a 1.7-fold increase in protein expression ( $P < 0.01$). Conversely, shRNA interference targeting *TNKS2* Led to an 8-fold decrease in gene expression and a 3-fold decrease in protein expression ($P < 0.01$) in NCI-H647 cells. Furthermore, the cell apoptosis rate was significantly reduced (50%) and cell migration rate was increased (35%) in the TNKS2 overexpression

group than in the control group ($P < 0.05$). In contrast, sh*TNKS2* promoted apoptosis by more than one fold and reduced migration by 60% ($P < 0.05$). Immunofluorescence analysis revealed enhanced nuclear localization of β-catenin fluorescence signal associated with high TNKS2 expression levels. Western blot analysis investigating TNKS2/β-catenin-related proteins indicated consistent changes between TNKS2 and β-catenin expression in lung cancer cells, whereas Axin displayed an opposite trend ($P < 0.05$).

*CONCLUSION*
The obtained results revealed that TNKS2 may serve as an adverse prognostic factor and a potential therapeutic target in NSCLC.

**Key Words:** Apoptosis; Migration; Lung Cancer; Proliferation; Tankyrase 2



**Core Tip:** This study provides a comprehensive overview of the role of tankyrase 2 (TNKS2) overexpression, which facilitates β-catenin activation and nuclear accumulation of β-catenin protein in non-small cell lung cancer (NSCLC) cells, in turn contributing to disease onset and progression. The findings obtained herein elucidate that TNKS2 is a putative molecular target candidate, which would serve as a prognostic indicator and a therapeutic agent for NSCLC patients.

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## INTRODUCTION

Lung cancer has the highest fatality rate among all cancers worldwide[1,2], and non-small cell lung cancer (NSCLC) is the most common histological subtype, accounting for approximately 85% of all lung cancer cases worldwide[3]. Despite considerable advancements in lung cancer diagnosis and therapy (such as surgery, chemotherapy, and radiation therapy), delays and difficulties in the early diagnosis of NSCLC contribute to its high mortality rates. At first diagnosis, more than 60% of NSCLC cases are at an advanced stage. Consequently, the 5-year survival rate for NSCLC remains less than 20%[4,5]. Non-small-cell lung cancer is a heterogeneous molecular disease. The molecular mechanisms underlying this type of cancer are complex and have not yet been fully elucidated. Currently, a widely accepted pathogenic mechanism of NSCLC is the alteration of gene expression, leading to dysregulation of signaling pathways and abnormal molecular biological behaviors. Therefore, further research on novel molecular markers of NSCLC is required.

Dysregulated β-catenin expression plays a causal role in the pathogenesis of NSCLC[6]. In the canonical activation of the Wnt/β-catenin signal, β-catenin is phosphorylated and degraded, after which it undergoes nuclear translocation and interacts with TCF4/LEF, promoting the transcription of downstream target genes[7]. The poly (ADP-ribose) polymerase (PARP) tankyrase (TNKS) plays a key role in the carcinogenic Wnt/β-catenin signaling pathway, which can not only promote the accumulation of cytoplasmic β-catenin but also participate in the process of driving the transcription of proto-oncogenes[8]. TNKS are a family of enzymes belonging to the PARP superfamily which is located on chromosome 8 and contains 1327 amino acids. TNKS' primary structure includes four domains: The C-terminal catalytic PARP domain that mediates the poly(ADP-ribose) addition to its substrates, a sterile alpha module responsible for the homo- and hetero-oligomer formation, the ankyrin domain divided into five clusters (ARC 1-5) which serve as the substrate binding site, and the His, Pro, and Ser rich domain with unknown function at the N-terminus[9]. TNKS performs various functions in different physiological processes: Mitosis, telomere maintenance, proteasome regulation, GLUT4 vesicles and translocation, viral replication, and Wnt/b-catenin signaling[10].

Expression of the *TNKS2* gene, which is located on 10q23.32, enhances β-catenin activation *via* induction of axin degradation[8]. The Wnt/β-catenin pathway is a promising target for NSCLC[11]. TNKS2 is widely distributed and is involved in the regulation of various physiological and pathological processes, including cell growth[12], signal transduction[13], proliferation and apoptosis[14]. It is also closely associated with the occurrence and development of various diseases, including cancer. However, the expression pattern of TNKS2 in NSCLC cells has not been elucidated. Therefore, in the present study, we explored the underlying mechanism of TNKS2 expression in regulating the biological behavior of NSCLC cells.

## MATERIALS AND METHODS

### Cell culture

The NSCLC cell lines were purchased from the Cell Bank of the Chinese Academy of Sciences. TNKS2 expression was

high in the H647 cells and low in the A549 cells, as confirmed by real-time reverse transcriptase-polymerase chain reaction (RT-qPCR) and western blot analyses. Briefly, H647 cells were cultured in an RPMI-1640 medium (Corning, Shanghai, China) supplemented with 10% fetal bovine serum (FBS; Gibco, NY, United States). A549 cells were cultured in DMEM (Corning) containing 10% FBS. All the cells were grown in an incubator with saturated humidity at 37 °C and 5% $CO_2$.

### Transfection

A549 and H647 cells were seeded in 12-well plates (Fisher Scientific, United States) at a density of approximately 40%. On day 2 of the culture, H647 cells were infected with the corresponding shRNA (shRNA sequences designed by Invitrogen Life Technologies). The shRNA sequences (Table 1) were designed to target different coding regions of the human *TNKS2* mRNA sequence [GenBank Accession No. NM_025235.3], and A549 cells were infected with a lentivirus for TNKS2 overexpression. After 48 h of infection, the cells were selected with 1 μg/mL of puromycin for 48 h. Transfection efficiency was assessed by quantitative polymerase chain reaction (qPCR) and Western blotting.

### RT-qPCR

RNA was extracted from the cells using an RNAiso Plus kit (TaKaRa, 9108). Eppendorf tubes and tips were soaked in 0.1% diethylpyrocarbonate, and the total RNA was digested using DNase I (TaKaRa, 2270A). After determining RNA purity and concentration, it was reverse-transcribed to cDNA using the RevertAid First-Strand cDNA Synthesis Kit (Thermo, K1622). The reverse transcription system included 5 μL total RNA, 4 μL 5 × TransScript All-in-One SuperMix for qPCR, 1 μL gDNA Remover, and 10 μL Rnase-free double-distilled water (dd$H_2O$). qPCR was performed using the Sso Advance Universal SYBR Green Supermix (Bio-Rad, cat 172-5274). The qPCR reaction system is listed as follows: SybrGreen qPCR Master Mix (2 ×) 10 μL, forward primer (10 uM) 0.4 μL, reverse primer (10 uM) 0.4 μL, ddH20 7.2 μL, and template (cDNA) 2 μL. The primer sequences are shown in Table 1. The PCR conditions were set as follows: 3-min predenaturation at 95 °C, followed by 45 cycles, each consisting of 7-s denaturation at 95 °C, 10-s annealing at 57 °C, and 15-s extension at 72 °C. The relative expression levels were calculated by the $2^{-\Delta\Delta Ct}$ method, using homo *GAPDH* as the internal reference.

### Western blot analysis

Cells were lysed in a RIPA buffer (CWBIO, CW2334S) containing protease and phosphatase inhibitors (CWBIO, CW2383S). Protein samples were quantified using a bicinchoninic acid assay protein assay kit (Sigma-Aldrich). Briefly, the protein samples (10 μg) were subjected to 10% SDS-PAGE and transferred onto polyvinylidene fluoride membranes (Bio-Rad). The membranes were blocked with 5% non-fat milk for 1 h at 37 °C. The membranes were then incubated with primary antibodies against TNKS2 (1:1000 dilution; Santa Cruz Biotechnology, sc-365897) or the loading control GAPDH (1:2000; Abcam, ab8245) at 4 °C overnight, before incubation with a secondary goat anti-mouse IgG (1:1000; Biosharp, BL001A). Immunoreactivity was detected using an ECL kit and analyzed using ImageJ software (NIH, United States).

### Flow cytometry

An annexin V-APC/7-ADD apoptosis kit (Lianke, 70-AP105-100) was used to detect apoptosis. After the cells in the logarithmic growth phase were trypsinized, they were centrifuged at 500 × *g* for 5 min. The cells were seeded in wells of a six-well plate (8 × $10^4$ cells/well) and incubated overnight. On day 2, they were centrifuged at 500 × *g* for 5-10 min at room temperature (22-25 °C) and collected for flow cytometry. After washing twice with 1 × phosphate-buffered saline (PBS; precooled at 4 °C), the cells were centrifuged at 500 × *g* for 5-10 min. They were then resuspended in 1 × binding buffer (diluted with dd$H_2O$), and the cell density was adjusted to 1 × $10^6$-1 × $10^7$ cells/mL. Thereafter, 100 μL of the cell suspension was transferred to a flow tube, so that each tube contained approximately 1 × $10^5$ to 1 × $10^6$ cells. Following this, 5 μL Annexin V-APC and 10 μL 7-ADD were added to each tube, and the mixture was incubated at room temperature for 15 min in darkness. Subsequently, 300 μL of 1 × binding buffer was added to the cells, and they were transferred to a flow tube and tested on a NovoCyte Flow Cytometer (Agilent, United States).

### Carboxyfluorescein succinimidyl ester detection of cell proliferation

The cells were resuspended in PBS containing 5% fetal calf serum (FCS) before seeding into the wells of a six-well plate (Thermo, United States) at a density of 1 × $10^7$/well. A carboxyfluorescein diacetate succinimidyl ester (CFSE) working solution was prepared by adding 1 μL of 5 mmol/L CFSE solution to 1 mL of PBS containing 10% FCS. Thereafter, 1 mL of the CFSE working solution was added to a tube containing 1 mL of the cell suspension, and the mixture was incubated at 37 °C for 5-10 min. After washing thrice with a complete medium and centrifugation, ice-cold RPMI-1640 medium containing 10% FBS was added to the cells, which were then centrifuged at 500 × *g* for 5 min. Cell proliferation was determined by flow cytometry.

### Wound healing assay

Logarithmic-phase cells were centrifuged at 500 × *g* for 5 mi and seeded into the wells of a six-well plate (5 × $10^5$/well). After the cells were cultured to 80%-90% confluence, a 10 μL pipette tip was used to make a scratch through the cell layer. After washing with PBS to remove any non-adherent cells, the cells were cultured in a medium containing 5% serum for 24 h. At 0 and 24 h of culture, photographs of the cells (100 ×) were obtained using the Leica Application Suite software, and the scratch distance (width) was recorded. The relative cell migration rate was calculated as follows: Experimental group (scratch distance at 0 h-scratch distance at 24 h)/control group (scratch distance at 0 h-scratch distance at 24 h).

**Table 1 Sequences of shRNA and primers for real-time reverse transcriptase-polymerase chain reaction**

| Sequences of shRNA | | RT-qPCR primers | |
|---|---|---|---|
| shNC | 5′-GCTCAATCCCGACAGTAGAGT-3′ | *TNKS2* | *GAPDH* |
| sh*TNKS2* #1 | 5′- GCGGAAAGACGTAGTTGAATA-3′ | Forward 5′-GCTGAGCCAACCATCCGAAAT-3′ | Forward 5′-GGAGCGAGATCCCTCCAAAAT-3′ |
| sh*TNKS2* #2 | 5′- GACCCCAATGCTCG AGATAAT-3′ | Reverse 5′-ACTTGCGTGGCAGTTGACA-3′ | Reverse 5′-GGCTGTTGTCATACTTCTCATGG-3′ |

RT-qPCR: Real-time reverse transcriptase-polymerase chain reaction.

### *Immunofluorescence staining*

For immunofluorescence analysis, cells were centrifuged at 500 × *g* for 5 min and spread on glass coverslips at a density of approximately 40%. The coverslips were placed in the wells of a 24-well plate. The cells were subjected to immunofluorescence analysis the following day. Briefly, they were fixed with 4% paraformaldehyde for 10 min at room temperature, incubated with 0.2% Triton X-100 solution for 10 min at room temperature, and then blocked with 5% bovine serum albumin solution at 37 °C for 1 h. Thereafter, the cells were incubated with primary antibodies against β-catenin (1:250 dilution; Abcam, ab2365) and TNKS2 (1:100 dilution; Abcam, ab155545) in a humidified box at 4 °C. The following day, the wet box was incubated at room temperature for 30 min and the cells were washed thrice with 1 × PBS. They were then incubated with 100 μL goat anti-rabbit IgG H&L (APC) (Abcam, pre-adsorbed secondary antibody) at 37 °C for 1 h in darkness. The nuclei were counterstained with 4′,6-diamidino-2-phenylindole. The cells were mounted using a mounting tablet containing an anti-quenching agent. Staining results were evaluated using an Eclipse Ti-S fluorescence microscope (Nikon, Japan).

### *Statistical analysis*

All assays were independently repeated at least thrice. The representative results are presented. Data are expressed as the mean ± SD. Comparisons between different groups were performed using Student's *t*-tests or one-way analysis of variance. Statistical significance was set at $P < 0.05$. All the statistical analyses were performed using GraphPad Prism software (version 8.0; GraphPad, Inc., San Diego, CA, United States).

## RESULTS

### *Construction of an H647 cell line stably transfected with TNKS2 shRNA and A549 cell line transfected with a lentiviral vector for TNKS2 overexpression*

To study the role of TNKS2 in NSCLC, we first selected H647 cells, which express a high level of TNKS2, to construct a stably transfected TNKS2 interference cell line, and A549 cells, which have a low TNKS2 expression level, to be transfected with a TNKS2-overexpression lentivirus (Figure 1A-C). To avoid the off-target effects of shRNA, two shRNAs were synthesized. RT-qPCR and Western blot analyses revealed that TNKS2 mRNA and protein levels were significantly downregulated after transfection with sh*TNKS2*. Of the two shRNAs examined, a better interference effect was observed for sh*TNKS2*#2. Therefore, sh*TNKS2*#2 was used for H647 cells and shNC was used as a control in the assay. A549 cells were transfected with a TNKS2-overexpression lentiviral vector or empty control vector. The transfected cells were selected using puromycin. RNA and proteins were extracted from the collected cells for subsequent use in RT-qPCR and Western blot analyses, respectively. The results of RT-qPCR and Western blot analyses revealed the successful construction of a stable TNKS2-overexpressing A549 cell line and a TNKS2 interference H647 cell line, as shown by TNKS2 overexpression at the mRNA and protein levels (Figure 1D and E).

### *Varying expression of TNKS2 does not affect NSCLC cell proliferation*

The proliferative capacity of NSCLC cells transfected with sh*TNKS2* and TNKS2-overexpression lentiviral vectors was assessed. Compared to the rate of proliferation seen in control cells, the proliferative ability of H647 cells was the same as that of cells with silenced TNKS2, whereas the proliferative ability of A549 cells with TNKS2 overexpression was the same as that of A549 cells (Figure 2A).

### *TNKS2 overexpression inhibits apoptosis of NSCLC cells*

Using flow cytometry analysis, we investigated the effects of TNKS2 knockdown or overexpression on apoptosis in NSCLC cells. Our results revealed that TNKS2 knockdown led to a significant increase in the apoptosis rate of H647 cells compared to that in the control group. By contrast, TNKS2 overexpression distinctly inhibited the apoptosis of A549 cells compared to that in the control group (Figure 2B). These results indicated that TNKS2 expression is negatively correlated with apoptosis in NSCLC cells.

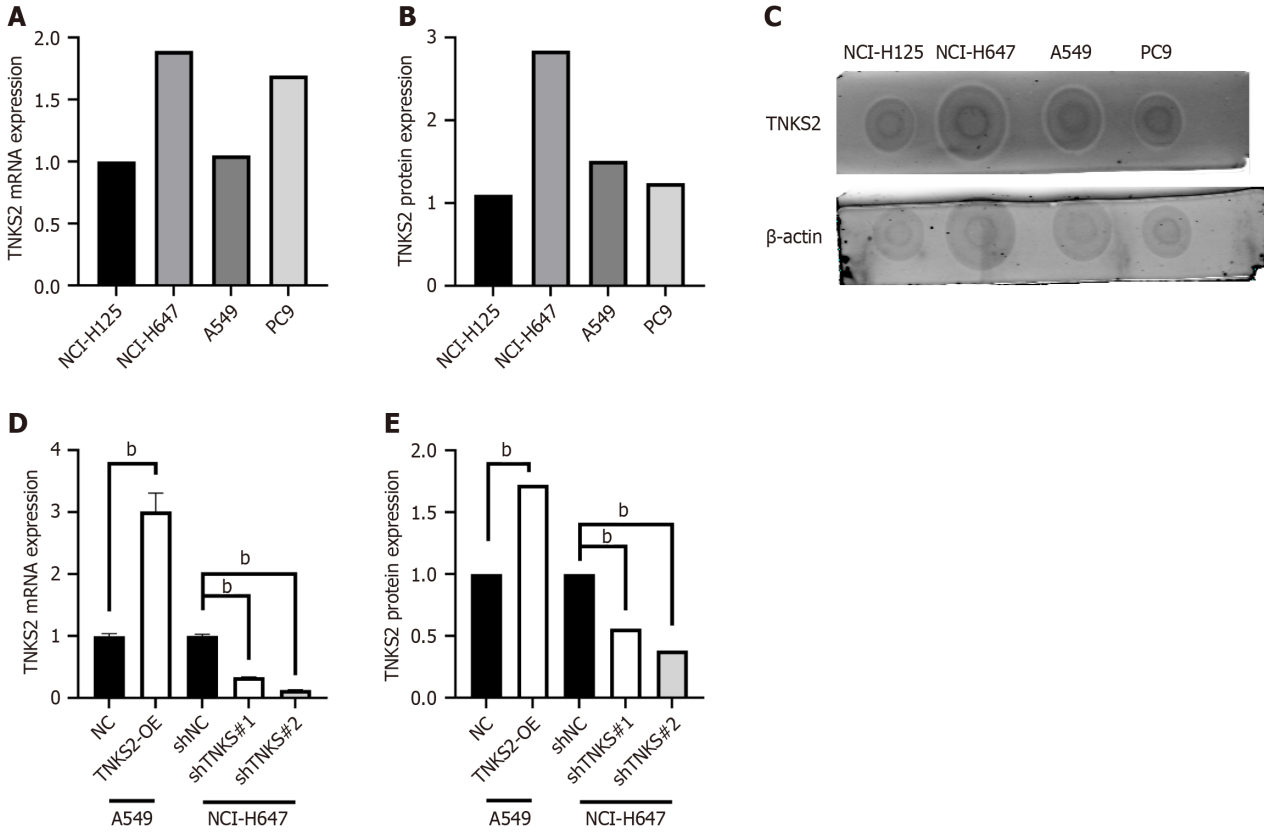



**Figure 1 Successful construction of cancer cell lines with TNKS2 knockdown or overexpression.** A: Real-time reverse transcriptase-polymerase chain reaction (RT-qPCR) analysis of tankyrase 2 (*TNKS2*) mRNA expression in all four lung cancer cell lines; B and C: Dot-blot analysis of TNKS2 protein expression in all four lung cancer cell lines; D: Construction of stably transfected non-small cell lung cancer cell lines with TNKS2 interference and overexpression. Efficiency of TNKS2 interference or overexpression was assessed in H647 and A549 cells utilizing RT-qPCR; E: Efficiency of TNKS2 interference or overexpression was assessed in H647 and A549 cells *via* Western blot analysis. $^{a}P < 0.05$; $^{b}P < 0.01$.

### *TNKS2 increases migration ability of NSCLC cells*

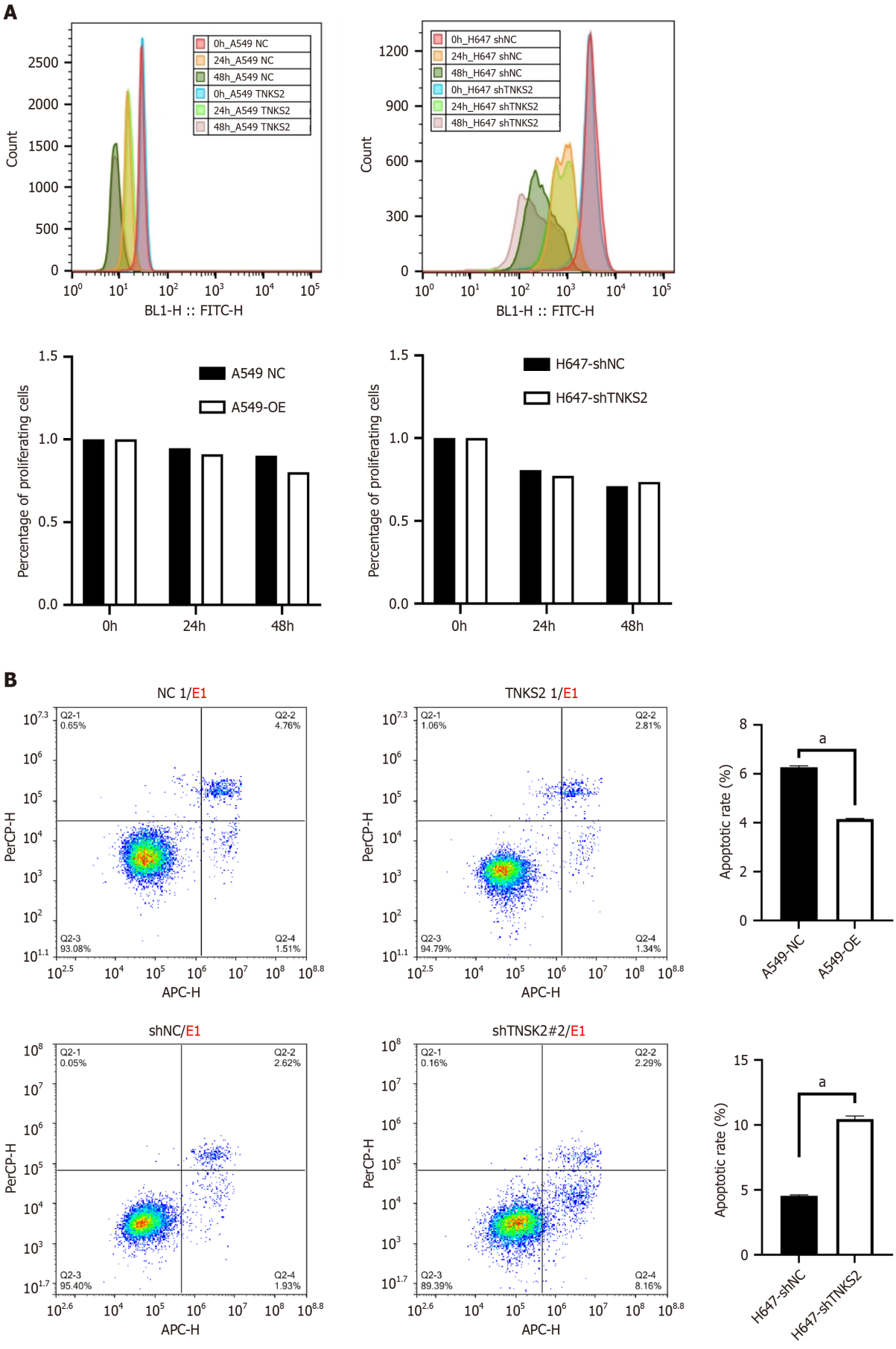
The scratch test was used to determine the influence of TNKS2 silencing or overexpression on the migratory ability of NSCLC cells. Our results showed that TNKS2 knockdown significantly reduced the scratch-healing ability of H647 cells, indicating that cell migration was weakened by TNKS2 knockdown. In contrast, TNKS2 overexpression significantly enhanced the scratch-healing ability of A549 cells, indicating that the migratory ability of A549 cells was enhanced by TNKS2 overexpression (Figure 2C).

### *TNKS2 overexpression enhances β-catenin signaling activation in NSCLC cells*

We used immunofluorescence staining to evaluate the expression of TNKS2 in NSCLC cells transfected with sh*TNKS2* or TNKS2-overexpression vectors. TNKS2 expression notably decreased in sh*TNKS2*-transfected H647 cells. β-catenin protein expression was decreased in both the nucleus and cytoplasm (Figure 3A). Furthermore, immunofluorescence staining was conducted to detect the effects of TNKS2 overexpression on the expression and location of β-catenin. Conversely, its expression was upregulated in A549 cells transfected with a TNKS2-overexpression vector. Upon TNKS2 overexpression, β-catenin protein expression significantly increased in both the nucleus and cytoplasm (Figure 3B). The proteins were then extracted, and the expression of TNKS2/β-catenin-related proteins (axin and β-catenin) was analyzed by Western blotting. β-catenin expression was upregulated with the elevated expression of TNKS2, whereas axin expression was downregulated (Figure 4). These results indicated that TNKS2 overexpression promotes β-catenin activation.

## DISCUSSION

Research to discover prognostic markers for NSCLC has attracted increasing attention; however, continued studies are required to identify accurate, simple, and rapid markers for NSCLC[15-17]. Optimal prognostic markers for lung cancer may be determined by conducting studies with rigorous design and large sample sizes. WNT/β-catenin signaling pathway activation has emerged as a potential therapeutic target in NSCLC[18], and there are several candidate therapeutic target proteins in the WNT/β-catenin signaling pathway[19-21]. One study conducted a focused analysis of 500 microarray probes in the Wnt receptor signaling pathway. TNKS1 and *TNKS2* expression levels were 1.30- and 1.43-fold higher in the NSCLC cells than in adjacent normal lung cells in a murine model[22]. In the present study, we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Percentage of proliferating cells
A549 NC
A549-OE
H647-shNC
H647-shTNKS2
0h
24h
48h
B
NC 1/E1
TNKS2 1/E1
shNC/E1
shTNSK2#2/E1
PerCP-H
APC-H
Q2-1 0.65%
Q2-2 4.76%
Q2-3 93.08%
Q2-4 1.51%
Q2-1 1.06%
Q2-2 2.81%
Q2-3 94.79%
Q2-4 1.34%
Q2-1 0.05%
Q2-2 2.62%
Q2-3 95.40%
Q2-4 1.93%
Q2-1 0.16%
Q2-2 2.29%
Q2-3 89.39%
Q2-4 8.16%
Apoptotic rate (%)
a
A549-NC
A549-OE
H647-shNC
H647-shTNKS2

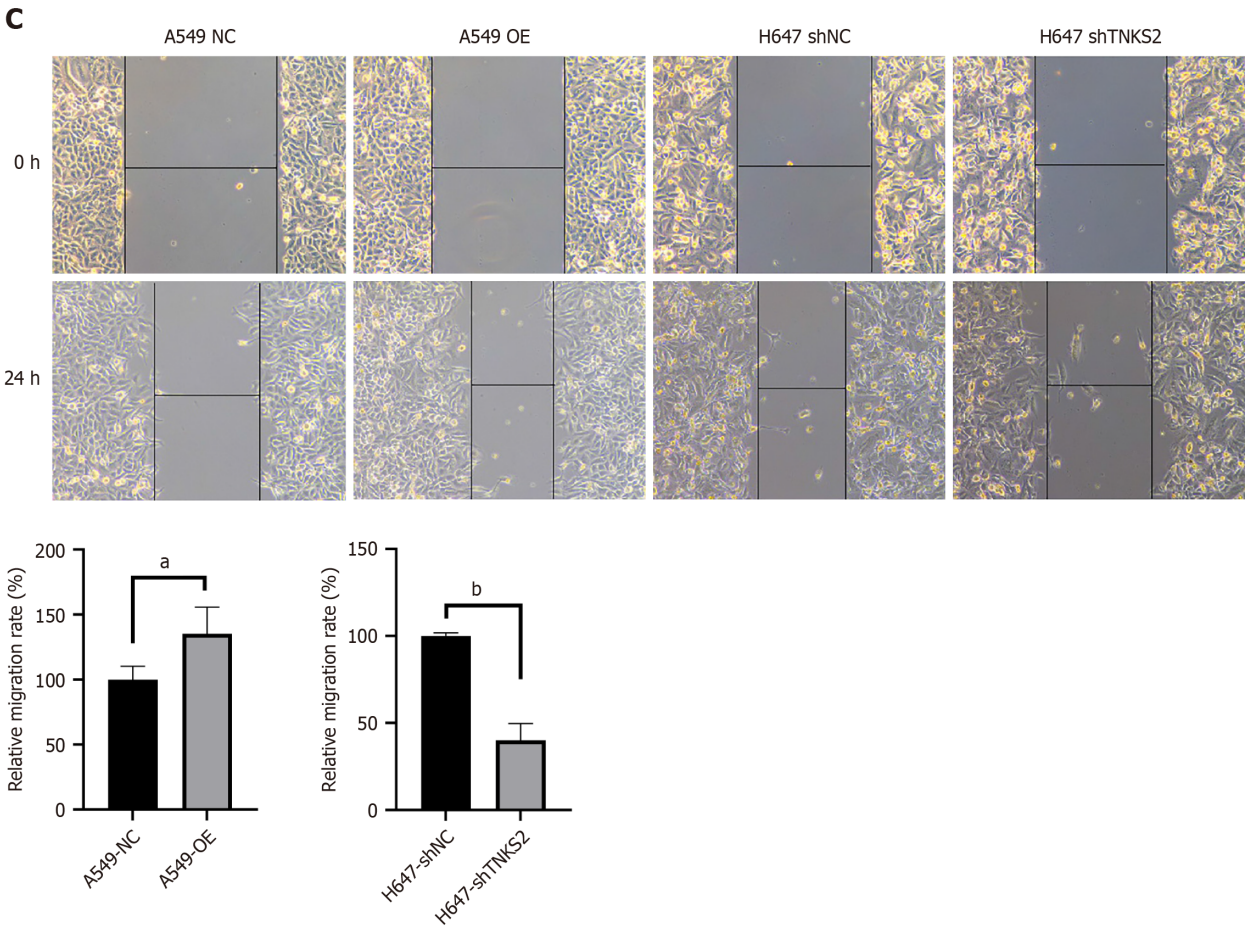


**Figure 2 Effects of tankyrase 2 knockdown or overexpression on proliferation of non-small cell lung cancer cells.** A: Proliferative ability of H647 cells transfected with sh*TNKS2* and A549 cells with tankyrase 2 (TNKS2) overexpression; B: Apoptosis of non-small cell lung cancer cells assessed by flow cytometry. Apoptotic rate of sh*TNKS2*-transfected H647 cells and TNKS2-overexpressing A549 cells was calculated; C and D: Migration ability of H647 cells transfected with sh*TNKS2* and A549 cells transfected with a TNKS2 overexpression vector at 24 h after transfection (magnification, × 100). $^{a}P < 0.05$; $^{b}P < 0.01$.

provided preliminary evidence to support the hypothesis that TNKS2 overexpression promotes the malignant behavior of lung cancer cells. These results suggest that TNKS2 is a feasible and clinically relevant target for the diagnosis and treatment of NSCLC.

By constructing shRNA and overexpression vectors that alter the expression of TNKS2, we established NSCLC cell lines with stable knockdown or overexpression of TNKS2 and studied the correlation between TNKS2 expression and the biological behavior of NSCLC cells. Low TNKS2 expression led to increased apoptosis in NSCLC cells, while high expression had the opposite effect. Apoptosis is the orderly and autonomous death of cells that is controlled by gene expression to maintain a stable internal environment[23-25]. Unlike cell necrosis, apoptosis is not a passive process but an active one that involves the activation, expression, and regulation of a series of genes. It is not a phenomenon of autologous injury under pathological conditions but a process in which death is induced to actively achieve better adaptation to the living environment. From the perspective of cell apoptosis, tumors occur because of the inhibition of cell apoptotic mechanisms and the inability to clear abnormal cells through apoptosis[26]. Our findings revealed that TNKS2 inhibits apoptosis and induces the proliferation of NSCLC cells.

Malignant tumors easily metastasize through various channels, such as lymphatic vessels and blood circulation[27-29]. Migration and invasion are key processes in tumor metastasis[30-32]. In the present study, we performed a wound-healing assay to determine the effect of TNKS2 on tumor cell migration. Our results revealed that high TNKS2 expression enhanced the migration of NSCLC cells, whereas low TNKS2 expression had the opposite effect. Previous studies have consistently shown that TNKS2 accelerates the growth and invasiveness of triple-negative breast[30] and cervical cancer cells[33]. TNKS2 can dissociate the destruction complex containing axins, thereby enhancing the stability of β-catenin[34]. Activation of β-catenin contributes to the malignant phenotype of NSCLC. Herein, the knockdown and overexpression of TNKS2 inhibited and promoted, respectively, the expression of nuclear β-catenin protein in NSCLC cells, consequently confirming the antitumor potential of inhibiting these molecules in the WNT/β-catenin pathway.

The present study has certain limitations. It only included *in vitro* experiments. As the lungs are the major respiratory organs of the human body, the growth environment and oxygen concentration of lung cancer cells cultured *in vitro* are different from those of lung tissues *in vivo*. Therefore, our results do not reflect the specific effects of TNKS2 on cells *in vivo*. Additionally, future studies need to include a more detailed and in-depth analysis to explore the mechanism of TNKS2/β-catenin or other pathways.

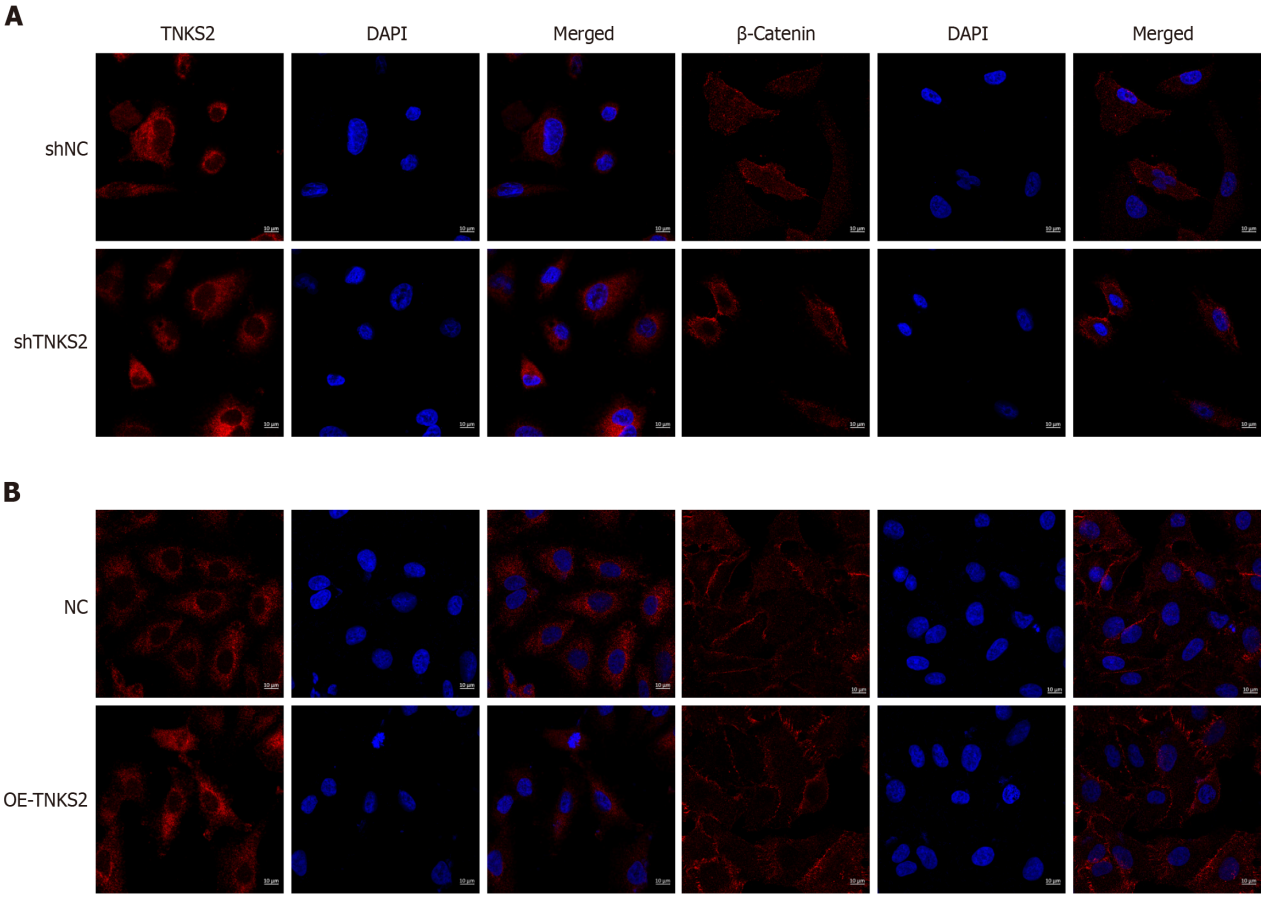


**Figure 3 Immunofluorescence images obtained using confocal laser scanning microscopy.** A: Tankyrase 2 (TNKS2) and β-catenin expression in sh*TNKS2*-transfected H647 cells; B: TNKS2-overexpressing A549 cells (magnification, × 200). Nuclei were stained blue; the target protein was stained red.

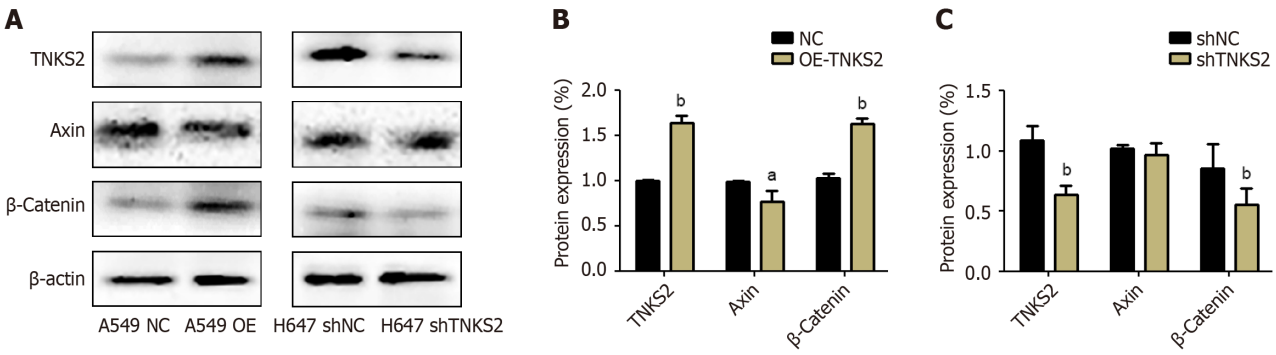


**Figure 4 Expression of TNKS2/β-catenin-related proteins assessed by Western blot assay.** A: Expression levels of tankyrase 2 (TNKS2), axin, and β-catenin proteins; B: Protein expression of TNKS2-overexpressing A549 cells compared with NC-overexpressing A549 cells was analyzed by gray value; C: Protein expression of sh*TNKS2*-transfected H647 cells compared with shNC H647 cells was analyzed by gray value. $^{a}P < 0.05$; $^{b}P < 0.01$.

The results of the present study provide insights into the biological functions of TNKS2 in NSCLC cells. Furthermore, the obtained findings indicate that TNKS2 may affect the proliferation, apoptosis, and migration of lung cancer cells through the TNKS2/β-catenin pathway. These findings may be useful in the development of therapies targeting TNKS2 for the treatment of NSCLC.

## CONCLUSION

Taken together, our results demonstrate that TNKS2 is a promising prognostic factor and therapeutic target for NSCLC. Furthermore, TNKS2 overexpression is associated with the malignant behavior of NSCLC cells (as shown in Figure 5); however, the underlying molecular mechanisms require further investigation.

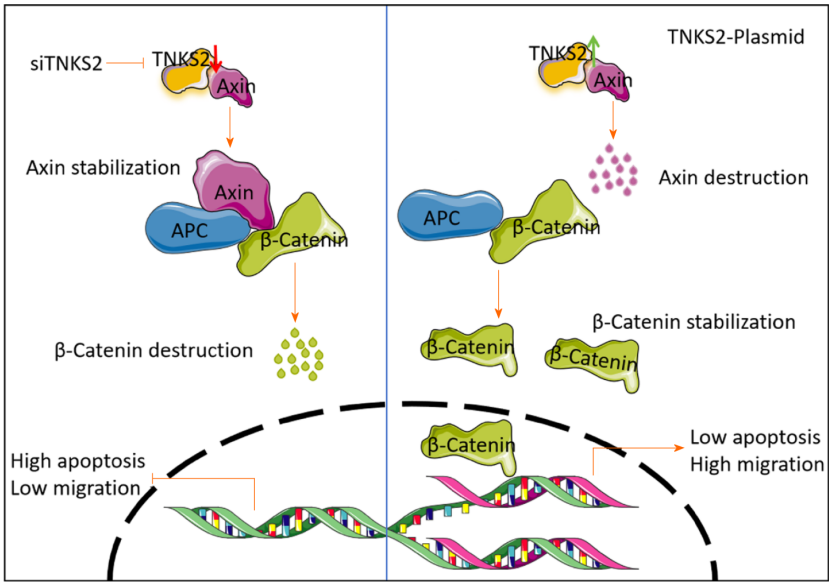


**Figure 5 Tankyrase 2 overexpression promotes the malignant behavior of lung cancer cells.** Tankyrase 2 dissociates the destruction complex containing axins, thereby enhancing the stability of β-catenin. Activated β-catenin translocates into the nucleus, where it activates target genes, which contribute to the malignant behavior of lung cancer cells.

## FOOTNOTES

**Author contributions:** Wang Y and Zhang YJ designed the research study; Zhang YJ performed the research, analyzed the data, and wrote the manuscript; Wang Y revised the manuscript. All the authors have read and approved the final manuscript.

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