# Adenovirus-mediated *p53* gene therapy reverses resistance of breast cancer cells to adriamycin

Xiaodong Qi, Zhikun Chang, Jin Song, Gui Gao and Zheng Shen

**The aim of this study was to determine whether adenovirus-mediated *p53* gene (*Ad-p53*) transfection can enhance adriamycin cytotoxicity and reverse adriamycin resistance in human breast cancer cells and explore its effect on the expression of *MDR1* gene and permeability-glycoprotein (P-gp). Human breast cancer cell lines, MCF-7 and MCF-7/ADR, were used in in-vitro studies. After infection with *Ad-p53*, the cytotoxicity of adriamycin was evaluated using the Cell Counting Kit-8 assay. The expression of MDR1 mRNA was detected by quantitative real-time PCR. The expression of P-gp was analyzed using western blotting. In in-vivo studies, MCF-7/ADR tumor cells were inoculated subcutaneously in athymic nude mice. After 14 days of inoculation, tumor size was measured. Apoptosis and expression of P-gp in the tumor tissue were analyzed by fluorescence activated cell sorting and western blotting. After transfection with a multiplicity of infection of 50 for *Ad-p53*, chemosensitivity of MCF-7/ADR cells increased by 18.1 times ($P=0.001$), and 50% inhibitory concentration ($IC_{50}$) of adriamycin decreased from $4.54 \pm 0.91$ to $0.26 \pm 0.11$ mg/l. Real-time PCR showed that MDR1 mRNA decreased from 1.32 to 0.85 ($P=0.001$). Western blotting analysis showed that P-gp also decreased. In in-vivo studies, *Ad-p53* combined with adriamycin dramatically inhibited the growth of subcutaneous xenograft of MCF-7/ADR. The fluorescence activated cell sorting assay showed that there were more apoptotic cells in tumor tissues treated with *Ad-p53* and adriamycin. The expression of P-gp was significantly decreased in tumor tissues. This study suggests that *Ad-p53* can reverse MCF-7/MDR cell resistance to adriamycin. The reversal effect was associated with inhibition of P-gp expression and induction of apoptosis. *Anti-Cancer Drugs* 22:556–562 © 2011 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

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## Introduction

Breast cancer is one of the most chemoresponsive solid tumors and can respond to structurally diverse chemotherapeutic drugs either as a single drug or as various combinations of these drugs. However, breast tumor cells gradually develop acquired resistance to multiple chemodrugs after starting chemotherapy. Multidrug resistance (MDR) in breast cancer is the main obstacle for chemotherapy. MDR usually presents as cross-resistance to multiple chemotherapeutic drugs with different structures and mechanisms [1]. The nature of MDR seems to be multifactorial and complex. One of the most crucial mechanisms is expression of the *MDR1* gene and its product, permeability-glycoprotein (P-gp) [2]. *p53* gene is a typical tumor suppressor gene. Mutations in *p53* gene are found in approximately 50% of all human malignant tumors [3]. *p53* gene mutation not only relates to tumorigenesis, but is also associated with poor prognosis, tumor progression, and tumor resistance to chemotherapeutic drugs [4,5]. Many studies showed a positive correlation between a mutation of p53 and expression of P-gp [6]. p53 protein is involved in the apoptotic process induced by chemotherapeutic drugs or ionizing radiation. Thus, mutation of p53 may lose its induction of apoptosis and enhance resistance of tumor cells to a variety of chemotherapeutic drugs.

Gene therapy by adenovirus-mediated introduction of wild-type human *p53* gene into tumor cells with mutation of the *p53* gene has shown to enhance chemosensitivity both *in vitro* and *in vivo* [7–11]. In this study, we explored whether adenovirus-mediated wild-type *p53* gene (*Ad-p53*) transfection can reverse MCF-7/ADR cell resistance to adriamycin both *in vitro* and *in vivo*, and introduction of a wild-type *p53* gene can increase chemosensitivity of the tumor cells to adriamycin. The mechanism of reversal was explored.

## Materials and methods

### Cell lines and culture conditions

The human breast cancer cell line, MCF-7, with a wild-type *p53* gene was derived from a human breast adenocarcinoma. The cell line, MCF-7/ADR, which is an adriamycin-resistant MCF-7 subcell line and contains a mutant *p53* gene, was derived from MCF-7 by selection with adriamycin. MCF-7 and MCF-7/ADR cell lines were

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provided by the Chinese Academy of Medical Sciences Cancer Institute. Monolayer cultures of MCF-7 and MCF-7/ADR were maintained in RPMI 1640 medium with 10% fetal calf serum (Gibco Chemical Co., Grand Island, New York, USA), 100 U/ml penicillin, and 100 U/ml streptomycin. The MCF-7/ADR cells were passed into a drug-free medium 2 weeks before use. All cells were incubated at 37°C in a humidified atmosphere supplied with 5% carbon dioxide.

### Agents

*Ad-p53*, with a replication-defective, E1- and E3-region-deleted adenoviral vector, was obtained from Shenzhen SiBiono GeneTech Co. Ltd. (Shenzhen, China). Adenovirus titer in plaque-forming units was determined by plaque formation assays using human embryonic kidney 293 cells. The multiplicity of infection (MOI) was defined as the ratio of plaque-forming units to total number of cancer cells to be infected. Adriamycin was purchased from Pharmacia and Upjohn Company (Bridgewater, New Jersey, USA).

### Cell growth assay

MCF-7/ADR cell growth was assessed by using trypan blue staining. MCF-7/ADR cells were washed with PBS three times and harvested, and were then plated at a density of $2 \times 10^4$ cells/well in a 24-well plate with each well containing 2 ml of medium. After growing for 24 h, the cells were infected with *Ad-p53* at an MOI of 50 and then separately cultured for 1, 2, 3, 4, and 5 days at 37°C with 5% carbon dioxide. Cell growth for each group was measured by counting cells in quadruplicate wells daily for 5 consecutive days. Viability of cells was assessed by trypan blue stain exclusion.

### Fluorescence activated cell sorting analysis of wild-type p53 expression

The expression of wild-type p53 protein was examined in MCF-7 and MCF-7/ADR cell lines using fluorescence activated cell sorting (FACS) analysis before and after *Ad-p53* transfection. MCF-7/ADR cells were infected with *Ad-p53* at an MOI of 50 for 48 h. Then these cells were harvested in a tube with approximately $1 \times 10^6$ cells per tube and fixed in 70% ethanol at 4°C for 2 h. After washing with PBS three times, the cells were incubated with fluorescein isothiocyanate (FITC)-primary anti-p53 antibody at a 1:100 dilution in the dark at a room temperature for 20 min. FITC-anti-mouse immunoglobulin G (IgG) (at a 1/100 dilution) was added as a control. After washing with PBS, the expression of wild-type p53 protein was examined by FACS (FACS Calibur, Becton Dickinson, San Jose, California, USA) and analyzed using CellQuest software (San Jose, California, USA).

### Drug sensitivity test

Chemosensitivity in cell lines MCF-7 and MCF-7/ADR was determined using CCK-8. Cell line MCR-7 or MCF-7/ADR was seeded at a density of $1 \times 10^4$ cells per well in a 96-well microtiter plate with each well containing 200 µl of medium. The cells were grown for 24 h, and were then transfected with *Ad-p53* at an MOI of 50 and cultured for 24 h. Then, cells were removed from the old medium and washed twice with PBS, and cultured in 200 µl of fresh medium containing several concentrations of adriamycin (0, 0.01, 0.1, 1.0, 10, 100 mg/l) for 48 h. Then, 10 µl/well CCK-8 solution was added in each well and the cells were incubated at 37°C for 3 h. Absorbance of the samples was analyzed using a Microplate Reader (BMG LABTECH GmbH, Offenburg, Germany) at a wavelength of 450 nm. The rate of cell viability was defined as a percentage of the absorbance measured in untreated control cells. The 50% inhibitory concentration ($IC_{50}$) of adriamycin with or without *Ad-p53* infection was calculated based on an equation of linear regression. The resistance ratio to adriamycin was defined as $IC_{50}$ in MCF-7/ADR/$IC_{50}$ in MCF-7. The ratio of reversal of cell resistance to adriamycin was determined as $IC_{50}$ in MCF-7/ADR without *Ad-p53* infection/$IC_{50}$ in MCF-7/ADR with *Ad-p53* infection.

### Determination of MDR1 mRNA expression by real-time PCR

*MDR1* gene expression before and after *Ad-p53* infection was determined by measuring mRNA level from total RNA using MDR1-special primer pairs with RT-PCR. After infection with *Ad-p53* at an MOI of 50 for 48 h, total RNA was extracted from cells with TRIzol reagent (Gibco Chemical Co.). The PCR mixture contains 3 µg of total RNA, M-MuLV RNase $H^+$ reverse transcriptase, 300 ng/µl random primers (1 µl), $2 \times$ RT buffer (10 µl), and RNase-free water (4 µl). MDR1 forward primer was 5′-CCCATCATTGCAATAGCAGG-3′ and reverse primer was 5′-GTTCAAACTTCTGCTCCTGA-3′. PCR amplification was carried out by predenaturing at 95°C for 15 min followed by running 35 cycles in the following conditions: denaturing at 94°C for 30 s, annealing at 50°C for 25 s, and extending at 72°C for 30 s followed by a final extending step at 72°C for 5 min and melting step at 95°C for 20 min. As an internal control, RT-PCR for β-actin was carried out using 5′-GTGGACATCCGCAAAGAC-3′ as a forward primer and 5′-GAAAGGGTGTAACGCAACT-3′ as a reverse primer in the same condition as described above. The threshold cycle value (Ct) was used to estimate the MDR1 expression level and was analyzed by Sequence Detection System Software (Life Technologies, Carlsbad, California, USA). The higher the Ct the lower the gene expression level. The following is a calculation using β-actin as internal control:

$$\Delta Ct = \frac{Ct \text{ of target gene}}{Ct \text{ of } \beta\text{-actin}}$$

The relative expression of target gene (the ratio of target gene to β-actin) $= 2^{-\Delta Ct}$.

### Western blot analysis of permeability-glycoprotein expression

Before and after *Ad-p53* infection, the expression of P-gp in MCF-7/ADR cell line was assayed by western blotting. The cells were plated at a density of $10^6$ in 25 $cm^3$ flasks and infected with *Ad-p53* at an MOI of 50 for 48 h. Total proteins were harvested by radioimmunoprecipitation assay lysate with 1 mmol/l phenylmethylsulfonyl fluoride and quantified using BCA protein quantitation kit (Pierce, Bonn, Germany). The protein was aliquoted and stored at −20°C until use. The protein samples (each one with 80 μg of total protein extract) were electrophoresed through 1 g/l SDS/acrylamide gel and the gel was run at 120 V for 1.5 h. The separated proteins were transferred onto polyvinylidene fluoride membrane at 100 V for 105 min by electroblotting. The membrane was blocked in 5% skimmed milk in Tris buffered saline (TBS)/Tween 20 under room temperature for 1 h. After multiple washes with TBS/Tween 20, the membranes were incubated with primary antibody against P-gp at a dilution of 1/100 at 4°C overnight. Then, multiple washes with TBS/Tween 20 were carried out before incubation with horseradish peroxidase-conjugated secondary anti-mouse IgG at a dilution of 1/10 000 at 37°C for 1 h. Signals were detected using the enhanced chemiluminescence system.

### In-vivo experiment

Athymic nude mice (Balb/c nu/nu, females, 4–6 weeks of age) were purchased from the Experimental Animal Department of Capital Medical University in Beijing. MCF-7/ADR cells were used to establish subcutaneous tumor in mice. Briefly, a total of $5 \times 10^6$ cells in 100 μl of PBS were injected subcutaneously into the right flank of each mouse. After growing for approximately 14 days, the tumor size reached a volume of approximately $5 \times 5 \times 5$ $mm^3$ and then these mice were randomly selected for treatment with either normal saline, *Ad-p53* alone, adriamycin alone, or *Ad-p53* and adriamycin. Each experimental group consisted of five mice. Mice were treated as indicated in Table 1.

Tumor dimensions were measured three times per week using a vernier caliper (Guilin Guanglu Measuring Instrument Co., Guilin, Guangxi Province, China). Tumor volume was calculated using an equation: $V\ (mm^3) = a \times b^2/2$, where $a$ is the largest diameter and $b$ is the smallest diameter. Mouse weight before and after treatment was measured. After 44 days of initial treatment, the mice were killed and tumors were excised. The local invasion and metastasis were observed and documented.

**Table 1 Treatment groups and treatment schedule**

| Treatment group | Treatment |
|---|---|
| Control ($n=5$) | Intratumoral injection of 0.1 ml normal saline for six injections on alternate days |
| *Ad-p53* ($n=5$) | Intratumoral injection of *Ad-p53* at a dose of $5 \times 10^{10}$ viral particles six times on alternate days |
| Adriamycin ($n=5$) | Intravenous injection of adriamycin diluted in 0.2 ml of normal saline at a dose of 4 mg/kg/mouse on every 4 days for four times |
| *Ad-p53* plus adriamycin ($n=5$) | *Ad-p53* group treatments plus adriamycin group treatments |

### Fluorescence activated cell sorting analysis

Tumor tissues were harvested, cut into small particles using a pair of scissors and then manually ground and washed with cold PBS twice. The released tumor cells were resuspended at a concentration of $1 \times 10^6$/ml and transferred into a tube. After adding 5 μl of annexin V-FITC (20 mg/ml) and 5 μl of propidium iodide (10 mg/ml) and gently mixing, the cells were incubated in dark for 15 min. Then 500 μl of binding buffer was added into the tube and mixed. The proportion of apoptotic cells from each treatment group was determined by FACS analysis (FACS Calibur, Becton Dickinson). Cell debris and fixation artifacts were excluded by appropriate gating.

### Western blot

Tumor tissues were frozen in liquid nitrogen and stored at −80°C. The expression of P-gp with different treatments was examined in tumor tissues by western blotting. Total proteins were extracted by the radioimmunoprecipitation assay lysate with 1 mmol/l phenylmethylsulfonyl fluoride and quantified using the BCA protein quantitation kit. The protein samples were electrophoresed through 1 g/l SDS/acrylamide gel running at 120 V for 1.5 h. The separated proteins were transferred onto a polyvinylidene fluoride membrane at 100 V for 70 min by electroblotting. The membrane was blocked in 5% skimmed milk in TBS/Tween 20 under room temperature for 1 h. After multiple washes in TBS/Tween 20, the membranes were incubated with primary antibody against P-gp at a 1/1000 dilution at 4°C overnight. After multiple washes with TBS/Tween 20 the membranes were incubated with fluorescent-conjugated secondary anti-mouse IgG at a 1/3000 dilution at 37°C for 1 h. As an internal control, β-actin was detected using its primary antibody at a 1/1000 dilution. Membranes were scanned with an Odyssey fluorescence scanner (LI-COR Biosciences, Lincoln, Nebraska, USA). Densitometric analysis was carried out using the Odyssey scanning software (LI-COR Biosciences).

### Statistical analysis

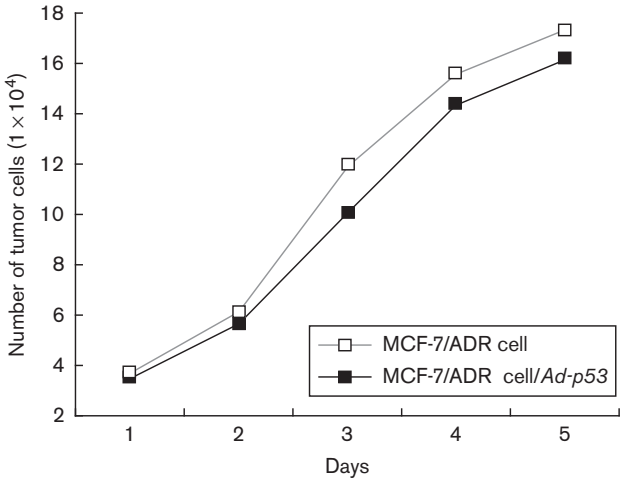
Statistical analysis was carried out using the SPSS 11.5 software (IBM Corporation, Somers, New York, USA). The data were expressed as mean ± standard deviation. Student's $t$-test was used for comparing the means from two groups and one-way analysis of variance was used for comparing the means of several groups. The difference was considered statistically significant when $P$ value was 0.05 or less.

## Results

### Effect of *Ad-p53* infection on MCF-7/ADR cell growth

The effect of *Ad-p53* infection on MCF-7/ADR cell growth is shown in Fig. 1. The inhibition ratio of cell

**Fig. 1**




Effect of *Ad-p53* transfection on the growth of MCF-7/ADR tumor cells. The MCF-7/ADR tumor cells were plated at density of $2 \times 10^4$cells/well and were infected with *Ad-p53* at a multiplicity of infection of 50. Cell growth in each treatment group was measured by counting cells daily in quadruplicate. The growth of MCF-7/ADR cells was significantly inhibited by *Ad-p53* transfection ($P<0.05$).

**Table 2 Effect of *Ad-p53* infection on the sensitivity of MCF-7/ADR to adriamycin**

| Cell groups | $IC_{50}$ (μg/ml) |
|---|---|
| MCF-7 | 0.04 ± 0.01 |
| MCF-7/ADR | 4.54 ± 0.91 |
| MCF-7/ADR + *Ad-p53* | 0.26 ± 0.11 |

growth was defined as the number of live cells in the control group minus number of live cells in the *Ad-p53* infection group divided by number of live cells in control group, and then times 100%. After infection with *Ad-p53*, the cells were separately cultured for 1, 2, 3, 4, and 5 days and cell growth was inhibited by 5.3, 7.4, 15.3, 7.7, and 6.0%, respectively. Cell growth was significantly inhibited after infection with *Ad-p53* ($P < 0.05$). The most effective inhibition occurred on the third day.

### Effect of *Ad-p53* infection on sensitivity of MCF-7/ADR cells to adriamycin

The sensitivity of MCF-7 and MCF-7/ADR cells to adriamycin was evaluated using the CCK-8 assay as shown in Table 2. The $IC_{50}$ of adriamycin was 0.04 ± 0.01 μg/ml for MCF-7 cells and 4.54 ± 0.91 μg/ml for MCF-7/ADR cells. It showed that the MCF-7/ADR cell line was 118.1-fold more resistant to adriamycin than the parental MCF-7 cell line ($P = 0.001$). After *Ad-p53* infection at an MOI of 50 for 24 h, the $IC_{50}$ of adriamycin was decreased to 0.26 ± 0.11 μg/ml. Treatment with *Ad-p53* decreased the resistance of MCF-7/ADR to adriamycin by 18.1-fold ($P = 0.001$).

### Expression of wild-type p53 protein in MCF-7/ADR cells after *Ad-p53* infection

FACS analysis was carried out to determine whether *Ad-p53* can efficiently express wild-type p53 protein in MCF-7/ADR cells. The percentage of p53 protein expression in MCF-7 and MCF-7/ADR cell lines was 0.76 and 10.24%, respectively. FACS analysis showed a higher percentage (36.20%) of wild-type p53 protein expression in the MCF-7/ADR cell line 48 h after *Ad-p53* infection.

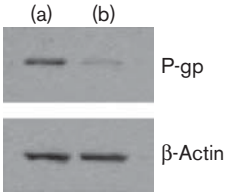
### Effect of *Ad-p53* transfection on endogenous MDR1 mRNA expression

To investigate whether *Ad-p53* transfection can inhibit endogenous MDR1 mRNA expression, MCF-7/ADR cells were infected with *Ad-p53* at an MOI of 50. After 48 h, MDR1 mRNA was detected by RT-PCR. As shown in Fig. 2 and Table 3, MCF-7/ADR cells had a higher MDR1 mRNA expression than MCF-7, which was in accordance with their higher chemoresistance. There was a significant decrease in MDR1 mRNA expression 48 h after *Ad-p53* infection in MCF-7/ADR cells, suggesting that wild-type p53 expression can inhibit endogenous MDR1 mRNA expression in MCF-7/ADR cells ($P < 0.05$).

### Effect of *Ad-p53* infection on P-gp expression in MCF-7/ADR cells

As seen in Fig. 2, 48 h after *Ad-p53* infection, there was a decrease in P-gp expression in MCF-7/ADR cells, suggesting that transduction of *Ad-p53* could inhibit P-gp expression in MCF-7/ADR cells effectively.

**Fig. 2**




Effect of *Ad-p53* transfection on p-glycoprotein (P-gp) expression in MCF-7/ADR cells. After infection of *Ad-p53* for 48 h, there was a decrease in P-gp expression. (a) untransfected MCF-7/ADR cells; (b) *Ad-p53* transfected MCF-7/ADR cells.

**Table 3 Expression of MDR1 mRNA in MCF-7, MCF-7/ADR, and MCF-7/ADR cells transfected with *Ad-p53***

| Cell groups | MDR1/β-actin |
|---|---|
| MCF-7 | 1.03 ± 0.02 |
| MCF-7/ADR | 7.35 ± 0.30* |
| MCF-7/ADR plus *Ad-p53* | 4.66 ± 0.22** |

Comparing with MCF-7.
*$P<0.05$.
**$P<0.001$.

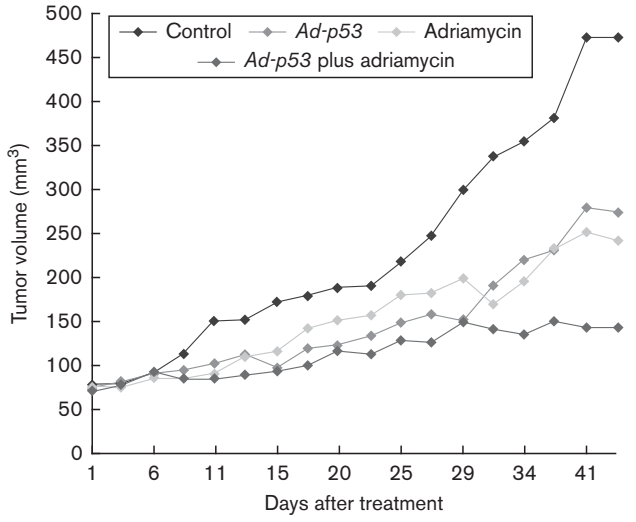
### Effect of *Ad-p53* treatment on tumor growth of xenograft

Subcutaneous MCF-7/ADR tumors generated in female Balb/c nude mice were treated by intratumoral injection with *Ad-p53* and intravenous injection with adriamycin. Tumor growth was measured regularly during a 6-week period after treatment. The tumor volumes of control, *Ad-p53*, adriamycin, and the *Ad-p53* and adriamycin group on day 44 were $473 \pm 69.6\,mm^3$, $274 \pm 50.4\,mm^3$, $240 \pm 73.8\,mm^3$, and $142 \pm 44.8\,mm^3$, respectively. Treatment with *Ad-p53* alone, adriamycin alone, or *Ad-p53* and adriamycin suppressed tumor volume by 42.05, 49.21 and 69.97%, respectively. In *Ad-p53*, adriamycin, and the *Ad-p53* and adriamycin group, tumor growth of *Ad-p53*, adriamycin, and *Ad-p53* plus adriamycin group was significantly suppressed compared with the control ($P < 0.05$). As shown in Fig. 3, the *Ad-p53* and adriamycin group showed a more significant inhibition on tumor growth compared with *Ad-p53* alone and adriamycin alone ($P < 0.05$).

### Induction of apoptosis in tumor tissue after *Ad-p53* infection

FACS analysis of tumor section showed a significant increase of apoptotic cells in the tumor tissue treated with *Ad-p53* alone and *Ad-p53* and adriamycin compared with control and adriamycin alone ($P < 0.05$). As shown in Figs 4 and 5, *Ad-p53* and adriamycin significantly increased the number of apoptotic cells compared with *Ad-p53* alone ($P < 0.05$).

**Fig. 3**




The effects of normal saline, *Ad-p53* alone, adriamycin alone, or *Ad-p53* plus adriamycin on the xenograft tumor growth in mice. Mice with implanted tumor were randomly assigned to treatment of normal saline, *Ad-p53*, adriamycin, or *Ad-p53* plus adriamycin; five mice in each treatment group. Tumor growth was significantly inhibited by *Ad-p53*, adriamycin, and *Ad-p53* plus adriamycin compared with the normal saline group ($P<0.05$). *Ad-p53* plus adriamycin showed most significant inhibitory effect ($P<0.05$).

### Effect of *Ad-p53* infection on P-gp expression in tumor tissue

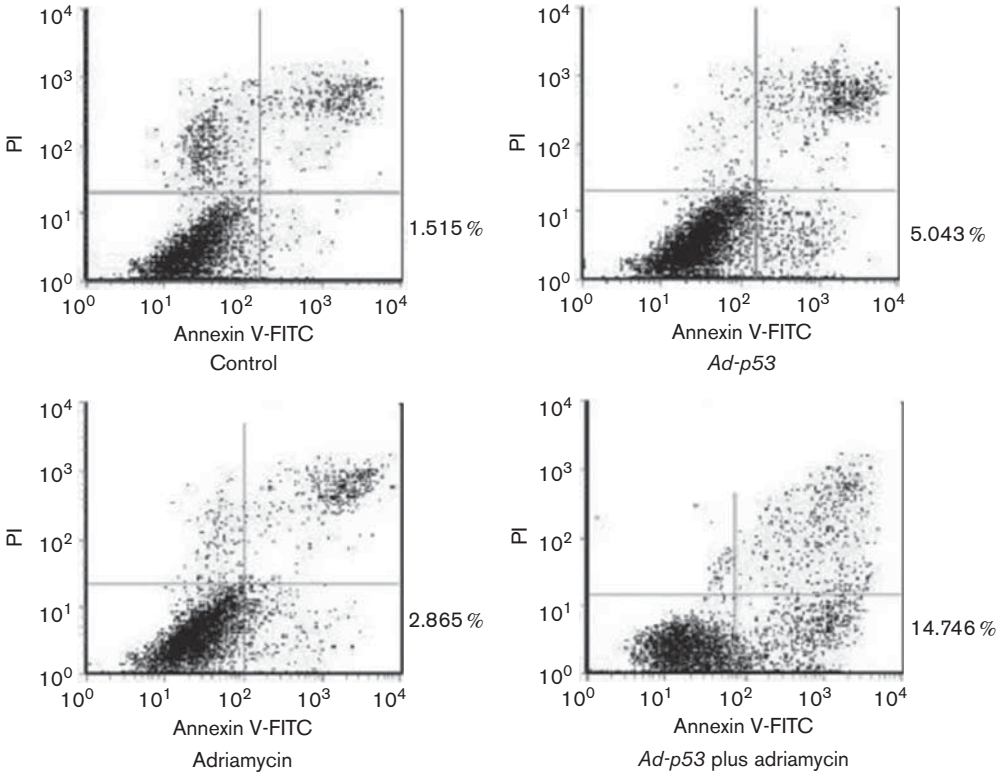
In-vitro data indicated that *Ad-p53* infection reversed the resistance of tumor cells to adriamycin, which was associated with the suppression of P-gp expression. Then, we explored the effect of *Ad-p53* treatment on P-gp expression in a tumor tissue. P-gp in the tumor tissue obtained from athymic mice was analyzed using western blotting. As shown in Fig. 6 and Table 4, P-gp expression decreased in the tumor tissue treated with *Ad-p53* alone and *Ad-p53* plus adriamycin, and *Ad-p53* plus adriamycin had a more significant inhibition than *Ad-p53* alone.

## Discussion

The mechanism of MDR is multifactorial and complex, and one of them is the overexpression of the *MDR1* gene and its P-gp product [2]. P-gp is considered to be an ATP-dependent membrane pump involved in pumping out chemotherapeutic drugs from the cytosol inside the tumor cells. Overexpression of P-gp in malignant cells could result in increased efflux and reduce the intracellular accumulation of toxic agents, such as 5-fluorouracil, anthracyclines, vinca alkaloids, and epipodophyllotoxins. Clinical data have shown that MDR1/P-gp was expressed in 26–46% of the untreated patients with breast cancer and the expression of MDR1/P-gp was significantly increased after chemotherapy, which was linked with resistance to chemotherapeutic drugs and poor prognosis [12]. Several agents, such as $Ca^{2+}$ channel blocker, verapamil, calmodulin inhibitor, and trifluoperazine, have been described to affect *MDR1* gene expression or MDR phenotype. These agents have been shown to reverse the MDR phenotype through direct competition with chemotherapeutic drugs for P-gp binding. Treatment with these agents could result in increased intracellular concentrations of cytotoxic drugs. However, these agents could induce significant side effects, which limited their clinical benefits. Therefore, new strategies for the reversal of MDR with lesser toxicity should be developed.

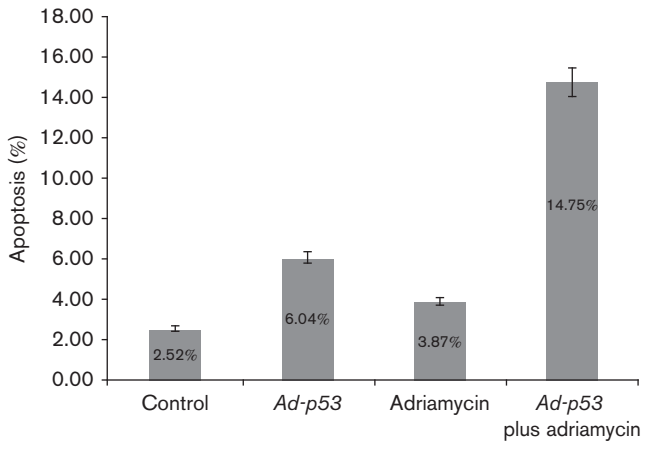
The wild-type *p53* tumor suppressor gene is involved in the control of cell growth and apoptosis in response to a variety of stress signals [13]. As a DNA-binding protein, p53 protein could positively regulate the expression of downstream genes including *Gadd45*, *p21* (*WAF1/CIP1*), *Bax*, *Fas*, and others [14]. However, the *p53* gene is functionally inactivated in approximately 50% of all human malignancies [3]. Lack of a functional p53 usually leads to increased genomic instability, accelerated tumor progression, and elevated cellular resistance to anticancer therapy [15]. Several studies have suggested that p53 status in tumor cells may be an important response determinant to chemotherapy [4,5]. A number of in-vitro and in-vivo studies have shown that the introduction of the wild-type *p53* gene could increase the tumor sensitivity of chemotherapeutic drugs in a spectrum of

**Fig. 4**



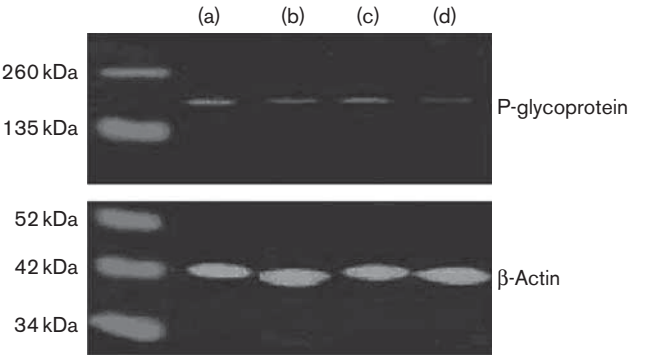

The effects of normal saline, *Ad-p53* alone, adriamycin alone, or *Ad-p53* plus adriamycin on induction of apoptosis in the implanted tumor. *Ad-p53* alone and *Ad-p53* plus adriamycin induced a higher proportion of apoptotic cells ($P<0.05$) compared with normal saline treatment. The *Ad-p53* plus adriamycin significantly increased number of apoptotic cells in comparison with *Ad-p53* alone ($P<0.05$).

**Fig. 5**




Using column graph to show the effects of normal saline, *Ad-p53* alone, adriamycin alone, or *Ad-p53* plus adriamycin on induction of apoptosis in the implanted tumor.

**Fig. 6**




The effects of normal saline, *Ad-p53* alone, adriamycin alone, or *Ad-p53* plus adriamycin on P-glycoprotein expression using western blotting analysis. Treatment with *Ad-p53* plus adriamycin showed a significant decrease of P-glycoprotein expression. (a) Normal saline, (b) *Ad-p53*, (c) adriamycin, (d) *Ad-p53* plus adriamycin.

cancers, such as lung cancer, ovarian cancer, pancreatic cancer, colon cancer, bladder cancer, and others, with the mutant *p53* gene [7–9,16].

The human breast cancer cell line, MCF-7/ADR, was selected from MCF-7 cells through a culture in the presence of adriamycin and was resistant to adriamycin,

**Table 4 Expression of P-gp protein in different treatment groups**

| Treatment groups | P-gp/β-actin |
|---|---|
| Control | 1.24 ± 0.18 |
| *Ad-p53* | 1.01 ± 0.04[a] |
| Adriamycin | 0.86 ± 0.09 |
| *Ad-p53* plus adriamycin | 0.63 ± 0.11[b] |

[a]Comparing with control group, $P<0.05$.
[b]Comparing with adriamycin group, $P<0.05$.

which was toxic to MCF-7 parental cells. MCF-7/ADR cells overexpress MDR1 mRNA and their *p53* gene is mutated [17,18]. Similar to a number of earlier studies on the other types of malignant tumor cells, our studies have shown that *Ad-p53* infection increased the sensitivity of MCF-7/ADR cells to adriamycin. The infection of *Ad-p53* significantly decreased the $IC_{50}$ of adriamycin from $4.54 \pm 0.91$ μg/ml to $0.26 \pm 0.11$ μg/ml. The results of in-vivo experiments showed that intratumoral injection of *Ad-p53* alone could suppress tumor growth. *Ad-p53* and adriamycin had a more significant inhibition on tumor growth and showed a synergistic effect.

Earlier studies have shown that mutated p53 and P-gp were always coexpressed in breast cancer, which was associated with MDR and poor prognosis [6]. Mutated p53 can upregulate P-gp expression by activating the promoter of the *MDR1* gene. Transcriptional activation of the MDR1 promoter by mutant p53 required an *Ets* binding site, and mutant p53 and Ets-1 synergistically activated MDR1 transcription [19]. Our in-vitro and in-vivo studies suggested that infection of *Ad-p53* can significantly downregulate MDR1 transcription and P-gp expression in breast cancer cell lines. The combination of *Ad-p53* with adriamycin significantly strengthened inhibition of P-gp expression. These results indicate that *Ad-p53* infection reversing resistance to adriamycin is closely associated with the inhibition of MDR1/P-gp expression.

The alteration in cell apoptosis was not only related to tumorigenesis but was also associated with drug resistance. One of the cell killing mechanisms of chemotherapy and radiotherapy is through p53-dependent apoptosis. Loss of functional p53 in tumor cells results in tumor resistance to several of chemotherapeutic drugs. Our results showed that *Ad-p53* infection enhanced apoptosis induced by adriamycin in the MCF-7/ADR cell-derived tumor, which indicated reversal of MCF-7/ADR cell resistance to adriamycin.

In this study, only one antitumor agent was tested using an available cell line. Thus, the result could not be generalized to other cell lines or other chemical agents. Only an association, and not a cause-and- effect relationship, was found between *Ad-p53* synergistic antitumor effect and expression of MDR1/P-gp. To determine the effect of *Ad-p53* on MDR/P-gp in other tumor cells, more tumor cell lines and more chemical agents must be tested. In the FACS analysis, cutting and grinding methods could not release all tumor cells from the tumor tissue. Therefore, the FACS result might not represent the cell status from whole tumor tissue.

In summary, our in-vitro and in-vivo results showed that *Ad-p53* gene therapy could increase MCF-7/ADR cell chemosensitivity to adriamycin. Enhanced cytotoxicity was associated with inhibition of expression of MDR1/P-gp.

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