TRIAP1 knockdown sensitizes non-small cell lung cancer to ionizing radiation by disrupting redox homeostasis

Chun-cheng Hao¹, Jia-ning Luo¹, Cui-yang Xu¹, Xin-yu Zhao¹, Zhen-bin Zhong¹, Xiao-nan Hu¹, Xiao-ming Jin² & Xiaofeng Ge¹

¹ Department of Radiotherapy, Harbin Medical University Cancer Hospital, Harbin City, China
² Department of Pathology, Harbin Medical University, Harbin City, China

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Non-small cell lung cancer; radioresistance; redox homeostasis; TRIAP1.

Abstract

Background: Radioresistance of some non-small cell lung cancer (NSCLC) types increases the risk of recurrence or metastasis in afflicted patients, following radiotherapy. As such, further improvements to NSCLC radiotherapy are needed. The expression of oncogene TP53-regulated inhibitor of apoptosis 1 (TRIAP1) in NSCLC is increased following irradiation. Furthermore, gene set enrichment analysis (GSEA) has suggested that TRIAP1 might be involved in maintaining redox homeostasis. This in turn might enhance cell radioresistance.

Methods: In this study we irradiated human NSCLC cell lines (A549 and H460), while knocking down TRIAP1, to determine whether a disrupted redox homeostasis could attenuate radioresistance.

Results: Irradiation notably increased both mRNA and protein levels of TRIAP1. In addition, TRIAP1 knockdown decreased the expression of several antioxidant proteins, including thioredoxin-related transmembrane protein (TMX) 1, TMX2, thioredoxin (TXN), glutaredoxin (GLRX) 2, GLRX3, peroxiredoxin (PRDX) 3, PRDX4, and PRDX6 in A549 and H460 cells. In addition, silencing TRIAP1 impaired the radiation-induced increase of the aforementioned proteins. Continuing along this line, we observed a radiation-induced reduction of cell viability and invasion, as well as increased apoptosis and intracellular reactive oxygen species following TRIAP1 knockdown.

Conclusions: In summary, we identified TRIAP1 as a key contributor to the radioresistance of NSCLC by maintaining redox homeostasis.

Introduction

Lung cancer remains the leading cause of cancer-related deaths worldwide. Non-small cell lung cancer (NSCLC) is the most common type of this malignancy, accounting for approximately 85% of all lung cancer cases.¹ NSCLC has a very poor prognosis, with five-year survival rates below 20%. Radiotherapy is one of the major therapeutic strategies to counter early stage and locally advanced NSCLC.² Radiation exposure generates reactive oxygen species (ROS) via the radiolysis of water, which can in turn cause damage to biological macromolecules such as DNA. Such genetic damage has been associated with the activation of cell cycle checkpoints, which may in turn delay cellular growth and could potentially activate apoptotic pathways, thus leading to cell death. However, the response of NSCLC to radiation treatment is variable. Some NSCLC cells show intrinsic radioresistance, which can lead to recurrence or metastasis in lung cancer patients, following radiotherapy.³ This phenomenon is a challenge for the further improvement of NSCLC radiotherapy.

TP53-regulated inhibitor of apoptosis 1 (TRIAP1) is an evolutionarily conserved protein, mainly regulated by TP53. Whereas TP53 is a well-known tumor suppressor, TRIAP1 functions as an oncogene because it mechanistically inhibits the interaction of cytochrome c with the apoptotic protease activating factor 1, thus preventing cancer cell apoptosis.⁴,⁵ A previous transcriptomics study found that radiation significantly increased TRIAP1 expression in...
A549 cells. Furthermore, gene set enrichment analysis (GSEA) in this study highlighted an implication of TRIAP1 in the regulation of redox homeostasis, which implies that TRIAP1 might be linked to the radioresistance of NSCLC cells by maintaining redox homeostasis. Our study explored the role of TRIAP1 in the radiosensitivity of NSCLC cells and the underlying mechanisms.

**Methods**

**Cell culture**

We used human NSCLC cell lines A549 and H460, derived respectively from lung carcinoma and large-cell lung cancer (American Type Culture Collection, Rockville, Maryland, US). We cultured the cells in RPMI 1640 medium (Invitrogen, Carlsbad, California, USA), supplemented with 10% fetal bovine serum (Gibco, CA, USA), 100 U/mL penicillin (Gibco), and 100 μg/mL streptomycin (Gibco) in a 5% CO₂ humidified incubator at 37°C.

**Ionizing radiation (IR) exposure**

We X-irradiated A549 and H460 cells using a linear accelerator (Primus K, Siemens, Munich, Bayern, Germany) with 6 MV photons/100 cm focus-surface distance (2.0 or 4.0 Gy/minute). Cell viability, apoptosis, and other assays were performed 48 hours after irradiation.

**Short hairpin RNA transfection**

TRIAP1-targeting short hairpin RNA (shRNA) and negative control shRNA were purchased from GenePharma (Shanghai, China). We cultured A549 and H460 cells in 12-well plates until confluence was approximately 80%. Next, we performed cell transfection with Lipofectamine 2000 (Invitrogen) according to the manufacturer’s instructions. Twenty-four hours after transfection, we evaluated the expression of TRIAP1 in A549 and H460 cells using quantitative real-time polymerase chain reaction (PCR) and western blot. The cells with the TRIAP1 knockdown were in turn irradiated or kept as control.

**PCR analysis**

We extracted RNA from cells using TRIzol (Invitrogen), followed by reverse transcription using the SuperScript First-Strand Synthesis System (Invitrogen). PCR conditions were as follows: 95°C for five minutes, followed by 38 cycles of 95°C for 15 seconds and 60°C for 40 seconds. The primer sequences that were used in this assay are listed in Table 1. We determined the relative mRNA levels using the 2−ΔΔCt method with normalization against glyceraldehyde-3-phosphate dehydrogenase (GAPDH, internal control).

**Western blot assay**

Cell lysates were prepared using ice-cold radioimmunoprecipitation assay buffer (Invitrogen) and we subsequently measured the protein concentrations using a
protein assay kit (Beyotime biotechnology company, Nanjing, China). Next, we separated the protein samples by sodium dodecyl sulfate–polyacrylamide gel electrophoresis, followed by a transfer onto nitrocellulose membranes. To detect our proteins of interest we incubated the membranes (4°C, overnight) with the following primary antibodies: anti-TRIAP1 antibody (1:500, Abcam, Cambridge, Massachusetts, UK), anti-thioredoxin-related transmembrane protein 1 (TMX1) antibody (1:500, Abcam), anti-TMX2 antibody (1:500, Abcam), anti-thioredoxin (TXN) antibody (1:500, Abcam), anti-glutaredoxin (GLRX) 2 antibody (1:500, Abcam), anti-GLRX3 antibody (1:500, Abcam), anti-peroxiredoxin (PRDX) 3 antibody (1:500, Abcam), anti-PRDX4 antibody (1:500, Abcam), anti-PRDX6 antibody (1:500, Abcam) and anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) antibody (1:2000, Abcam). This was followed by an incubation (one hour, room temperature) with horseradish peroxidase-conjugated secondary antibodies (Santa Cruz Biotechnology). We visualized the immune complexes using an enhanced chemiluminescence kit (Abcam) and quantified the protein bands by densitometry, using the Quantity One software (Bio-Rad Laboratories).

**Cell viability measurement**

We measured cell viability with a cell counting kit-8 (CCK8). Following an incubation (one hour, 37°C) of cells with CCK8 solution (Boster, Wuhan, China), we measured absorbance at 450 nm using a multimode plate reader (EnSpire, PerkinElmer, USA).

**Apoptosis assay**

We harvested A549 and H460 cells using trypsin and suspended the cells in binding buffer. Fluorescein isothiocyanate-conjugated annexin V and propidium iodide (Beyotime biotechnology company) were added to the cell suspension and incubated (15 minutes, room temperature) in a dark chamber. In turn, we quantified the stained cells using a flow cytometer (FACSCalibur; Becton Dickinson Biosciences, San Jose, California).

**Invasion assay**

We performed the invasion assays in transwell plates containing polycarbonate filters (8.0 µm pore size). First, we coated the transwell inserts with 50 uL of 1 mg/mL Matrigel matrix (Becton Dickinson, Franklin Lakes, NJ, USA) and stored the inserts at 37°C (four hours) to elicit gelling, according to the manufacturer’s recommendations. We seeded A549 and H460 cells at a density of 1.5 × 10^5 cells in 200 uL of medium (without fetal bovine serum) in the upper chamber. Next, we removed the inserts and washed with phosphate-buffered saline (PBS). The nonmigrating cells in the upper chamber were removed using a cotton swab. The inserts were then fixed in cold methanol (10 minutes, room temperature) and stained with hematoxylin. Cells that invaded the lower surface were counted in 10 fields at 200× magnification.

**Endogenous ROS measurement**

We incubated (30 minutes, 37°C) A549 and H460 cells with 7'-dichlorodihydrofluorescein diacetate (c-H2DCFDA, 10 µM, Invitrogen), a ROS-detecting molecular probe. When ROS is present, oxidation of c-H2DCFDA takes place and forms a fluorescent compound. This fluorescence was measured at an excitation wavelength of 480 nM and an emission wavelength of 525 nM, using a FAC Scan flow cytometer (Becton Dickinson, San Jose, CA).

**Statistical analysis**

Data are expressed as mean ± standard deviation. All statistical differences were analyzed using one-way analysis of variance, followed by a Tukey post-hoc test. A P-value of <0.05 was considered statistically significant.

**Results**

**TRIAP1 expression is negatively correlated with overall survival of lung adenocarcinoma patients**

To the best of our knowledge, the expression and role of TRIAP1 in NSCLC has not been systemically investigated until now. However, the TCGA database has already collected a vast amount of information regarding gene expression and its correlation with patient survival for a variety of tumors. By data mining the TCGA database (http://ualcan.path.uab.edu/analysis.html), we discovered that TRIAP1 expression is upregulated in lung adenocarcinoma, lung squamous cell carcinoma, and various other cancer types (Fig 1a,b). Moreover, Asian lung adenocarcinoma patients showed a relatively higher TRIAP1 expression than Caucasian and African-American patients (Fig 1c). TRIAP1 expression appeared more prominent as clinical stages of lung adenocarcinoma became more advanced (Fig 1d). We used the Kaplan-Meier plotter (http://geopia.cancer-pku.cn/) to investigate the correlation between individual TRIAP1 mRNA levels and overall survival (OS) of lung adenocarcinoma patients. Here, we observed that TRIAP1 expression is negatively correlated with the OS of lung adenocarcinoma patients (Fig 1e).
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TRIAP1 is a regulator of cell redox homeostasis according to GSEA

High throughput sequencing data (http://www.linkedomics.org/admin.php) highlighted various genes that are correlation with TRIAP1 expression in lung adenocarcinoma. GSEA of these genes might predict the biological processes influenced by TRIAP1. It is likely that TRIAP1 affects various biological processes, such as maintenance of redox homeostasis, which might be crucial for cancer cell radioresistance (Fig 2a). Twenty five genes that were correlated with TRIAP1 expression are involved in the maintenance of redox homeostasis. Of these, there are eight genes with an inverse correlation between expression and OS in lung adenocarcinoma patients, as is indicated in the Kaplan-Meier plot (http://gepia.cancer-pku.cn/) (Fig 2b). Data that we extracted from the TCGA database (http://starbase.sysu.edu.cn/index.php) further confirmed a positive correlation between TRIAP1 and these eight genes regarding their expression, as was demonstrated via Pearson correlation analysis (Fig 2c). As a result, we decided to further study these eight genes (TMX1, TMX2, TXN, GLRX2, GLRX3, PRDX3, PRDX4, and PRDX6).

Irradiation promoted TRIAP1 expression in NSCLC cells

As was indicated by PCR, the mRNA levels of TRIAP1 were increased in A549 and H460 cells following exposure to 2 Gy ($P < 0.01$) and 4 Gy ($P < 0.01$ or $P < 0.05$). Of note, the increase was most prominent with the 2 Gy dose (Fig 3a). Transfection with shRNA-TRIAP1 decreased TRIAP1 mRNA expression in both A549 and H460 cells ($P < 0.01$, Fig 3b) and prevented the previously described radiation-induced (2 Gy) increase of TRIAP1 mRNA. Western blot results highlighted a very significant increase in TRIAP1 protein expression (in both A549 and H460 cells) after irradiation with 2 Gy ($P < 0.001$, Fig 3c). Conversely, TRIAP1 protein levels in both A549 and H460 cells were decreased after transfection with shRNA-TRIAP1 ($P < 0.01$). The treatment of shRNA-TRIAP1 in combination with IR did not
Figure 2 Legend on next page.
significantly decrease TRIAP1 protein expression compared to control.

**TRIAP1 mediated upregulation of various antioxidative proteins in NSCLC cells following irradiation**

PCR analysis demonstrated that mRNA levels of TMX1, TMX2, TXN, GLRX2, GLRX3, PRDX3, PRDX4, and PRDX6 in A549 and H460 cells were significantly increased after irradiation \((P < 0.05, \text{or} \ P < 0.001, \text{Fig 4a})\). Next, we observed that TRIAP1 knockdown was associated with decreased TMX1, TMX2, TXN, GLRX2, GLRX3, PRDX4, and PRDX6 mRNA levels in A549 cells. In addition, TMX1, GLRX2, GLRX3, PRDX3, PRDX4, and PRDX6 mRNA was reduced in H460 cells \((P < 0.05 \text{ or} \ P < 0.01)\). Furthermore, TRIAP1 knockdown prevented the radiation-induced increase of TMX1, TMX2, TXN, GLRX2, PRDX3, PRDX4, and PRDX6 in A549 cells. Similarly, the radiation-induced expression of TMX1, TMX2, TXN, GLRX2, GLRX3, PRDX3, and PRDX6 was prevented in H460 cells \((P < 0.05 \text{ or} \ P < 0.01 \text{ vs. irradiation group})\). Building upon these findings, we explored expression at the protein level as well, using western blot. Both A549 and H460 cells showed radiation-induced increases in TMX1, TMX2, TXN, GLRX2, GLRX3, PRDX3, PRDX4, and PRDX6 protein levels \((P < 0.05 \text{ or} \ P < 0.01)\), while knocking down TRIAP1 in H460 cells reduced the amount of TMX1, GLRX2, GLRX3, PRDX3, PRDX4, and PRDX6 proteins \((P < 0.05 \text{ or} \ P < 0.01)\). Radiation-induced increases of TMX1, TMX2, TXN, GLRX2, GLRX3, PRDX4, and PRDX6 proteins in A549 cells were inhibited by TRIAP1 knockdown \((P < 0.05 \text{ or} \ P < 0.01 \text{ vs. irradiation group})\). Furthermore, in H460 cells, it was apparent that TRIAP1 knockdown prevented the radiation-induced increases of TMX1, TMX2, TXN, GLRX2, GLRX3, PRDX3, and PRDX6 proteins \((P < 0.05 \text{ or} \ P < 0.01 \text{ vs. irradiation group})\).

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**Figure 2** GSEA demonstrated the involvement of TRIAP1 in the regulation of cell redox homeostasis. (a) High throughput sequencing data (http://www.linkedomics.org/admin.php) highlighted various genes of which the expression is correlated with TRIAP1 expression in lung adenocarcinoma. GSEA of these genes suggested that various biological processes might be influenced by TRIAP1, such as the maintenance of redox homeostasis. This process might be an important factor in cancer radiosensitivity. (b) A total of 25 genes that were correlated with TRIAP1 expression are involved in the maintenance of redox homeostasis. From these, it was the expression of eight specific genes that is inversely correlated with the OS of lung adenocarcinoma patients, as indicated by the Kaplan-Meier curve (http://gepia.cancer-pku.cn/). (a) Low TMX1 TPM, (b) High TMX1 TPM; (c) Low TMX2 TPM, (d) High TMX2 TPM, (e) Low TXN TPM, (f) High TXN TPM, (g) Low GLRX2 TPM, (h) High GLRX2 TPM, (i) Low GLRX3 TPM, (j) High GLRX3 TPM, (k) Low PRDX3 TPM, (l) High PRDX3 TPM, (m) Low PRDX4 TPM, (n) High PRDX4 TPM, and (o) Low PRDX6 TPM. (q) Data from the TCGA database (http://starbase.sysu.edu.cn/index.php) further confirmed the positive correlation between TRIAP1 and these eight genes, regarding their expression, via Pearson correlation analysis. **(a)** Regression \((y = 0.2399x + 2.7822)\) and \((r = 0.207, P\text{-value} = 1.72e-06)\); **(b)** Regression \((y = 0.3382x + 3.7549)\) and \((r = 0.401, P\text{-value} = 9.33e-22)\); **(c)** Regression \((y = 0.4282x + 6.806)\) and \((r = 0.375, P\text{-value} = 4.72e-19)\); **(d)** Regression \((y = 0.2828x + 3.7549)\) and \((r = 0.397, P\text{-value} = 2.86e-21)\); **(e)** Regression \((y = 0.5347x + 1.3424)\) and \((r = 0.485, P\text{-value} = 1.99e-32)\); **(f)** Regression \((y = 0.5690x + 3.0335)\) and \((r = 0.531, P\text{-value} = 1.23e-39)\); **(g)** Regression \((y = 0.7181x + 2.9036)\) and \((r = 0.464, P\text{-value} = 2.17e-29)\); **(h)** Regression \((y = 0.3831x + 5.0952)\) and \((r = 0.373, P\text{-value} = 7.50e-19)\).
Figure 4 TRIAP1 mediated the upregulation of various antioxidative proteins in NSCLC cells following irradiation. Bioinformatics analysis showed that antioxidative proteins (TMX1, TMX2, TXN, GLRX2, GLRX3, PRDX3, PRDX4, and PRDX6) were positively regulated by TRIAP1. To validate this prediction, TRIAP1 was knocked down by transfecting shRNA which targets TRIAP1, before irradiation. The mRNA and protein levels of these antioxidative proteins were assessed respectively by (a) PCR and (b) Western blot. 

*P < 0.05, **P < 0.01 and ***P < 0.001 vs. control groups; #P < 0.05 and ##P < 0.01 vs. radiation group.
TRIAP1 knockdown increased the toxicity of ionizing radiation to NSCLC cells

We found that 2 Gy exposure decreased cellular (A549 and H460) viability (P < 0.05, Fig 5a). Comparably, silencing TRIAP1 also reduced A549 and H460 cell viability (P < 0.05). Combining TRIAP1 knockdown and 2 Gy irradiation resulted in an even more pronounced inhibition of cell viability, as compared to both treatments separately (P < 0.01). In addition, 2 Gy irradiation increased the rate of apoptosis in both A549 and H460 cells (P < 0.05, Fig 5b). Here, knocking down TRIAP1 also led to an increased rate of apoptosis in both A549 and H460 cells (P < 0.01).

Figure 5 TRIAP1 knockdown increased the toxicity of radiation exposure to NSCLC cells. TRIAP1 was knocked down by transfecting shRNA, which targeted TRIAP1, before irradiation. Next, (a) cell viability (a) A549, and (a) H460, (b) apoptosis rate (a) A549, and (a) H460, (c) cell invasion (a) A549, and (a) H460, and (d) intracellular reactive oxygen species levels were assessed (a) A549, and (a) H460. *P < 0.05 and **P < 0.01 ***P < 0.001 vs. control groups; #P < 0.05 and ##P < 0.01 vs. radiation group.
TRIAP1 knockdown in combination with 2 Gy irradiation further aggravated the rate of apoptosis in both A549 and H460 cells (P < 0.001). Furthermore, 2 Gy irradiation decreased the invasion of H460 cells (P < 0.05, Fig 5c), while no such effect was observed in A549 cells. TRIAP1 knockdown reduced the number of invading A549 and H460 cells (P < 0.05). Silencing TRIAP1 prior to 2 Gy irradiation caused a marked inhibition of invading A549 and H460 cells (P < 0.01).

In this study, we also evaluated the impact of TRIAP1 knockdown and 2 Gy irradiation (potentially both combined) on cellular ROS levels. We determined that 2 Gy irradiation increased the ROS levels in A549 and H460 cells (P < 0.05, Fig 5d), and TRIAP1 knockdown increased ROS levels in H460 cells, while ROS levels in A549 cells remained unaltered. Silencing TRIAP1 prior to 2 Gy irradiation notably increased ROS levels in A549 and H460 cells (P < 0.01).

Discussion

We performed a bioinformatics analysis on data collected from the TCGA database, which demonstrated that TRIAP1 is upregulated in lung adenocarcinoma and that its expression level is inversely correlated with overall patient survival. This suggests that TRIAP1 might play a cancer-promoting role in lung adenocarcinoma. Previous studies demonstrated that both microRNA-18a and microRNA-320b target TRIAP1. Of note, abnormal down-regulation of these microRNAs results in an elevated TRIAP1 expression, thus promoting the progression of ovarian cancer and nasopharyngeal carcinoma. The scientific consensus states that the cancer-promoting role of TRIAP1 is probably associated with its inhibitory effect on apoptosis. Fook-Alves et al. discovered that permanent silencing of TRIAP1 promoted late apoptosis in multiple myeloma cells, by increasing caspase 9 and APAF1 expression. In addition, stable transfection of TRIAP1 expressing plasmids in breast cancer cells increased the number of doxorubicin-resistant clones, while shRNA targeting TRIAP1 had an adverse effect.

In our study, we demonstrated that TRIAP1 expression in NSCLC was increased following irradiation. The increase of TRIAP1 expression is probably a response of cells to radiation. Radiation has been reported to change cell signal, epigenetic modification, expression of transcription factors and miRNAs, which are probably implicated in the change of TRIAP1 expression in response to radiation. The solid mechanism is needed to be identified in further studies. A GSEA study showed that TRIAP1 expression is correlated with several proteins that play key roles in maintaining the redox homeostasis. As such, TRIAP1 might also be implicated in the modulation of cellular redox equilibrium. Because this function of TRIAP1 has not previously been reported, we decided to further investigate which of these proteins are regulated by TRIAP1 and whether the regulatory effect on redox equilibrium is involved in the radioresistance of NSCLC. Knocking down TRIAP1 in NSCLC cells decreased the expression of TMX1, TMX2, TXN, GLRX2, GLRX3, PRDX3, PRDX4, and PRDX6 at both the mRNA and protein level, suggesting that these proteins are indeed regulated by TRIAP1.

TMX1, TMX2, and TXN are important members of the thioredoxin antioxidant system. TMX1 and TMX2 belong to the TMX subfamily and are localized to the membrane of the endoplasmic reticulum (ER). These proteins participate in various redox reactions, via the reversible oxidation of its active center (dithiol) to a disulfide. This will in turn catalyze dithiol-disulfide exchange reactions. TMXs have antiapoptotic properties in response to ER stress. Radiation-induced ROS leads to the accumulation of misfolded or abnormal proteins in the ER, which causes ER stress. Because ER stress is a critical inducer of apoptosis, previous studies focused on the link between ER stress and the enhanced radiosensitivity of cancer cells. Of note, Matsuo et al. found that overexpression of TMXs in 293 cells significantly attenuated ER stress and delayed the onset of ER stress-induced cell death. These findings suggest that TMX1 and TMX2 might protect NSCLC against radiation-induced apoptosis. TXN1, also known as Trx1, is a small reductase with a highly conserved redox-active motif (-Cys-Gly-Pro-Cys-). TXN1 overexpression in NSCLC is indicative of a more aggressive tumor phenotype, which in turn is associated with bad prognostic features and a poorer outcome. Inhibiting the expression and function of TXN1 has been shown to reduce the radiosensitivity of various cancer types.

GLRX2 and GLRX3 are thiol-disulfide oxidoreductases, which consist of an N-terminal Trx homology region followed by two tandem repeats of glutaredoxin domains. They play a pivotal role in protecting the cell from oxidative stress-induced apoptosis. For example, GLRX2 overexpression can prevent H2O2-induced apoptosis by protecting the mitochondrial activity of complex I. Furthermore, cancer cells with GLRX2 overexpression are more resistant to the doxorubicin and phenylarsine oxide, which induce oxidative stress. More importantly, GLRX2 can maintain an antioxidant and antiapoptotic state, even when other antioxidant enzymes such as superoxide dismutase 1 and thioredoxin reductase might be dysfunctional. Decreased levels of GLRX3 render cancer cells susceptible to death following oxidative stress. Furthermore, GLRX3 expression has also been associated with the migration, metastasis, and progression of oral squamous cell carcinoma and nasopharyngeal carcinoma.
The aforementioned studies suggest that the upregulation of GLRX2 and GLRX3 by TRIAP1 confers resistance of NSCLC to ROS-induced death following radiation.

The PRDX family of antioxidant enzymes comprises six identified mammalian isoforms (PRDX1-6). These proteins primarily protect cells from oxidative damage via the oxidation of a cysteine residue, which then forms sulfenic acid. This is subsequently reduced by thioredoxin. In addition to its antioxidant role, overexpression of PRDXs in cancer tissue appears strongly correlated with more aggressive cancer behavior, tumor metastasis and tumor-node-metastasis (TNM), indicating a possible role in the progression of tumorigenesis.\(^\text{31-34}\) The cancer-promoting effects of the PRDXs are probably associated with the regulation of several redox-sensitive cellular processes, such as cell proliferation, apoptosis, and cell signaling.\(^\text{31-34}\) In our study, TRIAP1 mediated the radiation-induced upregulation of PRDX3, PRDX4, and PRDX6 in NSCLC. They might enhance the cellular antioxidant capacity and promote the survival of NSCLC following irradiation.

TRIAP1 knockdown is associated with a more profound inhibition of radiation-induced cell viability, reduced cell invasion and an increased apoptosis rate of NSCLC cells. This suggests that the toxic effects of radiation are enhanced. Ketteler et al.\(^\text{35}\) unveiled that TRIAP1 (secreted by fibroblasts) was taken up by adjacent prostate cancer cells, PC3 and LNCaP, resulting in a pronounced cancer homeostasis.\(^\text{36}\) Our in-depth analysis will investigate the mechanism of regulation of antioxidant proteins by TRIAP1 in near future.

In summary, this study is the first to demonstrate that TRIAP1 contributes to the scavenging of radiation-induced ROS by upregulating several antioxidant proteins. Because TRIAP1 knockdown enhanced the radiosensitivity of NSCLC cells, we conclude that TRIAP1 is a key contributor to the radioresistance of NSCLC by maintaining redox homeostasis.

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Disclosure

The authors declare there are no potential conflicts of interest.

References

1 Huang G, Zhang J, Wang X, Chen Y, Liu D, Guo S. Clinicopathological and prognostic significance of Nanog expression in non-small cell lung cancer: A meta-analysis. *Onco Targets Ther* 2019; 12: 3609–17.
2 Zhang H, Zhang DX, Ju T, Zhou J. The effect of postoperative radiotherapy on the survival of patients with resectable stage III-N2 non-small-cell lung cancer: A systematic review and meta-analysis. *Neoplasma* 2019; 66(5): 717–26.
3 Li Q, Zong Y, Li K et al. Involvement of endothelial CK2 in the radiation induced perivascular resistant niche (PVRN) and the induction of radioresistance for non-small cell lung cancer (NSCLC) cells. *Biol Res* 2019; 52 (1): 22.
4 Li Y, Tang X, He Q et al. Overexpression of mitochondria mediator gene TRIAP1 by miR-320b loss is associated with progression in nasopharyngeal carcinoma. *PLoS Genet* 2016; 12 (7): e1006183.
5 Liu P, Qi X, Bian C et al. MicroRNA-18a inhibits ovarian cancer growth via directly targeting TRIAP1 and IPMK. *Oncol Lett* 2017; 13 (6): 4039–46.
6 Yang HJ, Kim N, Seong KM, Youn H, Youn B. Investigation of radiation-induced transcriptome profile of radioresistant non-small cell lung cancer A549 cells using RNA-seq. *PLOS One* 2013; 8 (3): e59319.
7 Fook-Alves VL, de Oliveira MB, Zanatta DB, Strauss BE, Colleoni GW. TP53 regulated inhibitor of apoptosis 1 (TRIAP1) stable silencing increases late apoptosis by upregulation of caspase 9 and APAF1 in RPMI8226 multiple myeloma cell line. *Biochim Biophys Acta* 2016; 1862 (6): 1105–10.
8 Adams C, Cazzanelli G, Rasul S et al. Apoptosis inhibitor TRIAP1 is a novel effector of drug resistance. *Oncol Rep* 2015; 34 (1): 415–22.
9 Cheng JCH, Chou CH, Kuo ML, Hsieh CY. Radiation-enhanced hepatocellular carcinoma cell invasion with MMP-9 expression through PI3K/Akt/NF-κB signal transduction pathway. *Oncogene* 2006; 25 (53): 7009–18.
10 Deborah AA, Kristina MG, Wayne DL, Douglas MR, Steven FZ. Radiation-induced epigenetic DNA methylation modification of radiation-response pathways. Epigenetics 2013; 8 (8): 839–48.

11 Cho S, Cinghu S, Yu JR, Park WY. Helicase-like transcription factor confers radiation resistance in cervical cancer through enhancing the DNA repair capacity. J Cancer Res Clin Oncol 2010; 137 (4): 629–37.

12 Shin S, Cha HJ, Lee EM et al. Alteration of miRNA profiles by ionizing radiation in A549 human non-small cell lung cancer cells. Int J Oncol 2009; 35 (1): 81–6.

13 Matsuo Y, Nishinaka Y, Suzuki S et al. TMX, a human transmembrane oxidoreductase of the thioredoxin family: The possible role in disulfide-linked protein folding in the endoplasmic reticulum. Arch Biochem Biophys 2004; 423 (1): 81–7.

14 Yoshihara E, Chen Z, Matsuo Y, Masutani H, Yodoi J. Thiol redox transitions by thioredoxin and thioredoxin-binding protein-2 in cell signaling. Methods Enzymol 2010; 474: 67–82.

15 Kim JY, Cho JH, Kim EM et al. β-Apocriopodophyllin functions as a radiosensitizer targeting ER stress in non-small cell lung cancer. Biomed Pharmacother 2019; 113: 108769.

16 Wang C, Song X, Shang M et al. Curcumin exerts cytotoxicity dependent on reactive oxygen species accumulation in non-small-cell lung cancer cells. Future Oncol 2019; 15 (11): 1243–53.

17 Seo MJ, Lee DM, Kim JY et al. Gambogic acid triggers vacuolization-associated cell death in cancer cells via disruption of thiol proteostasis. Cell Death Dis 2019; 10 (3): 187.

18 Shah SS, Rodriguez GA, Musick A et al. Targeting glioblastoma stem cells with 2-deoxy-D-glucose (2-DG) potentiates radiation-induced unfolded protein response (UPR). Cancers (Basel) 2019; 11 (2): 1.

19 Kakolyris S, Giatromanolaki A, Koukourakis M et al. Thioredoxin expression is associated with lymph node status and prognosis in early operable non-small cell lung cancer. Clin Cancer Res 2001; 7 (10): 3087–91.

20 Rodman SN, Spence JM, Ronnfeldt TJ et al. Enhancement of radiation response in breast cancer stem cells by inhibition of thioredoxin- and glutathione-dependent metabolism. Radiat Res 2016; 186 (4): 385–95.

21 Wang H, Jiang H, Corbet C et al. Piperlongumine increases sensitivity of colorectal cancer cells to radiation: Involvement of ROS production via dual induction of glutathione and thioredoxin systems. Cancer Lett 2019; 450: 42–52.

22 Wang H, Bouzakoura S, de Mey S et al. Auranoﬁn radiosensitizes tumor cells through targeting thioredoxin reductase and resulting overproduction of reactive oxygen species. Oncotarget 2017; 8 (22): 35728–42.

23 Wu H, Xing K, Lou MF. Glutaredoxin 2 prevents H2O2-induced cell apoptosis by protecting complex I activity in the mitochondria. Biochim Biophys Acta 2010; 1797: 1705–15.

24 Enoksson M, Fernandes AP, Prast S, Lillig CH, Holmgren A. Overexpression of glutaredoxin 2 attenuates apoptosis by preventing cytochrome c release. Biochem Biophys Res Commun 2005; 327: 774–9.

25 Lillig CH, Lonn ME, Enoksson M, Fernandes AP, Holmgren A. Short interfering RNA-mediated silencing of glutaredoxin 2 increases the sensitivity of HeLa cells toward doxorubicin and phenylarsine oxide. Proc Natl Acad Sci U S A 2004; 101: 13227–32.

26 Ferri A, Fiorenzo P, Nencini M et al. Glutaredoxin 2 prevents aggregation of mutant SOD1 in mitochondria and abolishes its toxicity. Hum Mol Genet 2010; 19 (22): 4529–42.

27 Zhang H, Du Y, Zhang X, Lu J, Holmgren A. Glutaredoxin 2 reduces both thioredoxin 2 and thioredoxin 1 and protects cells from apoptosis induced by auranoﬁn and 4-hydroxynonenal. Antioxid Redox Signal 2014; 21 (5): 669–81.

28 Pham K, Pal R, Qu Y et al. Nuclear glutaredoxin 3 is critical for protection against oxidative stress-induced cell death. Free Radic Biol Med 2015; 85: 197–206.

29 He F, Wei L, Luo W et al. Glutaredoxin 3 promotes nasopharyngeal carcinoma growth and metastasis via EGFR/Akt pathway and independent of ROS. Oncotarget 2016; 7 (24): 37000–12.

30 Li B, Chen M, Lu M, Xing X, Meng, Xiong P, Jun-Wu M. Glutaredoxin 3 promotes migration and invasion via the notch signalling pathway in oral squamous cell carcinoma. Free Radic Res 2018; 52 (4): 390–401.

31 Li S, Hu X, Ye M, Zhu X. The prognostic values of the peroxiredoxins family in ovarian cancer. Biosci Rep 2018; 38 (5): 20180667.

32 Hwang JA, Song JS, Yu DY et al. Peroxiredoxin 4 as an independent prognostic marker for survival in patients with early-stage lung squamous cell carcinoma. Int J Clin Exp Pathol 2015; 8 (6): 6627–35.

33 Chen L, Huang C, Yang X, Zhang Q, Chen F. Prognostic roles of mRNA expression of peroxiredoxins in lung cancer. Oncotargets Ther 2018; 11: 8381–8.

34 He Y, Xu W, Xiao Y et al. Overexpression of peroxiredoxin 6 (PRDX6) promotes the aggressive phenotypes of esophageal squamous cell carcinoma. J Cancer 2018; 9 (21): 3939–49.

35 Ketteler J, Panic A, Reis H et al. Progression-related loss of stromal caveolin 1 levels mediates radiation resistance in prostate carcinoma via the apoptosis inhibitor TRIAP1. J Clin Med 2019; 8 (3): 1.

36 Miliara X, Garnett JA, Tatsuta T et al. Structural insight into the TRIAP1/PRELI-like domain family of mitochondrial phospholipid transfer complexes. EMBO Rep 2015; 16 (7): 824–35.

37 Andryszik Z, Kim J, Tan AC, Espinoso JM. A genetic screen identifies TCF3/E2A and TRIAP1 as pathway-specific regulators of the cellular response to p53 activation. Cell Rep 2013; 3 (5): 1346–54.