TRIM52 regulates the proliferation and invasiveness of lung cancer cells via the Wnt/β-catenin pathway

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Abstract. As a major cause of cancer-associated mortalities, lung cancer is frequently diagnosed in males and females with an incidence ratio of 2.1:1. Tripartite motif 52 (TRIM52), an E3 ubiquitin ligase, has been reported to be involved in various biological functions, including cell proliferation and invasiveness. In the present study, an elevated TRIM52 level was observed in tumor tissues of patients with lung cancer and in lung cancer cell lines. The downregulation of TRIM52 in lung cancer cells significantly suppressed the proliferation of lung cancer cells, arrested the cell cycle at the G1 phase and was accompanied by a decrease in the levels of β-catenin, proliferating cell nuclear antigen, c-Myc and Cyclin D1 proteins. Additionally, TRIM52-induced cell proliferation and invasiveness, as well as the levels of cell cycle-associated proteins, were completely counteracted by the Wnt/β-catenin inhibitor XAV939. Based on these data, it was speculated that TRIM52 is critical for lung cancer progression and that downregulation of TRIM52 could inhibit cell proliferation by blocking cell cycle progression. It was also speculated that TRIM52 upregulation promotes proliferation and invasiveness through activation of the Wnt/β-catenin pathway. Thus, TRIM52 has the potential to be a therapeutic target for lung cancer.

Introduction

As the leading cause of cancer-associated mortalities globally, lung cancer is frequently diagnosed in males and females with an incidence ratio of ~2.1:1 in 2008 (1,2). In 2008, ~1.4 million people globally succumbed to lung cancer, which represented 18% of all cancer-associated mortalities (3). Generally, lung cancer is classified into two main types: Small cell lung cancer (SCLC); or non-SCLC (NSCLC) (4,5). Accumulating evidence has revealed that tobacco smoking is a major cause of lung cancer, as it is associated with ~90% of all lung cancer diagnoses (6-9). Furthermore, smokers have a 10-fold increased probability of developing lung cancer, compared with nonsmokers (10).

The Wnt/β-catenin pathway has been indicated to serve important roles in a number of cancer types. For example, it has been reported that the metastatic behavior of lung cancer cell lines is increased by increased Wnt/β-catenin signaling *in vitro* (11). A previous study demonstrated that Wnt family genes are frequently upregulated in multiple human cancer types, including NSCLC (12,13). Through β-catenin, oncogenic Wnt signaling is transduced. Wnt signaling promotes the accumulation of β-catenin, and elevated β-catenin translocates to the nucleus where it forms complexes with transcription factors (14). This in turn stimulates the expression of Wnt target molecules, including the oncogenes Cyclin D1 and c-Myc (15,16).

Tripartite motif-containing (TRIM) family proteins, with >80 members, contain three conserved domains, RING, B-box and a coiled-coil region, and are regarded as E3 ubiquitin ligases that are associated with human diseases, including intracellular immunity and cancer (17-20). It has been reported that TRIM proteins regulate multiple biological processes, including cell proliferation and invasion (21-23). Studies demonstrated an association between TRIM24 and TRIM29, and the progression of solid tumors (24,25). Elevated TRIM65 has also been observed in lung cancer, where it facilitates the growth of tumors (26,27); whereas, TRIM31 was reported to be downregulated in NSCLC, which indicates that it may function as a tumor suppressor (28). TRIM52 is a novel TRIM protein that contains only a unique expanded RING domain and a B-box2 domain (29). Previous studies demonstrated that TRIM52 could promote cell proliferation, migration and invasion in hepatocellular carcinoma through ubiquitination (30,31). Another study indicated that TRIM52 acts as an oncogene in ovarian cancer, where it is associated with the nuclear factor-κB pathway (32). However, the effect of TRIM52 in lung cancer remains largely unknown.

In the present study, a high expression of TRIM52 was observed in tumor tissues of patients with lung cancer and in lung cancer cell lines. The downregulation of TRIM52

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in lung cancer cell lines significantly inhibited cell proliferation by blocking cell cycle progression, which occurred concurrently with decreases in β-catenin, proliferating cell nuclear antigen (PCNA), c-Myc and Cyclin D1 expression. Furthermore, TRIM52-induced cell proliferation and invasion were completely counteracted by the Wnt/β-catenin inhibitor XAV939. These results indicated that TRIM52 downregulation inhibits lung cancer progression, possibly through inactivation of the Wnt/β-catenin signaling pathway.

Materials and methods

Tumor and adjacent normal tissues of patients with lung cancer. Following informed consent being obtained, 43 pairs of tumor and paracancer tissues from 43 patients with lung cancer treated at Longhua Hospital (Shanghai, China) were collected and immediately frozen in liquid nitrogen at -196℃. After the tissues were sectioned at 5 μm, the expression of TRIM52 was detected by immunohistochemistry, according to the subsequent protocol. All experiments in the present study were approved by the Ethics Committee of Shanghai University of Traditional Chinese Medicine (Shanghai, China).

Cell culture. A total of 5 cell lines derived from human lung cancer (H1975, H466, A549, H358 and H1299), and a cell line derived from the pulmonary epithelium (16HBE) were purchased from the Cell Bank of the Chinese Academy of Science (Shanghai, China). These cells were cultured in a 5% CO_2 humidified-incubator at 37℃ (Thermo Forma 3111; Thermo Fisher Scientific, Inc., Waltham, MA, USA) with RPMI-1640 medium (cat. no. SH30809.01B; HyClone; GE Healthcare Life Sciences; Logan, UT, USA) supplemented with 10% fetal bovine serum (cat. no. 16000-044; Gibco; Thermo Fisher Scientific, Inc.) and 1% antibiotic (x100; a mixture of penicillin and streptomycin; cat. no. P1400-100; Beijing Solarbio Science & Technology Co., Ltd., Shanghai, China). During incubation, the medium was replaced with fresh RPMI-1640 medium every two days according to cellular demand until the experiments. After selecting, three cell lines, H358, H1299 and H1975, were used for subsequent experiments.

Lentiviral construction. Short hairpin RNA (shRNA) sequences targeted to the TRIM52 gene (NM_032765.3) were synthesized and double strand-annealed to form the shRNA construct. The shRNA construct was inserted into Agel I/Ecol I restriction sites of a pLKO.1-puro vector (Addgene, Inc., Cambridge, MA, USA). Subsequently, the 894 bp full-length coding DNA sequence region of TRIM52 containing the EcoR I/BamH I restriction sites was synthesized by Genewiz, Inc. (Shanghai, China) and was then inserted into *Eco*RI/*Bam*HI restriction sites of a pLVX-Puro vector (Clontech Laboratories, Inc., Mountainview, CA, USA). pLKO.1-shTRIM52 and pLVX-Puro-TRIM52 were confirmed by DNA sequencing (Shanghai Meiji Biomedical Technology Co., Ltd., Shanghai, China). Subsequently, 0.5 μg core plasmid of pLKO.1-shTRIM52 or pLVX-Puro-TRIM52 and 1.5 μg mixed viral packaging plasmids psPAX2 and pMD2G (Addgene, Inc.) were added to 250 μl serum-free RPMI-1640 medium, and 9 μl Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) was added into a serum-free RPMI-1640 medium with a total volume of 250 μl, and then the two were mixed and transfected into 293T cells. The virus particles were obtained after 48 h of transfection.

Experimental grouping. In vitro, to regulate the expression of TRIM52 in lung cancer cell lines, lentivirus-mediated RNA interference or overexpression was used. H358 or H1299 cells were infected with RPMI-1640 medium (control), pLKO.1-puro vector (negative control lentivirus; shNC), or shTRIM52 lentivirus (shTRIM52-1, shTRIM52-2, shTRIM52-3 and shTRIM52-4), while H1975 cells were infected with RPMI-1640 medium (control), pLVX-Puro vector (Clontech Laboratories, Inc.) or TRIM52 recombinant lentivirus (oeTRIM52). After 48 h, the efficiency of knockdown or overexpression was detected by reverse transcription-quantitative polymerase chain reaction (RT-qPCR) and western blotting, according to the subsequent protocols. shTRIM52-1, shTRIM52-4 and oeTRIM52 lentiviruses were used for subsequent experiments.

Furthermore, H358 or H1299 cells were infected with RPMI-1640 medium, shNC, shTRIM52-1 or shTRIM52-4, and H1975 cells were treated with vector, oeTRIM52, Vector + 20 μM XAV939 (Wnt/β-catenin inhibitor; S1180; Selleck Chemicals, Shanghai, China) or oeTRIM52 + 20 μM XAV939. Assays to determine proliferation and cell cycle, and western blot analysis were then performed.

Immunohistochemistry. Following paraffin embedding, the tissue slides were fixed for 48 h in 10% formalin at 4℃ and then cut into 5 μm thick sections, which were baked at a constant temperature in an oven at 65℃ for 30 min, and then deparaffinized in two changes of xylenes (Sinopharm Chemical Reagent Co., Ltd., Shanghai, China) for 15 min each. The deparaffinized sections were then rehydrated in 100, 95, 85 and 75% ethanol solutions for 5 min each, which was followed by washing once in tap water for 10 min. Following antigen retrieval with 0.01 M sodium citrate buffer (pH 6.0) at ~95℃ for 15 min, the slides were incubated with 0.3% H_2O_2 for 10 min at room temperature in a humidified chamber and washed with 0.02 M phosphate-buffered saline (PBS). Subsequently, the slides were incubated with a rabbit antibody against TRIM52 (dilution 1:500; cat. no. NBP2-31651; Novus Biologicals, LLC, Littleton, CO, USA) at room temperature for 1 h in a humidified chamber. The slides were then incubated with a horseradish peroxidase-labeled broad-spectrum secondary antibody (dilution 1:1,000; cat. no. D-3004; Shanghai Long Island Biotechnology Co., Ltd., Shanghai, China) at room temperature for 25 min. At room temperature, following DAB staining for 5 min, a washing with tap water, (cat. no. FL-6001; Shanghai Long Island Biotechnology Co., Ltd.), the sections were stained with hematoxylin (cat. no. 714094; Zhuhai BASO Biotechnology Co., Ltd., Zhuhai, China) for 3 min at room temperature, exposed to 1% hydrochloric acid-alcohol at room temperature for 3 sec for differentiation and flushed with tap water once for 10 min. After drying, mounting and cover-slipping, the slides were imaged by an upright light microscope at x200 magnification (ECLIPSE Ni; Nikon Corporation, Tokyo, Japan) and were analyzed by an IMS image analysis system (DS-Ri2; Nikon Corporation).

RT-qPCR. RT-qPCR was performed to detect the TRIM52 mRNA level in cells. The total RNA in cells (H1975, H466, A549, H358 and H1299) that were or were not treated with lenti-viruses was extracted by TRIzol® reagent (cat. no. 1596-026; Invitrogen; Thermo Fisher Scientific, Inc.), and following quantification, the integrity of the RNA was confirmed by 1% agarose gel electrophoresis. The extracted RNA was reversed transcribed into cDNA using a Reverse Transcription kit (cat. no. K1622; Fermentas; Thermo Fisher Scientific, Inc.). With cDNA used as a template and a SYBR®-Green PCR kit (cat. no. K0223; Thermo Fisher Scientific, Inc.), RT-qPCR reactions were performed in an ABI 7300 Real-Time PCR system (ABI-7300; Applied Biosystems; Thermo Fisher Scientific, Inc.). The expression of TRIM52 mRNA normalized to GAPDH was analyzed by ABI Prism 7300 SDS software 1.4v (Applied Biosystems; Thermo Fisher Scientific, Inc.) and was calculated using the $2^{-\Delta\Delta Cq}$ method (33). The primer sequences were as follows: TRIM52, forward, 5'-GTGCCATCTGCT TGGATTAC-3', and reverse, 5'-TCATCTTCCTCCTCG TTCTG-3', and GAPDH, forward, 5'-AATCCCATCACC ATCTTC-3', and reverse, 5'-AGGCTGTTGTCATACTTC-3'. The RT-qPCR reaction conditions were as follows: 95°C for 10 min; 95°C for 15 sec and 60°C for 45 sec for 40 cycles; 95°C for 15 sec; 60°C for 1 min; 95°C for 15 sec; and 60°C for 15 sec (34).

Western blot analysis. Total proteins were extracted from lentivirus-treated H358, H1299 or H1975 cells by radioim-munoprecipitation assay buffer (Beijing Solarbio Science & Technology Co., Ltd.; R0010), which contained protease and phosphatase inhibitors, and were quantified by a Bicinchoninic Assay quantification kit (Thermo Fisher Scientific, Inc.; PICPI23223). Approximately 25 µg of proteins was separated by 10% SDS-PAGE (JRDUN Biotechnology Co., Ltd, Shanghai), followed by a semi-dry transfer onto polyvinylidene fluoride membranes (cat. no. HATF00010; EMD Millipore, Billerica, MA, USA) by electroblotting. After 1 h of blocking in 5% skim milk at room temperature (cat. no. BYL40422; BD Biosciences; Becton, Dickinson and Company, Franklin Lakes, NJ, USA), the blots were incubated with primary anti-bodies against TRIM52 (1:200; cat. no. sc-135589; Santa Cruz Biotechnology, Inc., Dallas, TX, USA), β-catenin (1:5,000; cat. no. ab32572; Abcam, Cambridge, UK), PCNA (1:1,000; cat. no. ab29; Abcam), c-Myc (1:1,000; cat. no. ab32072; Abcam), Cyclin D1 (1:5,000; cat. no. ab134175; Abcam) and β-actin (1:1,000; cat. no. 4970; Cell Signaling Technology, Inc., Danvers, MA, USA) with gentle shaking at 4°C overnight. Following the incubation with the horseradish peroxidase-conjugated secondary antibodies goat anti-rabbit (cat. no. A0208), donkey anti-goat (cat. no. A0181), and goat anti-mouse (cat. no. A0216) (1:1,000; Beyotime Institute of Biotechnology, Shanghai, China) for 1 h at 37°C, the blots were incubated with chemiluminescent detection reagent (cat. no. WBKLS0100; EMD Millipore) for 5 min in the dark. Bound proteins were then visualized using an Enhanced Chemiluminescent imaging system (Tanon-5200; Tanon Science and Technology Co., Ltd., Shanghai, China). Finally, using β-actin as the loading control, the relative protein levels were calculated by ImageJ software 1.47v (National Institutes of Health, Bethesda, MD, USA).

Proliferation assay. H358, H1299 or H1975 cells in a loga-rithmic growth phase were digested by 0.25% trypsin (Beijing Solarbio Science & Technology Co., Ltd.; P1300-100) and counted under a optical microscope at x10 magnification (cat. no. XDS-500C; Shanghai Cai Kang Optical Instrument Co., Ltd., Shanghai, China) to prepare a cell suspension of $3x10^4$ cells/ml. Subsequently, 100 µl of each cell suspension was inoculated in 96-well culture plates (cat. no. TR4001; TrueLine, Romeoville, IL, USA) in triplicate and cultured overnight at 37°C in a humidified 5% CO_2 incubator. After 0, 24, 48 and 72 h of treatment according to the experimental grouping, Cell Counting Kit-8 (CCK-8; cat. no. CP002; Signalway Antibody, College Park, MD, USA) reagent and serum-free RPMI-1640 medium were mixed at a volume ratio of 1:10, and 100 µl of the mixture was added to each well. The plates were incubated for 1 h at 37°C in a 5% CO_2 incubator. Using a microplate reader (cat. no. DNM-9602; Beijing Pulang New Technology Co., Ltd., Beijing, China), the absorbance value (optical density) at 450 nm was measured.

Cell cycle detection. Following treatment, according to the experimental grouping, H358, H1299 or H1975 cells were collected and centrifuged for 5 min at 1,000 x g at room temperature and were then resuspended in 300 µl of PBS supplemented with 10% fetal bovine serum. Subsequently, 700 µl absolute ethanol pre-cooled at -20°C was added to fix the cells for 24 h at 4°C. The next day, following centrifuga-tion at 1,000 x g at room temperature for 5 min, the fixed cells were washed with 1 ml pre-cooled PBS once. Subsequently, the cell pellets were slowly and fully resuspended in 100 µl 1 mg/ml RNase A solution (cat. no. R8020-25; Beijing Solarbio Science & Technology Co., Ltd.) and incubated in the dark for 30 min at 37°C. Finally, the cells were incubated with 400 µl 50 µg/ml propidium iodide solution in the cell cycle and apoptosis detection kit (cat. no. C001-200; Shanghai Qibao Xintai Biological Technology Co., Ltd., Shanghai, China), which was added to stain the nucleus, for 10 min in the dark at room temperature. Following staining, the cell cycle status of these cells was detected with a flow cytometer (BD Biosciences; Becton, Dickinson and Company; Accuri C6) and analyzed by FlowJo software 7.6.1v (Tree Star, Inc., Ashland, OR, USA).

Matrigel assay. Prior to inoculation, the 24-well plates and Transwell chambers (cat. no. 3422; Costar; Corning, Inc., Corning, NY, USA) were soaked in PBS for 5 min, and then the chambers were coated with 80 µl Matrigel and clotted for 30 min in an incubator at 37°C. Following overnight nutrient starvation in serum-free RPMI-1640 medium, the treated cells (H1975 and A549) were trypsinized and inoculated in the upper chamber ($5x10^4$ cells/well). RPMI-1640 medium with 10% fetal bovine serum was added to the lower chamber. After 24 h of incubation, the non-invading cells in the upper chamber were carefully scraped, and the cells that had invaded into the lower chamber were fixed in 4% formaldehyde (Sinopharm Chemical Reagent Co., Ltd., Shanghai, China) for 10 min at room temperature, followed by a 30 min incubation in 0.5% crystal violet (Beijing Solarbio Science & Technology Co., Ltd.; C8470) at room temperature. Subsequently, using an upright optical microscope (cat. no. XDS-500C; Shanghai Cai
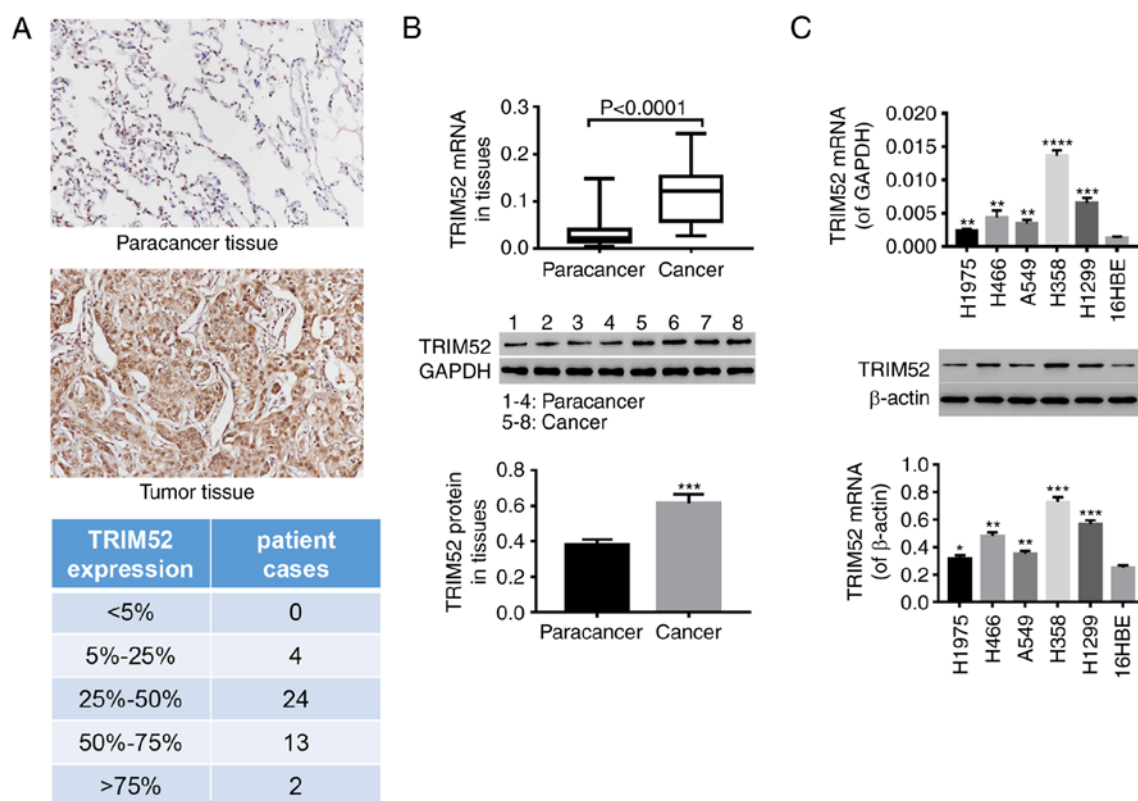
Figure 1. TRIM52 expression is elevated in the tumor tissues of patients with lung cancer and in lung cancer cell lines. A total of 43 pairs of tumor and paracancer tissues were collected from patients with lung cancer. (A) The TRIM52 level in tumor and paracancer tissues was detected by immunohistochemistry. Tissues were imaged under a microscope at a magnification of x200, and a table with all cases grouped according to the overall level of TRIM52 expression is presented. (B) Reverse transcription-quantitative polymerase chain reaction and western blotting were performed to analyze the expression of TRIM52 in tissues. (C) Reverse transcription-quantitative polymerase chain reaction and western blotting were performed to analyze the expression of TRIM52 in five lung cancer cell lines (H1975, H466, A549, H358 and H1299). The data are presented as the mean ± standard deviation. *$P<0.05$, **$P<0.01$, ***$P<0.001$ and ****$P<0.0001$, compared with paracancer tissues or 16HBE cells. TRIM52, tripartite motif 52.

Kang Optical Instrument Co., Ltd.), the invading cells were counted in 3 random fields at a magnification of x200.

Statistical analysis. The statistical analyses of all data in the present study were performed using GraphPad Prism 7.0 software (GraphPad Software, Inc., La Jolla, CA, USA). Student's t-test was used to evaluate the differences between two groups, while one-way analysis of variance followed by Tukey's multiple comparison was performed to evaluate the comparisons among ≥3 groups. Based on at least three independent experiments, quantitative data are shown as the mean ± standard deviation. $P<0.05$ was considered to indicate a statistically significant difference.

Results

TRIM52 expression is elevated in the tumor tissues of patients with lung cancer and in lung cancer cell lines. After 43 pairs of lung tumor and paracancer tissues were collected, immunohistochemistry (Fig. 1A) indicated that compared with the paracancer tissues, the TRIM52 level was notably increased in tumors. All cases were grouped according to the overall level of TRIM52 expression in tissues: <5% positivity; 5≤n<25; 25≤n<50; 50≤n<75; and ≥75%. The increased expression of TRIM52 was further demonstrated by RT-qPCR and western blot analysis (Fig. 1B and C). Statistical analysis of the

immunohistochemical results demonstrated that high expression of TRIM52 was observed in 97.7% of tumor tissues (data not shown). Additionally, the TRIM52 mRNA and protein levels in lung cancer cell lines (H1975, H466, A549, H358 and H1299) were significantly increased, compared with pulmonary epithelial cells (16HBE). The TRIM52 levels were increased in H358 and H1299 cells, compared with the other cell lines, while the levels were reduced in H1975 cells (Fig. 1C). These observations indicated that TRIM52 may be involved in the development and progression of lung cancer. A total of 3 lung cancer cell lines (H358, H1299 and H1975) were therefore selected for the following experiments.

Down- and upregulation of TRIM52 in lung cancer cell lines. To investigate the effect of TRIM52, shTRIM52 and oeTRIM52 lentiviral vectors were used to regulate the TRIM52 level in lung cancer cell lines. As depicted in Fig. 2, the levels of TRIM52 mRNA and protein were significantly downregulated by shTRIM52 infection in H358 (Fig. 2A) and H1299 (Fig. 2B) cells, and the effects of shTRIM52-1 and shTRIM52-4 were more notable. Furthermore, the TRIM52 level in H1975 cells was significantly upregulated by oeTRIM52 (Fig. 2C). Therefore, the shTRIM52-1, shTRIM52-4 and oeTRIM52 lentiviral vectors were selected for further study due to their more effective regulation of TRIM52 expression.
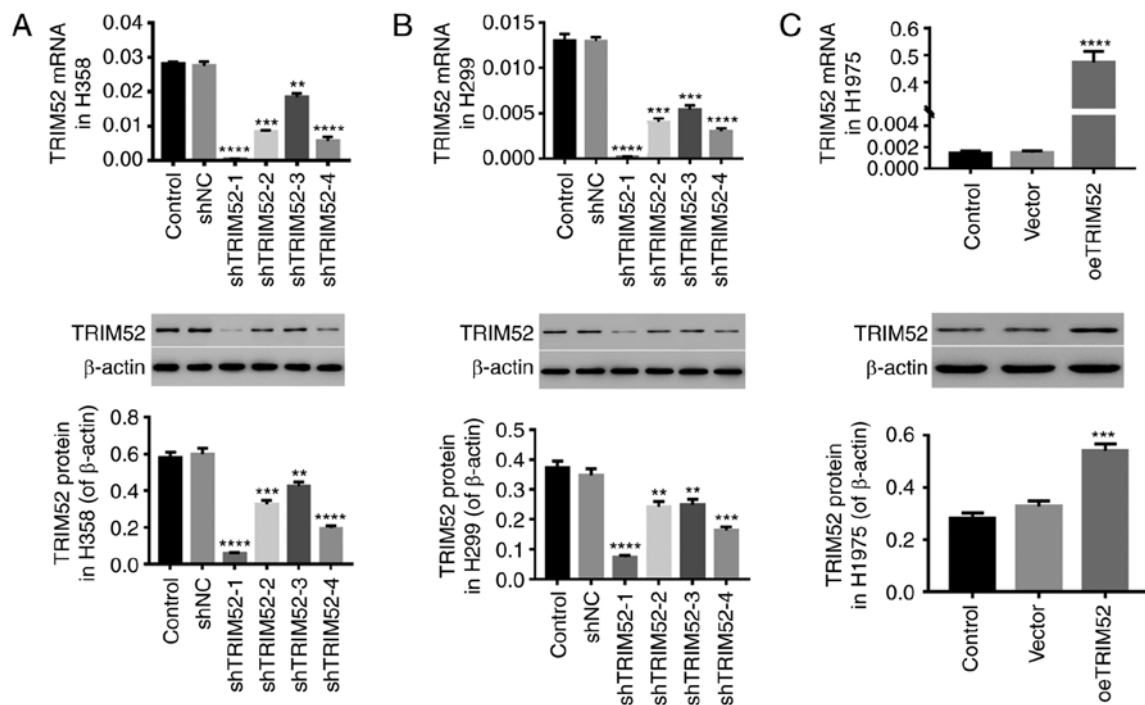
Figure 2. Down- and upregulation of TRIM52 in lung cancer cell lines. Lung cancer cells (H358, H1299 and H1975) were infected with lentiviruses of shNC/shTRIM52 or vector/oeTRIM52, while the cells treated with RPMI-1640 medium served as controls. The total RNA and proteins were extracted from the treated cells after 48 h. (A) TRIM52 mRNA (upper) and protein (lower) levels in TRIM52-slienced H358 cells were detected by reverse transcription-quantitative polymerase chain reaction and western blotting, respectively. (B) Additionally, TRIM52 expression in TRIM52-silenced H1299 cells was also detected. (C) The TRIM52 level in TRIM52-overexpressing H1975 cells was detected. All data are presented as the mean ± standard deviation. **P<0.01, ***P<0.001 and ****P<0.0001, compared with shNC or vector. TRIM52, tripartite motif 52; NC, control; sh, short hairpin.

Downregulation of TRIM52 inhibits lung cancer cell proliferation by cell cycle arrest. Following downregulation of the TRIM52 level in H358 and H1299 cells, cell proliferation and the cell cycle were evaluated. As depicted in Fig. 3, the proliferation of H358 and H1299 cells was notably inhibited when TRIM52 was downregulated (Fig. 3A). Furthermore, downregulation of TRIM52 significantly arrested the cell cycle at G1 phase in lung cancer cells, which reduced the proportion of cells in S/G2 phase (Fig. 3B). Additionally, the protein levels of β-catenin, PCNA, c-Myc and Cyclin D1 were significantly decreased in TRIM52-silenced H358 and H1299 cells (Fig. 3C). All results indicated that TRIM52 downregulation exerted an inhibitory effect on the proliferation of lung cancer cells by blocking cell cycle progression possibly via Wnt/β-catenin signaling.

TRIM52 regulates cell proliferation, cell cycle progression and invasion through the Wnt/β-catenin pathway. Wnt/β-catenin signaling activation has been reported to be a critical oncogenic event in the initiation and progression of tumors, and c-Myc and Cyclin D1 are two downstream Wnt/β-catenin signaling molecules (35). PCNA, a non-histone nuclear protein that functions in DNA synthesis, is a marker of cell proliferative activity in lung cancer (36) and has important prognostic value (37,38). The application of the Wnt/β-catenin inhibitor XAV939 has been reported in numerous studies (39,40). In the present study, the Wnt/β-catenin inhibitor XAV-939 was applied for further study. As depicted in Fig. 4, the upregulation of TRIM52 significantly promoted cell proliferation (Fig. 4A) and facilitated the entry of cells into the S phase from the

G1 phase (Fig. 4B), which was concurrent with increases in β-catenin, PCNA, c-Myc and Cyclin D1 expression (Fig. 4C). The upregulation of TRIM52 in normal epithelial 16HBE cells also induced cell proliferation and S-phase progression. Furthermore, the invasiveness of H1975 and A549 cells was significantly increased by TRIM52 upregulation (Fig. 4D). In contrast, treatment with XAV-939 completely counteracted the effect of TRIM52 upregulation in lung cancer cells, and a rescue effect of TRIM52 upregulation was observed upon Wnt/β-catenin inhibition. It has been reported that the activation of Wnt/β-catenin signaling is frequently observed in lung cancer and that it promotes the proliferation of lung cancer cells (11,41), which is consistent with the present results. These results further demonstrated that TRIM52 regulates the proliferation and invasiveness of lung cancer cells possibly through regulation of Wnt/β-catenin pathway activation.

Discussion

Increasing evidence demonstrates that TRIM proteins, including TRIM29 (42), TRIM16 (43) and TRIM15 (44), are of great importance in the development and progression of cancer. TRIM proteins were revealed to be involved in the regulation of various cellular processes, including cell proliferation, in cancer (45). A previous study indicated that TRIM59 is overexpressed in NSCLC, and promotes the proliferation and migration of NSCLC cells (46). In the present study, it was determined that TRIM52 was elevated in tumor tissues of patients with lung cancer and in tumor cell lines, which indicates that TRIM52 may act as an
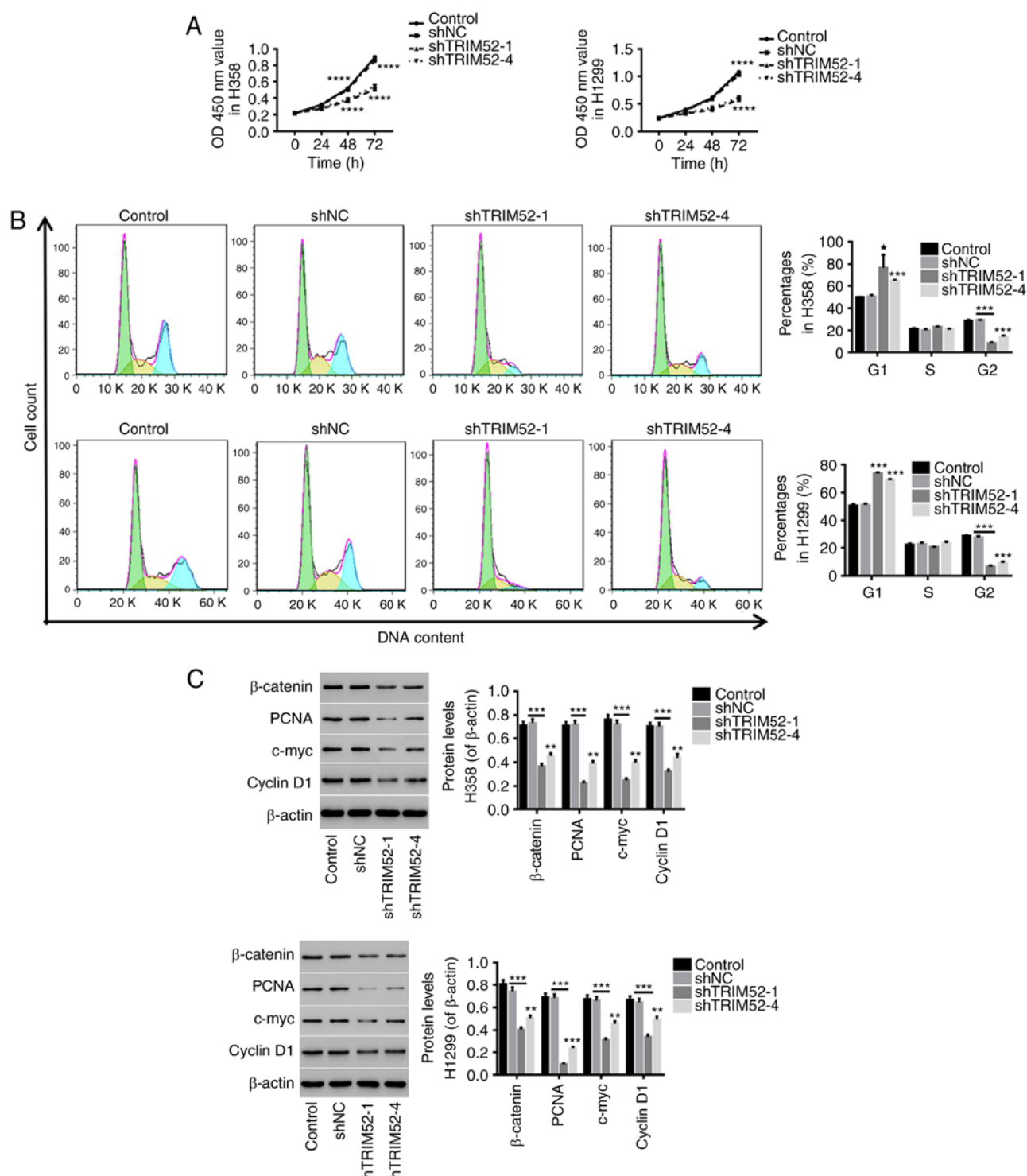
Figure 3. Downregulation of TRIM52 inhibits lung cancer cell proliferation via cell cycle arrest. Lung cancer cells (H358 and H1299) were infected with shNC/shTRIM52 lentiviruses, while the cells treated with RPMI-1640 medium served as controls. (A) The proliferation of TRIM52-slienced H358 and H1299 cells was determined at 0, 24, 48 and 72 h with a Cell Counting Kit-8 assay. (B) Subsequently, 48 h after infection, the cell cycle was detected by flow cytometry. (C) The protein levels of β-catenin, PCNA, c-Myc and Cyclin D1 were quantified by western blotting. Data are presented as the mean ± standard deviation. **P<0.01, ***P<0.001 and ****P<0.0001, compared with shNC. TRIM52, tripartite motif 52; NC, control; sh, short hairpin; PCNA, proliferating cell nuclear antigen.

oncogene in lung cancer. The downregulation of TRIM52 in lung cancer cells significantly inhibited cell proliferation by arresting cell cycle progression, and TRIM52 upregulation promoted proliferation and invasion. Notably, recent genomic analysis indicated that in certain genetic cancer cell backgrounds, an appropriate expression of TRIM52 may be essential for efficient proliferation and survival of certain cancer cell lines (47,48), which are in agreement with the present data. This indicates that the inhibitory effect of TRIM52 downregulation on the proliferation of lung cancer cells may contribute to novel treatments for lung cancer.
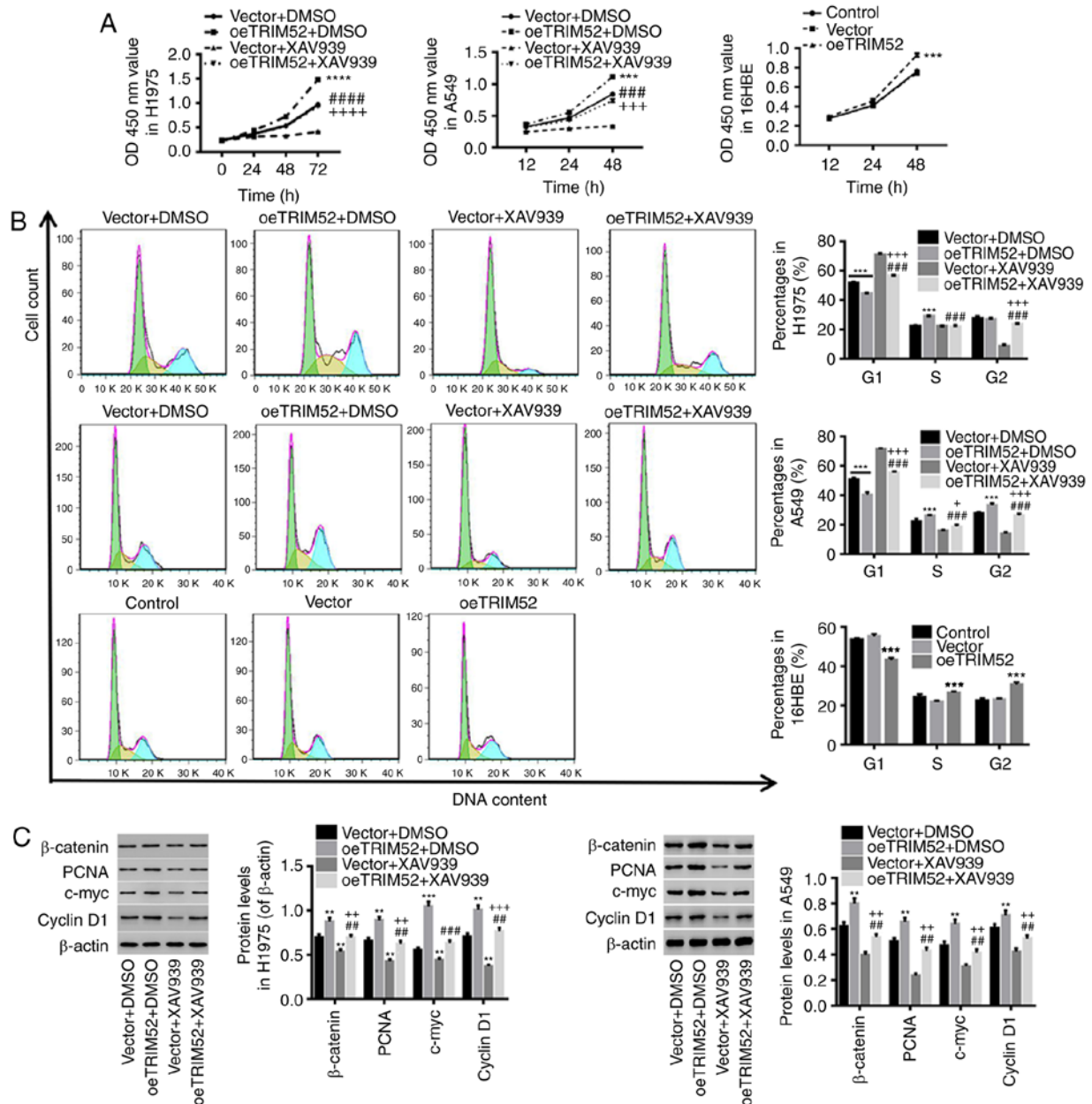
Figure 4. TRIM52 regulates proliferation, cell cycle and invasion through the Wnt/β-catenin pathway. H1975 and A549 cells were treated with vector/oeTRIM52 lentiviruses and 20 μM XAV939 (Wnt/β-catenin inhibitor), and 16HBE cells were treated with vector/oeTRIM52 lentiviruses. RPMI-1640 medium-treated cells served as controls. (A) The proliferation of treated-H1975, A549 and 16HBE cells was assessed with a Cell Counting Kit-8 assay. (B) Using flow cytometry, the cell cycle was evaluated after 48 h. (C) The levels of β-catenin, PCNA, c-Myc and Cyclin D1 proteins were also detected.

Furthermore, it was also investigated the mechanism that underlies TRIM52 in the regulation of lung cancer cell proliferation and invasion. It has been reported that aberrant activation of the Wnt/β-catenin pathway is associated with the development and progression of cancer (49-51). Control of Wnt/β-catenin signaling by disheveled binding antagonist of β-catenin 3 has potential as a therapeutic strategy for colorectal cancer (52). The proto-oncogene c-Myc has been reported to serve a primary role in the biological processes of tumors, including growth and apoptosis (53). When it forms a complex with its partner kinases, including cyclin dependent kinase 4 (CDK4) and CDK6, Cyclin D1, which is overexpressed in a variety of human cancer types, including breast and colon carcinoma cancer (16,54,55), allows cells to proceed into the S phase (56). Compared with Cyclin D1, PCNA has been reported to be elevated in the late G1 and S phases of the cell cycle (57-59). Additionally, in the present study, TRIM52-induced cell proliferation and S-phase cell cycle progression were counteracted by the β-catenin inhibitor XAV939. This was concurrent with decreased expression of β-catenin, PCNA, c-Myc and Cyclin D1 proteins, and a rescue effect of TRIM52 upregulation on Wnt/β-catenin inhibition. These observations are in agreement with those of previous reports, in that TRIM52 ablation increases the proportion of cells in the G0/G1-phase (30,31,60), which reveals that TRIM52 may regulate lung cancer cell proliferation through activation of the Wnt/β-catenin signaling pathway.
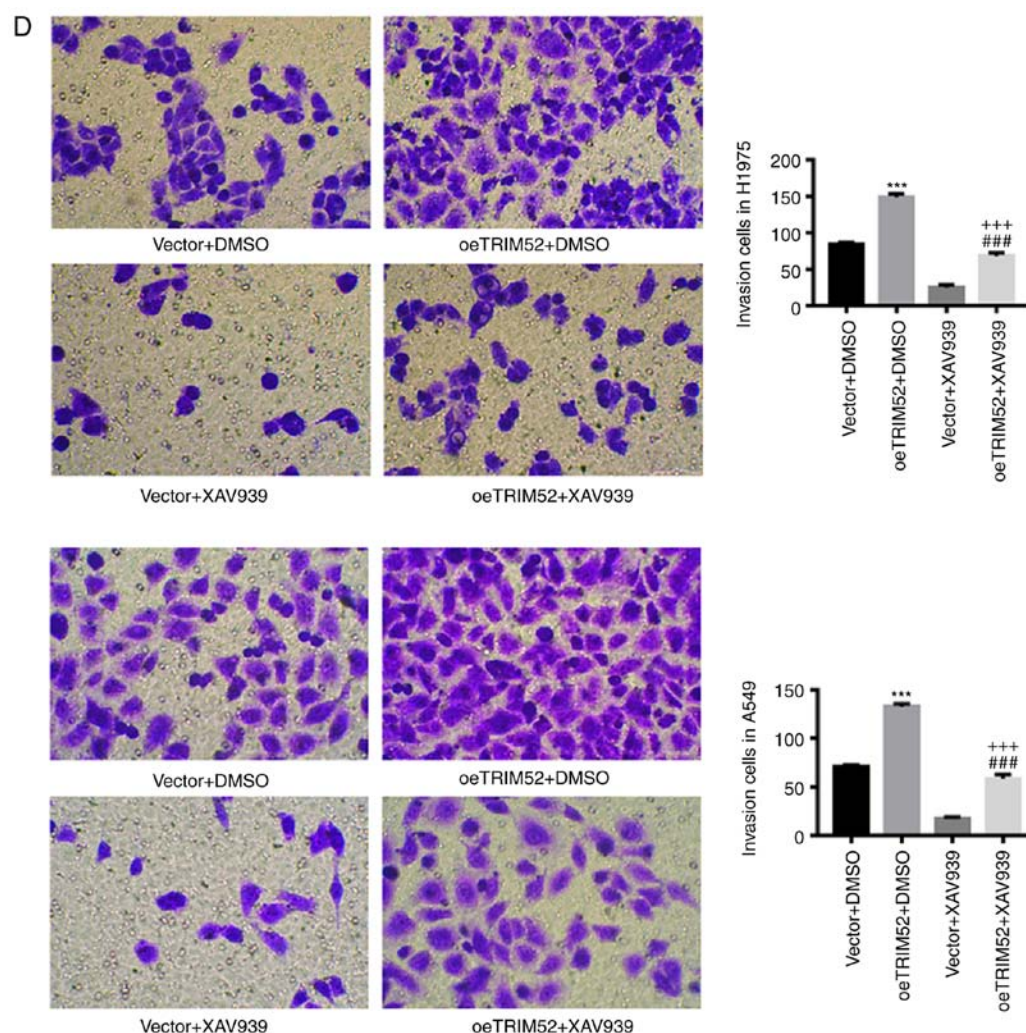
Figure 4. Continued. (D) Invasiveness of H1975 and A549 cells was determined with a Matrigel assay. Data are expressed as the mean ± standard deviation. **P<0.01, ***P<0.001 and ****P<0.0001, oeTRIM52 or oeTRIM52 + DMSO, compared with vector or Vector + DMSO. ##P<0.01, ###P<0.001 and ####P<0.0001, oeTRIM52 + XAV939, compared with oeTRIM52 + DMSO. +P<0.05, ++P<0.01, +++P<0.001 and ++++P<0.0001, oeTRIM52 + XAV939, compared with vector + XAV939. TRIM52, tripartite motif 52; DMSO, dimethyl sulfoxide; PCNA, proliferating cell nuclear antigen.

In summary, it was demonstrated that TRIM52 may act as an oncogene in lung cancer progression. The downregulation of TRIM52 significantly suppressed lung cancer cell proliferation via blocking cell cycle progression, and TRIM52 upregulation promoted proliferation and invasion, which may have occurred through activation of Wnt/β-catenin signaling. Therefore, targeting TRIM52 is a potential therapeutic strategy for the treatment of lung cancer.

Acknowledgements

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

XM and HL conceived and designed the study. XM, LZ and WX performed the experiments. XM and HL wrote the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

All experiments conducted in this study were approved by the Ethics Committee of Shanghai University of Traditional Chinese Medicine and written informed consent was obtained.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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