# Silencing of heat shock protein 27 increases the radiosensitivity of non-small cell lung carcinoma cells

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**Abstract.** Radiotherapy is a useful treatment for malignant tumors, including lung carcinoma; however, non-small cell lung carcinoma (NSCLC) is frequently insensitive to radiation. It has been reported that heat shock protein 27 (HSPB1) is a radioresistance-associated protein in nasopharyngeal carcinoma. In the present study, the role of HSPB1 in NSCLC cells induced by irradiation was investigated. The viability of cells was determined by a Cell Counting Kit-8 assay. The apoptotic activity, cell cycle distribution and mitochondrial membrane potential (MMP) of cells were evaluated via flow cytometry. Reverse transcription-quantitative polymerase chain reaction and western blot analyses were employed to measure the expression of various genes and proteins. It was observed that knockdown of HSPB1 with small interfering RNA (si-HSPB1) markedly decreased the viability of A549 NSCLC cells and induced cell cycle arrest in the G2/M phase following exposure to 6 Gy irradiation. Furthermore, it was revealed that si-HSPB1 significantly downregulated cyclin B1 and cyclin G1 expression. Additionally, si-HSPB1 promoted apoptosis and depolarized the MMP of cells exposed to 6 Gy irradiation. The expression levels of B-cell lymphoma-2 (Bcl-2), mitochondrial cytochrome $c$ (cyto $c$) and pro-caspase-8 were downregulated, whereas those of Bcl-2 associated X protein (Bax), cytosolic cyto $c$ and cleaved-caspase-8 were upregulated. Collectively, silencing of HSPB1 increased the radiosensitivity of NSCLC cells by reducing cell viability, depolarizing the MMP, arresting the cell cycle in the G2/M phase and promoting cell apoptosis. Therefore, HSPB1 may be a novel target for increasing radiosensitivity in the treatment of NSCLC.

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## Introduction

Lung cancer (LC) is the most common malignant tumor globally, and the leading cause of cancer-associated mortality (1). The incidence of LC is second to prostate cancer in all malignant tumors in men and second only to breast cancer in women (1). LC is separated into two subtypes, small cell lung carcinoma (SCLC) and non-small cell lung carcinoma (NSCLC); NSCLC accounts for 70-85% of total LC cases (2). Due to the absence of clinical symptoms during early stages, patients are frequently diagnosed with LC at advanced stages, therefore missing the ideal period for operation; the total surgical removal rate of NSCLC is only ~25% (3). Radiotherapy is an effective treatment for malignant tumors, including LC (4), and is frequently combined with exeresis and chemotherapy in the treatment of LC (5).

Radiotherapy mainly involves treatment with ionizing radiation (IR), which, by direct or indirect actions, leads to the damage of cellular DNA and subsequently results in cell death (6,7). The efficacy of radiotherapy depends on the sensitivity of tumor cells to radiation; tumors can become insensitive to radiation via the radioresistance of tumor cells or the protection of the tumor microenvironment, leading to the repair of damaged cells following IR (7,8). NSCLC cells frequently develop varying degrees of radiation tolerance in the middle and later stages of radiotherapy, reducing its efficacy (9-11). Therefore, it is important to identify the underlying mechanisms to increase the radiosensitivity of NSCLC tumors.

Heat shock proteins (HSPs) are a family of important proteins with molecular chaperone functions in stress situations, including cold, infection, starvation, trauma, poisoning, and particularly in high temperature conditions; HSPs are involved in the folding, assembly and modification of proteins, and promote the tolerance of cells to stress (12,13). HSP27 (also known as HSPB1) is an important member of the HSP family; HSP27 is involved in endoplasmic reticulum stress associated with protein misfolding, and is a regulator that contributes to the development of various types of tumors (14-16). HSP27 expression has been reported to be upregulated in various tumor cells, including NSCLC, hepatocellular carcinoma and prostate cancer cells (17-20). Additionally, it was observed that HSP27 was a radioresistance-associated protein in nasopharyngeal carcinoma (21);

however, whether HSP27 is involved in radiosensitivity in NSCLC remains unclear.

In the present study, the HSP27 gene was silenced in human NSCLC A549 cells, and the effects of HSP27 silencing on the viability, apoptosis, mitochondrial membrane potential (MMP) and cell cycle of irradiated cells was determined. Additionally, the underlying molecular mechanisms were investigated.

## Materials and methods

*Cell culture and transfection*. The human lung adenocarcinoma A549 cell line was purchased from American Type Culture Collection (Manassas, VA, USA). A549 cells were cultured in RPMI-1640 medium (Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) with 10% fetal bovine serum (Gibco; Thermo Fisher Scientific, Inc.), 100 U/ml penicillin and 100 $\mu$g/ml streptomycin (Beyotime Institute of Biotechnology, Shanghai, China) at 37°C with 5% $CO_2$. HSPB1-small interfering RNA (si-HSPB1) and siRNA negative control (NC) plasmids were obtained from Genewiz Inc. (Suzhou, China). The sequences of siRNA used in the current study were as follows: si-HSPB1 sense, 5'-UGAGACUGC CGCCAAGUAAUU-3' and antisense, 5'-UUACUUGGCGGC AGUCUCAUU-3'; si-NC sense, 5'-UAGCGACUAAACACA UCAAUU-3' and antisense, 5'-UUGAUGUGUUUAGUCGCU AUU-3'. A549 cells ($2x10^5$ cells/well) were transfected with si-HSPB1 (50 nM) or si-NC (50 nM) using Lipofectamine® 3000 (Thermo Fisher Scientific, Inc.). The mixture containing si-HSPB1 or si-NC plasmids (15 $\mu$g), transfection reagents and A549 cell was incubated for 48 h at 37°C. Following transfection for 48 h, the cells were harvested and used for the subsequent experiments.

*Cell Counting Kit-8 (CCK-8) assay*. Following transfection, cells ($2x10^3$ cells/well, 96-well plates) were subjected to irradiation (0 and 6 Gy) for 24, 48, and 72 h using a PRIMUS™ Linear Accelerator (Siemens AG, Munich, Germany); the dose rate was 300 cGy/min. Following treatment, CCK-8 reagent (MedChemExpress, Monmouth Junction, NJ, USA) was added to the cells, and cells were further incubated for 4 h at 37°C. The absorbance was detected at 450 nm using a light absorption microplate reader (SpectraMax iD3; Molecular Devices, LLC, Sunnyvale, CA, USA).

*Cell apoptosis assay*. Following transfection and irradiation, cells were washed three times with PBS. Subsequently, cells were incubated with 1X Annexin Binding Buffer (BestBio, Shanghai, China) on ice for 3 min and then stained using Annexin V-fluorescein isothiocyanate and propidium iodide (PI) (BestBio) in darkness for 25 min at room temperature. Cell apoptosis was detected using a flow cytometer (FACSCalibur; BD Biosciences, San Jose, CA, USA) equipped with CellQuest software (version 3.3; BD Biosciences).

*Mitochondrial membrane potential (MMP) assay*. Cells were digested by 0.25% EDTA-trypsin (Thermo Fisher Scientific, Inc.) for 5 min at 37°C and stained with JC-1 solution (MedChemExpress) for 20 min at 37°C. Then, cells were centrifuged at 800 x g for 2 min at 4°C. Cells were collected and the MMP was investigated using a flow cytometer.

*Cell cycle assay*. The cell cycle was investigated using a DNA Content Quantitation Assay (Cell Cycle) kit (Beijing Solarbio Science & Technology Co., Ltd., Beijing, China) according to the manufacturer's protocols. Briefly, following transfection and irradiation, cells were treated with 40X RNase A at 37°C for 20 min. Then, cells were stained with PI solution at 4°C for 15 min. Cell cycle distribution was analyzed using a flow cytometer.

*Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) assay*. TRIzol® reagent (Thermo Fisher Scientific, Inc.) was used to isolate total RNA from cells. cDNA was synthesized using the ABScript II cDNA First Strand Synthesis kit (ABclonal Biotech Co., Ltd., Wuhan, China) according to the manufacturer's protocols. The RT reaction was performed at 42°C for 15 min, followed by reverse transcriptase inactivation at 85°C for 15 sec. A SYBR Premix Taq™ II kit (Dalian Meilun Biotech Co., Ltd., Dalian, China) was used to amplify cDNA according to the manufacturer's protocols. The qPCR thermocycling conditions were: Preliminary denaturation at 95°C for 1 min, followed by 30 cycles of denaturation at 94°C for 30 sec, annealing at 56°C for 40 sec and extension at 72°C for 60 sec, followed by a final extension step at 72°C for 7 min. The primers were presented in Table I. GAPDH was used as an internal reference. The $2^{-\Delta\Delta Cq}$ method was (22) used to quantify relative gene expression.

*Western blot assay*. Total protein was isolated from cells using radioimmunoprecipitation assay buffer (Beijing Solarbio Science & Technology Co., Ltd.). Cytosolic and mitochondrial proteins were extracted using a Cytoplasmic and Mitochondrial Protein Extraction kit (Sangon Biotech Co., Ltd., Shanghai, China). The Bradford method was employed to determine the concentration of protein extracts. Proteins (30 $\mu$g/sample) were separated via 10% SDS-PAGE and then transferred to polyvinylidene difluoride membranes (EMD Millipore, Billerica, MA, USA). The membranes were blocked with 5% non-fat milk at 37°C for 1 h, and then incubated at 4°C overnight with primary antibodies, including anti-HSPB1 (1:500; cat. no. ab216610; Abcam, Cambridge, UK), anti-cyclin B1 (1:600; cat. no. ab32053; Abcam), anti-cyclin G1 (1:800; cat. no. sc-8016; Santa Cruz Biotechnology, Inc., Dallas, TX, USA), anti-B-cell lymphoma-2 (Bcl-2; 1:1,000; cat. no. MA5-11757, Invitrogen; Thermo Fisher Scientific, Inc.), anti-Bcl-2 associated X protein (Bax; 1:1,000; cat. no. ab53154; Abcam), anti-pro-caspase-8 (1:700; cat. no. MAB704; R&D Systems, Inc., Minneapolis, MN, USA), anti-cytochrome *c* (cyto *c*; 1:1,000; cat. no. MAB897; R&D Systems, Inc.), anti-cleaved caspase-8 (1:600; cat. no. 9429; Cell Signaling Technology, Inc., Danvers, MA, USA), anti-cyto *c* oxidase IV (1:100; cat. no. ab33985; Abcam) and anti-GAPDH (1:800; cat. no. ab8245; Abcam). Membranes were then incubated at 37°C for 90 min with horseradish peroxidase-conjugated secondary antibodies [mouse anti-rabbit immunoglobulin G (IgG); 1:8,000; cat. no. 31464, Invitrogen; Thermo Fisher Scientific, Inc.; and goat anti-mouse IgG; 1:8,000; cat. no. ab97023, Abcam]. Protein bands were visualized using

Table I. Primer sequences.

| Primer | Sequence (5'-3') | Product size (bp) |
|---|---|---|
| HSPB1-Forward | AGTGGTCGCAGTGGTTAGG | 223 |
| HSPB1-Reverse | TCCTTGGTCTTGACCGTCAG | |
| Cyclin B1-Forward | TTGTGTGCCCAAGAAGATGC | 205 |
| Cyclin B1-Reverse | GAAGTGCAAAGGTAGAGGCC | |
| Cyclin G1-Forward | CCTTCTGTGTTGGCATTGTCT | 221 |
| Cyclin G1-Reverse | GTACGCCCAGAAACAATCCA | |
| Bcl-2-Forward | TTCTTTGAGTTCGGTGGGGT | 209 |
| Bcl-2-Reverse | CTTCAGAGACAGCCAGGAGA | |
| Bax-Forward | CATCATGGGCTGGACATTGG | 226 |
| Bax-Reverse | CCTCAGCCCATCTTCTTCCA | |
| GAPDH-Forward | CCATCTTCCAGGAGCGAGAT | 222 |
| GAPDH-Reverse | TGCTGATGATCTTGAGGCTG | |

Bcl-2, B-cell lymphoma-2; Bax, Bcl-2-associated X protein; HSPB1, heat shock protein 27.

enhanced chemiluminescence detection reagent (Thermo Fisher Scientific, Inc.) and the densitometry was performed using the Bio-Rad ChemiDoc system with Image Lab software version 6.0 (Bio-Rad Laboratories, Inc., Hercules, CA, USA).

*Statistical analysis.* All data were presented as the mean ± standard deviation. All experiments were performed in triplicate. Data were analyzed using GraphPad Prism 6.0 (GraphPad Software, Inc., La Jolla, CA, USA). Differences were analyzed using Student's t-tests or one-way analyses of variance followed by Tukey's post hoc test. $P<0.05$ was considered to indicate a statistically significant difference.

## Results

*Silencing of HSPB1 promotes the radiosensitivity of NSCLC cells by reducing viability, arresting the cell cycle, depolarizing the MMP and promoting apoptosis.* RT-qPCR and western blot analyses demonstrated that the expression levels of HSPB1 in A549 cells were significantly downregulated following transfection with si-HSPB1 compared with the NC (Fig. 1), with a knockdown efficiency of >40%. A CCK-8 assay revealed that irradiation with 6 Gy significantly decreased the viability of cells at 48 and 72 h compared with 0 Gy irradiation (Fig. 2A). Furthermore, irradiation with 6 Gy significantly increased the apoptotic rate by >10% compared with no irradiation (0 Gy), whereas the number of red fluorescent cells decreased by ~30% following irradiation (Fig. 2B-E). In Fig. 2B the upper right quadrant is the advanced apoptotic cells, and the lower right quadrant was the early apoptotic cells. The rate of apoptotic cells is the sum of the rate of early and advanced apoptotic cells. Furthermore, arrest of the cell cycle in the G2/M phase was markedly promoted by irradiation when compared with the corresponding 0 Gy group (Fig. 3). In si-HSPB1 group, the percentage of cells in S phase was notably decreased, whereas the percentage of cells in G2/M phase was markedly increased following irradiation with 6 Gy compared with the NC group. Furthermore, si-HSPB1 notably enhanced the effects of

radiation on the viability, apoptosis, cell cycle distribution and MMP of NSCLC cells (Figs. 2 and 3).

*Silencing of HSPB1 increases the radiosensitivity of NSCLC cells by altering the expression of cell cycle- and apoptosis-associated genes.* To investigate the mechanisms underlying the effects of irradiation and si-HSPB1 on the cell cycle, the expression of associated genes and proteins was determined by RT-qPCR and western blot assays. It was revealed that irradiation of A549 cells with 6 Gy significantly downregulated the mRNA levels of cyclin B1 and cyclin G1 compared with the corresponding 0 Gy control group (Fig. 4A and B). Additionally, transfection of cells with si-HSPB1 significantly decreased the levels of cyclin B1 and cyclin G1 mRNA compared with the NC, and promoted the effects of irradiation on expression. The expression of apoptosis-associated genes was also investigated. Irradiation significantly increased the expression of Bax and decreased that of Bcl-2 compared with 0 Gy, and transfection with si-HSPB1 exhibited similar effects on expression when compared with the NC group (Fig. 4C and D). Additionally, transfection of irradiated cells with si-HSPB1 further promoted the effects of irradiation. Furthermore, western blotting revealed that 6 Gy irradiation and si-HSPB1 similarly affected the expression of cyclin B1, cyclin G1, Bax and Bcl-2 at the protein level (Fig. 5). It was also demonstrated that irradiation and si-HSPB1 significantly decreased the expression of pro-caspase-8 and increased that of cleaved caspase-8 compared with their respective controls.

*Silencing of HSPB1 and irradiation increase the expression of cytosolic cyto c and reduce the expression of mitochondrial cyto c.* Increased mitochondrial membrane permeability is associated with the opening of the mitochondrial permeability transition pore (23). To investigate the molecular mechanisms underlying the effects of HSPB1 silencing and irradiation on the MMP, the expression of cyto c was determined by western blotting. It was revealed that 6 Gy irradiation significantly decreased the mitochondrial levels of cyto c and increased
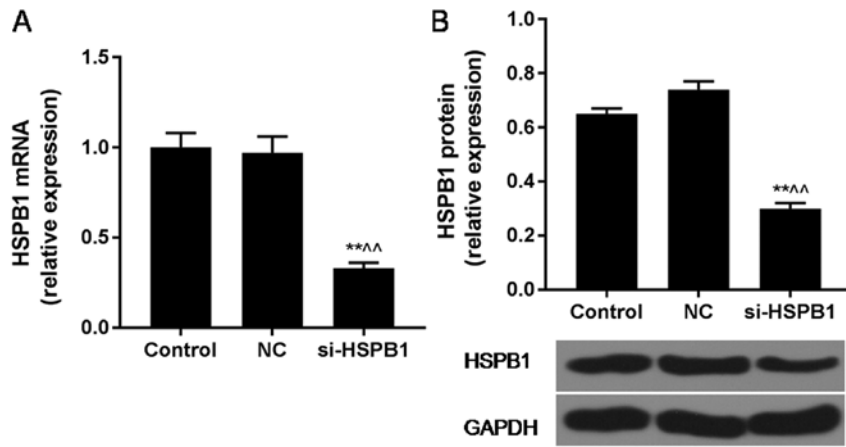
Figure 1. Transfection efficiency of HSPB1 in non-small cell lung carcinoma cells. (A) Expression of HSPB1 mRNA in A549 cells following transfection with si-HSPB1 and NC plasmids, as determined by reverse transcription-quantitative polymerase chain reaction analysis. (B) Expression of HSPB1 protein in transfected A549 cells, as determined by western blot analysis. Data are presented as the mean ± standard deviation. $^{**}$P<0.01 vs. control; $^{\wedge\wedge}$P<0.01 vs. NC. HSPB1, heat shock protein 27; NC, negative control; si-HSPB1, small interfering RNA specific for HSPB1.
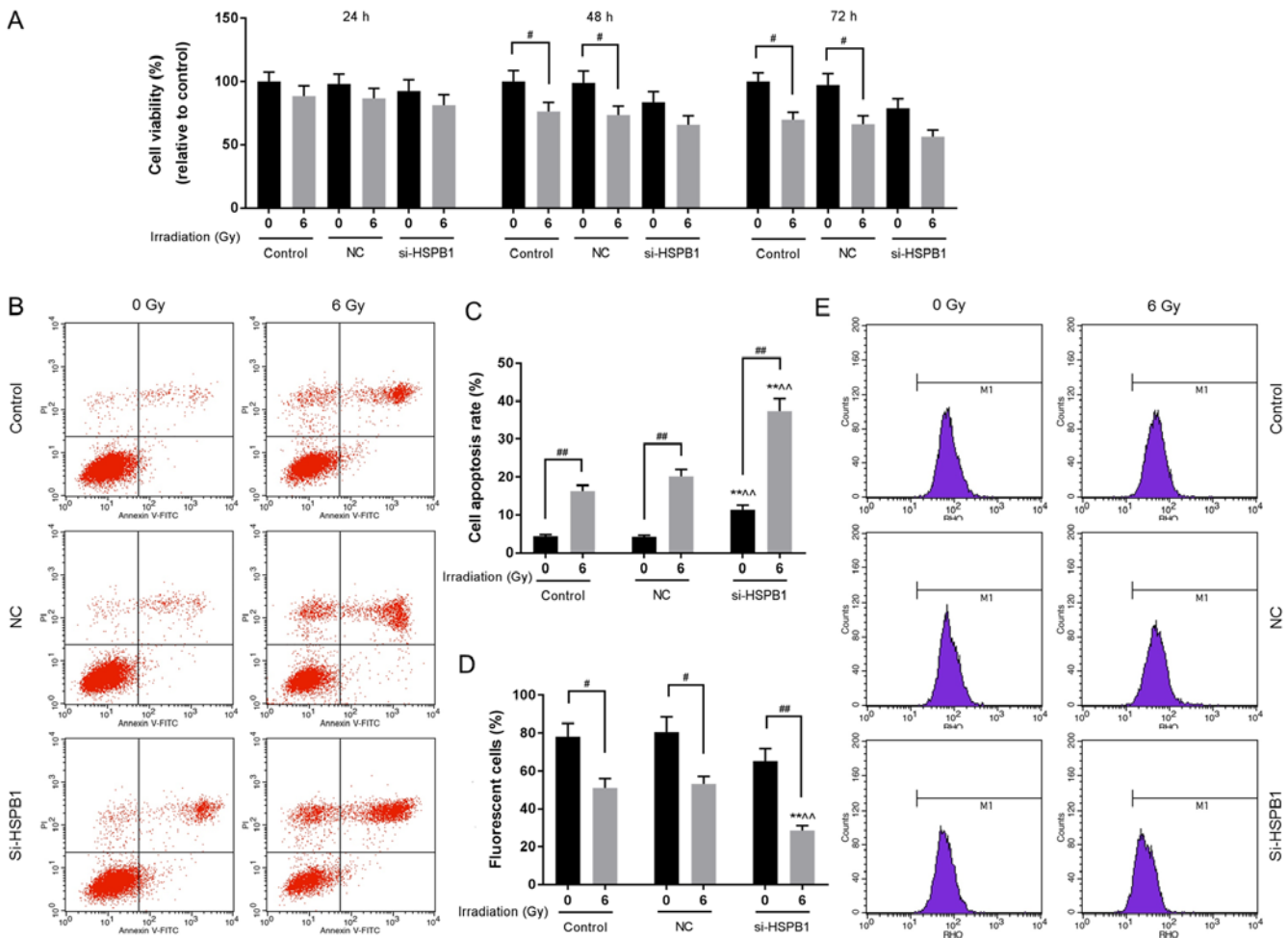


Figure 2. Silencing HSPB1 increases the radiosensitivity of non-small cell lung carcinoma cells by decreasing cell viability, promoting apoptosis and depolarizing the MMP. (A) The viability of A549 cells following irradiation with 0 or 6 Gy, and transfection with control, NC or si-HSPB1, as determined by a Cell Counting Kit-8 assay. (B and C) Apoptosis and (D and E) MMP of A549 cells following irradiation and transfection, as determined by flow cytometry. Data are presented as the mean ± standard deviation. $^{\#}$P<0.05 and $^{\#\#}$P<0.01, as indicated; $^{**}$P<0.01 vs. 0 Gy + NC; $^{\wedge\wedge}$P<0.01 vs. 6 Gy + NC. FITC, fluorescein isothiocyanate; HSPB1, heat shock protein 27; MMP, mitochondrial membrane potential; NC, negative control; PI, propidium iodide; si-HSPB1, small interfering RNA specific for HSPB1.

those of cytosolic cyto *c* compared with 0 Gy; si-HSPB1 also had similar significant effects on cyto *c* expression (Fig. 6).

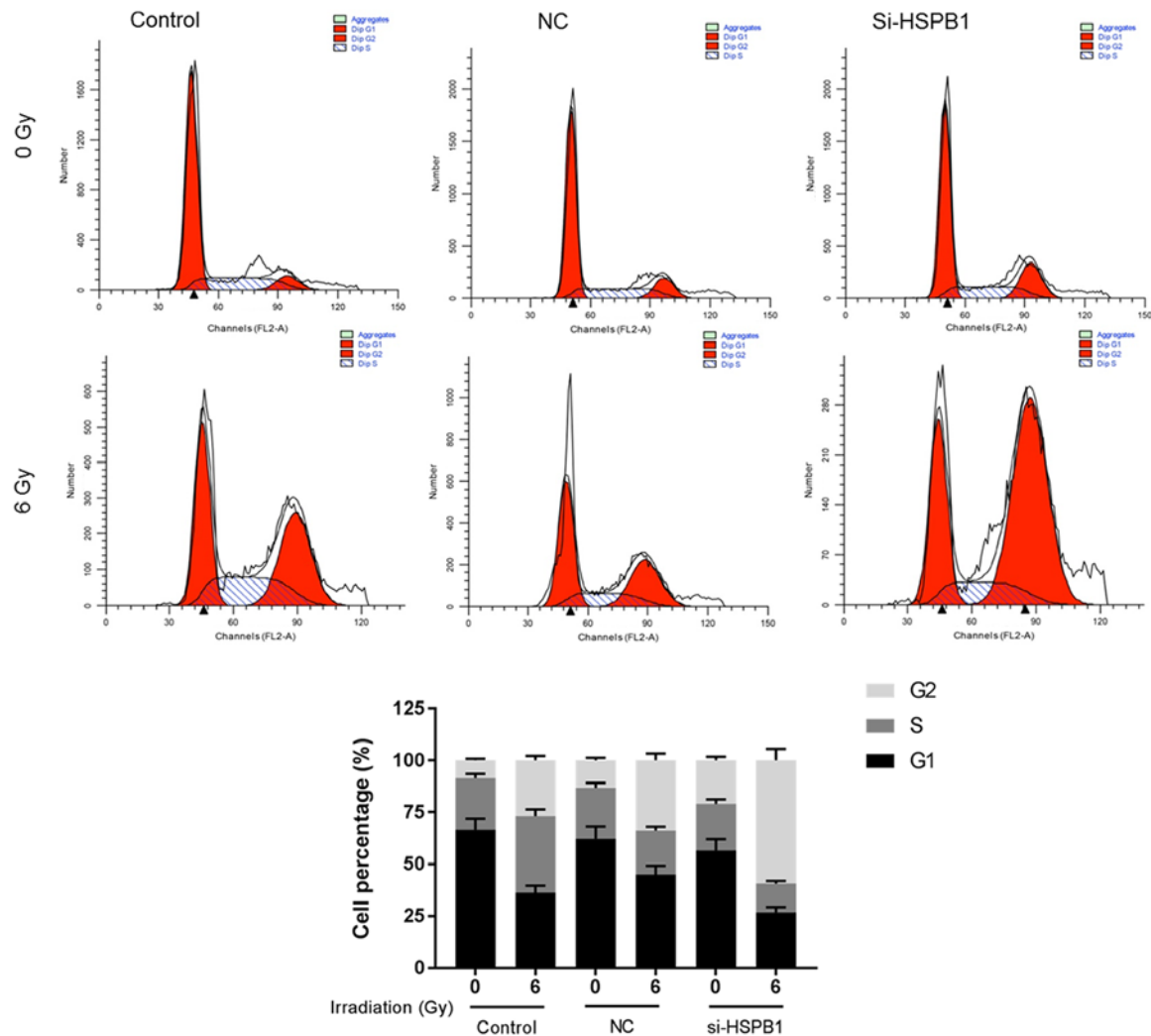Furthermore, the effects of irradiation were promoted by transfection with si-HSPB1 (Fig. 6).

Figure 3. Silencing of HSPB1 increases the radiosensitivity of non-small cell lung carcinoma cells by inducing cell cycle arrest. The cell cycle distribution of A549 cells following irradiation with 0 or 6 Gy, and transfection with control, NC or si-HSPB1, as determined by flow cytometry. Data are presented as the mean ± standard deviation. HSPB1, heat shock protein 27; NC, negative control; si-HSPB1, small interfering RNA specific for HSPB1.

## Discussion

At present, radiation therapy is an effective treatment of LC in clinical settings (4,5); however, radiosensitivity and radioresistance serve important roles in the therapeutic effects of radiotherapy (24-26). Compared with SCLC, NSCLC is frequently insensitive to radiation, reducing the efficacy of radiotherapy as a treatment of NSCLC (27). Therefore, the aims of the present study were to investigate the mechanisms underlying the radioresistance of NSCLC cells and identify novel targets for improving radiosensitivity. It has been reported that HSPB1 was upregulated in NSCLC (20). In addition, it was demonstrated that knockdown of HSPB1 increased the radiosensitivity of nasopharyngeal carcinoma and head-neck cancer cells (21,28). Therefore, it was hypothesized that silencing HSPB1 may increase the radiosensitivity of NSCLC cells.

Transfection with si-HSPB1 markedly decreased the viability of A549 cells following irradiation. One of the main effects of radiation on tumor cells is the induction of apoptosis (29). Additionally, depolarization of the MMP is associated with apoptosis (30). Thus, the apoptosis and

MMP of A549 cells following irradiation and silencing of HSPB1 was investigated. It was demonstrated that irradiation promoted the apoptosis and depolarized the MMP of NSCLC cells, with si-HSPB1 further enhancing the effects of irradiation. These findings indicated that downregulation of HSPB1 increased the radiosensitivity of NSCLC via the induction of apoptosis. The molecular mechanisms underlying radiosensitization include alterations in the tumor microenvironment, fixation of free radicals, cell cycle arrest, inhibition of DNA damage repair and the promotion of apoptosis (31-33). Cell cycle distribution is an important factor influencing radiosensitivity. The sensitivity of tumor cells to irradiation depends on their cell cycle phase (34); cells in the G2/M phase are more sensitive to radiation than those in other phases (35-37). It has been reported that drugs and gene manipulations increased the radiosensitivity of NSCLC cells by promoting G2/M phase cell cycle arrest (38-41). Consistent with these previous studies, the present study demonstrated that HSPB1 silencing markedly increased irradiation-induced cell cycle arrest in the G2/M phase.

As important regulators of the cell cycle, the roles of cyclins in the development and progression of tumors have
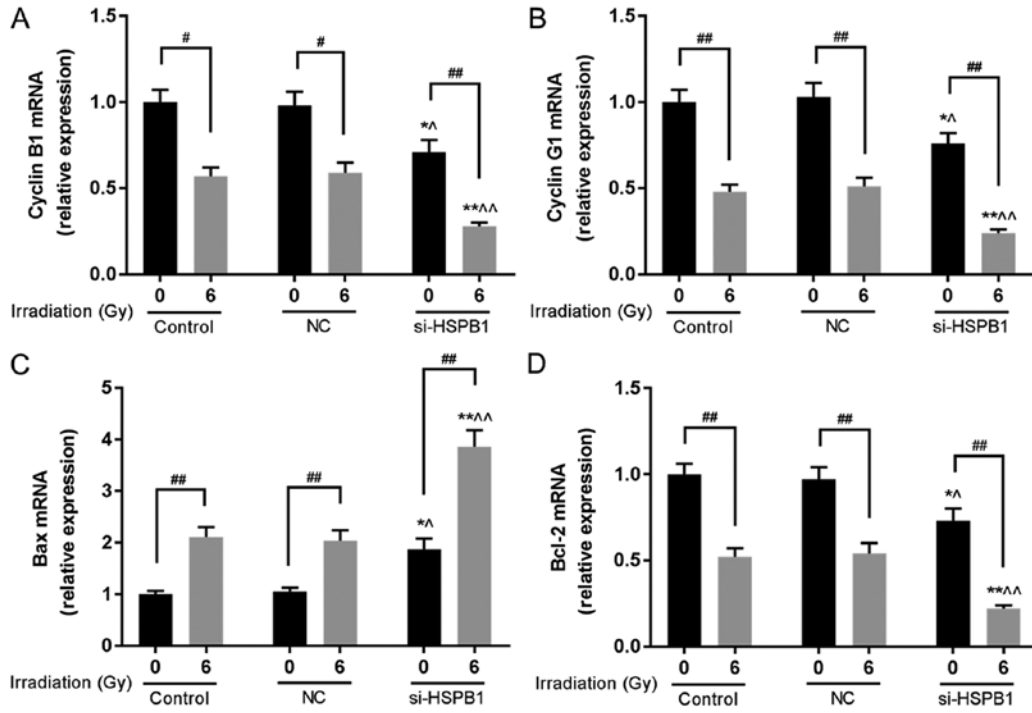
Figure 4. Silencing of HSPB1 increases the radiosensitivity of non-small cell lung carcinoma cells by regulating the expression of cell cycle- and apoptosis-associated genes. mRNA expression of (A) cyclin B1, (B) cyclin G1, (C) Bax and (D) Bcl-2 in A549 cells following irradiation with 0 or 6 Gy, and transfection with control, NC or si-HSPB1, as determined by reverse transcription-quantitative polymerase chain reaction. Data are presented as the mean ± standard deviation. #P<0.05 and ##P<0.01, as indicated; *P<0.05 and **P<0.01 vs. 0 Gy + NC; ^P<0.05 and ^^P<0.01 vs. 6 Gy + NC. Bcl-2, B-cell lymphoma-2; Bax, Bcl-2-associated X protein; HSPB1, heat shock protein 27; NC, negative control; si-HSPB1, small interfering RNA specific for HSPB1.
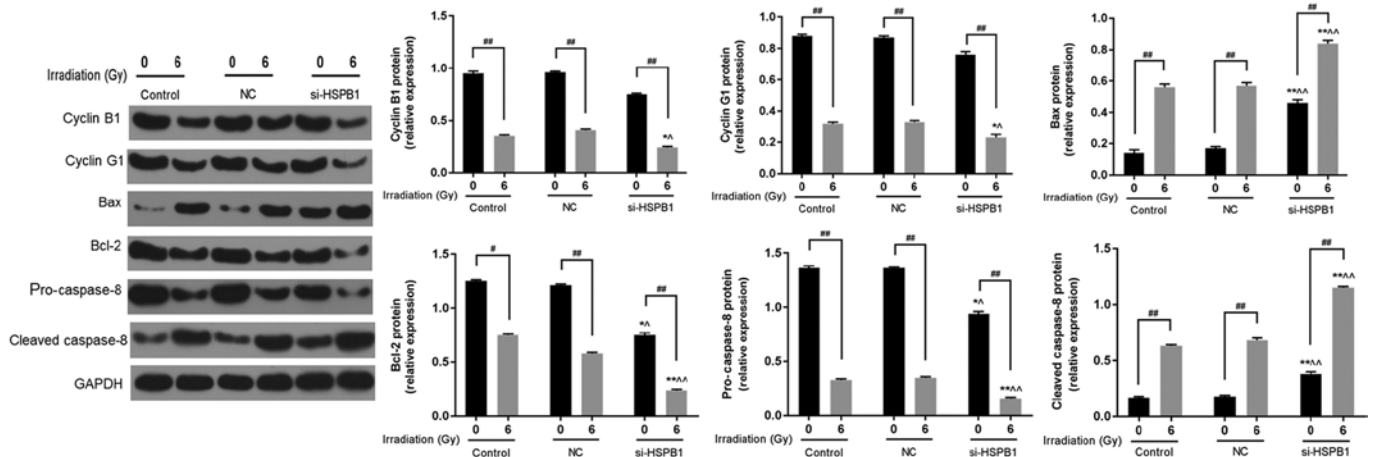


Figure 5. Silencing of HSPB1 increases the radiosensitivity of non-small cell lung carcinoma cells by regulating the expression of cell cycle- and apoptosis-associated proteins. Representative western blot and protein expression of cyclin B1, cyclin G1, Bax, Bcl-2, pro-caspase-3 and cleaved caspase-3 in A549 cells following irradiation with 0 or 6 Gy, and transfection with control, NC or si-HSPB1. Data are presented as the mean ± standard deviation. #P<0.05 and ##P<0.01, as indicated; *P<0.05 and **P<0.01 vs. 0 Gy + NC; ^P<0.05 and ^^P<0.01 vs. 6 Gy + NC. Bcl-2, B-cell lymphoma-2; Bax, Bcl-2-associated X protein; HSPB1, heat shock protein 27; NC, negative control; si-HSPB1, small interfering RNA specific for HSPB1.

been investigated; the abnormal expression of cyclins is a common mechanism underlying the dysregulation of cell cycle in malignant tumors (42-44). Cyclins are involved in cell cycle arrest (45,46). Cyclin B1 and cyclin G1 regulate G2/M phase transition (47). In the present study, cyclin B1 and cyclin G1 levels were detected by RT-qPCR and western blot analyses in A549 cells transfected with si-HSPB1 and exposed to radiation. It was demonstrated that the combination of si-HSPB1 and irradiation significantly decreased the expression levels

of cyclin B1 and cyclin G1, suggesting that silencing HSPB1 increases the radiosensitivity of HSPB1 cells by arresting the cell cycle in the G2/M phase via the downregulation of cyclins.

Apoptosis is associated with radiosensitivity; increased apoptosis indicates elevated radiosensitivity (48-50). Numerous molecules and pathways are involved in apoptotic processes, including the mitochondria, which serve key roles (51-53). For example, the voltage-dependent anion channel 1 (VDAC1) plays a key role in mitochondrial mediated apoptosis, VDAC1
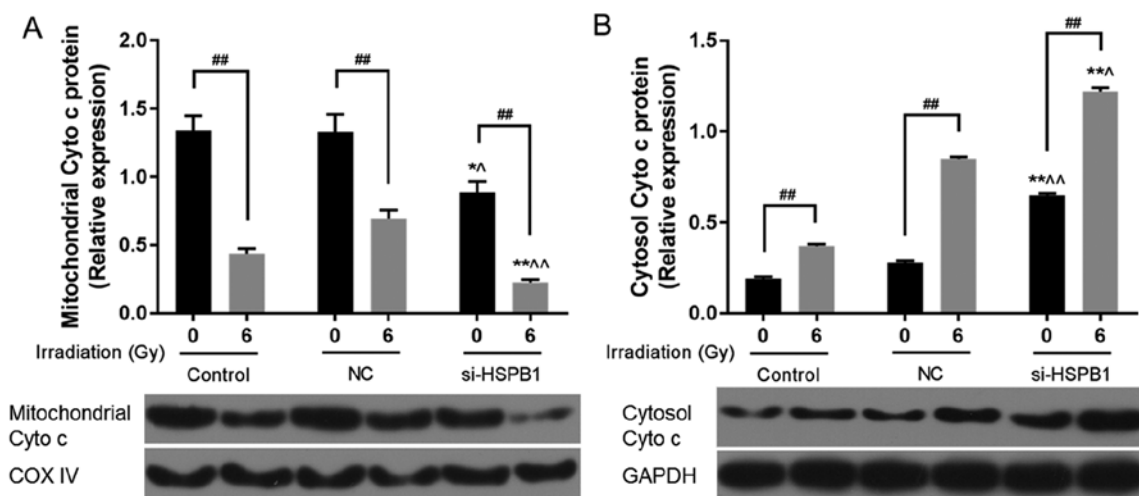
Figure 6. Silencing of HSPB1 increases the radiosensitivity of non-small cell lung carcinoma cells by regulating the expression of cytosolic and mitochondrial cyto *c*. (A and B) Representative western blots and the protein expression of mitochondrial cyto *c* and cytosolic cyto *c* in A549 cells following irradiation with 0 or 6 Gy, and transfection with control, NC or si-HSPB1. ##P<0.01, as indicated; *P<0.05 and **P<0.01 vs. 0 Gy + NC; ^P<0.05 and ^^P<0.01 vs. 6 Gy + NC. cyto *c*, cytochrome *c*; COX IV, cyto *c* oxidase IV; HSPB1, heat shock protein 27; NC, negative control; si-HSPB1, small interfering RNA specific for HSPB1.

oligomerization leads to the formation of a large pore that allows the release of pro-apoptotic proteins to cytosol, thereby activating apoptosis (51); Mitogen-activated protein kinases/extracellular signal-regulated kinase pathway has been reported to be associated with cell differentiation and apoptosis (54). Depolarization of the MMP is an early event in apoptotic cascades, leading to a series of biochemical altera-tions in the mitochondrial membrane that induce proapoptotic pathways (55,56). In the present study, it was observed that downregulation of HSPB1 increased apoptosis and altered the MMP in A549 cells following irradiation.

Apoptosis pathways can be divided into extracellular and intracellular apoptotic pathways (57,58). The external pathway is mainly regulated by caspase-8, which is acti-vated by apoptotic receptors on the surface of the cell membrane (59,60). The internal pathway involves Bcl-2 and Bax, which regulate the permeability of the outer membrane of mitochondria (61). Bcl-2 exhibits antiapoptotic effects and prevents the release of cyto *c* from mitochondria into the cytoplasm (62). Activation of the proapoptotic protein Bax accelerates cell death (58). Therefore, the expression of Bcl-2, Bax, cyto *c* and caspase-8 was investigated in A549 cells subjected to si-HSPB1 transfection and irradiation. It was demonstrated that si-HSPB1 upregulated the levels of Bax, cytosol cyto *c* and cleaved caspase-8, and downregu-lated those of Bcl-2, mitochondrial cyto *c* and pro-caspase-8 in cells exposed to irradiation. These findings indicated that HSPB1 silencing promoted the apoptosis of NSCLC cells following radiation treatment.

There are certain points to consider for future experi-ments. NSCLC accounts for the majority of lung cancers (63); however, the present study did not investigate the effects of HSPB1 silencing on SCLC cells, which should be determined in future studies. Additionally, a previous study reported that HSPs did not regulate the radioresistance of NSCLC cells, with no effect following the downregulation of HSP72 or heat shock treatment (64). The reasons for the discrepancy between the absence of a reported effect of heat shock treatment on

NSCLC cell radiosensitivity in the previous study and the increase in radiosensitivity following silencing of HSPB1 in the present study require further investigation.

In conclusion, the results of the present study demonstrated that silencing HSPB1 increased the radiosensitivity of NSCLC cells via reductions in cell viability, depolarization of the MMP, cell cycle arrest in the G2/M phase and the promotion of apoptosis. Therefore, HSPB1 may be a potential target for the treatment of radiation-insensitive NSCLC.

## Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

## Authors' contributions

LX made substantial contributions to the conception and design of the present study; XL, YZ and HZ performed data acquisition, analysis and interpretation; LX drafted the article or critically revised it for important intellectual content; all authors agreed to be accountable for all aspects of the work and ensuring that questions related to the accuracy or integrity of the work were appropriately investigated and resolved; all authors approved the final published version.

## Ethics approval and consent to participate

Not applicable.

**Patient consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

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