Differential Effects of *WRAP53* Transcript Variants on the Biological Behaviours of Human Non-Small Cell Lung Cancer Cells

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Abstract

Background: The WD40-encoding RNA antisense to p53 (*WRAP53*) gene, an antisense gene of *TP53,* has 3 different transcriptional start sites that yield 3 transcript variants. One of these variants *WRAP53-1β* encodes a WD repeat-containing protein WRAP53β, whereas *WRAP53-1α* is a noncoding RNA that regulates p53 mRNA levels. These variants are involved in the progression of non-small cell lung cancer (NSCLC). However, how the different transcript variants regulate NSCLC cell behaviours is to be elucidated.

Methods: Wild-type p53 NSCLC A549 cells and p53-mutated H1975 cells were transfected with *WRAP53-1α* and *WRAP53-1β* siRNAs, and their behaviours were examined colony formation, cell viability, apoptosis, cell cycle, wound healing, and cell invasion assays.

Results: *WRAP53-1α* knockdown increased the mRNA and protein levels of p53, whereas depletion of *WRAP53-1β* had no effect on p53 expression. *WRAP53-1α* knockdown suppressed colony formation and proliferation of A549 cells, but had the opposite effects on H1975 cells. However, *WRAP53-1β* knockdown promoted A549 cell growth. Depletion of *WRAP53*-1*α* and *WRAP53-1β* promoted apoptosis in A549 but not H1975 cells. *WRAP53-1α* knockdown increased the proportion of A549 but not H1975 cells at the G0/G1 phase. However, *WRAP53-1β* knockdown decreased the proportion of cells at the G0/G1 phase in A549 cells. Depletion of *WRAP53-1α* suppressed A549 cell migration and invasion, and promoted H1975 cell migration and invasion. However, depletion of *WRAP53-1β* had the opposite effects.

Conclusions: The 2 *WRAP53* transcript variants exerted opposite functions in NSCLC cells and regulated NSCLC cell behaviours in a p53-dependent manner.

Background

Alternative splicing is a crucial process in gene regulation, enabling a single gene to encode different protein products. It occurs in approximately 95% of multi-exon genes in eukaryotes, and is involved in various disorders including cancers [1, 2]. The different transcript variants of a single gene usually have different and even opposite biological functions in physiological and pathological processes.

Mutations in the tumour suppressor p53 cause loss of its tumour suppressor function, promoting malignant transformation. *TP53* mutations are found in over 50% of human cancers[3], and *TP53* is the most commonly mutated gene in non-small cell lung cancer (NSCLC)[4]. Previous studies reported that mutant p53 plays important roles in the occurrence and progression of NSCLC[5]. Furthermore, recent studies suggested that *TP53* mutation was a negatively prognostic factor in advanced NSCLC, and that different mutations had different prognostic values[6]. High expression levels of mutated p53 were correlated with poor survival, regardless of epidermal growth factor receptor mutations[7].

The *TP53* 5'-untranslated region flanking gene, WD40-encoding RNA antisense to p53 (*WRAP53*), has 3 different transcriptional start sites that yield 3 different variants: 1α, 1β, and 1γ. The start site for the 1α

variant corresponds to the first exon of p53 in a cis-antisense manner. *WRAP53-1α* is an antisense transcript that stabilizes *TP53* [8]. The *WRAP53-1β* transcript is not complementary to *TP53,* but encodes the WD repeat-containing protein WRAP53β, also known as WD repeat domain 79 and telomerase Cajal body protein 1, which is involved in multiple cellular processes. WRAP53β was reported to regulate the maintenance of the nuclear organelles known as Cajal bodies via recruiting motor neuron proteins, small Cajal body-specific RNAs, and telomerases[9-11]. WRAP53β also targeted telomerase to telomeres, promoting their elongation[9]. In addition, WRAP53β was reported to facilitate DNA damage repair via recruiting the ubiquitin ligase ring finger protein 8 to DNA breaks during both homologous recombination and non-homologous end joining. The effects of WRAP53β on DNA damage repair were p53-independent, as it also functioned in p53-deficient cells[12, 13].

The effects of WRAP53 on cancer progression are controversial*. WRAP53* is believed to be associated with cancer pathogenesis, because *WRAP53-1α* regulates the expression of *TP53*. Loss of WRAP53β impairs telomere maintenance and DNA repair, increasing genomic instability and the probability of carcinogenesis. It is conceivable that *WRAP53-1α and WRAP53-1β* play different roles in NSCLC cells. In order to test this hypothesis, we examined the distinct biological functions of these 2 *WRAP53* transcript variants in cell function assays *in vitro*.

Methods

Cell culture

NSCLC cell lines, A549 and H1975, were purchased from the Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). Both cell lines were cultured in RPMI-1640 medium (Hyclone, USA) supplemented with 10% foetal bovine serum (Hyclone), 100 U/mL penicillin, and 100 mg/mL streptomycin (Sangon, China). All cells were maintained in a humidified incubator (Sanyo, Japan) at 37 °C and 5% CO_2.

RNA extraction and quantitative real-time polymerase chain reaction

Total RNA was isolated using TRIzol (Vazyme, China). The RNA concentrations and the A260/A280 ratios were assessed with a Nanodrop spectrophotometer (Thermo, USA). Total RNA (500 ng) was reverse-transcribed using the HiScript® Q RT SuperMix for qPCR kit (Vazyme) according to the manufacturer's instructions. Quantitative real-time polymerase chain reaction (qRT-PCR) was performed using the ChamQ TM SYBR ® qPCR Master Mix (Vazyme) on the CFX Connect^{TM} RealTime PCR Detection System (Bio-Rad, USA). Glyceraldehyde-3-phosphate dehydrogenase was used as the reference for normalization. The relative fold change of mRNAs was calculated using the $2^{-\Delta\Delta Ct}$ method. The primers used for qRT-PCR are presented in Table 1.

RNA interference

Small interfering RNAs (siRNAs) targeting *WRAP53-1α* and *WRAP53-1β* [8] and negative control (NC) siRNAs were purchased from Genepharm Technologies (GenePharma, China). Transient transfection was performed using jetPRIME transfection reagent (Polyplus-transfection® SA, France) following the manufacturer's recommendations. The cells were transfected with NC or target siRNAs at 30-50% confluence.

Immunoblotting

Cells were lysed in RIPA cell lysis buffer (Beyotime, China) supplemented with phosphatase inhibitors and protease inhibitors (Sigma, USA). The lysates were then centrifuged (13300 rpm) at 4 °C for 15 min. The supernatants were collected, and protein concentration was measured using a bicinchoninic acid kit (Beyotime). Equal amount of proteins were applied to 10-12% sodium dodecyl sulphate-polyacrylamide separating gels and transferred to polyvinylidene fluoride membranes. After blocking with 5% non-fat milk, the membranes were incubated with primary antibodies at 4 °C overnight. After washing, the membranes were incubated with horseradish peroxidase-conjugated secondary antibodies at room temperature for 1 h. An enhanced chemiluminescence kit (Bio-Rad) was used to detect the proteins under a gel imaging analyser (Bio-Rad). Antibodies used for immunoblotting are listed in Table 2.

Colony formation assay

Tumour cells were plated in 6-well plates (500 cells/well) and cultured for 2 weeks. Cell colonies were fixed with 4% paraformaldehyde (Sangon) for 20 min and stained with 0.5% crystal violet (Beyotime) at room temperature for 30 min. After washing with water, the numbers of colonies in each well were counted under a microscope. This assay was repeated 3 times and performed in triplicates.

Cell viability assay

Cells were seeded into 96-well plates (3000 cells/well in 100 μL medium). After culturing for 24, 48, 72, and 96 h, the supernatant was replaced with 100 μL serum-free medium with 10 μL CCK-8 (Dojindo, Japan) and incubated at 37 °C in the dark for 1 h. The absorbance was measured at 450 nm by a microplate reader (Rayto, China). Each group had 5 wells, and the experiment was conducted 3 times.

Apoptosis assay

Cells were harvested 48 h after transfection, washed twice with cold phosphate-buffered saline (PBS), and stained with fluorescein isothiocyanate-conjugated annexin V (BioLegend, China) for 20 min and propidium iodide (BD, USA) in the dark for 5 min. The stained cells were assessed by flow cytometry and analysed by CytExpert 2.3 software (Beckman, China). Each experiment was conducted at least 3 times.

Cell cycle analysis

After washing with cold PBS, cells were treated with a cell cycle staining kit (MultiSciences, China). Flow cytometry was used to detect the percentage of cells at different cell cycle phases. This assay was repeated at least 3 times.

Wound healing assay

Cells were seeded in 6-well plates and transfected with NC or *WRAP53* siRNAs. When the cells were confluent, they were scratched with a 10-μL pipette tip and washed with PBS gently to remove cell debris. After that, 2 mL serum-free medium was added into each well, and pictures were taken using a phase contrast microscope (Nikon, Japan) at 0, 24, and 48 h. The migration rate was calculated as:

Wound closure (%) = (distance of initial scratch - distance of closed scratch) / distance of initial scratch.

Cell invasion assays

Cells (1×10^5 cells/well) transfected with NC or *WRAP53* siRNAs were seeded in the upper chambers of transwell units pre-coated with 100 μL Matrigel (Becton-Dickinson, USA, dilution 1:40). After incubation for 24 h, the filters were fixed with 4% paraformaldehyde for 20 min and stained with 0.5% crystal violet for 20 min. The cells on the upper surface of the filter were completely removed using a cotton swab. The cells that invaded through the Matrigel and reached the lower surface of the filter were counted. Experiments were independently repeated 3 times.

Statistical analysis

All statistical analyses were performed with GraphPad software. All data are presented as the mean ± standard deviation. Unpaired 2-tailed student's t-test was used to compare 2 groups. $P < 0.05$ was considered statistically significant.

Results

Knockdown of *WRAP53-1α*, but not *WRAP53-1β*, induced p53 expression

Transcript-specific siRNAs were designed to specifically target *WRAP53-1α* and *WRAP53-1β*. There was no marked difference in *WRAP53-1α* mRNA levels between cells transfected with si-Control and si-WRAP531β, whereas there was significantly less *WRAP53-1α* mRNA in A549 and H1975 cells transfected with si-WRAP531α ($P < 0.05$; Figure 1A). Similarly, si-*WRAP53-1β* reduced *WRAP53-1β* RNA levels in A549 and H1975 cells ($P < 0.01$ and $P < 0.05$, respectively; Figure 1A). Knockdown of *WRAP53-1α* significantly

increased *TP53* mRNA levels in both A549 and H1975 cells, whereas knockdown of *WRAP53-1β* had no effect on *TP53* levels (Figure 1B). Immunoblotting showed that there was no difference in WRAP53β protein levels between cells transfected with si-Control and si-WRAP531α, whereas the protein levels of WRAP53β were decreased in cells transfected with si-WRAP531β. Moreover, knockdown of *WRAP53-1α* increased p53 protein levels in both A549 and H1975 cells, whereas knockdown of *WRAP53-1β* had no effect (Figure 1C).

WRAP53-1α and *WRAP53-1β* had opposite effects on NSCLC cell growth and proliferation

To investigate the functions of *WRAP53-1α* and *WRAP53-1β* in NSCLC cell growth, both A549 and H1975 cells were transfected with si-WRAP531α or si-WRAP531β. The colony formation assay revealed that knockdown of *WRAP53-1α* significantly decreased colony formation in A549 cells, whereas knockdown of *WRAP53-1β* significantly increased colony formation (Figure 2A). However, in H1975 cells, knockdown of *WRAP53-1α* promoted colony formation, and knockdown of *WRAP53-1β* had no significant effect (Figure 2B). The results of the cell viability assay indicated a significant decrease in A549 cell proliferation with *WRAP53-1α* knockdown, whereas *WRAP53-1β* deficiency significantly promoted A549 cell proliferation (Figure 2C). In H1975 cells, knockdown of *WRAP53-1α* induced cell proliferation, and depletion of *WRAP53-1β* had no significant effect (Figure 2C). These results suggested that *WRAP53-1α* and *WRAP53-1β* had opposite effects on NSCLC cell proliferation and function in a p53-dependent manner.

WRAP53-1α and *WRAP53-1β* deficiency induced apoptosis in NSCLC cells

Flow cytometry was used to measure apoptosis in NSCLC cells transfected with si-WRAP531α or si-WRAP531β. Knockdown of either *WRAP53-1α* or *WRAP53-1β* induced A549 cells apoptosis ($P < 0.05$; Figure 3A), but had no significant effect on H1975 cells (Figure 3B). Immunoblotting results indicated that knockdown of either *WRAP53-1α* or *WRAP53-1β* upregulated Bcl-2 associated X protein and downregulated B cell lymphoma 2 in A549 cells. However, these changes were mild in H1975 cells transfected with si-WRAP531α or si-WRAP531β (Figure 3C). These results suggested that *WRAP53-1α* and *WRAP53-1β* negatively regulated apoptosis in cells with wild-type p53 (A549) but not in cells with mutant p53 (H1975).

WRAP53-1α and *WRAP53-1β* had opposite effects on cell cycle progression in NSCLC cells

Cell cycle distribution was assessed by flow cytometry. In A549 cells, knockdown of *WRAP53-1α* induced a significant G0/G1 arrest, whereas knockdown of *WRAP53-1β* decreased the proportion of cells at the

G0/G1 phase (Figure 4A). However, in H1975 cells, knockdown of *WRAP53-1α* decreased the proportion of cells at the G0/G1 phase ($P < 0.05$), and depletion of *WRAP53-1β* had no significant effect on the cell cycle (Figure 4B). To verify the impacts of *WRAP53-1α* and *WRAP53-1β* on the cell cycle, the levels of cell cycle regulation and checkpoint proteins were measured by immunoblotting. Cyclin-dependent kinase 4 (CDK4) was downregulated by knockdown of *WRAP53-1α* and upregulated by depletion of *WRAP53-1β* in A549 cells. However, in H1975 cells, the levels of CDK4 were increased by knockdown of *WRAP53-1α,* but not affected by depletion of *WRAP53-1β* (Figure 4C). These results suggested that *WRAP53-1α* and *WRAP53-1β* had opposite effects on NSCLC cell cycle arrest, and the regulation was p53-dependent.

WRAP53-1α and *WRAP53-1β* had opposite effects on migration and invasion in NSCLC cells

The impacts of *WRAP53-1α* and *WRAP53-1β* on NSCLC cells migration and invasion were examined by wound healing and modified Boyden chamber assays. Knockdown of *WRAP53-1α* significantly suppressed A549 cell migration, whereas *WRAP53-1β* deficiency promoted A549 cell migration (Figure 5A). In H1975 cells, knockdown of *WRAP53-1α* induced cell migration ($P < 0.05$), and depletion of *WRAP53-1β* had no significant effect (Figure 5A). The results of the modified Boyden chamber assay demonstrated that knockdown of *WRAP53-1α* reduced A549 cell invasion, whereas *WRAP53-1β* deficiency increased A549 cell invasion (Figure 5B). In H1975 cells, knockdown of *WRAP53-1α* promoted cell invasion ($P < 0.05$), whereas depletion of *WRAP53-1β* had no significant effect (Figure 5B). In addition, the results of immunoblotting indicated that matrix metalloproteinase 9 (MMP9) was downregulated by knockdown of *WRAP53-1α* and upregulated by depletion of *WRAP53-1β* in A549 cells. However, in H1975 cells, MMP9 was upregulated by knockdown of *WRAP53-1α* and not affected by depletion of WRAP53-1β (Figure 5C). These results suggested that *WRAP53-1α* and *WRAP53-1β* had opposite effects on migration and invasion in NSCLC cells in a p53-dependent manner.

Discussion

In the present study, the 2 *WRAP53* transcript variants, *WRAP53-1α* and *WRAP53-1β*, were knocked down in A549 cells with wild-type p53 and H1975 cells with mutated p53. *WRAP53-1α* not only regulates wild-type p53 expression, but also regulates mutant p53 expression. Knockdown of *WRAP53-1α* had anti-tumor effects in A549 cells, but had the opposite effects on H1975 cells. *WRAP53-1α* regulates NSCLC cell behaviours in a p53-dependent manner. *WRAP53-1β* does not regulate expression of either wild-type or mutant p53. *WRAP53-1β* acts as a tumour suppressor in A549 cells, but has no effect in H1975 cells. Our results suggest that the 2 *WRAP53* transcript variants have distinct effects on p53 and NSCLC cells.

Natural antisense transcripts (NATs), a type of long non-coding RNAs (lncRNAs), occur naturally and play important roles in carcinogenesis, invasion, and metastasis[14]. The lncRNA *WRAP53-1α* is a naturally occurring p53 antisense transcript that acts as a crucial effector in several cancers[15, 16]. *WRAP53-1α* is upregulated by anti-cancer drugs, and miR-4732-5p has a binding site in the 5'-untranslated region of

WRAP53[17-19]. In addition, *WRAP53-1α* methylation is significantly associated with worse survival in NSCLC. It is worth noting that *WRAP53-1α* stabilizes *TP53* mRNA to increase the tumour suppressor activity of wild-type p53, leading to better prognosis. However, downregulation of *WRAP53-1α* by promoter methylation does not affect survival in p53-mutated tumours[20]. These results suggest that *WRAP53-1α* regulates p53 signalling.

WRAP53β also acts as a tumour suppressor to regulate various cancer cellular activities[21]. The significance of WRAP53β in tissue homeostasis is demonstrated by the finding that inherited mutations in WRAP53β lead to telomere dysfunction and dyskeratosis congenita, increasing the risk of tumorigenesis[22]. Moreover, single nucleotide polymorphisms and downregulation of WRAP53β are associated with various sporadic forms of cancer, including breast and ovarian cancer[23-26]. In addition, WRAP53β downregulation is correlated with resistance of head and neck cancer to radiotherapy[27], as well as disruption of the DNA damage response in ovarian tumours[28].

Previous studies indicated that WRAP53β was a potential oncoprotein, whose overexpression led to transformation and promoted cancer cell survival, and whose downregulation induced massive cell death[29-32]. In addition, overexpression of WRAP53β was related to NSCLC progression. Knockdown of WRAP53β significantly inhibited NSCLC cell proliferation both *in vitro* and *in vivo* via inducing cell cycle arrest and apoptosis. WRAP53β induced cell cycle arrest at the G0/G1 phase and regulated the expression of G0/G1-related cyclins and cyclin-dependent kinase complexes. WRAP53β deficiency was also reported to induce apoptosis through the mitochondrial pathways[33]. WRAP53β colocalized and interacted with USP7, which reduced the ubiquitination of MDM2 and p53, thereby extending the half-life of these proteins and increasing their stability[34]. Moreover, WRAP53β exerted proliferative effects on NSCLC cells via stabilizing UHRF1[35]. Recent studies revealed that WRAP53β might be related to p53 mutations and acted as an independent biomarker to predict poor prognosis of patients with surgically resected NSCLC[36].

The involvement of *WRAP53* in disease progression is evident in lung cancer, but whether different targets of *WRAP53* variants exert different biological functions still requires further investigation. In the present study, we used different variant-specific siRNAs to knockdown *WRAP53-1α* and *WRAP53-1β* and evaluated the functions of these variants in NSCLC cells. First, significant associations were found between *WRAP53-1α* and p53 expression, whereas *WRAP531β* expression was not associated with p53 expression, which was consistent with previous reports[8]. However, knockdown of *WRAP531α* upregulated p53 in A549 and H1975 cells, which conflicted with a previous study wherein *WRAP53-1α* stabilized p53[8, 20]. At the molecular level, NATs can have a concordant or discordant relationship with the sense transcript, and this interaction may result in higher stability or translational repression of the sense RNA[37]. Therefore, we believe that *WRAP53-1α* might play different roles based on the p53 mutation status.

We further demonstrated that *WRAP53-1α* had different effects on NSCLC cell behaviours. It is highly probable that the different mutation statuses of p53 led to these contradictory biological functions. The

tumour suppressor p53 is a cellular gatekeeper that guards against genetic abnormality and instability via sensing multiple stress signals, including DNA damage and oncogene activation[38]. In addition, mutations in p53 usually result in increased half-life and nuclear accumulation, which can promote cancer progression via subverting multiple tumour suppression pathways[39]. In A549 cells, knockdown of *WRAP53-1α* induced upregulation of wild-type p53 and activation of cell cycle arrest and apoptosis. However, in H1975 cells with p53 mutations, the knockdown of *WRAP53-1α* exerted the opposite effects on cell cycle arrest and had no effect on apoptosis.

Cell function assays indicated that *WRAP53-1β* acted as a tumour suppressor in A549 cells. Knockdown of *WRAP53-1β* induced slight apoptosis and promoted the G1/S phase transition in A549 cells, but had no effect on H1975 cells. These findings concerning the contribution of WRAP53β to cancer might occur in a p53-dependent manner, which was consistent with the results of animal models[36]. Inactivation of *WRAP53-1β* could help initiate tumour development by impairing telomere maintenance and DNA repair, leading to genomic instability. Our data suggested that *WRAP53-1β* was a putative tumour suppressor during the progression and metastasis of NSCLC, whereas *WRAP53-1α* might have a dual function. These findings suggest potential interactions between the 2 transcript variants. This study provides a foundation for further examining the mechanisms by which *WRAP53-1α* and *WRAP53-1β* exert their functions in NSCLC, but more in vivo validation is needed.

Conclusions

In summary, our results indicated that different transcript variants of *WRAP53* played different and even opposite roles in NSCLC cells. Specifically targeting *WRAP53* variants may contribute to NSCLC therapeutic strategies.

Abbreviations

NSCLC non-small cell lung cancer

WRAP53 WD40-encoding RNA antisense to p53

TP53 tumor protein 53

qRT-PCR Quantitative real-time polymerase chain reaction

siRNAs small interfering RNAs

NC negative control

CCK-8 cell counting kit-8

PBS phosphate-buffered saline

SD	standard deviation
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
Bax	Bcl-2 Associated X Protein
Bcl-2	B cell lymphoma 2
CDK4	cyclin-dependent kinase 4
MMP	matrix metalloproteinase 9
NATs	natural antisense transcripts
lncRNAs	long non-coding RNAs
USP7	ubiquitin-specific protease-7
MDM2	murine double minute 2
UHRF1	ubiquitin-like containing PHD and RING finger domains 1

Declarations

Ethics approval and consent to participate Not applicable

Consent for publication Not applicable

Availability of data and materials Not applicable

Competing interests The authors declare that they have no competing interests.

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Y Zhu, R Bai, Y Luo, Y Gao; (V) Data analysis and interpretation: Y Zhu, S Li, Z Huang; (VI) Manuscript writing: Y Zhu, Y Gong, C Xie; (VII) Final approval of manuscript: All authors.

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References

1. Venables JP: **Unbalanced alternative splicing and its significance in cancer**. *BioEssays : news and reviews in molecular, cellular and developmental biology* 2006, **28**(4):378-386.

2. Black DL: **Mechanisms of alternative pre-messenger RNA splicing**. Annual review of biochemistry 2003, 72:291-336.

3. Binayke A, Mishra S, Suman P, Das S, Chander H: **Awakening the "guardian of genome": reactivation of mutant p53**. Cancer Chemother Pharmacol 2019, 83(1):1-15.

4. Surget S, Khoury MP, Bourdon JC: **Uncovering the role of p53 splice variants in human malignancy: a clinical perspective**. Onco Targets Ther 2013, 7:57-68.

5. Shi J, Hua X, Zhu B, Ravichandran S: **Somatic Genomics and Clinical Features of Lung Adenocarcinoma: A Retrospective Study**. PLoS Med 2016, 13(12):e1002162.

6. Jiao XD, Qin BD, You P, Cai J, Zang YS: **The prognostic value of TP53 and its correlation with EGFR mutation in advanced non-small cell lung cancer, an analysis based on cBioPortal data base**. *Lung cancer (Amsterdam, Netherlands)* 2018, **123**:70-75.

7. Zhu WY, Hu XF, Fang KX, Kong QQ, Cui R, Li HF, He JY, Zhang YK, Le HB: **Prognostic value of mutant p53, Ki-67, and TTF-1 and their correlation with EGFR mutation in patients with non-small cell lung cancer**. *Histology and histopathology* 2019, **34**(11):1269-1278.

8. Mahmoudi S, Henriksson S, Corcoran M, Méndez-Vidal C, Wiman KG, Farnebo M: **Wrap53, a Natural p53 Antisense Transcript Required for p53 Induction upon DNA Damage**. *Mol Cell* 2016, **64**(5):1009-1009.

9. Venteicher AS, Abreu EB, Meng Z, McCann KE, Terns RM, Veenstra TD, Terns MP, Artandi SE: **A human telomerase holoenzyme protein required for Cajal body localization and telomere synthesis**. *Science* 2009, **323**(5914):644-648.

10. Tycowski KT, Shu M-D, Kukoyi A, Steitz JA: **A conserved WD40 protein binds the Cajal body localization signal of scaRNP particles**. *Mol Cell* 2009, **34**(1):47-57.

11. Mahmoudi S, Henriksson S, Weibrecht I, Smith S, Söderberg O, Strömblad S, Wiman KG, Farnebo M: **Correction: WRAP53 Is Essential for Cajal Body Formation and for Targeting the Survival of Motor Neuron Complex to Cajal Bodies**. *PLoS Biol* 2018, **16**(9):e3000030-e3000030.

12. Henriksson S, Rassoolzadeh H, Hedström E, Coucoravas C, Julner A, Goldstein M, Imreh G, Zhivotovsky B, Kastan MB, Helleday T *et al*: **The scaffold protein WRAP53β orchestrates the ubiquitin response critical for DNA double-strand break repair**. *Genes Dev* 2014, **28**(24):2726-2738.

13. Henriksson S, Farnebo M: **On the road with WRAP53β: guardian of Cajal bodies and genome integrity**. *Front Genet* 2015, **6**:91-91.

14. Nie L, Wu HJ, Hsu JM, Chang SS, Labaff AM, Li CW, Wang Y, Hsu JL, Hung MC: **Long non-coding RNAs: versatile master regulators of gene expression and crucial players in cancer**. *American journal of translational research* 2012, **4**(2):127-150.

15. Kamel MM, Matboli M, Sallam M, Montasser IF, Saad AS, El-Tawdi AHF: **Investigation of long noncoding RNAs expression profile as potential serum biomarkers in patients with hepatocellular carcinoma**. *Transl Res* 2016, **168**:134-145.

16. Braicu C, Zimta A-A, Harangus A, Iurca I, Irimie A, Coza O, Berindan-Neagoe I: **The Function of Non-Coding RNAs in Lung Cancer Tumorigenesis**. *Cancers (Basel)* 2019, **11**(5):605.

17. Yuan JM, Li XD, Liu ZY, Hou GQ, Kang JH, Huang DY, Du SX: **Cisplatin induces apoptosis via upregulating Wrap53 in U-2OS osteosarcoma cells**. *Asian Pac J Cancer Prev* 2011, **12**(12):3465-3469.

18. Bug M, Dobbelstein M: **Anthracyclines induce the accumulation of mutant p53 through E2F1-dependent and -independent mechanisms**. *Oncogene* 2011, **30**(33):3612-3624.

19. Pouladi N, Kouhsari SM, Feizi MH, Gavgani RR, Azarfam P: **Overlapping region of p53/wrap53 transcripts: mutational analysis and sequence similarity with microRNA-4732-5p**. *Asian Pac J Cancer Prev* 2013, **14**(6):3503-3507.

20. Kim DS, Lee WK, Park JY: **Promoter methylation of Wrap53α, an antisense transcript of p53, is associated with the poor prognosis of patients with non-small cell lung cancer**. *Oncol Lett* 2018, **16**(5):5823-5828.

21. Jain BP, Pandey S: **WD40 Repeat Proteins: Signalling Scaffold with Diverse Functions**. *Protein J* 2018, **37**(5):391-406.

22. Zhong F, Savage SA, Shkreli M, Giri N, Jessop L, Myers T, Chen R, Alter BP, Artandi SE: **Disruption of telomerase trafficking by TCAB1 mutation causes dyskeratosis congenita**. *Genes Dev* 2011, **25**(1):11-16.

23. Garcia-Closas M, Kristensen V, Langerød A, Qi Y, Yeager M, Burdett L, Welch R, Lissowska J, Peplonska B, Brinton L *et al*: **Common genetic variation in TP53 and its flanking genes, WDR79 and ATP1B2, and susceptibility to breast cancer**. *Int J Cancer* 2007, **121**(11):2532-2538.

24. Mędrek K, Magnowski P, Masojć B, Chudecka-Głaz A, Torbe B, Menkiszak J, Spaczyński M, Gronwald J, Lubiński J, Górski B: **Association of common WRAP 53 variant with ovarian cancer risk in the Polish**

population. *Mol Biol Rep* 2013, **40**(3):2145-2147.

25. Sedaie Bonab A, Pouladi N, Hosseinpourfeizi MA, Ravanbakhsh Gavgani R, Dehghan R, Azarfam P, Montazeri V, Fakhrjou A: **Single-strand conformational polymorphism analysis of a common single nucleotide variation in WRAP53 gene, rs2287499, and evaluating its association in relation to breast cancer risk and prognosis among Iranian-Azeri population**. *Med Oncol* 2014, **31**(9):168-168.

26. Pouladi N, Abdolahi S, Farajzadeh D, Hosseinpour Feizi MA: **Haplotype and linkage disequilibrium of TP53-WRAP53 locus in Iranian-Azeri women with breast cancer**. *PLoS One* 2019, **14**(8):e0220727-e0220727.

27. Garvin S, Tiefenböck K, Farnebo L, Thunell LK, Farnebo M, Roberg K: **Nuclear expression of WRAP53β is associated with a positive response to radiotherapy and improved overall survival in patients with head and neck squamous cell carcinoma**. *Oral Oncol* 2015, **51**(1):24-30.

28. Hedström E, Pederiva C, Farnebo J, Nodin B, Jirström K, Brennan DJ, Farnebo M: **Downregulation of the cancer susceptibility protein WRAP53β in epithelial ovarian cancer leads to defective DNA repair and poor clinical outcome**. *Cell Death Dis* 2015, **6**(10):e1892-e1892.

29. Mahmoudi S, Henriksson S, Farnebo L, Roberg K, Farnebo M: **WRAP53 promotes cancer cell survival and is a potential target for cancer therapy**. *Cell Death Dis* 2011, **2**(1):e114-e114.

30. Sun C-k, Luo X-b, Gou Y-p, Hu L, Wang K, Li C, Xiang Z-t, Zhang P, Kong X-l, Zhang C-l *et al*: **TCAB1: a potential target for diagnosis and therapy of head and neck carcinomas**. *Mol Cancer* 2014, **13**:180-180.

31. Rao X, Huang D, Sui X, Liu G, Song X, Xie J, Huang D: **Overexpression of WRAP53 is associated with development and progression of esophageal squamous cell carcinoma**. *PLoS One* 2014, **9**(3):e91670-e91670.

32. Zhu Y, Ding L, Chen B-F, Song J-G, Yao Y-S: **Oncogenic Activity of Wrap53 in Human Colorectal Cancer In Vitro and in Nude Mouse Xenografts**. *Med Sci Monit* 2018, **24**:6129-6136.

33. Sun Y, Yang C, Chen J, Song X, Li Z, Duan M, Li J, Hu X, Wu K, Yan G *et al*: **Overexpression of WDR79 in non-small cell lung cancer is linked to tumour progression**. *J Cell Mol Med* 2016, **20**(4):698-709.

34. Sun Y, Cao L, Sheng X, Chen J, Zhou Y, Yang C, Deng T, Ma H, Feng P, Liu J *et al*: **WDR79 promotes the proliferation of non-small cell lung cancer cells via USP7-mediated regulation of the Mdm2-p53 pathway**. *Cell Death Dis* 2017, **8**(4):e2743-e2743.

35. Chen J, Sheng X, Ma H, Tang Z, Yang C, Cao L, Sun Y, Deng T, Feng P, Hu B *et al*: **WDR79 mediates the proliferation of non-small cell lung cancer cells by regulating the stability of UHRF1**. *J Cell Mol Med* 2018, **22**(5):2856-2864.

36. Peng J, Zhan Y, Feng J, Fan S, Zang H: **Expression of WDR79 is associated with TP53 mutation and poor prognosis in surgically resected non-small cell lung cancer**. *J Cancer* 2019, **10**(13):3046-3053.

37. Werner A, Swan D: **What are natural antisense transcripts good for?** *Biochem Soc Trans* 2010, **38**(4):1144-1149.

38. Green DR, Kroemer G: **Cytoplasmic functions of the tumour suppressor p53**. *Nature* 2009, **458**(7242):1127-1130.

39. Yue X, Zhao Y, Xu Y, Zheng M, Feng Z, Hu W: **Mutant p53 in Cancer: Accumulation, Gain-of-Function, and Therapy**. *Journal of molecular biology* 2017, **429**(11):1595-1606.

Tables

Table 1 Primer sequences and siRNA sequences used in this study.

Gene	sequences or target sequence (5'→3')
GAPDH-F	GGAGCGAGATCCCTCCAAAAT
GAPDH-R	GGCTGTTGTCATACTTCTCATGG
Wrap531α-F	CCGGAGCCCAGCAGCTACCTG
Wrap531α-R	CATGGCGACTGTCCAGCTTTG
Wrap531β-F	AGGAGGGAAGCACAGTATGAAGAC
Wrap531β-R	GGCATCAGTTCAGAGTCCGCA
P53-F	ACGACGGTGACACGCTTCCC
P53-R	AGGGGGCTCGACGCTAGGAT
siControl sense	UUCUCCGAACGUGUCACGUTT
siControl Antisense	ACGUGACACGUUCGGAGAATT
siWrap531α sense	AAAACCCCAATCCCATCAACC
siWrap531α Antisense	AAGGTTGATGGGATTGGGGTT
siWrap531β sense	AATCGGAAGGTGGACCGAAAT
siWrap531β Antisense	AAATTTCGGTCCACCTTCCGA

Table 2 Antibodies used in this study.

Antibody	Company	Dilution
GAPDH (10494-1-AP)	Proteintech	1:2500
WRAP53 (14761-1-AP)	Proteintech	1:1000
P53 (10442-1-AP)	Proteintech	1:1000
E-Cadherin (20874-1-AP)	Proteintech	1:1000
Vimentin (10366-1-AP)	Proteintech	1:1000
MMP9 (10375-2-AP)	Proteintech	1:1000
BAX (60267-1-Ig)	Proteintech	1:5000
BCL-2 (60178-1-Ig)	Proteintech	1:1000
CDK2 (10122-1-AP)	Proteintech	1:1000
CDK4 (11026-1-AP)	Proteintech	1:1000
CyclinE1 (11554-1-AP)	Proteintech	1:1000
CyclinD1 (60186-1-Ig)	Proteintech	1:5000
P21 (10355-1-AP)	Proteintech	1:1000
Tubulin (10094-1-AP)	Proteintech	1:1000

Figures

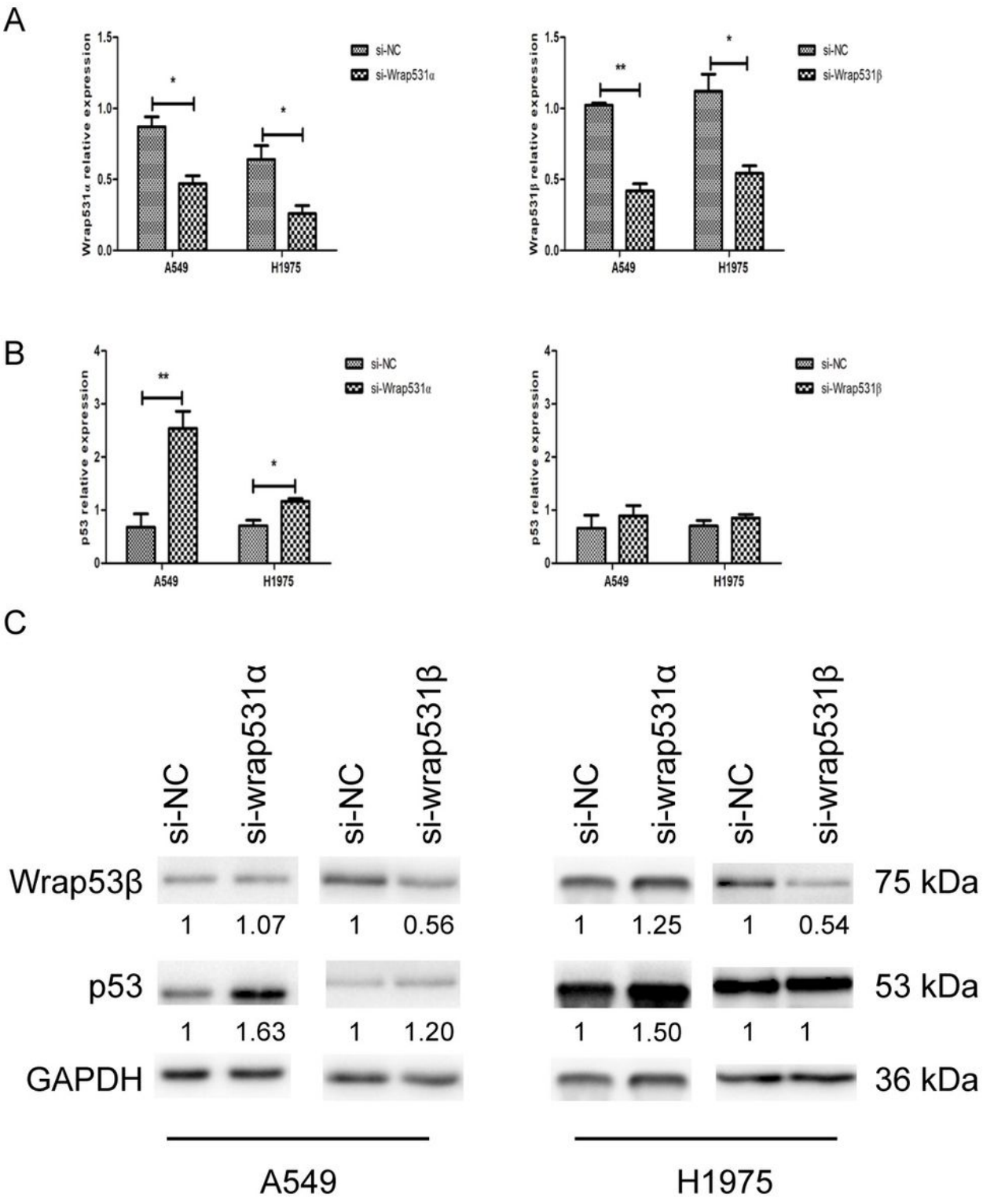

Figure 1

Knockdown of WRAP53-1α increases p53 expression. (A) qRT-PCR analysis of WRAP53-1α and WRAP53-1β mRNA levels in A549 and H1975 cells transfected with negative control siRNA (si-NC), WRAP53-1α siRNA (si-WRAP531α), or WRAP53-1β siRNA (si-WRAP531β). (B) qRT-PCR analysis of TP53 mRNA levels in A549 and H1975 cells transfected with siNC, siWRAP531α, or siWRAP531β. (C) Immunoblotting of

WRAP53β and p53 in A549 and H1975 cells transfected with si-NC, si-WRAP531α, or si-WRAP531β. GAPDH was used as an internal control. N = 3; *, P < 0.05; **, P < 0.01.

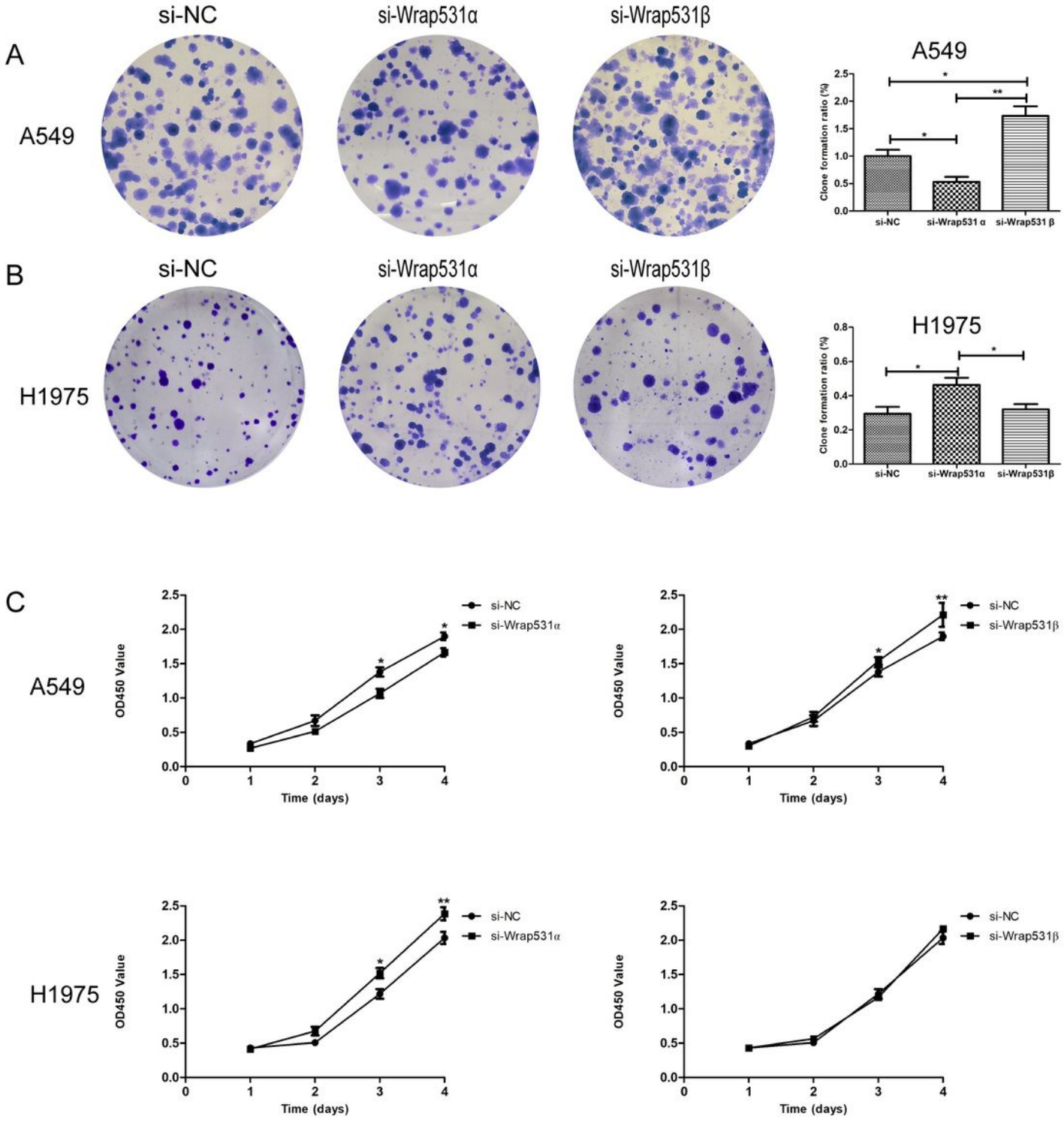

Figure 2

WRAP53-1α and WRAP53-1β have opposite effects on NSCLC cell proliferation. (A) Representative images of colony formation assays using A549 cells transfected with WRAP53 or control siRNA. Knockdown of WRAP53-1α reduced colony formation, whereas depletion of WRAP53-1β increased colony

formation. (B) Knockdown of WRAP53-1α increased colony formation in H1975 cells. (C) Knockdown of WRAP53-1α reduced A549 cell proliferation, whereas depletion of WRAP53-1β increased proliferation. In H1975 cells, knockdown of WRAP53-1α increased proliferation, and depletion of WRAP53-1β had no effect. N = 3; *, P < 0.05; **, P < 0.01.

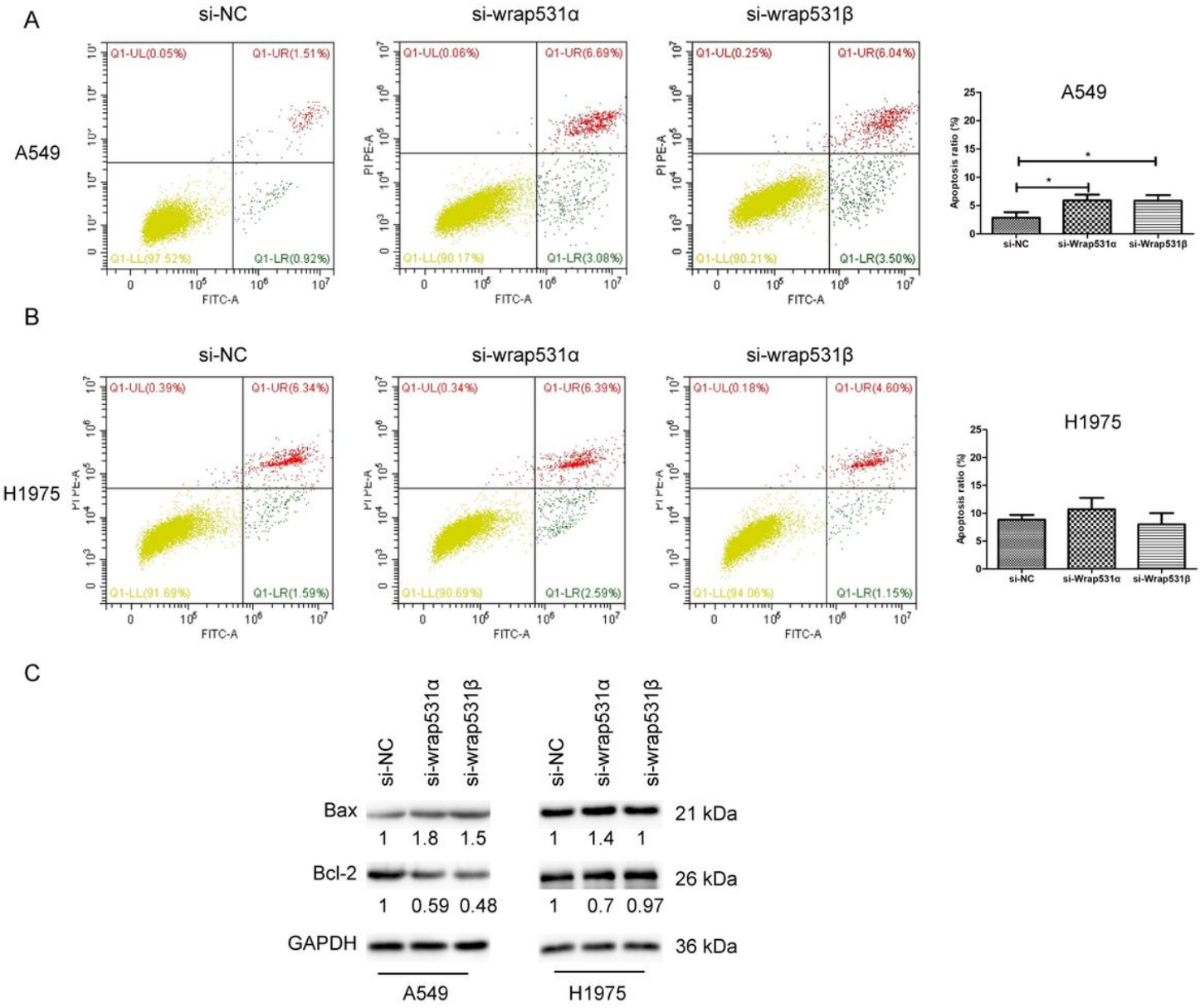

Figure 3

Knockdown of WRAP53-1α and WRAP53-1β promotes apoptosis in A549 cells but not in H1975 cells. (A) Knockdown of WRAP53-1α and WRAP53-1β increased apoptosis in A549 cells. (B) Flow cytometry analysis indicated that there was no significant difference in apoptosis in H1975 cells transfected with control, WRAP53-1α, or WRAP53-1β siRNA. (C) Protein levels of the apoptosis-related markers, Bax and Bcl-2, were assessed by immunoblotting. N = 3; *, P < 0.05.

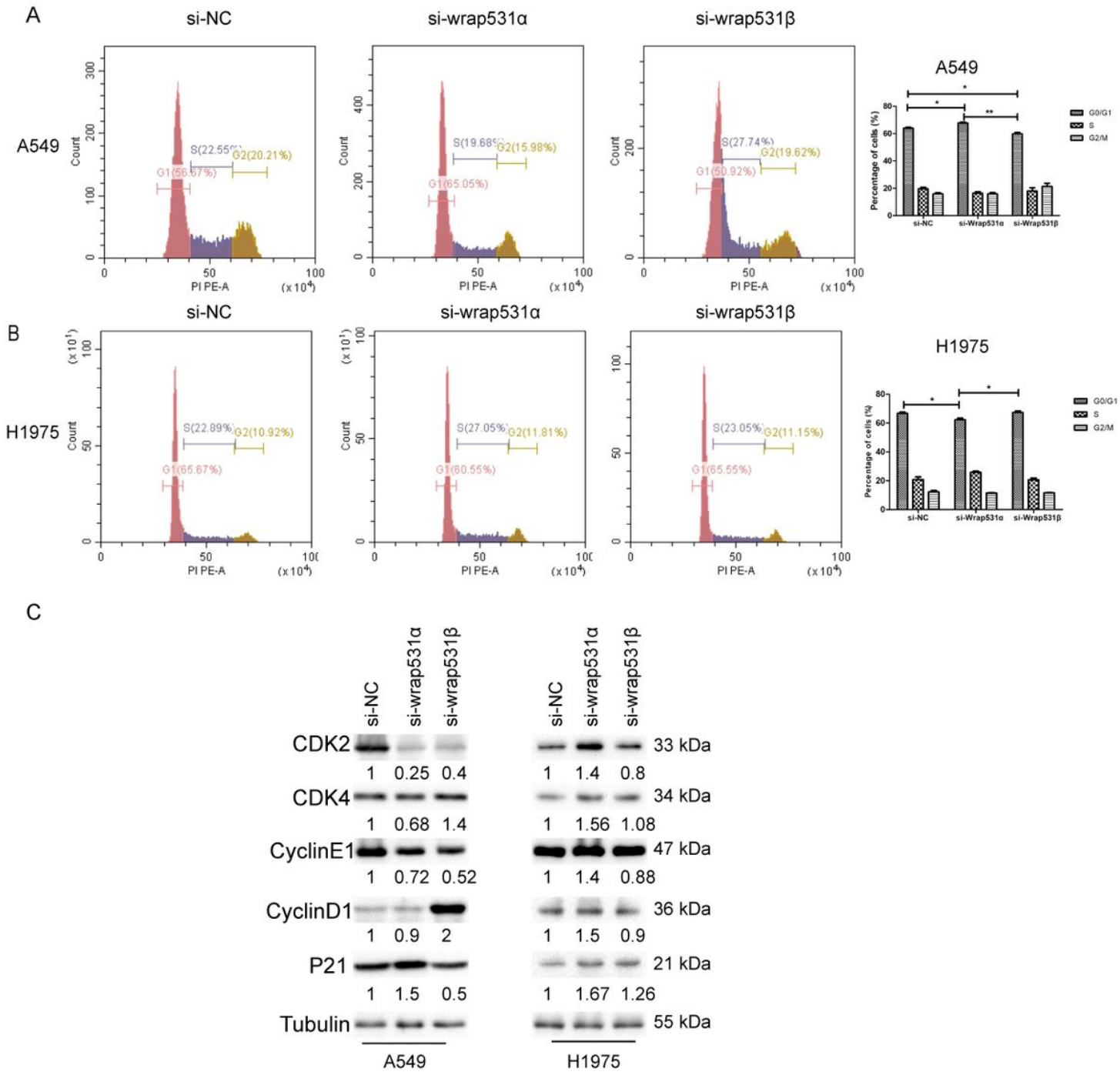

Figure 4

WRAP53-1α and WRAP53-1β have opposite effects on the cell cycle in NSCLC cells. (A) In A549 cells, knockdown of WRAP53-1α increased the proportion of cells at the G0/G1 phase, whereas WRAP53-1β deficiency increased the proportion of cells at the G0/G1 phase. (B) In H1975 cells, knockdown of WRAP53-1α increased the proportion of cells at the G0/G1 phase. (C) Protein levels of cell cycle regulation and checkpoint proteins were assessed by immunoblotting. N = 3; *, P < 0.05; **, P < 0.01.

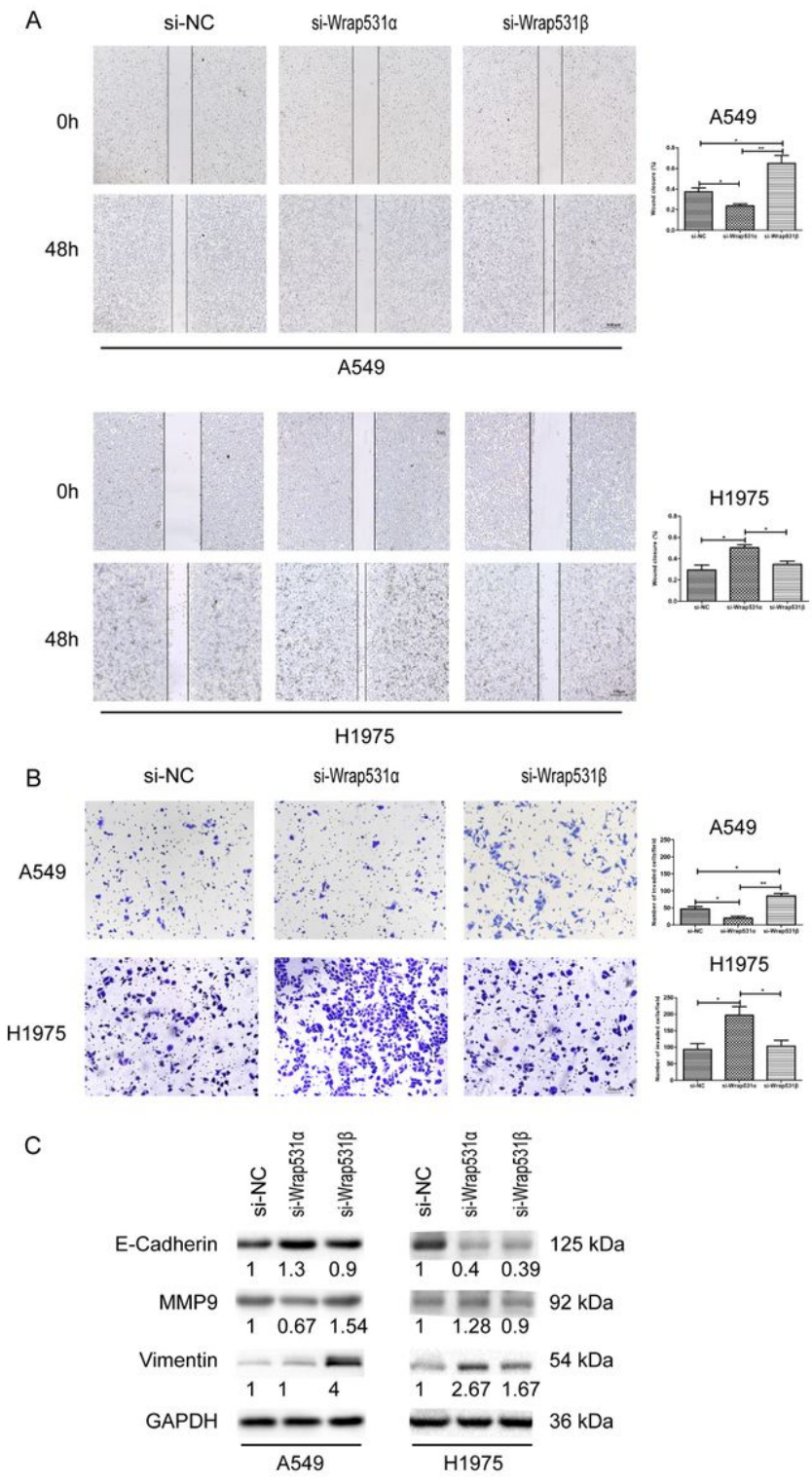

Figure 5

WRAP53-1α and WRAP53-1β have opposite effects on the invasion and migration of NSCLC cells. (A) Wound healing assay indicated that knockdown of WRAP53-1α blocked wound closure, and that WRAP53-1β knockdown promoted wound closure in A549 cells. Knockdown of WRAP53-1α accelerated wound closure in H1975 cells. The images were taken at 100× magnification. The scale bar is 100 μm. (B) Modified Boyden chamber assays indicated that knockdown of WRAP53-1α reduced cell invasion,

and that knockdown of WRAP53-1β increased cell invasion in A549 cells. Knockdown of WRAP53-1α increased invasion in H1975 cells. (C) Protein levels of the invasion-related markers E-cadherin, MMP9, and vimentin were assessed by immunoblotting. The images were taken at 100× magnification. The scale bar is 100 μm. N = 3; *, P < 0.05; **, P < 0.01.

Supplementary Files

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