

Original Article

Wrap53-1 α and Wrap53-1 β exert distinct p53-dependent effects on radiosensitivity in lung adenocarcinoma

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Abstract: This study elucidates the distinct roles of Wrap53 isoforms (1 α /1 β) in lung adenocarcinoma (LUAD) radiosensitivity through integrated bioinformatics and functional analyses. Bioinformatics revealed Wrap53-associated enrichment in DNA repair and p53 pathways, with TCGA data linking its expression to prognosis and chemotherapy response. Functional studies demonstrated isoform- and p53 context-dependent effects: In p53-wild type A549 cells, Wrap53-1 α silencing upregulated p53, enhanced radiosensitivity through Bax/Bcl-2-mediated apoptosis, and impaired DNA repair through RAD51 recombinase (RAD51)/Breast cancer susceptibility gene 1 (BRCA1) regulation. Conversely, in p53-mutant H1975 cells, silencing either Wrap53-1 α or Wrap53-1 β individually increased colony formation. Wrap53-1 α was found to modulate post-radiation G2/M arrest through cyclin-dependent kinase 1 (CDK1) regulation, with effects contingent on p53 status. Importantly, Wrap53 expression correlated with differential chemosensitivity. High Wrap53 tumors showed increased cisplatin sensitivity but resistance to docetaxel, while low-expressing tumors were resistant to paclitaxel and vinca alkaloids. Our findings reveal a previously unappreciated inverse correlation between Wrap53-1 α and p53 in LUAD cells and establish a molecular network where Wrap53 isoforms and p53 status collectively determine LUAD radiosensitivity.

Keywords: Wrap53-1 α , Wrap53-1 β , p53 dynamics, radio-sensitivity, lung adenocarcinoma

Introduction

Lung adenocarcinoma (LUAD) is the main subtype of non-small cell lung cancer (NSCLC) [1]. Current research efforts are focused on identifying more effective therapeutic targets to improve treatment outcome and survival rate for NSCLC patients. Radiotherapy remains a cornerstone of NSCLC treatment, serving both as an adjunct to local surgical intervention and as a component of combined-modality therapy with systemic agents such as chemotherapy, targeted therapy, and immunotherapy.

WD repeat-containing antisense to TP53 (Wrap53), officially designated as gene ID 55135, is located on chromosome 17p13. Its mRNA spans 1,837 bp, and its open reading frame comprises 1,647 bp [2]. The gene harbors three alternative initial exons, giving rise to two functionally distinct products: the antisense transcript Wrap53-1 α and the protein

Wrap53 β [3]. Exon 1 α shares up to 227 bp of complementarity with the first exon of TP53, producing Wrap53-1 α , a natural endogenous antisense transcript that maintains basal p53 mRNA levels and participates in p53-mediated DNA damage responses [4, 5]. Exon 1 β , located downstream of exon 1 α , encodes Wrap53 β , a WD40 repeat protein that localizes to both the cytoplasm, where it facilitates the translocation of survival motor neuron 1 protein, and the nucleus, specifically within Cajal bodies [6]. Upon DNA damage, Wrap53 β rapidly relocates to damage sites in an Ataxia telangiectasia-mutated-, Histone 2AX (H2AX)-, and Mediator of DNA damage checkpoint 1 (MDC1)-dependent manner, where it independently interacts with Ring finger protein 8 (RNF8) and MDC1 through its WD40 domain to promote their recruitment to double-strand breaks (DSBs) [7]. This directs RNF8-mediated ubiquitination of phosphorylated H2AX, thereby orchestrating the assembly of downstream DNA

repair factors [8]. Functionally, Wrap53 β knock-down leading to spontaneous DNA break accumulation and delayed radiation-induced cell cycle arrest [9, 10], whereas its overexpression enhances DSB repair capacity. Beyond its role in DNA repair, Wrap53 β is also critical for telomere maintenance [11], and mutations in its WD40 domain disrupt telomerase trafficking to telomeres, resulting in telomere shortening [12].

Wrap53 is upregulated in LUAD cells and tissues. Silencing Wrap53 significantly suppresses LUAD growth [13, 14]. Mechanistically, Wrap53 induces G0/G1 phase cell cycle arrest by downregulating G0/G1-associated cyclins and cyclin-dependent kinase complexes, while its knockdown triggers apoptosis through the mitochondrial pathway. Conversely, Wrap53 has also been shown to promote LUAD cell proliferation through multiple mechanisms, including co-localization and interaction with ubiquitin-specific protease 7 (USP7) in the nucleus to activate the USP7-mediated MDM2-p53 signaling axis, and stabilization of ubiquitin-like with PHD and ring finger domains 1 (UHRF1) [15]. These seemingly paradoxical functions suggest that Wrap53 plays a complex, context-dependent role in LUAD tumorigenesis.

The aforementioned studies indicate that Wrap53 is involved in the progression of LUAD. However, the molecular mechanisms underlying Wrap53's function in LUAD tissues and its prognostic significance remain incompletely understood. To elucidate the biological roles and molecular pathways of Wrap53 in LUAD, we systematically investigated gene regulatory networks associated with Wrap53-mediated regulation. We examined the Wrap53 expression in LUAD patients, as well as their sensitivity to anti-tumor drugs. Furthermore, we explored the relationship between distinct Wrap53 transcript variants and radiosensitivity.

Materials and methods

Download of TCGA database

The LUAD dataset, including corresponding clinical feature data, was downloaded and curated from The Cancer Genome Atlas (TCGA) (<https://www.cancer.gov/ccg/research/genome-sequencing/tcga>). This dataset comprised 498

LUAD patient samples. Only samples with both RNA-seq data and complete clinical information were retained for subsequent analysis. Clinical information that was unavailable or ambiguous was treated as missing values.

Analysis of functional and pathway enrichment

The Gene Ontology (GO) terms associated with Wrap53 and their corresponding annotations were retrieved from the GO database.

Samples were stratified into high- and low-expression groups based on the median Wrap53 expression level. Differential expression analysis was performed using the Limma package in R (version 4.2.1), with $|\text{Fold Change}| > 1.5$ and $P < 0.05$ set as thresholds for identifying significantly upregulated and downregulated genes. To elucidate the biological functions associated with the target mRNAs, the ClusterProfiler package was employed to conduct enrichment analyses, including GO annotation, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis, and HALLMARK gene set enrichment. Visualization was performed using the ggplot2 package. HALLMARK gene set enrichment analysis (GSEA) was carried out with the fgsea package in R. Genes were pre-ranked according to fold change between Wrap53 high- and low-expression groups. Significantly enriched gene sets were identified using a false discovery rate (FDR) cutoff of < 0.25 , consistent with standard GSEA practice.

Survival analysis

Clinical characteristics were correlated with Wrap53 gene expression levels. Univariate and multivariate Cox proportional hazards regression analyses were performed using the Survival package in R. Forest plots visualizing the results of both univariate and multivariate Cox analyses were generated using the forestplot package.

Samples were stratified into four groups according to the median expression levels of Wrap53 and TP53, and Kaplan-Meier (KM) survival analysis was carried out using the Survival and Survminer packages. Additionally, samples were classified into four groups based on the median Wrap53 expression level and

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TP53 mutational status (mutant vs. wild type), followed by KM survival analysis using the same packages.

Drug sensitivity analysis

IC₅₀ values for commonly used anti-tumor drugs in LUAD cell lines were obtained from the Genomics of Drug Sensitivity in Cancer (GDSC) database (<https://www.cancerrxgene.org/>). Wrap53 expression levels were dichotomized into high- and low-expression categories, and drug sensitivity analysis was conducted using the oncoPredict package. Results were visualized with the ggplot2 and ggpvr packages.

Cell culture

NCI-H1975 and A549 cells were cultured in RPMI-1640 medium (HyClone) containing 10% fetal bovine serum. All lines were obtained from the Chinese Academy of Sciences (CAS) Cell Bank.

Cell transfection

siRNAs against Wrap53-1 α , Wrap53-1 β , and a negative control were from Shanghai GenePharma. Transfection used jetPRIME reagent at 37°C (Polyplus-transfection® SA, France). The target sequences were as follows: si-Wrap53-1 α , AAAACCCCAATCCCATCAACC (sense) and AAGGTTGATGGGATTGGGGTT (antisense); si-Wrap53-1 β , AATCGGAAGGTGGACCGAAAT (sense) and AAATTCGGTCCACCTTCCGA (antisense); si-NC, UUCUCCGAACGUGUCACGUTT (sense) and ACGUGACACGUUCGAGAATT (antisense).

Quantitative PCR (q-PCR)

Total RNA was extracted using an RNA extraction kit (Vazyme, Nanjing, China) and reverse-transcribed into cDNA (Vazyme, Nanjing, China). SYBR Green-based quantitative PCR was performed on a Roche 480 detection system with GAPDH serving as the internal control. Primer sequences were as follows: Wrap53-1 α forward, 5'-CCGGAGCCCAGCAGC-TACCTG-3' and reverse, 5'-CATGGCGACTGTC-CAGCTTTG-3'; Wrap53-1 β forward, 5'-AGGAG-GGAAGCACAGTATGAAGAC-3' and reverse, 5'-GGCATCAGTTCAGAGTCCGCA-3'; GAPDH forward, 5'-GGAGCGAGATCCCTCCAAAAT-3' and reverse, 5'-GGCTGTTGCATACTTCTCATGG-3'.

Western blot

Equal amounts of protein (30 μ g) were separated and transferred onto PVDF for 90 min. Blocked membranes were incubated overnight with the following primary antibodies: WRAP53 β (14761-1-AP, Proteintech), BAX (60267-1-Ig, Proteintech), p53 (10442-1-AP, Proteintech), CDK1 (19532-1-AP, Proteintech), BCL-2 (60178-1-Ig, Proteintech), Cyclin B1 (55004-1-AP, Proteintech), BRCA1 (ab16781, Abcam), RAD51 (14961-1-AP, Proteintech), γ -H2AX (7631, CST), Tubulin (10094-1-AP, Proteintech), and GAPDH (10494-1-AP, Proteintech). Then the membranes were incubated with secondary antibodies for 1 h and subsequently developed using enhanced chemiluminescence (ECL) substrate (Fdbio, China).

Clone formation assay

Radiosensitivity was assessed by colony formation assay. Transfected cells were dissociated into single-cell suspensions and seeded into six-well plates at varying densities (100, 200, 400, 800, and 1,000 cells per well). Cells were then irradiated using a PXI X-RAD 225Cx small-animal irradiation platform at doses of 0, 1, 2, 4, 6, and 8 Gy, corresponding to different seeding densities. After irradiation, cells were cultured for 14 days. Colonies were fixed with 4% paraformaldehyde and stained with 0.5% crystal violet solution for 30 min. Colonies containing ≥ 50 cells were counted as individual clones.

Flow cytometry

Transfected cells were exposed to 4 Gy irradiation and subsequently cultured for an additional 48 h. Cells were stained with FITC-conjugated Annexin V for 20 min, and then stained with PI solution (Bestbio, China) for 5 min, then CytExpert 2.3 software was used (Beckman Coulter). For cell cycle analysis, the pre-prepared staining solution was added to resuspend the cells fully. The suspension was incubated at 37°C for 30 min, and cell cycle distribution was evaluated using CytExpert 2.3 software.

Statistical analysis

Statistical analyses were performed using GraphPad Prism 5.0 software. Data were presented as mean $\pm$ SD. For comparisons

Table 1. Clinical information of the TCGA dataset

Clinical characteristic	Group	N	Proportion (%)
Gender	Male	230	46.2
	Female	268	53.8
Age (years)	≤ 66	246	49.4
	> 66	242	48.6
	Unknown	10	2
Tumour stage	I	267	53.6
	II	118	23.7
	III	80	16.1
	IV	25	5
	Unknown	8	1.6
M stage	M0	330	66.3
	M+	24	4.8
	Unknown	144	28.9
N stage	N0	323	64.9
	N+	164	32.9
	Unknown	11	2.2
T stage	T1	167	33.5
	T2	265	53.2
	T3	45	9
	T4	18	3.6
	Unknown	3	0.7
DFS status: recurrence or non-recurrence	non-recurrence	241	48.4
	recurrence	183	36.7
	Unknown	74	14.9

DFS: Disease-free survival.

between two groups, an independent-sample t-test was used following confirmation of normality and homogeneity of variance. For comparisons involving three groups, one-way analysis of variance (ANOVA) followed by Tukey's post hoc test was applied. For colony formation assays involving multiple radiation doses across different transfection groups, two-way ANOVA followed by Bonferroni's *post hoc* test was employed to assess the main effects of transfection and radiation dose as well as their interaction.

Results

GO terms associated with Wrap53

Querying the GO database revealed that Wrap53 was involved in several core biological processes, including Cajal body formation (GO:0030576) and the localization of associated RNAs (e.g., telomerase RNA and scaRNA), DNA repair mechanisms (positive regulation:

GO:0006281, GO:2000781), regulation of telomerase activity and telomere maintenance (GO:0051973, GO:0007004), and the regulation of protein localization to specific structures such as telomeres and Cajal bodies (GO:1904851, GO:1904867).

Clinical characteristics of the TCGA cohort

Clinical and pathologic data of LUAD patients were retrieved from the TCGA database. Only patients with complete records for survival time, survival status, age, sex, tumor stage (T), nodal involvement (N), metastasis (M), and overall cancer stage were retained for subsequent analysis (**Table 1**).

Enrichment analysis of signaling pathways and functional annotations associated with Wrap53 in LUAD

GO analysis revealed that Wrap53-based DEGs were significantly enriched in microtubule and

cilia movement, microtubule bundle formation, and hormone activity-related molecules in LUAD (**Figure 1A**). Specifically, upregulated DEGs were enriched in cilia- or microtubule-dependent cell movement and microtubule bundle formation, whereas downregulated DEGs were enriched in regulation of T cell/leukocyte-mediated cytotoxicity, chemical homeostasis at the multicellular organism level (**Figure 1B**).

KEGG demonstrated that upregulated DEGs were enriched in pathways including the cell cycle, neuroactive ligand-receptor interaction, motor proteins, cytochrome P450 metabolism, and the p53 signaling pathway in LUAD. In contrast, downregulated DEGs showed significant enrichment in pathways related to protein digestion and absorption, viral protein interactions, hematopoietic cell lineage, and cytokine-cytokine receptor interactions (**Figure 1C**).

HALLMARK gene set enrichment analysis indicated that the Wrap53 low-expression group was associated with biological processes such as heme synthesis and degradation, immune signaling, inflammatory signaling pathways, KRAS signaling, cell proliferation, NF- κ B signaling, and TNF- α signaling. Conversely, the Wrap53 high-expression group was associated with E2F pathway, regulation of the G2/M checkpoint, DNA repair, and MYC signaling pathways (**Figure 1D** and **1E**).

Relationship between Wrap53 expression and prognosis of LUAD

Integrating Wrap53 gene expression levels with clinical characteristics, univariate Cox regression analysis revealed that overall stage, N, M, and T stage were significantly associated with survival outcome. Specifically, the hazard ratio increased progressively with advancing stage. Furthermore, multivariate Cox regression analysis demonstrated that N stage and T stage remained independently significant predictors of survival after adjusting for other covariates (**Figure 2A**).

Samples were stratified into four groups based on the median expression levels of Wrap53 and TP53: TP53 high/Wrap53 high, TP53 high/Wrap53 low, TP53 low/Wrap53 high, and TP53 low/Wrap53 low. KM survival analysis revealed no significant differences

among the four groups, either overall or in pairwise comparisons (**Figure 2B**).

Additionally, samples were categorized into four groups according to the median Wrap53 expression level and TP53 mutational status (mutant vs. wild-type): TP53 mutant/Wrap53 high, TP53 mutant/Wrap53 low, TP53 wild-type/Wrap53 high, and TP53 wild-type/Wrap53 low. KM survival analysis similarly showed no significant differences among the four groups, either overall or in pairwise comparisons (**Figure 2C**).

Association between the expression of Wrap53 and drug sensitivity

Wrap53 expression levels were dichotomized into high- and low-expression groups. Drug sensitivity analysis revealed that Wrap53 was associated with sensitivity to cisplatin, temozolomide, and etoposide, whereas low Wrap53 expression was associated with enhanced sensitivity to crizotinib. In addition, high Wrap53 expression was correlated with resistance to docetaxel, vinblastine, and vinorelbine, while low Wrap53 expression was associated with resistance to paclitaxel (**Figure 3A** and **3B**).

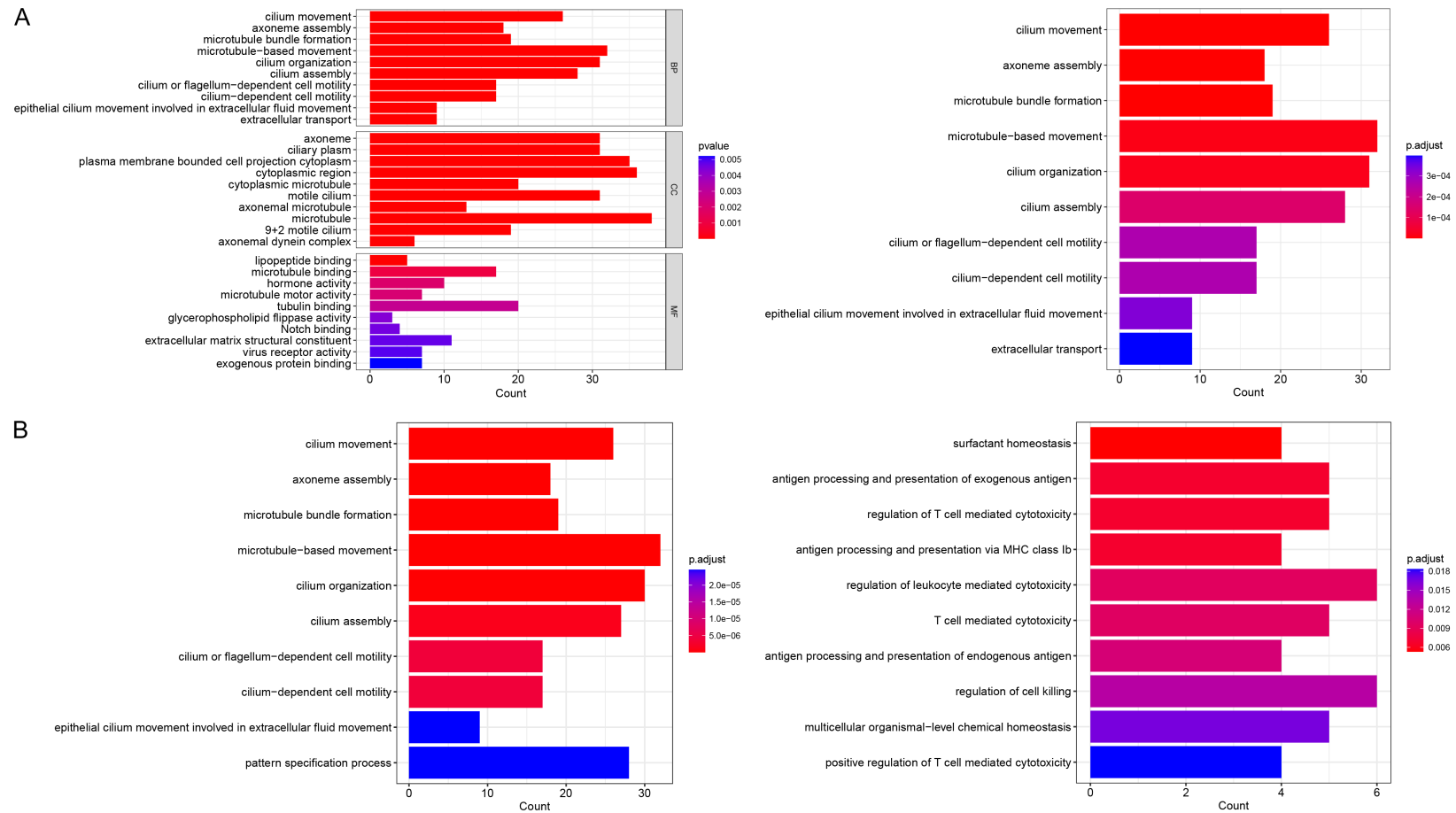
Inhibition of Wrap53-1 α correlates with increased p53 expression, whereas inhibition of Wrap53-1 β had no effect on p53 expression

Transfection with si-Wrap53-1 α significantly decreased Wrap53-1 α mRNA in A549 and H1975 cells, while si-Wrap53-1 β selectively reduced Wrap53-1 β levels without affecting Wrap53-1 α expression (**Figure 4A**, **4B**). Knockdown of Wrap53-1 α , but not Wrap53-1 β , increased p53 mRNA levels (**Figure 4C**). si-Wrap53-1 β , but not si-Wrap53-1 α , reduced Wrap53 β protein levels. Conversely, si-Wrap53-1 α upregulated p53 protein, while si-Wrap53-1 β had no effect (**Figure 4D**).

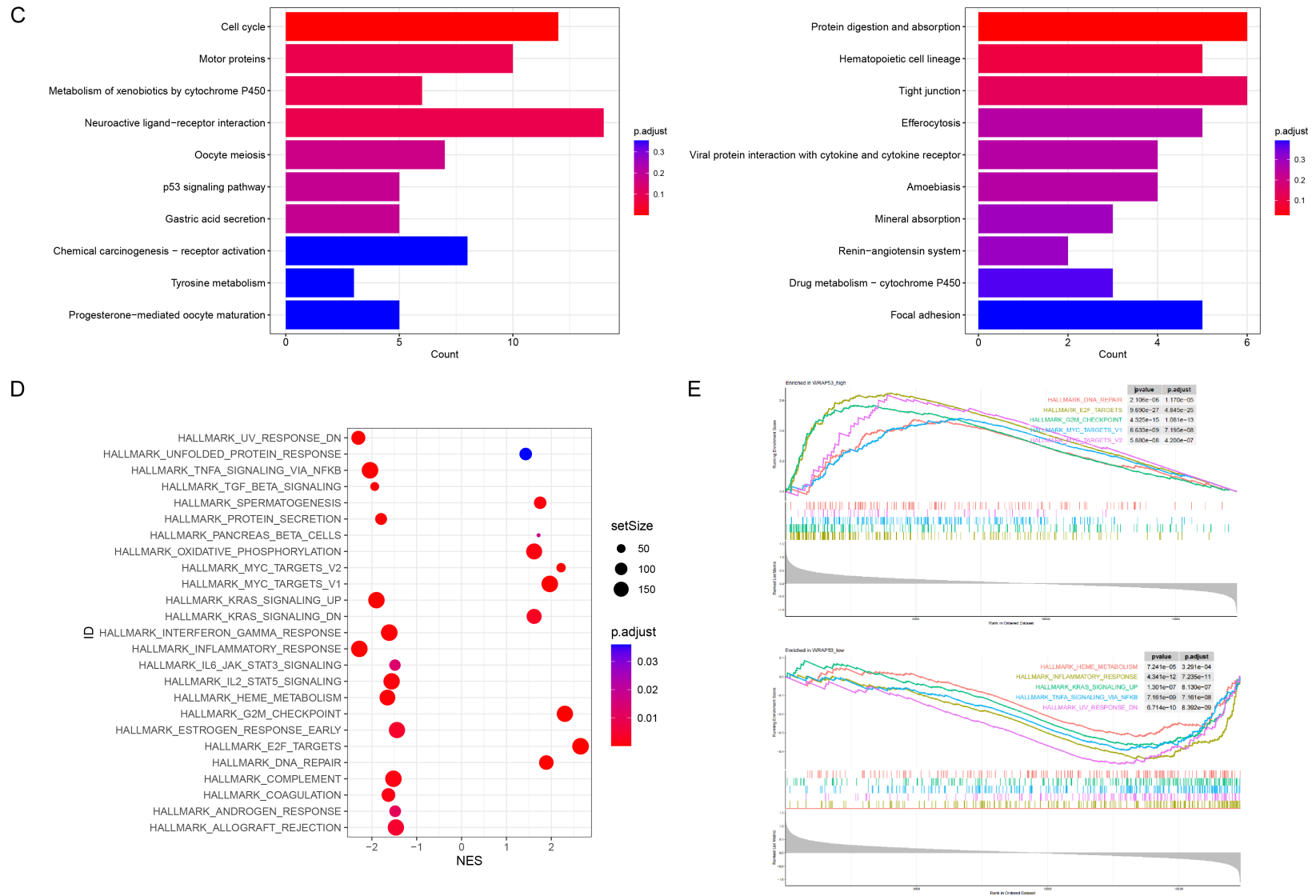
Wrap53-1 α and Wrap53-1 β exerted distinct influences on the radiosensitivity

si-Wrap53-1 α reduced colony formation in A549 cells, while si-Wrap53-1 β increased it (**Figure 5A**). In H1975 cells, both siRNAs promoted colony formation (**Figure 5B**). Wrap53-1 α modulates the radiosensitivity of LUAD cells and that its regulatory effect is contingent upon p53 mutational status.

Wrap53-1 α /1 β exert p53-dependent effects on radiosensitivity

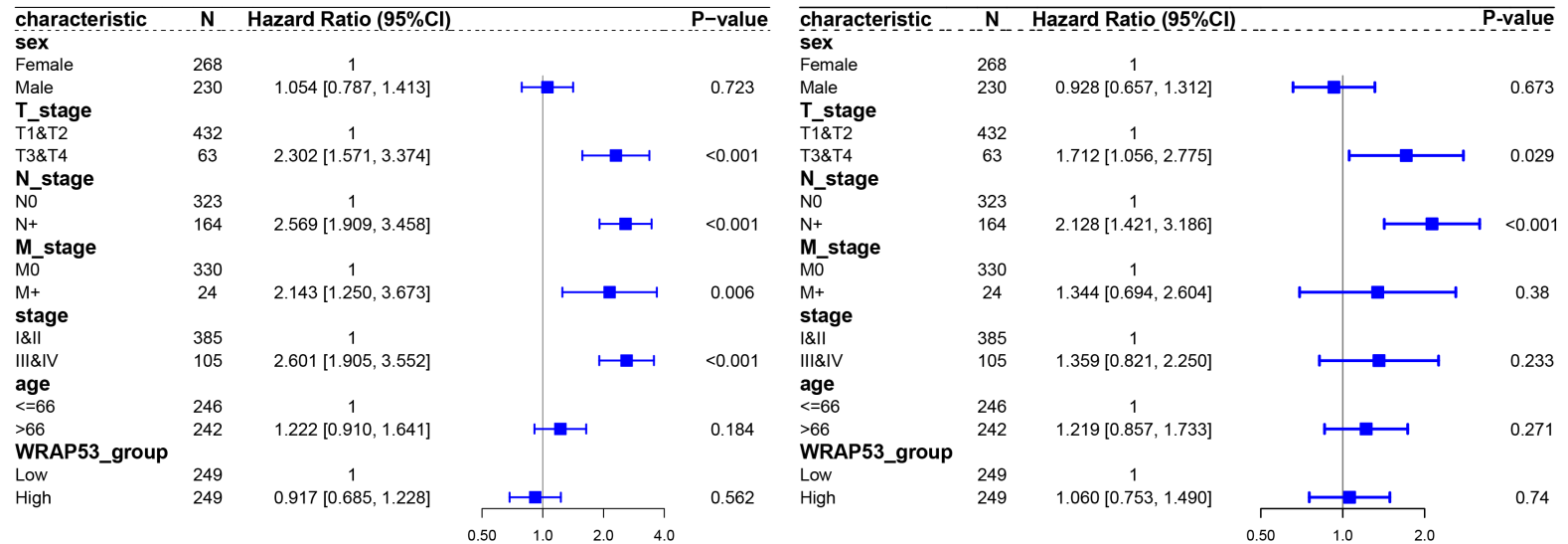


Wrap53-1 α /1 β exert p53-dependent effects on radiosensitivity

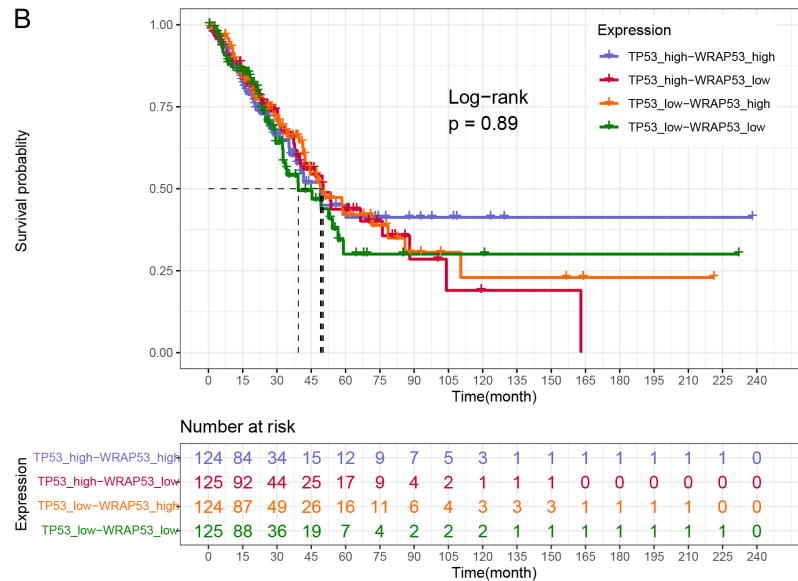


Wrap53-1α/1β exert p53-dependent effects on radiosensitivity

A



B



C

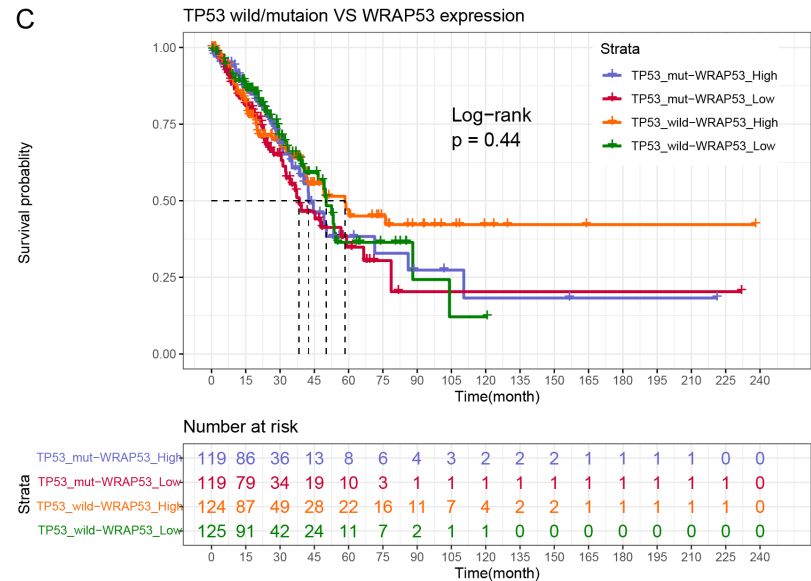


Figure 2. Relationship between Wrap53 expression and prognosis of LUAD. A. Univariate (left) and multivariate (right) Cox regression forest plots. B. Kaplan-Meier survival curves stratified by high/low expression of Wrap53 and TP53. C. Kaplan-Meier survival curves stratified by Wrap53 expression and TP53 mutational status (wild-type vs. mutant).

Wrap53-1 α /1 β exert p53-dependent effects on radiosensitivity

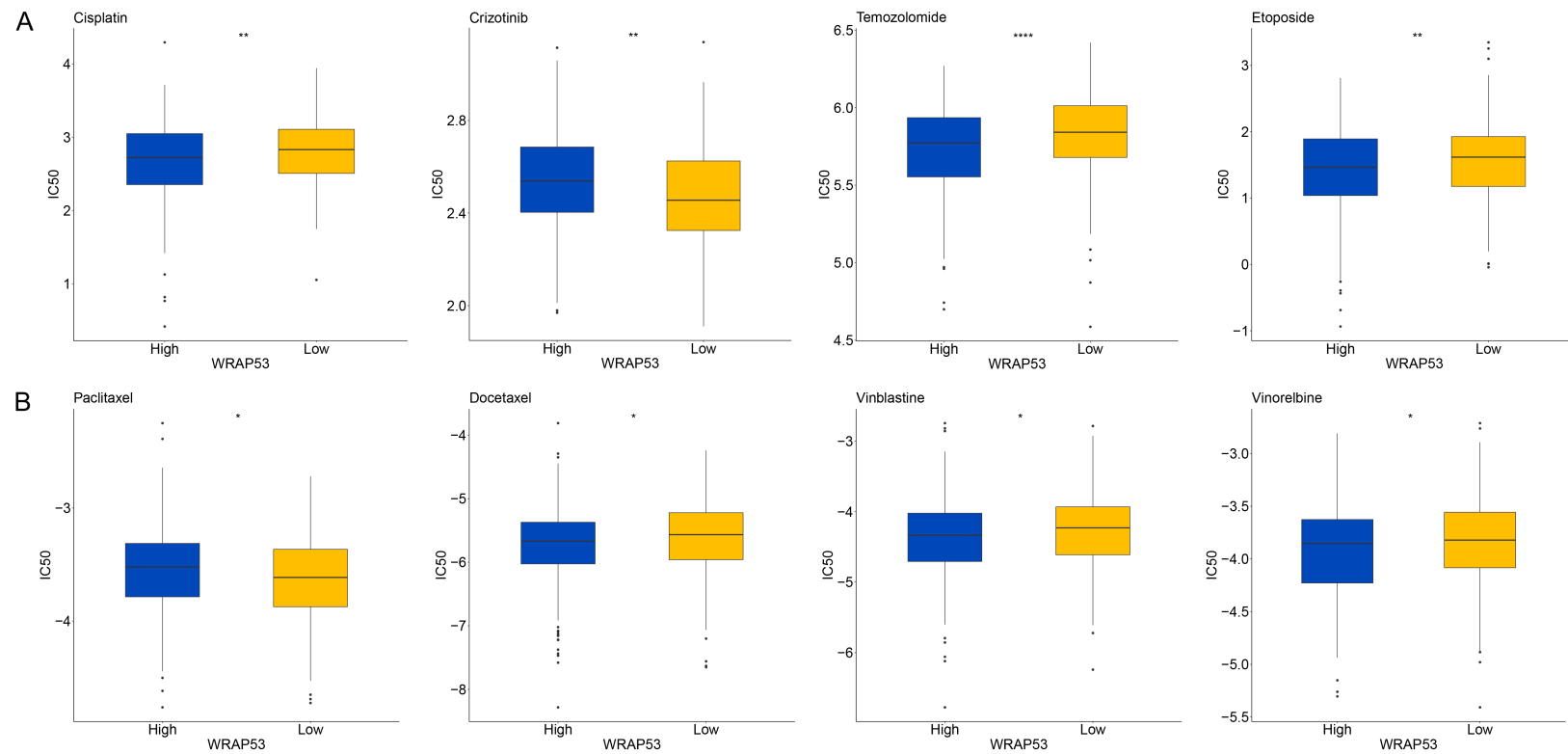


Figure 3. Association between the expression of Wrap53 and drug sensitivity. A. Effect of high versus low Wrap53 expression on drug sensitivity across different drugs. B. Impact of high versus low Wrap53 expression on drug resistance across different drugs. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.

Wrap53-1 α /1 β exert p53-dependent effects on radiosensitivity

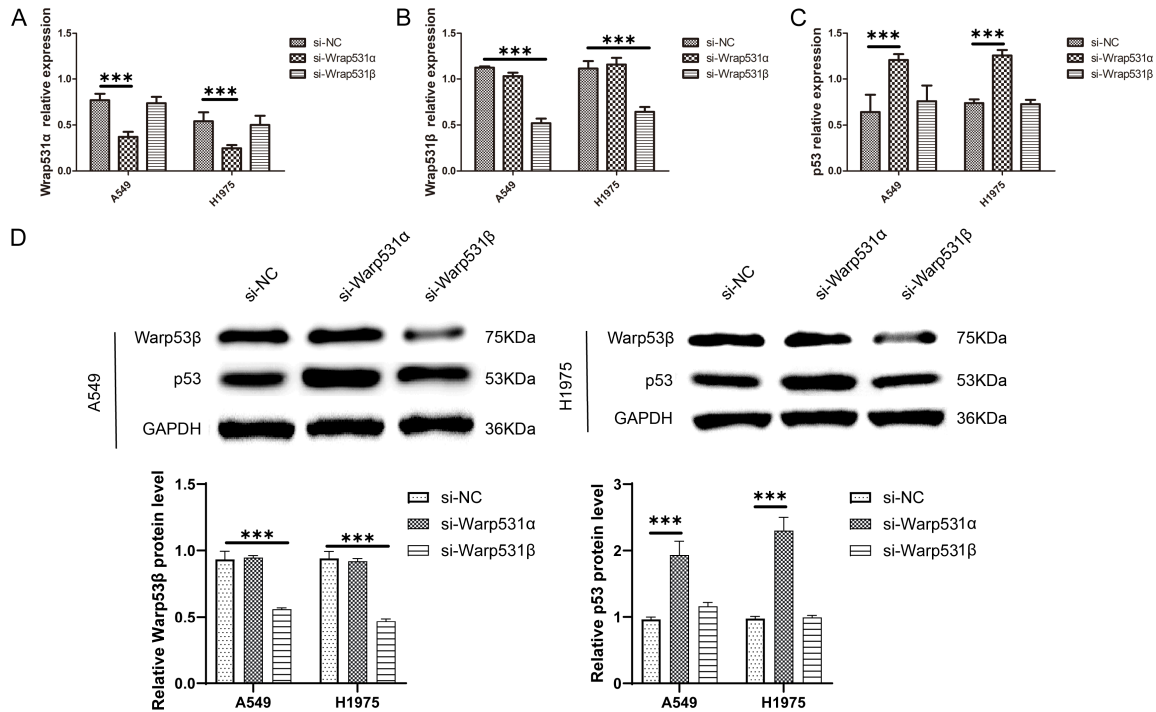


Figure 4. Inhibition of Wrap53-1 α led to the induction of p53 expression, whereas inhibition of Wrap53-1 β had no effect on p53 expression. A, B. qRT-PCR analysis of Wrap53-1 α and Wrap53-1 β mRNA levels in A549 and H1975 cells transfected with si-NC, si-Wrap53-1 α , or si-Wrap53-1 β without irradiation. C. qRT-PCR analysis of p53 mRNA levels in A549 and H1975 cells transfected with si-NC, si-Wrap53-1 α , or si-Wrap53-1 β without irradiation. D. Western blot analysis of Wrap53 β and p53 protein levels in A549 and H1975 cells transfected with si-NC, si-Wrap53-1 α , or si-Wrap53-1 β without irradiation. GAPDH served as the loading control. N = 3; ***P < 0.01.

Inhibition of Wrap53-1 α induced apoptosis in LUAD cells following radiation exposure

Flow cytometry analysis demonstrated that silencing Wrap53-1 α triggered apoptosis post-irradiation, whereas silencing Wrap53-1 β did not have a significant impact on apoptosis (**Figure 6A** and **6B**). Western blot analysis revealed that knockdown of Wrap53-1 α upregulated Bax expression and downregulated Bcl-2 expression. In contrast, si-Wrap53-1 β group showed no significant changes (**Figure 6C**).

Wrap53-1 α and Wrap53-1 β exert distinct impacts on cell cycle arrest in LUAD cells following radiation exposure

Silencing of Wrap53-1 α and Wrap53-1 β decreased G2/M phase arrest in A549 cells (**Figure 7A**), whereas in H1975 cells, silencing of both transcripts increased the G2/M phase (**Figure 7B**). In A549 cells, knockdown of Wrap53-1 α and Wrap53-1 β upregulated CDK1 expression, while in H1975 cells, knockdown of

both transcripts downregulated CDK1 expression (**Figure 7C**). These findings indicate that the regulation of post-radiation cell cycle arrest by Wrap53-1 α and Wrap53-1 β in LUAD cells is influenced by TP53 mutational status.

Wrap53-1 α and Wrap53-1 β played distinct roles in DNA damage repair in LUAD cells following radiation exposure

Western blot analysis showed that silencing Wrap53-1 α and Wrap53-1 β following radiation exposure increased γ -H2AX protein levels while concurrently reducing RAD51 and BRCA1 protein levels. While in H1975 cells, neither si-Wrap53-1 α nor si-Wrap53-1 β led to a detectable increase in γ -H2AX protein levels at 48 h post-irradiation relative to si-NC controls; instead, a marginal reduction was observed in both knockdown groups. Furthermore, transfection with si-Wrap53-1 α significantly upregulated RAD51 and BRCA1 protein expression in H1975 cells, whereas no notable differences in RAD51 or BRCA1 levels were observed

Wrap53-1 α /1 β exert p53-dependent effects on radiosensitivity

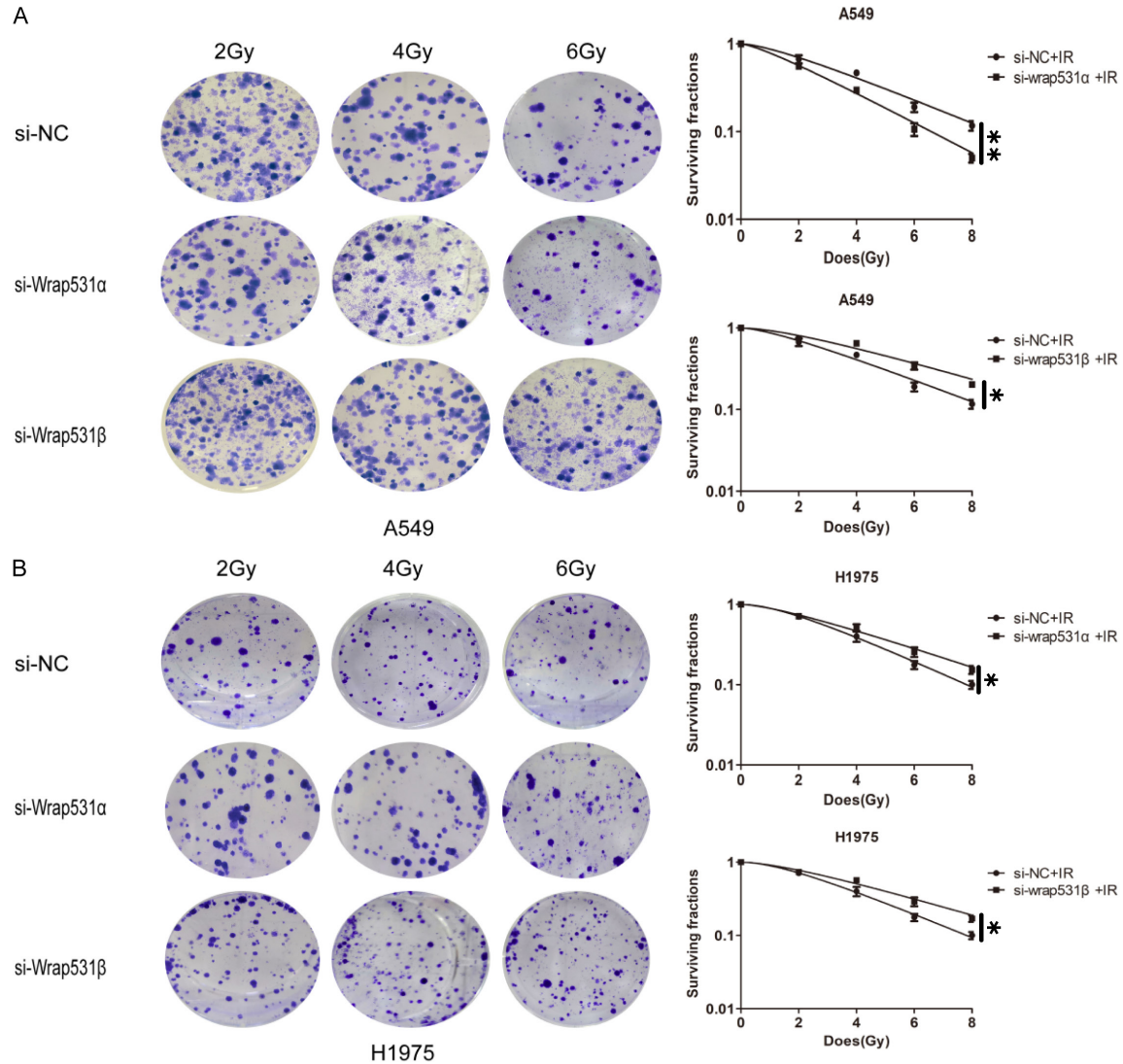


Figure 5. Wrap53-1 α and Wrap53-1 β exerted distinct influences on the radiosensitivity of LUAD cells. (A, B) Cell clone formation was performed in A549 (A) and H1975 (B) cells transfected with si-NC, si-Wrap53-1 α , or si-Wrap53-1 β . N = 3; *P < 0.05, **P < 0.01.

between si-NC and si-Wrap53-1 β groups following irradiation (**Figure 8**). These results suggest that the modulation of DNA damage repair by Wrap53-1 α and Wrap53-1 β transcripts in irradiated LUAD cells is dependent on p53 mutational status.

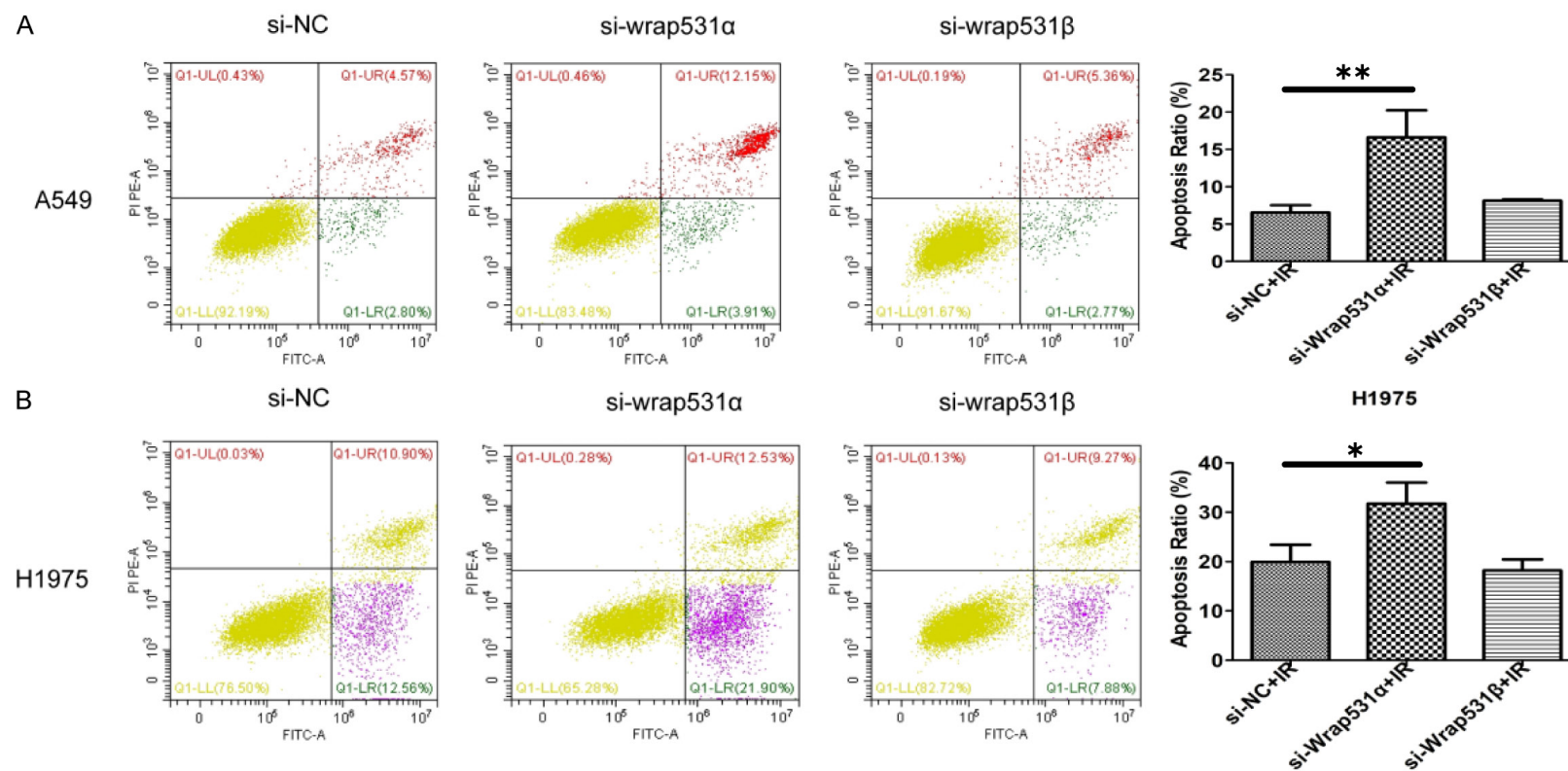
Discussion

In this study, GO analysis revealed that Wrap53-associated DEGs in LUAD were enriched in antigen processing and presentation, T cell-mediated cytotoxicity, and multicellular chemical homeostasis. KEGG and HALLMARK analyses further demonstrated enrichment in

the cell cycle, p53 signaling, DNA repair, E2F/G2/M checkpoint activation, and immune/inflammatory signaling pathways. The complex interplay among these pathways suggests that Wrap53's functional role may vary with LUAD subtype and disease stage.

Of particular interest, the linkage between Wrap53 and cytochrome P450 metabolism identified by KEGG analysis was corroborated by our drug sensitivity analysis. This is consistent with prior reports that chemotherapeutic agents differentially modulate Wrap53 and p53 expression in a drug-context-dependent manner [4, 16-19]. Several factors may recon-

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C

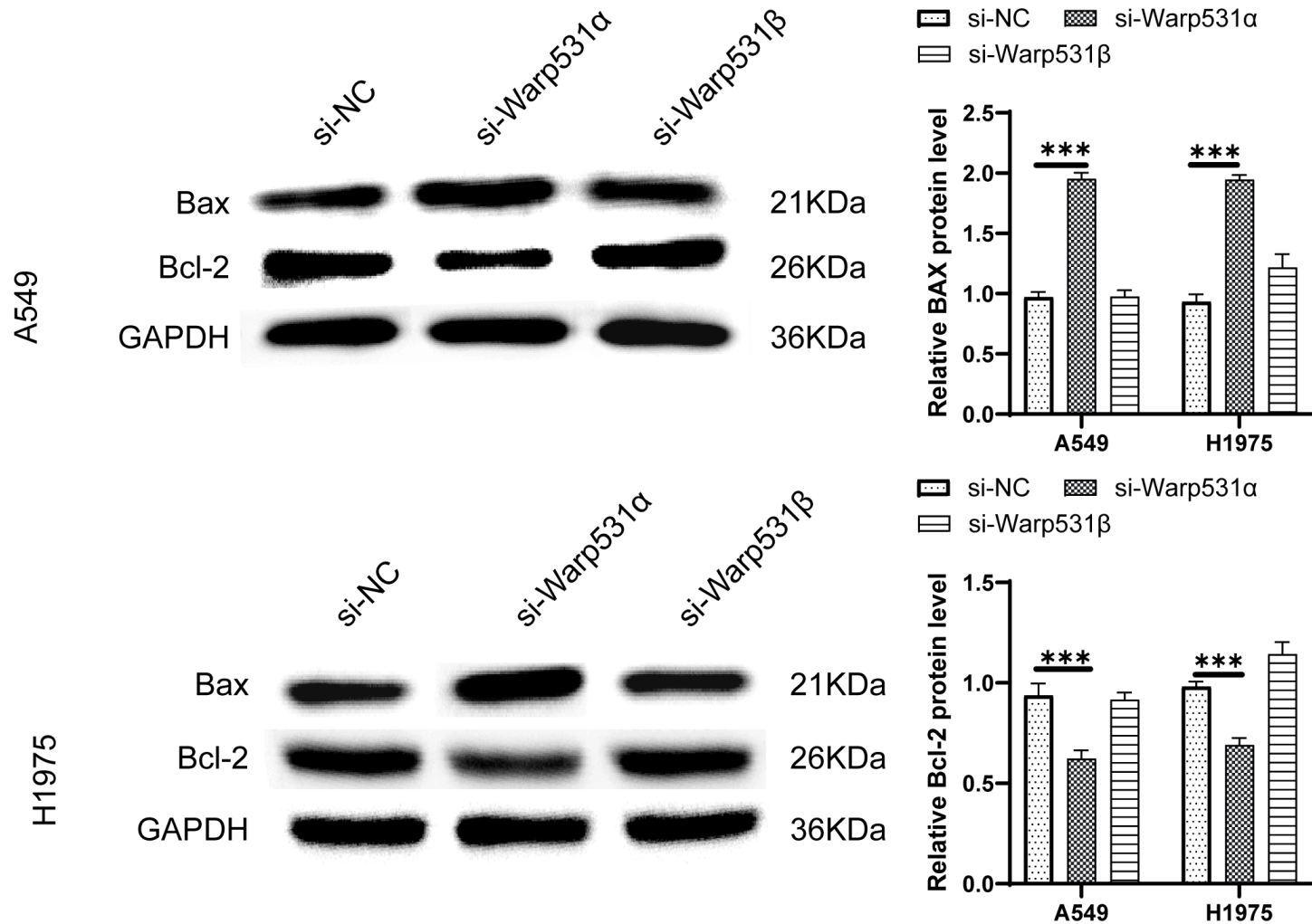


Figure 6. Inhibition of Wrap53-1 α induced apoptosis in LUAD cells following radiation exposure. (A, B) Flow cytometry analysis of apoptosis (Annexin V/PI) in (A) A549 and (B) H1975 cells after 4 Gy irradiation (IR). (C) Western blot analysis was performed to examine the protein levels of apoptosis-related markers Bax and Bcl-2. N = 3; *P < 0.05, **P < 0.01, ***P < 0.001.

Wrap53-1 α /1 β exert p53-dependent effects on radiosensitivity

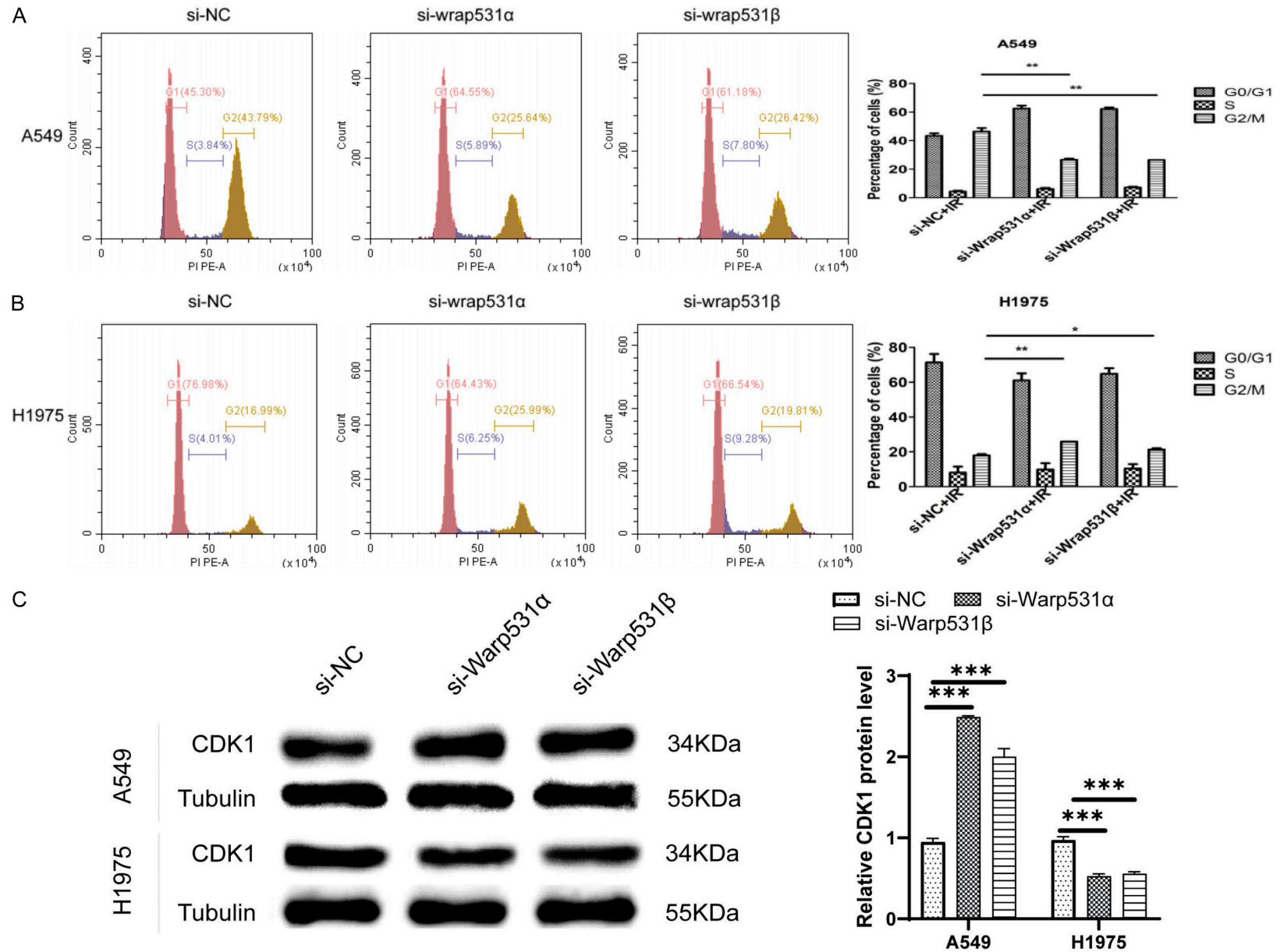


Figure 7. Wrap53-1 α and Wrap53-1 β exert distinct effects on cell cycle arrest in LUAD cells following radiation exposure. (A, B) Cell cycle distribution in (A) A549 and (B) H1975 cells transfected as indicated after 4 Gy irradiation. (C) Western blot analysis was performed to examine the protein levels of cell cycle regulators and checkpoint proteins. N = 3; *P < 0.05, **P < 0.01, ***P < 0.001. CDK1: Cyclin Dependent Kinase 1.

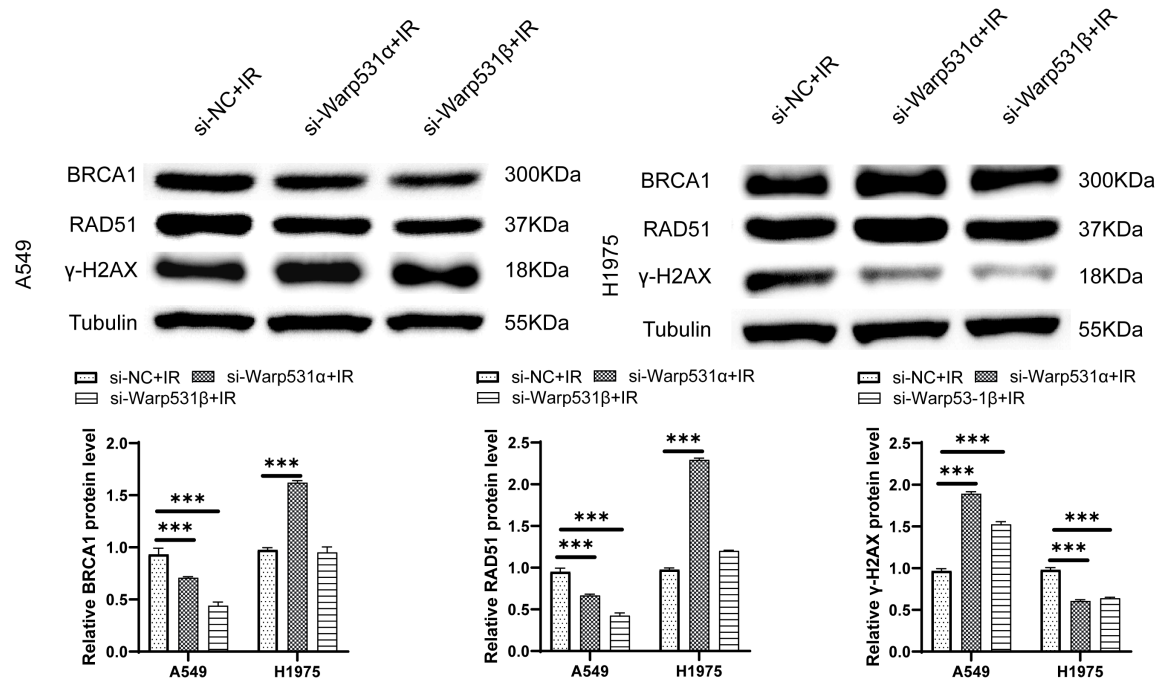


Figure 8. Wrap53-1 α and Wrap53-1 β played distinct roles in DNA damage repair in LUAD cells following radiation exposure. Western blot analysis of γ -H2AX, RAD51 recombinase (RAD51) and Breast cancer susceptibility gene 1 (BRCA1) in A549 and H1975 cells transfected with si-NC, si-Wrap53-1 α , or si-Wrap53-1 β . ***P < 0.001.

cile the apparent discrepancy between our observation that high Wrap53 expression correlates with cisplatin sensitivity while Wrap53-1 α silencing enhances radiosensitivity in A549 cells. The GDSC drug sensitivity data reflect basal chemosensitivity in unirradiated cells, whereas our functional experiments specifically measured clonogenic survival following ionizing radiation, a fundamentally distinct genotoxic stress that activates the DNA damage response and alters the cellular context in which Wrap53 operates. Moreover, the GDSC analysis captures total Wrap53 expression without distinguishing between the 1 α and 1 β isoforms, which our data show can exert divergent effects. Interestingly, although docetaxel and paclitaxel both belong to the taxane family, high Wrap53 expression correlated with resistance to docetaxel whereas low Wrap53 expression correlated with resistance to paclitaxel. This divergent association may reflect the known pharmacologic differences between the two agents: docetaxel exhibits higher affinity

for β -tubulin and distinct drug efflux pump susceptibility compared to paclitaxel, which could differentially interact with Wrap53-mediated cellular pathways. A similar drug-specific effect within the same class has also been observed for Wrap53-associated chemosensitivity to different topoisomerase II inhibitors [16], further supporting the context-dependent nature of Wrap53's role in drug response.

With respect to clinical relevance, prior studies identified Wrap53 β as an independent adverse prognostic factor in NSCLC [20]. In the present study, Cox regression revealed that Wrap53 expression was associated with T and N stage, but survival analysis did not identify a significant prognostic association for Wrap53 alone or in combination with TP53 status, possibly due to limited sample size. The intricate Wrap53-p53 interplay underscores the need for further mechanistic studies to guide therapeutic development.

It is important to note that our observation, that silencing Wrap53-1 α leads to increased p53 levels, appears to contradict the canonical function of Wrap53-1 α in stabilizing p53 mRNA [4]. This canonical function showed that Wrap53-1 α depletion decreased p53 levels in unperturbed colon cancer cells, the opposite of our finding in irradiated LUAD cells. Given that researchers demonstrated Wrap53 β acts as a scaffold protein in DNA repair, it is plausible that under radiation, the concurrent activity of both isoforms creates a regulatory environment distinct from that in unperturbed cells [7]. Furthermore, antisense transcript-mediated regulation can be bidirectional and context-dependent [5, 20]. It is also noteworthy that Wrap53-1 β knockdown did not alter p53 levels, arguing against a generalized off-target effect. Therefore, our data suggest an inverse relationship between Wrap53-1 α and p53 in this specific context, warranting further investigation.

Wrap53 expression has been linked to radiosensitivity across multiple cancer types, yet the contributions of individual transcript variants remain largely unexplored [21-26]. We found that radiosensitizing effects of Wrap53-1 α and Wrap53-1 β were isoform-specific and p53 status-dependent. In p53 wild-type A549 cells, Wrap53-1 α knockdown enhanced radiosensitivity, as evidenced by reduced colony formation, decreased G2/M arrest, increased DNA damage, and induction of Bax/Bcl-2-mediated apoptosis, whereas in p53-mutant H1975 cells, it exerted the opposite effect. Conversely, Wrap53-1 β knockdown promoted radioresistance in both cell lines through distinct mechanisms: impaired DNA repair with reduced G2/M in A549, and enhanced G2/M without significant DNA repair changes in H1975 cells. Although both si-Wrap53-1 α and si-Wrap53-1 β reduced G2/M arrest in A549, only si-Wrap53-1 α triggered significant apoptosis. This suggests that the differential effect on apoptosis, rather than cell cycle arrest alone, determines the ultimate radiosensitivity outcome in these cells. Notably, in A549 cells, si-Wrap53-1 β increased γ -H2AX levels and reduced RAD51/BRCA1 expression at 48 h post-irradiation, indicating impaired DNA double-strand break repair. Paradoxically, this did not translate into enhanced radiosensitivity, as clonogenic survival was increased. This dis-

crepancy may reflect a delay rather than a complete failure of DNA repair, or the engagement of compensatory survival pathways that ultimately promote long-term clonogenic growth despite early DNA damage signaling. Wrap53-1 α knockdown induced apoptosis in both cell lines, but since H1975 cells harbor the TP53 R273H mutation with potential gain-of-function activities, whether the upstream mechanisms are identical remains to be determined. Wrap53-1 β silencing did not significantly affect apoptosis.

Wrap53-1 α modulates p53 expression and radiosensitivity in a p53-context-dependent manner, whereas Wrap53-1 β primarily contributes to DNA repair and cell cycle checkpoint regulation. Our data demonstrate that Wrap53-1 α /1 β regulate G2/M arrest in opposing directions depending on p53 status, suggesting that targeting specific Wrap53 transcripts may offer a therapeutic window for radiosensitization, particularly in p53-mutant tumors that rely on G2 arrest due to deficient G1 checkpoint function [27-29].

Several limitations should be acknowledged. First, our functional experiments were conducted in only two LUAD cell lines; validation in a broader panel and *in vivo* models is warranted. Second, rescue experiments were not performed, precluding definitive exclusion of off-target effects. Third, γ -H2AX levels were assessed at a single time point, which does not distinguish between altered repair kinetics and changes in initial DNA damage. Fourth, the retrospective TCGA analysis and limited sample size may account for the lack of significant prognostic associations. Future studies incorporating time-course analyses, isoform-specific rescue experiments, and *in vivo* models will be essential to extend these findings. Ultimately, a deeper understanding of Wrap53 isoform biology may inform precision radiotherapy strategies that consider both the specific Wrap53 transcript targeted and the p53 status of the tumor.

Conclusion

Wrap53-1 α and Wrap53-1 β exerted distinct, p53 status-dependent effects on radiosensitivity in LUAD cells. Wrap53-1 α modulated p53 expression and radiosensitivity in a context-dependent manner, while Wrap53-1 β primarily

regulated DNA repair and cell cycle checkpoint control. These findings highlight the importance of considering individual Wrap53 transcript variants, rather than total Wrap53 expression, in the development of precision radiotherapy strategies for LUAD.

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Disclosure of conflict of interest

None.

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