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Adenovirus-mediated transfer of siRNA against peroxiredoxin I enhances the radiosensitivity of human intestinal cancer

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ABSTRACT

Peroxiredoxin I (Prx-I), a key member of the peroxiredoxin family, reduces peroxides and equivalents through the thioredoxin system. Our previous work has shown that expression of Prx-I in mammalian cells increases following ionizing radiation (IR), and suppression of its expression enhances radiation-induced cell death *in vitro*, suggesting that inhibition of Prx-I might be an important pretreatment for cancer radiotherapy. To test this hypothesis *in vivo*, we suppressed the expression of Prx-I in the human intestinal cancer cell line SW480 by adenovirus-mediated transfer of siRNA. Our results showed that expression of Prx-I in SW480 cells was dramatically reduced by recombinant Ad-SiPrxI, which resulted in decreased cell growth and increased cell death by IR. Significantly more cell apoptosis was detected by flow cytometry analysis when Prx-I expression was knocked down. To evaluate the effect of recombinant Ad-SiPrxI *in vivo*, xenografts were pretreated with adenovirus before IR. Tumor growth in mice was inhibited when the xenografts were pretreated with Ad-SiPrxI before IR. Our results suggest that pretreatment with recombinant adenovirus to inhibit Prx-I expression can enhance the radiosensitivity of cancer cells, and thus might be a potential application in clinical therapy.

1. Introduction

Ionizing radiation (IR) has been well demonstrated to be effective as a therapeutic agent against cancer. However, certain side effects and complications have been encountered which limit its applications in cancer radiotherapy. One of the important reasons for this is that some cancer cells are not sensitive to IR because of their genetic backgrounds. Although some genes related to DNA repair, cell cycle regulation, cell growth and so on, are suspected to have roles in radiation sensitivity, the genetic factors responsible for cancer radiosensitivity remain elusive. IR promotes many important cellular processes, such as DNA damage, apoptosis, signal transduction and oxidative stress [1]. One of the early effects of IR is that it produces reactive oxygen species (ROS). Oxidative stress induced by IR produces a variety of highly reactive free radicals that damage cells, initiate signal transduction pathways and alter gene expression. ROS can induce cellular antioxidant defense enzymes such as superoxide dismutase and

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glutathione peroxidase [2]. The relationship between IR and antioxidant enzymes has been thoroughly investigated. Antioxidant enzymes can attenuate radiation injuries [3]. Thus, different cancer cells have different radiosensitivities, partially because of different expression of antioxidant enzymes.

Peroxiredoxin (Prx), a newly defined family of highly conserved antioxidant enzymes, has been shown to play a critical role in peroxide detoxification [4,5]. Six Prx isoforms have been found in mammalian cells, which can be divided into two subgroups based on conserved cysteine residues. Peroxiredoxin I (Prx-I) contains two conserved cysteine residues, and has been found in abundance in the cytoplasm of cells as homodimers. From the results of biochemical and physiological studies, Prx-I has been implicated in a number of cellular functions, such as cell proliferation and differentiation, enhancement of natural killer cell activity and intracellular signaling, in addition to its antioxidant activity [6–8].

Increased Prx-I expression has been observed in various kinds of cancer cells, indicating it may be involved in cancer development or progression. Noh et al. found by Western immunoblotting analysis that Prx-I, as well as Prx-II and Prx-III, was over-expressed in human breast cancer tissues compared to normal tissues [9]. Using comparative proteome analysis between human normal (BEAS 2B) and malignant (A549) lung epithelial cells, Chang et al. found that Prx-I was reproducibly increased more than two-fold [10]. Additionally, Prx-I expression levels in the follicular neoplasm and thyroiditis groups were significantly higher than those of the control group, although there was not a statistically significant difference compared to those of the papillary carcinoma group [11]. All of these data suggest that Prx-I is involved in tumor development and progression, and might be a new target for gene therapy of tumor cells.

Our previous work demonstrated that the expression of Prx-I was up-regulated by IR in mouse intestinal epithelia and cultured IEC-6 cells [12,13]. This was consistent with studies from other researchers [14]. We speculated that increased Prx-I expression post-irradiation was just a compensation for increased ROS after IR. We also found knock down of Prx-I expression enhanced radiation-induced cell death *in vitro*. To further confirm the relationship between Prx-I expression and radiosensitivity in SW480 cancer cells *in vivo*, in this study we sought to inhibit the expression of Prx-I by RNA interference, and evaluate its effect on IR-induced cell death.

To achieve this goal, we utilized an adenovirus-mediated gene delivery system, as viral delivery systems are the most advanced in terms of preclinical development and clinical potential [15]. Because of their many advantages, adenovirus vectors have now been developed as tools for gene transfer into mammalian cells and for gene therapy applications. The recombinant adenovirus DNA is transfected into 293 cell lines that express the deleted viral genes in trans, so that viral particles can be easily produced and isolated. In this study, we used adenovirus as the gene delivery vehicle to efficiently transfer genetic material to cancer cells.

2. Materials and methods

2.1. Cell culture and mice

The human SW480 cell line and 293 cell line were obtained from the Shanghai Cell Bank (http://www.ctcccas.ac.cn/xibao). These cells were cultivated in growth medium (DMEM, Life Technologies, Gaithersburg, MD, USA) supplemented with 10% fetal bovine serum (Hyclone, Logan, USA), 100 units/mL of penicillin and 100 mg/mL of streptomycin at 37 °C in 5% CO_2.

Male Balb/c nude mice, 6–8 weeks of age, were obtained from The Animal Facility of the Third Military Medical University (Chongqing, China). Animals were housed under controlled temperature, humidity and day–night cycles with food and water. The Institutional Animal Care and Use Committee approved all animal protocols.

2.2. Plasmid constructs

Synthesized oligonucleotides were annealed and ligated to the XbaI/SalI sites of pAVU6+27 to produce pAVU6-PrxI or pAVU6-Con (as previously described [16]). These plasmids contain an expression cassette driven by the U6 promotor that produces dsRNA as a stem-loop structure in mammalian cells. A HindIII fragment of pAVU6-PrxI or its control, pAVU6-Con, was inserted into pAdTrack (Stratagene, http://www.stratagene.com), yielding the shuttle vectors pAdTrack-SiPrxI and pAdTrack-SiCon, respectively (Fig. 1). The inserted sequences were confirmed by dideoxy sequencing. We employed the efficient homologous recombination system as described [17]. The shuttle vectors were linearized with PmeI and transformed into BJ5183 cells (which express pAdEasy-1) by electroporation. Positive clones were selected and confirmed by PacI digestion and PCR. Plasmids from correct clones were amplified by transforming into DH10B cells. Adenoviral DNA (Ad-SiCon and Ad-SiPrxI) was prepared by a standard alkaline lysis procedure, and was linearized with PacI and purified by ethanol precipitation.

2.3. Production of adenovirus and cell infection

The 293packaging cell line was grown in DMEM supplemented with 10% FBS, 100 U/mL penicillin and 100 mg/mL streptomycin in a humidified atmosphere containing 5% CO_2 in air at 37 °C. Twenty-four hours before transfection, cells in monolayer culture during the logarithmic growth phase were trypsinized and plated in a six-well plate. Cells were transfected with Lipofectamine 2000 according to the manufacturer's instructions (Invitrogen, USA). After 4 h, the medium containing the transfection mix was replaced with fresh medium. Transfected cells were incubated for an additional period of 7–10 days and the medium was refreshed every 2–3 days. Viruses were harvested, amplified and titered according to the manufacturer's instructions (Stratagene, CA, USA).

SW480 cells were cultivated as previously described [16]. On the day before virus infection, 1.0×10^5 SW480 cells were plated in each well of a six-well plate. On the following day, the cells were incubated with recombinant virus particles (Ad-SiCon or Ad-SiPrxI) at a multiplicity of infection (MOI) of 10 or

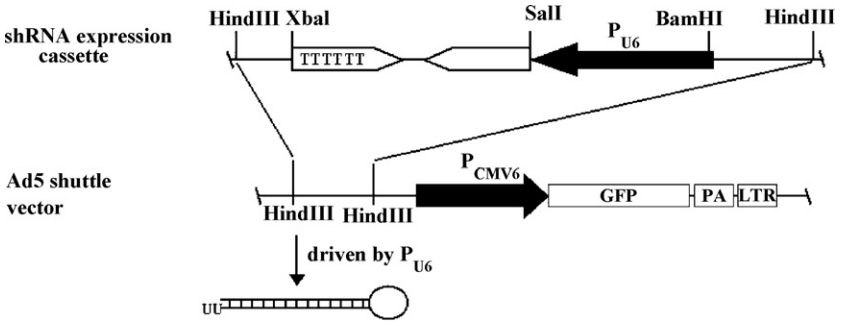

Fig. 1 – Schematic diagram of the target shRNA-expressing cassette of the Ad5 shuttle vector. An inverted repeat corresponding to the target sequences was inserted under the control of P_{U6}, along with a transcriptional termination signal of six Ts. The shRNA-expressing cassette was then subcloned into the multiple cloning site of the Ad5 shuttle vector pAdTrack. Transcription of the shRNA-coding insert was driven by P_{U6}.

50 at 37 °C. After adsorption for about 2 h, 2 mL of fresh growth medium was added and cells were placed in the incubator for an additional period of 2 and 4 days. Total protein was isolated and the expression of Prx-I was detected by Western blotting. The efficiency of infection was observed every day by fluorescence microscopy at 470 nm.

2.4. *Effect of adenovirus on cell growth*

All experiments were repeated at least three times. For growth curves, 1×10^4 SW480 cells were plated into 96-well dishes and fed every second day. Then, the cells were infected by recombinant virus or its control at a MOI of 10. Two and four days later, 20 μL of MTT solution (10 mg/mL) was added into the culture media, and the plate was returned to the incubator and cultivated for another 4 h. After the culture medium was drained out, 200 μL of DMSO was added into each well. The plates were read on a Dynatech MR600 microplate reader at 540 nm.

To determine the cell survival after irradiation, 1×10^4 SW480 cells were plated into 96-well dishes and fed every second day. After being infected by virus, the cells were irradiated at a dose of 10 Gy. Then, the irradiated cells were further cultivated for 2 and 4 days, and the absorbance was detected as described above. All data were normalized relative to the corresponding untreated cells.

2.5. *Flow cytometry analysis*

Three days post-viral infection, SW480 cells were trypsinized and washed with cold PBS. The harvested cells were fixed in 75% ethanol overnight at 4 °C, washed with PBS, digested by DNase-free RNase A (at a final concentration of 1 mg/mL) for 30 min at room temperature and stained with 50 mg/mL propidium iodide (PI) in PBS (1 h on ice in the dark). Flow cytometry analysis was performed on a FACScan, and data were analyzed by using Multi Cycle software (Becton Dickinson). The presence of apoptotic cells was detected by determination of the SubG1 population.

2.6. *Western blotting*

SW480 cells were infected by adenoviruses and harvested at the indicated time points, washed once with cold phosphate-buffered saline (PBS, pH 7.2) and lysed in lysis buffer (150 mmol/L NaCl, 50 mmol/L Tris–HCl, pH 7.4, 2 mmol/L EDTA, 1% NP-40) containing protease inhibitors. The protein concentration was determined using the Bradford dye-binding assay with bovine serum albumin as the standard. Total protein (30 μg per lane) was resolved on SDS-polyacrylamide gel and transferred onto a nitrocellulose membrane and incubated with anti-Prx-I and anti-GAPDH antibodies (Santa Cruz, CA, USA), followed by incubation with the corresponding secondary antibodies. The bands were visualized by using the enhanced chemiluminescence system (Pierce, Rockford, IL, USA).

2.7. *Tumor model*

Male Balb/c nude mice were inoculated subcutaneously with 1×10^6 human SW480 cells in the dorsal aspect of the neck. Three weeks after tumor inoculation with SW480 cells, all of the mice developed a large tumor (#100 mg). Mice with established 3-week-old tumors were randomly divided into four groups: groups 1 and 2 were intratumorally injected with adenovirus (Ad-SiCon and Ad-SiPrxI, respectively); group 3 was given a single dose of localized irradiation (20 Gy), and the final group was irradiated, followed by intratumoral injection of Ad-SiPrxI adenovirus. Each adenovirus-treated group received 10^9 viral particles per injection, and the injection was repeated the next day. All experiments used 5 mice per group. The tumor volume (TV) was measured every 3 days post-irradiation (Day 0), and was calculated according to the formula: $TV = L \times W^2/2$, where L and W are the major and minor dimensions, respectively.

2.8. *Immunohistochemistry*

Tumors xenografts were excised after the mice were sacrificed, and were then fixed with 4% paraformaldehyde in PBS for 24 h. All lobes were frozen and sectioned sagittally. Tissue sections were cut to 20 μm thickness and used for immunohistochemistry. The sections were placed into 0.1% H_2O_2 in methanol at room temperature for 20 min, and then incubated with goat anti-Prx-I or anti-GFP at 4 °C overnight. The next day, the slides were incubated with horseradish peroxidase (HRP) conjugated anti-goat-IgG

(1:200) (Zhongshan Company, Beijing) at 37 °C for 60 min. Positive cells were stained with DAB substrate. At the end of each step, the slides were thoroughly washed three times with PBS for 5 min each. The slides were microscopically examined for the percentage of positively stained cells and the distribution of positive cells.

2.9. TUNEL staining

Apoptosis was measured using a terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL) assay in frozen and paraformaldehyde-fixed tissue sections. Tumors xenografts from mice were fixed with paraformaldehyde for a few days before sectioning. The TUNEL staining of apoptotic cells was accomplished using the In Situ Cell Death Detection Kit (Roche Applied Science, Germany) according to the manufacturer's instruction, and the apoptotic index was calculated by dividing the number of apoptotic cells by the total number of cells. The cells were counterstained with hematoxylin. Positive controls were included with each group of samples stained.

2.10. Statistical analysis

Results were expressed as mean ± S.D., and the mean values were compared by using ANOVA. Means, standard errors and P values were calculated using SPSS version 10 for Windows. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Inhibition of Prx-I expression by adenovirus-mediated siRNA

The plasmid pAVU6-PrxI contains an expression cassette driven by the U6 promoter, and it produces dsRNA as a stem-loop structure in mammalian cells. This plasmid can efficiently inhibit Prx-I expression in SW480 cells. The U6 expression cassette was successfully ligated to the pAdTrack vector, and recombinant adenoviral DNA was generated in *E. coli* strain BJ5183. Next, it was transfected into 293 cells to produce virus particles. GFP expression was visible 24 h after transfection in 20–30% of the cells, representing the fraction of the population that was transfected. The comet-like foci of GFP expression began to appear 4–5 days after transfection. Viruses were amplified in 293 cells for several generations, and the final titers of the viruses particles was about 10^{10-11} expression-forming units (efu)/mL on 293 cells. Thus, sufficient viruses were produced for further experiments.

Next, the suppression of Prx-I expression by recombinant adenovirus was assessed in cultured SW480 cells. SW480 cells were infected at a MOI of 10–50, and almost all the cells could be observed under the fluorescence microscope the next day. Then, the Prx-I expression was detected by Western blotting. Prx-I expression in SW480 cells was significantly decreased after Ad-SiPrxI adenovirus infection, compared with that of the control adenovirus (Fig. 2). More inhibition of Prx-I expression was coincident with higher MOI and longer time after adenovirus infection.

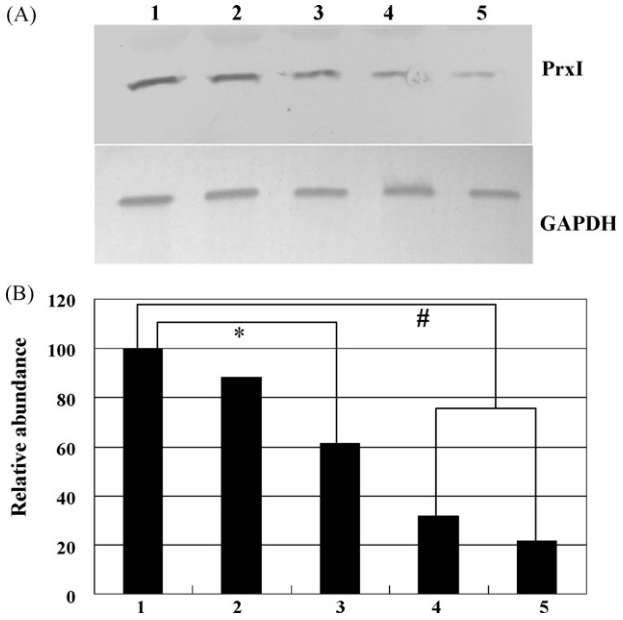

Fig. 2 – The effect of siRNA-expressing adenovirus on Prx-I expression in cultured SW480 cells. (A) Cell lysates were prepared at the indicated time and analyzed by Western blotting for both Prx-I and GAPDH expression. (B) Quantitation of the relative Prx-I protein level. Lane (1) Ad-SiCon infected for 2 days at a MOI of 50; lane (2) Ad-SiPrxI infected for 2 days at a MOI of 10; lane (3) Ad-SiPrxI infected for 2 days at a MOI of 50; lane (4) Ad-SiPrxI infected for 4 days at a MOI of 10; lane (5) Ad-SiPrxI infected for 4 days at a MOI of 50. Statistical significance: $^{*}P < 0.05$; $^{\#}P < 0.01$.

3.2. Decreased cell growth and increased radiation-induced cell injury by adenovirus-mediated siRNA

To determine the biological function of Prx-I in cell proliferation, we first compared the cell growth of SW480 cells infected with Ad-SiPrxI and Ad-SiCon. Cell proliferation was determined by MTT assay. As shown in Fig. 3A, the cell growth of Ad-SiPrxI infected SW480 cells was significantly delayed compared with that of Ad-SiCon. The proliferating time of SW480 was prolonged because of lower Prx-I expression. This indicates that Prx-I plays a role in cell proliferation, which is coincident with our previous result. We further asked whether down-regulation of Prx-I would sensitize the cells to radiation. Cultivated SW480 cells in 96-well plates were infected with Ad-SiPrxI and Ad-SiCon, and then were irradiated at dose of 10 Gy. Fig. 3B shows that the cell survival of Ad-SiPrxI-infected SW480 cells was significantly decreased after radiation, compared to that of the control. Two days after 10 Gy of irradiation, about 85% of Ad-SiCon-infected SW480 cells survived, but only 68% of Ad-SiPrxI-infected SW480 cells did. Four days after irradiation, the difference between the Ad-SiCon and Ad-SiPrxI groups became more significant. These data indicate sublethal radiation-induced damage is more severe when the expression of Prx-I is inhibited.

As the biological function of Prx-I has been linked to cell proliferation, we next assessed the effect of IR on the cell cycle

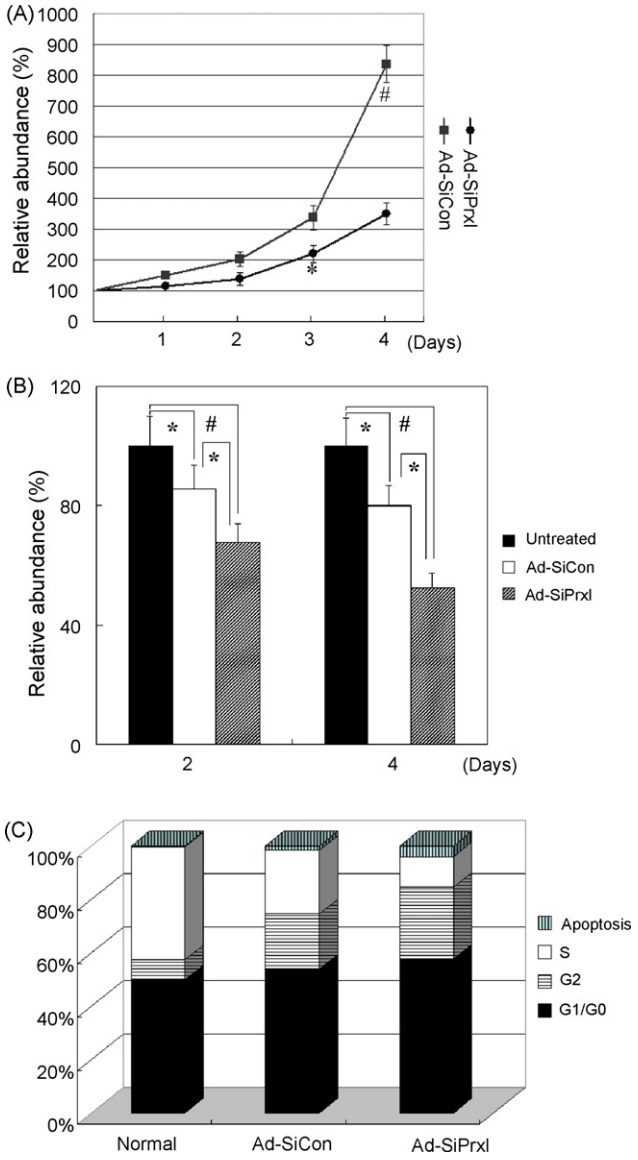

Fig. 3 – Effect of Prx-I on cell growth and cell survival after 10 Gy irradiation. (A) Cell growth curves of SW480 cells infected by Ad-SiCon and Ad-SiPrxI, respectively. Statistical significance of the difference between Ad-SiCon- and Ad-SiPrxI-infected cells: 3d, $P < 0.05$; 4d, $P < 0.01$. (B) Cell survival of SW480 infected by Ad-SiCon and Ad-SiPrxI, respectively, compared with that of untreated cells. SW480 cells were infected and then irradiated at a dose of 10 Gy. Two and four days later, cells survival was determined by MTT assay. Uninfected cells were used as a negative control. $^{*}P < 0.05$; $^{\#}P < 0.01$. (C) Cell cycle distribution of SW480 cells infected by Ad-SiCon and Ad-SiPrxI, respectively, and irradiated at a dose of 10 Gy.

distribution and apoptotic cell death after radiation. Ad-SiPrxI- and Ad-SiCon-infected SW480 cells were irradiated at a dose of 10 Gy and analyzed by flow cytometry. Twenty-four hours after irradiation, G2-M checkpoint arrest was found in both samples. Twenty-one of the Ad-SiCon-infected SW480 cells were blocked in G2 phase, and 24% of these cells were found in S phase. However, 28% of the Ad-SiPrxI-infected SW480 cells were blocked in G2 phase, and 12% of the cells were in S phase. Decreased cells in S phase were coincident with a reduced capacity to replicate because of Prx-I expression. A higher G2/G1 ratio was observed in Ad-SiPrxI-infected SW480 cells than that in Ad-SiCon-infected SW480 cells. Also, more apoptotic cell death was observed in irradiated Ad-SiPrxI-infected SW480 cells than in Ad-SiPrxI-infected SW480 cells.

3.3. *GFP and Prx-I expression and cell apoptosis in tumor xenografts*

To validate the efficiency of adenovirus in tumor xenografts, Ad-SiCon and Ad-SiPrxI adenovirus were intratumorally injected and detected. A high background was observed under the fluorescence microscope in frozen and paraformaldehyde-fixed tissue sections. GFP-positive cells were barely distinguished from the background. Thus, GFP-positive cells were alternatively detected using an anti-GFP antibody. GFP-positive cells were found in all sections from Ad-SiCon and Ad-SiPrxI adenovirus-infected tumor xenografts, indicating infection of adenoviruses *in vivo* were efficient (data not shown). Then, Prx-I expression was determined by immunohistochemical staining of these tissue sections. The results showed stronger positive staining on Ad-SiCon-infected tumors and weaker staining on Ad-SiPrxI-infected tumors (Fig. 4). Inhibition of Prx-I expression was up to 50% compared between the two groups. This suggests that treatment with Ad-SiPrxI adenovirus can efficiently attenuate Prx-I expression.

Because inhibition of Prx-I expression caused a significant growth delay, the possibility of Ad-SiPrxI-induced apoptosis *in vivo* was examined. But only a negligible amount of apoptosis was detected in the Ad-SiCon- and Ad-SiPrxI-infected tumor xenografts, and the difference between these groups was not significant.

3.4. *Tumor growth retardation by combined adenovirus infection and localized radiation therapy*

As inhibition of Prx-I expression could increase the radiosensitivity of SW480 cells *in vitro*, we evaluated the effect of combining adenovirus infection and localized radiation therapy. Nude mice with established tumors xenografts were treated with adenovirus (Ad-SiCon and Ad-SiPrx), followed by local radiotherapy. In the Ad-SiCon infection groups, tumors grew progressively to an average volume of 300 mm^2; all mice were sacrificed at Day 17. As shown in Fig. 5, the results of a tumor growth curve showed that tumor growth was retarded by radiation alone, consistent with others' experiments. Ad-SiPrxI adenovirus infection significantly inhibited the growth of tumor xenografts, compared with Ad-SiCon-infected controls. Moreover, the therapeutic effect of radiation was more significant than that of Ad-SiPrxI adenovirus. Furthermore, the radiation effect on tumor xenografts was enhanced by combined adenovirus infection. However, when compared with Ad-SiCon-infected controls, the retarded tumor growth by Ad-SiPrxI infection only lasted until Day 9, because after

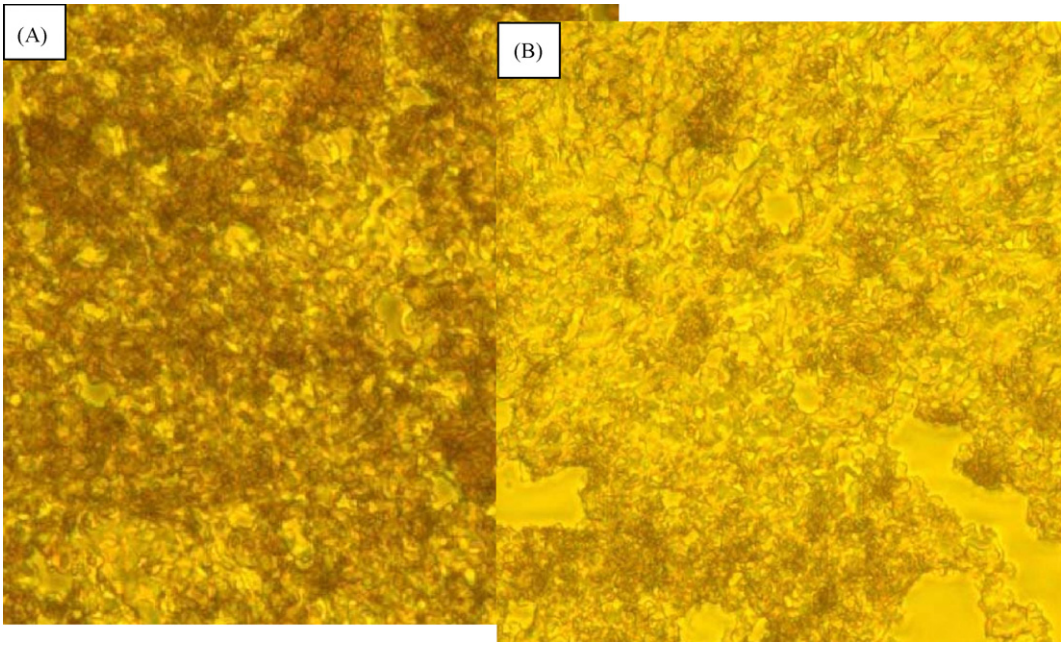

Fig. 4 – Typical Prx-I staining of tumor xenografts. Mice with established tumors were intratumorally injected with Ad-SiCon (A) and Ad-SiPrxI (B) adenovirus, respectively. Tumors xenografts were excised and detected as described in Section 2.

that it increased rapidly. This might reflect clearance of adenovirus *in vivo* [18].

4. Discussion

The novel findings in this study are that: (1) Prx-I expression could efficiently be inhibited by adenovirus-mediated siRNA and (2) inhibition of Prx-I expression sensitizes SW480 cells to radiation *in vivo*. Prx-I is a versatile protein and has been implicated in a number of cellular functions. The most striking feature of Prx-I is related to cancers. Although mice lacking Prx-I are viable and fertile, these mice had a shortened lifespan owing to the development of severe hemolytic anaemia and several malignant cancers, including lymphomas, sarcomas and carcinomas, suggesting that this protein functions as a tumor suppressor [19]. Other researchers have observed increased Prx-I expression in various kinds of cancer cells, indicating Prx-I is a potential new tumor marker for cancer cells, and possibly a potential therapeutic target. Chen et al. found that blocking Prx-I expression with antisense DNA in lung cancer cells resulted in significant growth inhibition, suggesting that Prx-I expression by tumor cells might be important for tumorigenesis [14]. Furthermore, they found Prx-I antisense DNA-transfected A549 showed a three-fold delay in the generation of palpable tumors, suggesting that inactivation of Prx-I may be a promising approach to improve the treatment outcome of patients with lung cancer [20]. In our previous work, we found a decrease of Prx-I expression by siRNA could give rise to cell growth delay, further confirming its function in cell proliferation. In this study, we also confirmed that inhibiting Prx-I expression enhanced radiotherapy of SW480 cancer cells *in vivo*.

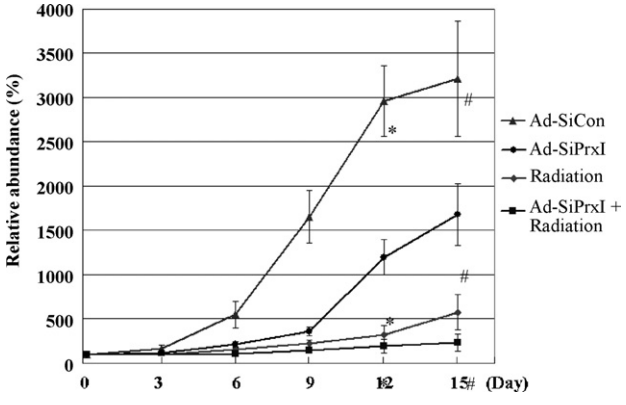

Fig. 5 – Injection of Ad-SiPrxI reduced *in vivo* tumor growth of SW480 xenografts. Tumor growth curves of Ad-SiCon-, Ad-SiPrxI-, radiation- and Ad-SiPrxI plus radiation-treated SW480 xenografts. SW480 cells (1×10^6) were injected into the subcutaneous space of nude mice, and tumor size was measured every 3 days after each tumor cell type was seeded into the animals. Tumor growth curves in nude mice were determined by relative tumor volumes. $^{*}P < 0.05$; $^{\#}P < 0.01$.

It has been suggested that Prx-I is a radiation responsive gene. Lee et al. found that Prx-I is transiently up-regulated in the irradiated testis [21]. Their result suggests that the relative radiation-resistance of Leydig and Sertoli cells can be attributed in part to the antioxidant function of the Prx system in these cells. The highly homologous gene Prx-II was increased in tissues isolated from the patients who did not respond to radiation therapy. Furthermore, treatment with a Prx-II antisense DNA decreased the expression of Prx-II,

enhancing the radiation sensitivity of cells [22]. In our previous work, we also found by two-dimensional electrophoresis that Prx-I protein was increased in epithelial cells post-radiation *in vitro* and *in vivo*. All of these data suggest that Prx-I expression can be induced in many kinds of cells. However, some researchers have found that the expression of Prx-I and Prx-II at the mRNA and protein level was not induced in K562 myeloid leukemia cells exposed to ionizing radiation, even 72 h after gamma irradiation [23].

RNAi was first achieved in a transient fashion by electroporation of *in vitro* synthesized dsRNA [24]. Another more valuable approach utilizing inducible, stably maintained constructs that express stem-loop transcripts has recently been described. In contrast to the success of RNAi in mammalian cell culture, there have been few reports of the effective use of siRNA in animal models [25]. Given the unknown complexity of animals and the current lack of understanding of the molecular basis of RNAi in vertebrates, it is likely that the main challenge for developing *in vivo* RNAi is the delivery of duplex RNA intact to the target tissue. To achieve this, virus-mediated siRNA technology has been developed. Adenovirus, lentivirus and retrovirus have been applied as transporters of siRNA [26]. Because of its many advantages, adenovirus technology is the most important. For example, Chen et al. used adenovirus-mediated RNAi against either structural protein 1D (Ad5-NT21) or polymerase 3D (Ad5-POL) of foot-and-mouth disease virus (FMDV), and their result showed delivery of these shRNAs significantly reduced the susceptibility to FMDV infection [27]. Our result showed the expression of Prx-I in SW480 cells was knocked down by adenovirus-mediated siRNA. The inhibition of Prx-I expression was associated with MOI and duration of adenovirus infection.

As some kinds of cancers are radiation resistant, it is very important to find new strategies to overcome this shortcoming. New efforts to enhance the effects of radiation therapy are underway, including the use of radiosensitizers. Motexafin gadolinium (xcytrin) is one such novel agent with several unique properties that enhance the cytotoxic potential of radiation therapy, as well as several chemotherapeutic agents [28]. Large amounts of small chemicals are screened as radiosensitizers. However, few chemicals have been selected as perfect radiosensitizers. Gene therapy strategies are a new choice to enhance the effectiveness of cancer radiotherapy [29]. Despite some formidable obstacles, the successful application of gene therapy in the treatment of cancer might occur when combined with radiation therapy. ROS are major mediators of radiation damage, and numerous studies have shown that levels of redox-regulating compounds can affect cellular radiation responses. Antioxidant enzymes scavenge ROS derived from ionizing radiation. These enzymes play important roles in radiation. The newly discovered Prx antioxidant enzymes appear to contribute in this respect, although the extent of their contribution is only now being realized. In this study, adenovirus-mediated Prx-I siRNA could sensitize cancer cells to radiation, indicating that Ad-SiPrxI might be a new kind of radiosensitizer. Our current study and others indicate that Prx-I might be considered for predicting tumor response to radiation therapy, and also as a target for rendering tumor cells more radioresponsive. Prx-I will help to develop more effective radiation therapy for cancer.

Conflict of interest

The authors declare that they have no competing financial interests.

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