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Involvement of Mitochondrial Ribosomal Protein MRPS28 in Carbon Ion Irradiation-Induced A549 Cell Apoptosis

Authors: Guomin Huang, Zhang Jinhua, Dou Zhihui, Bao Xingting, Liu Jiadi, Xu Duling, Li Hongyan, Zhang Hong, Zhang Hong

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Abstract

Objective: To investigate the mechanism by which mitochondrial ribosomal protein MRPS28 influences carbon ion irradiation-induced apoptosis in A549 cells. Methods: siRNA-mediated knockdown of MRPS28 was employed to establish a stable MRPS28-knockdown A549 cell line; cells were irradiated with carbon ion beams and X-rays; cell proliferation rate was assessed using CCK-8 assay; clonogenic survival assay was used to determine cell survival rate; apoptosis, reactive oxygen species (ROS) levels, and mitochondrial membrane potential were measured by flow cytometry; related genes were analyzed by qRT-PCR; and apoptosis-related proteins were examined by Western blot. Results: Exposure of non-small cell lung cancer A549 cells to carbon ion irradiation led to decreased MRPS28 expression; compared with the control group, MRPS28 knockdown reduced A549 cell proliferation and clonogenic survival, increased intracellular ROS content, decreased mitochondrial membrane potential, and induced apoptosis via the p53-mediated mitochondrial apoptosis pathway; combined MRPS28 knockdown and carbon ion irradiation significantly reduced A549 cell proliferation and survival, and promoted post-irradiation apoptosis. Limitations: Low MRPS28 expression increases the sensitivity of A549 cells to carbon ion irradiation, but the underlying mechanism requires further investigation. Conclusion: These findings preliminarily demonstrate that low MRPS28 expression is involved in carbon ion irradiation-induced apoptosis in A549 cells and enhances the radiosensitivity of A549 cells to carbon ion beams.
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Full Text

Preamble

Mitochondrial Ribosomal Protein MRPS28 Participates in Carbon Ion Irradiation-Induced Apoptosis of A549 Cells

Guomin Huang[1,2,3,4,5], Jinhua Zhang[1,2,3,4,5], Zhihui Dou[1,2,3,4,5], Xingting Bao[1,2,3,4,5], Jiadi Liu[1,2,3,4,5], Duling Xu[1,2,3,4,5], Hongyan Li[1,2,3,4,5],, *Hong Zhang*[1,2,3,4,5],

[1](Department of Medical Physics, Institute of Modern Physics, Chinese Academy of Sciences, Lanzhou, 730000, China)
[2](Key Laboratory of Heavy Ion Irradiation Biology and Medicine of Chinese Academy of Sciences, Lanzhou, 730000, China)
[3](Key Laboratory of Basic Research on Heavy Ion Irradiation Application in Medicine, Lanzhou, 730000, China)
[4](College of Nuclear Science and Technology, University of Chinese Academy of Sciences, Beijing, 101408, China)
[5](Advanced Energy Science and Technology Guangdong Laboratory, Huizhou, 516029, China)

Corresponding authors:
Hong Zhang, E-mail: zhangh@impcas.ac.cn;
Hongyan Li, E-mail: lihy@impcas.ac.cn

Purpose: To investigate the mechanism by which mitochondrial ribosomal protein MRPS28 affects carbon ion irradiation-induced apoptosis in A549 cells.

Methods: siRNA was used to knock down MRPS28 and establish a stable MRPS28-knockdown A549 cell line. Cells were irradiated with carbon ion beams and X-rays. Cell proliferation was assessed using CCK-8 assay, clonogenic sur-

vival assay measured cell survival rates, and flow cytometry detected apoptosis, reactive oxygen species (ROS), and mitochondrial membrane potential. qRT-PCR examined relevant genes, while Western blot analyzed apoptosis-related proteins.

Results: Exposure of non-small cell lung cancer A549 cells to carbon ion irradiation led to decreased MRPS28 expression. Compared to the control group, MRPS28 knockdown reduced A549 cell proliferation and clonogenic survival, increased intracellular ROS levels, decreased mitochondrial membrane potential, and induced apoptosis through the p53-mediated mitochondrial apoptotic pathway. MRPS28 knockdown combined with carbon ion irradiation significantly reduced A549 cell proliferation and survival while promoting apoptosis after irradiation.

Limitation: While MRPS28 downregulation increased the radiosensitivity of A549 cells to carbon ion irradiation, the specific underlying mechanism requires further investigation.

Conclusion: This study preliminarily demonstrates that MRPS28 downregulation participates in carbon ion irradiation-induced apoptosis in A549 cells and enhances A549 cell sensitivity to carbon ion beams.

Keywords: Carbon ion irradiation; A549 cells; Mitochondrial ribosomal protein; Apoptosis; p53

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Author biography: Guomin Huang (1995-), female, from Fengjie, Chongqing, Ph.D. candidate; engaged in biophysics research; E-mail: huangguomin@impcas.ac.cn.

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Limitation: MRPS28 down-regulation increased the sensitivity of A549 cells to carbon ion irradiation, but the specific mechanism requires further investigation.

Conclusion: This study preliminarily demonstrates that MRPS28 down-regulation is involved in carbon ion irradiation-induced apoptosis in A549 cells and enhances A549 cell sensitivity to carbon ion.

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1 Introduction

Heavy ions possess unique physical characteristics including large mass, minimal scattering during penetration, and high linear energy transfer (LET), enabling highly precise radiotherapy that minimizes damage to normal tissue before reaching the tumor, concentrates high energy deposition at the tumor site, and exhibits rapid energy attenuation after passing through the lesion [1]. Among cancer-related deaths in China over the past 50 years, lung cancer has shown the highest mortality rate and fastest growth [2]. Radiotherapy represents a crucial treatment modality for lung cancer, with approximately 60%-70% of patients requiring radiation during their treatment course, including definitive radiotherapy for early-to-mid stage disease and palliative radiotherapy for advanced stages. Although radiotherapy techniques for lung cancer are relatively mature, heavy ion irradiation represents a specialized form of radiation therapy whose mechanisms remain incompletely understood, prompting extensive research into its anticancer mechanisms [3, 4].

Mitochondrial ribosomes reside within mitochondria and share structural similarities with cytoplasmic ribosomes, comprising large and small subunits. A total of 89 mitochondrial ribosomal proteins (MRPs) constitute the mitochondrial ribosome, and emerging evidence indicates that MRPs play roles in apoptosis. Three apoptosis-associated MRPs have been identified to date: MRPS29, MRPL41, and MRPS30. MRPS29 participates in mitochondrial network dynamics during apoptosis, with its overexpression promoting cell death [5]. MRPL41 exhibits multiple extended conformations and interacts with 16S rRNA and surrounding MRPs, including the pro-apoptotic protein MRPS30 [6]. Studies have shown that MRPL41 overexpression enhances p53

stability and facilitates its translocation to mitochondria, thereby promoting p53-mediated apoptosis [7]. Additionally, MRPs can serve as biomarkers for tumor development and progression [8]. Database queries using GEPIA (http://gepia.cancer-pku.cn) reveal aberrant expression of multiple MRPs across various tumor tissues, with MRPS28, MRPL20, and MRPS16 showing high expression in lung cancer, which correlates with poorer prognosis. MRPS28 is a protein component of the small subunit of the mitochondrial ribosome. Aberrant MRPS28 expression affects mitochondrial translation processes [9], and studies have demonstrated its important role in mitochondrial metabolism [10]; however, the involvement of MRPS28 in apoptosis and radiosensitivity remains unexplored.

Our study revealed that carbon ion irradiation of A549 cells decreased MRPS28 expression while increasing apoptosis rates. By designing siRNA and constructing stable MRPS28-knockdown A549 cell lines, we demonstrated that MRPS28 downregulation participates in carbon ion irradiation-induced apoptosis. These findings contribute positively to understanding the mechanisms of heavy ion radiotherapy for lung cancer and may provide theoretical support for advancing heavy ion radiotherapy in non-small cell lung cancer.

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2 Materials and Methods

2.1 Cell Culture

Human non-small cell lung cancer A549 cells were purchased from Shanghai Fuxiang Biological Technology Co., Ltd. and cultured in DMEM medium supplemented with 10% fetal bovine serum (FBS; Cellmax, Shanghai, China) at 37°C in a 5% CO_2 humidified incubator. All experiments utilized cells in logarithmic growth phase.

2.2 Carbon Ion Irradiation

Carbon ion beams were provided by the Heavy Ion Research Facility in Lanzhou (HIRFL) at the Institute of Modern Physics, Chinese Academy of Sciences, and the medical heavy ion accelerator at Lanzhou Heavy Ion Hospital. The HIRFL terminal energy was 80 MeV/u with LET of 50 keV/ m; the Lanzhou Heavy Ion Hospital accelerator operated at 100 MeV/u with LET of 50 keV/ m.

2.3 Proteomic Analysis

At 48 h after 4 Gy carbon ion irradiation of A549 cells, proteins were extracted, snap-frozen in liquid nitrogen, and ground into powder. Protein quantification was performed using BCA assay. Appropriate amounts of protein from each sample were digested with trypsin using the FASP (Filter-Aided Sample Preparation) method, followed by desalting of peptides using C18 columns. After

lyophilization, peptides were resuspended in 40 L dissolution buffer and quantified (OD_{280}). Mass spectrometry data were analyzed for identification and quantification using Mascot 2.2 and Proteome Discoverer 1.4 software.

2.4 MRPS28-Specific siRNA Transient Transfection of A549 Cells

siRNA sequences were designed as shown in Table 1 .

Table 1 siRNA Sequences

Name	Sequence (5'-3')
MRPS28-Homo-58 si-58	Sense: CUGCGAGUGUUUCUCUUCUTTAntisense: AGAAGAGAAACACUCGCAGTT
MRPS28-Homo-122 si-122	Sense: GUAGUUCCAAUGCCAAGGATTAntisense: UCCUUGGCAUUGGAACUACTT
MRPS28-Homo-314 si-314	Sense: GACGGAUCUUUCAUAUUGUTTAntisense: ACAAUAUGAAAGAUCCGUCTT

Cationic liposome-mediated transfection was employed to downregulate MRPS28 expression in A549 cells. Cells were seeded at 1.5×10^{5} cells per well in $ $35 culture dishes. Transfection was performed using GenePharma transfection reagent and siRNA diluted in Opti-MEM serum-free medium. After 8 h transfection, the medium was replaced with complete culture medium. Cells were harvested for RNA extraction at 24, 48, and 72 h post-transfection, and for protein extraction at 48 h.

2.5 CRISPR/Cas9 Construction of Stable MRPS28-Knockdown A549 Cell Line

Step 1: A549 cells were seeded in 6-well plates. When cells reached 70-80% confluence, lentiviral stock was diluted to 1×10^{8} TU/mL. The viral stock was produced using a three-plasmid second-generation lentiviral packaging system. The dual gRNA expression vector (dual gRNA sequences shown in Table 2), packaging plasmid (psPAX2), and envelope plasmid (pMD2.G) were co-transfected into 293T cells. Virus and Polybrene (final concentration 5 g/mL) were added to the culture medium. After 24 h infection, the medium was replaced with regular complete medium. Blasticidin selection was applied at 72-96 h post-infection.

Step 2: Selected cells were seeded in 6-well plates at 70-80% confluence (approximately 1×10^{6} cells). Virus (1×10^{8} TU/mL) and Polybrene (final concentration 5 g/mL) were added to the medium. The viral stock was produced using a three-plasmid second-generation lentiviral packaging system. Cas9 expression vector, packaging plasmid (psPAX2), and envelope plasmid (pMD2.G) were co-transfected into 293T cells. After 24 h infection, the medium

was replaced with regular complete medium. Puromycin selection was applied at 72-96 h post-infection.

Table 2 Dual gRNA Vector Construction for Human MRPS28 Knockdown

gRNA Design	gRNA Sequence	PAM Sequence
Upstream gRNA 1EX158-1fw	gAGGCGGTTTCGCGAGCGCGT	Not specified
Upstream gRNA 1IN30-9fw	gCGGGAATTACGCCAACGACG	Not specified
Downstream gRNA 2IN1408-1fw	GTTATGTAAACGGCTACTGA	Not specified
Downstream gRNA 2IN255-3re	GTGTGGAATGGCTTATTATC	Not specified

2.6 qRT-PCR Analysis

After 8 h transfection with different siRNA fragments, A549 cells were switched to normal medium and cultured for 24, 48, and 72 h before RNA extraction using TRIzol reagent. RNA was reverse-transcribed to cDNA using TaKaRa PrimeScript™ RT reagent Kit with gDNA Eraser under the following conditions: 42°C for 30 min, followed by 85°C for 5 min to inactivate the reverse transcriptase. qRT-PCR was performed using TaKaRa TB Green™ Premix Ex Taq™ II in a 20 L reaction mixture containing 2 L cDNA template, 10 L SYBR Green, 0.8 L PCR Forward Primer (10 M), 0.8 L PCR Reverse Primer (10 M), and ddH$_2$O to volume. Cycling conditions were: 95°C denaturation for 30 s, 60°C annealing for 30 s, 72°C extension for 30 s, for 40 cycles. Five replicate wells were used per sample.

Table 3 qPCR Primer List

Gene	Forward Primer (5'-3')	Reverse Primer (5'-3')
MRPS28	CTGAGAGTGGATCCGAAAGTGGTA	CTTTGCAGGTCCCATCTGTGTAA
β-actin	TGGCACCCAGCACAATGAA	CTAAGTCATAGTCCGCCTAGAAGCA

2.7 Western Blot Analysis

Stable MRPS28-knockdown A549 cells were cultured for 48 h before protein extraction. Cells were lysed on ice for 30 min in RIPA buffer (1% PMSF), followed by centrifugation at 13,000 rpm for 15 min at 4°C. Protein concentration was determined using BCA assay. Twenty-five micrograms of protein were separated on 10% SDS-PAGE gels and transferred to PVDF membranes at constant current (150 mA). Membranes were blocked with 5% skim milk for 1 h at room temperature. Primary antibodies were diluted 1:1000 and incubated overnight at 4°C. After washing, membranes were incubated with corresponding secondary antibodies (1:10,000 dilution) for 1 h at room temperature, followed by extensive washing and chemiluminescent detection.

2.8 Clonogenic Survival Assay

For validation of stable MRPS28-knockdown cell lines, cells were seeded at 300 cells per dish in $ $60 culture dishes. After 14 days of culture, colonies were fixed with 4% paraformaldehyde and stained with 1% crystal violet. Colonies containing $ $50 cells were counted under a microscope.

For combined knockdown and irradiation experiments, different treatment groups were seeded at varying densities in $ $60 dishes: negative control (200 cells), negative control + 2 Gy (600 cells), negative control + 4 Gy (1500 cells), knockdown group (500 cells), knockdown + 2 Gy (1000 cells), and knockdown + 4 Gy (2000 cells). After 16 days of culture, colonies were fixed, stained, and counted as described above.

2.9 Flow Cytometric Analysis of Apoptosis

Cells were seeded at 1.5$×10^{5}$ cells per well in $ $35 culture dishes and transfected with siRNA using GenePharma transfection reagent diluted in Opti-MEM. After 8 h, the medium was replaced with complete medium and cells were cultured for an additional 48 h. A549 cells were harvested using EDTA-free trypsin, centrifuged at 800 rpm for 4 min, and washed twice with PBS. Cells were stained using the Annexin V-FITC apoptosis detection kit according to the manufacturer's instructions. Apoptosis rates were analyzed using FlowSight flow cytometer (Amnis, Seattle, WA) with IDEAS Application v6.0 software.

Stable MRPS28-knockdown A549 cells were cultured for 48 h and apoptosis rates were measured using the same protocol.

2.10 Flow Cytometric Detection of Reactive Oxygen Species (ROS)

A549 cells were transfected with si-314 (the fragment showing highest apoptosis rate) for 8 h, then switched to normal medium for 48 h. Cells were collected and resuspended in DCFH-DA solution (final concentration 10 mol/L) at a density of 1-20$×10^{5}$ cells/mL, incubated at 37°C for 20 min. After washing three times with serum-free medium to remove unincorporated DCFH-DA, ROS levels were measured by flow cytometry.

Stable MRPS28-knockdown A549 cells were cultured for 48 h and ROS content was measured using the same procedure.

2.11 Flow Cytometric Analysis of Mitochondrial Membrane Potential

Mitochondrial membrane potential was assessed using JC-1 fluorescent probe. A549 cells transfected with si-314 for 48 h were resuspended in 0.5 mL culture medium, mixed with 0.5 mL JC-1 staining working solution, and incubated at 37°C for 20 min. After washing twice with JC-1 staining buffer (1×), cells were resuspended in 200 L JC-1 staining buffer (1×) for flow cytometric analysis.

Stable MRPS28-knockdown A549 cells were cultured for 48 h and mitochondrial membrane potential was measured using the same protocol.

2.12 Statistical Analysis

Data were analyzed using GraphPad Prism 8. Results are presented as mean ± standard error. Comparisons between two groups were performed using t-tests, while multiple group comparisons employed one-way ANOVA. $p < 0.05$ was considered statistically significant.

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3 Results

3.1 Carbon Ion Irradiation Alters MRPs Expression in A549 Cells

Proteomic analysis revealed that carbon ion irradiation altered the expression of 48 MRPs in A549 cells, with 14 upregulated and 34 downregulated (Figure 1 [Figure 1: see original paper]). Notably, MRPS28 expression was downregulated.

Figure 1 Effects of carbon ion irradiation on mitochondrial protein expression. (A) Volcano plot. (B) Cluster heatmap.

3.2 MRPS28 Downregulation Inhibits A549 Cell Proliferation

CCK-8 assay was used to assess the effect of MRPS28 downregulation on A549 cell proliferation. As shown in Figure 2 [Figure 2: see original paper]A, transfection with different siRNA fragments significantly inhibited cell proliferation at 24, 48, and 72 h compared to the negative control (empty vector) group. Experiments using stable MRPS28-knockdown cell lines demonstrated significantly reduced proliferation rates at 24, 48, and 72 h (Figure 2B).

Figure 2 Effects of MRPS28 downregulation on A549 cell proliferation. $p < 0.05$ vs. negative control group.

3.3 MRPS28 Downregulation Reduces Clonogenic Survival of A549 Cells

To determine whether MRPS28 downregulation exerts long-term irreversible effects, clonogenic survival assays were performed using stable knockdown cell lines. As shown in Figure 3 [Figure 3: see original paper], MRPS28 downregulation significantly reduced the clonogenic survival capacity of A549 cells.

Figure 3 Effects of MRPS28 downregulation on clonogenic survival of A549 cells. $p < 0.05$ vs. negative control group.

3.4 MRPS28 Downregulation Decreases Mitochondrial Membrane Potential

Proteomic analysis indicated that carbon ion irradiation caused aberrant expression of multiple mitochondrial proteins, impairing mitochondrial function. Flow cytometric analysis revealed that both stable MRPS28 knockdown and si-314 transfection for 48 h significantly reduced mitochondrial membrane potential, as evidenced by increased JC-1 monomer content (Figure 4 [Figure 4: see original paper]). Decreased mitochondrial membrane potential represents an early hallmark of apoptosis.

Figure 4 Effects of MRPS28 downregulation on mitochondrial membrane potential. (A) Representative flow cytometry plots. (B, C) Statistical analysis of membrane potential. $p < 0.05$ vs. negative control group.

3.5 MRPS28 Downregulation Increases ROS Levels in A549 Cells

Mitochondria are the primary site of ROS production in animal cells. Carbon ion irradiation-induced mitochondrial membrane potential reduction and abnormal electron transport chain function may lead to altered ROS levels. Experiments using stable knockdown cells and si-314-transfected cells showed that MRPS28 knockdown significantly elevated intracellular ROS content (Figure 5 [Figure 5: see original paper]), disrupting cellular redox balance and promoting apoptosis.

Figure 5 Effects of MRPS28 downregulation on intracellular ROS content. (A) Representative flow cytometry plots. (B, C) Statistical analysis of ROS levels. $p < 0.05$ vs. negative control group.

3.6 MRPS28 Downregulation Promotes A549 Cell Apoptosis

The effect of MRPS28 downregulation on apoptosis was further investigated using stable knockdown cells and cells transfected with different siRNA fragments for 48 h. As shown in Figure 6 [Figure 6: see original paper]A and B, all three siRNA fragments promoted apoptosis. Additionally, stable MRPS28 knockdown significantly increased apoptosis rates compared to the negative control group (Figure 6C and D).

Figure 6 MRPS28 downregulation promotes apoptosis in A549 cells. (A, B) Effects of siRNA-mediated MRPS28 knockdown on apoptosis. (C, D) Effects of stable MRPS28 knockdown on apoptosis. $p < 0.05$ vs. negative control group.

3.7 MRPS28 Knockdown Induces Apoptosis via p53-Mediated Mitochondrial Pathway

Western blot analysis of stable MRPS28-knockdown A549 cells revealed that while total p53 protein expression decreased, phosphorylated p53 at the N-terminal Ser15 site (denoted as P-p53(ser15)) increased significantly (Figure 7 [Figure 7: see original paper]). Phosphorylation of p53 at Ser15 is associated

with apoptosis induction [11]. The Bax/Bcl-2 ratio increased markedly, indicating enhanced mitochondrial membrane permeability and cytochrome C (Cyto C) release into the cytoplasm, which initiates Caspase-mediated apoptosis. As shown in Figure 7B, expression of Caspase-3 and its cleaved form (c-Casp-3) increased significantly. These results demonstrate that MRPS28 downregulation induces apoptosis through the p53-mediated mitochondrial pathway.

Figure 7 MRPS28 downregulation participates in apoptosis via p53-mediated mitochondrial pathway. (A) Protein expression bands. (B-D) Statistical analysis of protein expression. $p < 0.05$ vs. negative control group. (P-p53(ser15) denotes p53 protein phosphorylated at N-terminal Ser15; Cyto C denotes cytochrome C; c-Casp-3 denotes cleaved Caspase-3).

3.8 MRPS28 Knockdown Enhances A549 Cell Sensitivity to Carbon Ion Irradiation

A549 cells were treated with 2 Gy or 4 Gy carbon ion irradiation combined with MRPS28 knockdown, and cell proliferation, clonogenic survival, and apoptosis were assessed.

(1) MRPS28 knockdown combined with carbon ion irradiation inhibits A549 cell proliferation. Cells were seeded at 5,000 cells per well in 96-well plates and proliferation rates were measured at 24, 48, and 72 h. Compared to irradiation alone, the combination treatment significantly reduced cell proliferation (Figure 8 [Figure 8: see original paper]) and clonogenic survival (Figure 9 [Figure 9: see original paper]).

Figure 8 Effects of MRPS28 knockdown combined with carbon ion irradiation on A549 cell proliferation. $p < 0.05$ vs. control group.

Figure 9 Effects of MRPS28 knockdown combined with carbon ion irradiation on clonogenic survival. (A) Representative colony images. (B) Statistical analysis of clonogenic survival. $p < 0.05$ vs. control group.

(2) MRPS28 knockdown combined with carbon ion irradiation increases A549 cell apoptosis. The effect of combined treatment on apoptosis was further examined. As shown in Figure 10 [Figure 10: see original paper], apoptosis rates were higher in the combination groups compared to the negative control group at 48 h post-irradiation, suggesting that MRPS28 knockdown enhances the efficacy of carbon ion irradiation and increases A549 cell radiosensitivity, though the precise mechanism requires further investigation.

Figure 10 Effects of MRPS28 knockdown combined with carbon ion irradiation on A549 cell apoptosis. (A) Representative flow cytometry plots. (B) Statistical analysis of apoptosis rates. $p < 0.05$ vs. control group.

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4 Discussion and Conclusion

Carbon ion irradiation damages cellular DNA. Mitochondria, as the only organelles with independent DNA, represent major targets of radiation damage alongside the nucleus. MRPs are regulated by both nuclear and mitochondrial genomes, underscoring their importance in the radiation stress response of A549 cells. Carbon ion irradiation downregulated multiple MRPs, primarily due to nuclear DNA damage reducing the overall transcription of MRP-encoding genes. Based on these proteomic findings, we used siRNA to downregulate MRPS28 in A549 cells and found that MRPS28 knockdown inhibited proliferation and reduced clonogenic survival. Further investigation revealed that MRPS28 downregulation disrupted mitochondrial ribosomal translation function, directly affected the electron transport chain on the mitochondrial membrane, caused significant ROS elevation, reduced mitochondrial membrane potential, increased membrane permeability, released cytochrome C, and activated apoptotic proteases to execute cell death.

MRPs are frequently overexpressed in tumor cells and correlate with poor prognosis. MRPL15 is highly expressed in ovarian cancer and associated with adverse patient outcomes, with mechanistic analyses suggesting its overexpression relates to gene amplification and hypomethylation, functioning through cell cycle, DNA repair, and mTOR signaling pathways [12]. MRPS23 overexpression promotes hepatocellular carcinoma cell proliferation [13], and similarly, increased MRPS18-A expression facilitates liver cancer development [14]. Functional studies of ribosomal proteins have revealed extra-translational roles in cell proliferation, DNA repair, apoptosis, intracellular homeostasis, and malignant transformation [15].

MRPS28 downregulation increased ROS levels, decreased mitochondrial membrane potential, and induced apoptosis via the p53-mediated mitochondrial pathway. Mitochondria are the primary source of ROS, and excessive ROS can trigger opening of the mitochondrial permeability transition pore, leading to membrane potential loss and cytochrome C release, which subsequently activates Caspase cascades to execute apoptosis. In apoptosis, ROS increase is closely linked to Ca^{2+} elevation. Studies have shown that in surfactin-induced apoptosis of breast cancer MCF-7 cells, ROS caused Ca^{2+} release from the endoplasmic reticulum and mitochondria, resulting in mitochondrial permeability transition pore opening and membrane potential reduction [16]. Conversely, mitochondrial permeability transition pore opening and membrane potential loss increase cytosolic Ca^{2+}, which subsequently elevates mitochondrial Ca^{2+}, creating a positive feedback loop that promotes mitochondrial apoptosis. ROS fluctuations also affect p53 expression by inducing oxidative DNA damage and promoting p53 activation. p53 regulates apoptosis and cell cycle progression in response to DNA damage, oncogene activation, and hypoxia. p53 mediates mitochondrial apoptosis through multiple mechanisms, including transcriptional activation of pro-apoptotic effectors (PUMA, NOXA, BID, Bax) and inhibition of anti-apoptotic genes (Bcl-2) [17]. Following MRPS28 downregulation,

phosphorylated p53 at Ser15 increased while Bcl-2 expression decreased, potentially activating the mitochondrial apoptotic pathway. The mechanism by which MRPS28 downregulation affects p53 expression requires further investigation. GST pull-down studies have shown interactions between p53 and six MRPs including MRPS28 [18], though these may be direct or indirect. MRPS28 downregulation affects expression of multiple other MRPs, potentially influencing p53 through these proteins. Protein interaction network analysis has revealed MRPS23-p53 interactions [19], and previous studies showed MRPL41 can trigger p53 mitochondrial localization and enhance p53 stability and apoptosis without direct MRPL41-p53 interaction [20].

Approximately 60% of non-small cell lung cancer patients receive radiotherapy; however, tumor cells gradually develop radiation resistance during treatment, leading to suboptimal therapeutic outcomes. Major factors affecting lung cancer radiotherapy include tumor hypoxia tolerance, expression of radiation resistance genes, and activation of resistance signaling pathways [21]. Combining carbon ion irradiation with MRPS28 knockdown resulted in lower proliferation and clonogenic survival rates and higher apoptosis rates compared to irradiation alone, demonstrating that MRPS28 downregulation enhances A549 cell sensitivity to carbon ion irradiation, though the detailed mechanism warrants further study.

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Author Contributions

Guomin Huang, Hongyan Li, Hong Zhang: Conceived the study and designed the research protocol;
Guomin Huang, Jiadi Liu, Duling Xu: Performed the experiments;
Guomin Huang, Jinhua Zhang, Zhihui Dou, Xingting Bao: Collected, processed, and analyzed data;
Guomin Huang, Jinhua Zhang, Zhihui Dou: Drafted the manuscript;
Guomin Huang, Hong Zhang, Hongyan Li: Revised the final manuscript.

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