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

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Abstract

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 ± 0.91 to 0.26 ± 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.

Key word: p53; tumor gene therapy; breast cancer cells; multidrug resistance; adriamycin

Introduction

Breast cancer is one of the most chemo-responsive solid tumors and can respond to structurally diverse chemotherapeutic drugs either as single drug or various combinations of these drugs. However, breast tumor cells gradually develop acquired

resistance to multiple chemo-drugs and tumor progression occurs. Multidrug resistance (MDR) in breast cancer is 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, P-glycoprotein (P-gp) [2]. P53 gene is a typical tumor suppressor gene. Mutations in p53 gene are found in about 50% of all human malignant tumors [3]. P53 gene mutation not only relates to tumorigenesis, but also are 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 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 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 transfection can reverse MCF-7/ADR cell resistance to adriamycin both in vitro and in vivo, and introduction of 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 wild-type p53 gene was derived from a human breast adenocarcinoma. The cell line MCF-7/ADR, which is an adriamycin-resistant MCF-7 sub-cell 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 provided by Chinese Academy of Medical Science 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), 100U/mL penicillin, 100U/mL streptomycin. The MCF-7/ADR cells were passed into drug-free medium 2 weeks prior to using. 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-regions deleted adenoviral vector, was obtained from Shenzhen SiBiono GeneTech Co. Ltd. Adenovirus titer in plaque-forming units (PFU) was determined by plaque formation assays using 293 cells. The multiplicity of infection (MOI) was defined as the ratio of PFU to total number of cancer cells to be infected. Adriamycin was purchased from Pharmacia & Upjohn Company.

Cell growth assay

MCF-7/ADR cell growth was assessed using trypan blue staining. MCF-7/ADR cells were washed with PBS for three times and harvested, and then were plated at a density of 2×10^4 cells/well in a 24-well plate with each well containing 2ml of medium. After growing for 24 hours, the cells were infected with Ad-p53 at 50 of MOI and then 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 five consecutive days. Viability of cells was assessed by trypan blue stain exclusion.

FACS 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 FACS analysis before and after Ad-p53 transfection. MCF-7/ADR cells were infected with Ad-p53 at 50 of MOI for 48 hours. Then these cells were harvested in a tube with about 1×10^6 cells per tube and fixed in 70% ethanol at 4 °C for 2 hours. After washing with PBS three times, the cells were incubated with FITC-primary anti-p53 antibody at a 1/100 dilution in dark at room temperature for 20 minutes. FITC-anti-mouse IgG (at a 1/100 dilution) was added as control. After washing with PBS, expression of wild-type p53 protein was examined by FACS (FACS Calibur, Becton Dickinson) and analyzed using CellQuest software.

Drug sensitivity test

Chemosensitivity in cell lines MCF-7 and MCF-7/ADR were determined using CCK-8. Cell line MCF-7 or MCF-7/ADR was seeded at a density of 1×10^4 cells per well in a 96-well microtiter plate with each well containing 200 μ l of medium. Let cells grow for 24 hours, then the cells were transfected with Ad-p53 at 50 of MOI and cultured for 24 hours. Then, after removing old medium and washing twice with PBS, and the cells were cultured in 200 μ l of fresh medium containing several concentrations of adriamycin (0, 0.01, 0.1, 1.0, 10, 100mg/L) for 48 hours. Then, 10ul/well CCK-8 solution was added in each well and the cells were incubated at 37 °C for 3 hours. Absorbance of the samples was analyzed using Microplate Reader 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 IC_{50} of adriamycin with or without Ad-p53 infection was calculated based on equation of linear regression. 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 real-time PCR. After infection with Ad-p53 at a MOI of 50 for 48 hours, total RNA was extracted from cells with Trizol Reagent (Gibco Chemical Co). The PCR mixture contains 3 μ g of total RNA, M-MuLV RNase H⁺ reverse transcriptase, 300ng/ μ l random primers (1 μ l), 2 \times RT buffer(10 μ l), and RNase-free water(4 μ l). MDR1

forward primer was 5' CCCATCATTGCAATAGCAGG3', and reverse primer was 5' GTTCAAACCTTCTGCTCCT GA 3'. PCR amplification was performed by pre-denaturing at 95°C for 15 minutes, and then run 35 cycles in the following conditions: denaturizing at 94°C for 30 seconds, annealing at 50°C for 25 seconds, and extending at 72°C for 30 seconds and followed by a final extending step at 72°C for 5 minutes and melting step at 95°C for 20 minutes. As internal control, RT-PCR for β -actin was carried out using 5' GTGGACATCCGCAAAGAC3' as a forward primer and 5' GAAAGGGTGTAACGCAACT3' 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 (SDS) software. The higher is the Ct, the lower the gene expression level. The following calculation using β -actin as internal control:

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

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

Western blot analysis of P-gp expression

Before and after Ad-p53 infection, the expression of P-gp in MCF-7/ADR cell line was assayed by WB. The cells were plated at a density of 10^6 in 25 cm^3 flasks and infected with Ad-p53 at a MOI of 50 for 48 hours. Total proteins were harvested by RIPA lysate with 1mmol/L phenylmethylsulfonyl fluoride and quantified using BCA protein quantitation kit. The protein was aliquoted and stored at -20°C until using. The protein samples (each one with 80 μg of total protein extract) were electrophoresed through 1g/L SDS/acrylamide gel and the gel was run at 120 volts for 1.5 hours. The separated proteins were transferred onto PVDF membrane at 100 volts for 105 minutes by electroblotting. The membrane was blocked in 5% skimmed milk in TBS/Tween-20 under room temperature for 1 hour. After multiple washes in 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 in TBS/Tween-20 were performed before incubation with horseradish peroxidase conjugated secondary anti-mouse IgG at a dilution of 1/10000 at 37°C for 1 hour. Signals were detected using the enhanced chemiluminescence system (ECL).

***In vivo* experiment**

Athymic nude mice (Balb/c nu/nu females, 4–6 weeks of age) were purchased from 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 5×10^6 cells in 100 μl of PBS were injected subcutaneously into the right flank of each mouse. After growing for about 14 days, the tumor size reached to a volume about $5 \times 5 \times 5 \text{ mm}^3$ and then these mice were randomly selected for treatment with either normal saline, Ad-p53 alone, adriamycin alone, or Ad-p53 plus adriamycin. Each experimental group consisted of 5 mice. Mice were treated as indicated in Table 1.

Tumor dimensions were measured three times per week using a vernier caliper.

Tumor volume was calculated using an equation: $V(\text{mm}^3) = a \times b^2 / 2$, 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 sacrificed and tumors were excised. The local invasion and metastasis were observed and documented.

FACS analysis

Tumor tissues were harvested, cut into small particles using a pair of scissors and then manually ground, and then were washed with cold PBS twice. The released tumor cells were re-suspended at a concentration of $1 \times 10^6/\text{ml}$ and transferred to a tube. After adding 5 μl of Annexin V-FITC (20mg/ml) and 5 μl of propidium iodide (10mg/ml) and gently mixing, the cells were incubated in dark for 15 minutes. Then added 500 μl of binding buffer in tube and mixed. The proportions 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 WB. Total proteins were extracted by RIPA lysate with 1mmol/L phenylmethylsulfonyl fluoride and quantified using BCA protein quantitation kit. The protein samples were electrophoresed through 1g/L SDS/acrylamide gel running at 120 volts for 1.5 hours. The separated proteins were transferred onto a PVDF membrane at 100 volts for 70 minutes by electroblotting. The membrane was blocked in 5% skimmed milk in TBS/Tween-20 under room temperature for 1 hour. 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 using TBS/Tween-20 the membranes were incubated with fluorescent conjugated secondary anti-mouse IgG at a 1/3000 dilution at 37°C for 1 hour. As internal control, β -actin was detected using its primary antibody at a 1/1000 dilution. Membranes were scanned with an Odyssey fluorescence scanner. Densitometric analysis was performed with Odyssey scanning software.

Statistical analysis

Statistical analysis was performed using SPSS 11.5 software. The data was expressed as mean \pm SD. Student's t-test was used for comparing means from two groups and one-way analysis of variance (ANOVA) was used for comparing means of several groups. The difference was considered statistically significant when $P \leq 0.05$.

Results

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

The effect of Ad-p53 infection on MCF-7/ADR cell growth as shown in Figure 1. The

inhibition ratio of cell growth was defined as number of live cells in control group minus number of live cells in the Ad-p53 infection group, and then divided by number of live cells in control group, and then times 100%. After 1, 2, 3, 4 and 5 days infection with Ad-p53, the growth inhibition ratio was 5.3%、7.4%、15.3%、7.7%、6.0% respectively. The 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 CCK-8 assay as shown in Table 2. The IC_{50} of adriamycin was (0.04 ± 0.01) $\mu\text{g/ml}$ for MCF-7 cells and (4.54 ± 0.91) $\mu\text{g/ml}$ for MCF-7/ADR cells. It showed MCF-7/ADR cell line was 118.1-fold more resistant to adriamycin than parental MCF-7 cell line ($P=0.001$). After Ad-p53 infection at a MOI of 50 for 24 hours, the IC_{50} of adriamycin was decreased to 0.26 ± 0.11 $\mu\text{g/ml}$. Treatment with Ad-p53 decreased the resistance of MCF-7/ADR to adriamycin by 18.1 times ($P=0.001$).

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

infection

FACS analysis was performed 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 were 0.76% and 10.24%, respectively. FACS analysis showed a higher percentage of wild-type p53 protein expression in MCF-7/ADR cell line 48 hours after Ad-p53 infection, which was 36.20%.

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 a MOI of 50. After 48 hours, MDR1 mRNA was detected by real-time RT-PCR. As shown in Figure 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 hours after Ad-p53 infection in MCF-7/ADR cells, suggesting 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 Figure 2, 48 hours after Ad-p53 infection, there was a decrease in P-gp expression in MCF-7/ADR cells, suggesting transduction of Ad-p53 could inhibit P-gp expression in MCF-7/ADR cells effectively.

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 following treatment. The tumor volumes of control, Ad-p53, adriamycin, and Ad-p53 plus adriamycin group on day 44 were $473 \pm 69.6 \text{ mm}^3$, $274 \pm 50.4 \text{ mm}^3$, $240 \pm 73.8 \text{ mm}^3$, and $142 \pm 44.8 \text{ mm}^3$, respectively. Treatment with Ad-p53 alone, adriamycin alone, or Ad-p53 plus adriamycin suppressed tumor volume by 42.05%, 49.21%, 69.97%, respectively. In Ad-p53, adriamycin and Ad-p53 plus adriamycin group, The tumor growth of Ad-p53, adriamycin, and Ad-p53 plus adriamycin group was significantly suppressed compared to the control ($P < 0.05$). As shown in Figure 3, the group of Ad-p53 plus adriamycin demonstrated more significant inhibition on tumor growth compared to 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 tumor tissue treated with Ad-p53 alone and Ad-p53 plus adriamycin compared to control and adriamycin alone ($P < 0.05$). As shown in Figure 4 and 5, Ad-p53 plus adriamycin significantly increased number of apoptotic cells comparing to Ad-p53 alone ($P < 0.05$).

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 suppression of P-gp expression. Then, we explored the effect of Ad-p53 treatment on P-gp expression in tumor tissue. P-gp in the tumor tissue removed from athymic mice was analyzed using WB. As shown in Figure 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 over expression of MDR1 gene and its P-gp product [2]. P-gp is considered as an ATP-dependent membrane pump involved in pumping out chemotherapeutic drugs from the cytosol inside tumor cells. Over expression of P-gp in malignant cells could result in increased efflux and reduce intracellular accumulation of toxic agents, such as 5-FU, anthracyclines, Vinca alkaloids, and epipodophyllotoxins. Clinical data has shown MDR1/P-gp expressed in 26%-46% of untreated patients with breast cancer and expression of MDR1/P-gp was significantly increased after chemotherapy, which 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 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. So new strategies of reversal of multidrug resistance with less toxicity should be developed.

The wild-type p53 tumor suppressor gene is involved in 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 expression of downstream genes, including Gadd45, p21 (WAF1/CIP1), Bax, Fas, et al [14]. However, p53 gene is functionally inactivated in about 50% of all human malignancies [3]. Lack of functional p53 usually leads to increased genomic instability, accelerated tumor progression, and elevated cellular resistance to anticancer therapy [15]. Several reports 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 introduction of wild-type p53 gene could increase tumor sensitivity to chemotherapeutic drugs in a spectrum of cancers with mutant p53 gene, such as lung cancer, ovarian cancer, pancreatic cancer, colon cancer, bladder cancer and others [7-9, 16].

Human breast cancer cell line MCF-7/ADR was selected from MCF-7 cells through culture in presence of adriamycin and was resistant to adriamycin, which was toxic to MCF-7 parental cells. MCF-7/ADR cells over-express MDR1 mRNA and their p53 gene is mutated [17, 18]. Similar to a number of previous studies on other types of malignant tumor cells, our studies demonstrated Ad-p53 infection increased sensitivity of MCF-7/ADR cells to adriamycin. The infection of Ad-p53 significantly decreased the IC₅₀ of adriamycin from (4.54±0.91) µg/ml to (0.26±0.11) µg/ml. The results of the *in vivo* experiments showed intratumoral injection of Ad-p53 alone could suppress tumor growth. Ad-p53 plus adriamycin had a more significant inhibition on tumor growth and demonstrated a synergistic effect.

Previous studies have shown mutated p53 and P-gp were always co-expressed in breast cancer, which was associated with MDR and poor prognosis [6]. Mutated p53 can up-regulate P-gp expression by activating the promoter of MDR1 gene. Transcriptional activation of 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 infection of Ad-p53 can significantly down-regulate 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 Ad-p53 infection reversing resistance to adriamycin is closely associated with inhibition of MDR1/P-gp expression

The alteration in cell apoptosis not only related in tumorigenesis but also associated with drug resistance. One of 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 demonstrated that Ad-p53 infection enhanced apoptosis induced by adriamycin in

MCF-7/ADR cells-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, not a cause-effect relationship, was found between Ad-p53 synergistic anti-tumor 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 FACS analysis, cutting and grinding method could not release all tumor cells from the tumor tissue. Therefore, FACS result might not represent the cell status from whole tumor tissue.

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

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Table

Treatment Group	Treatment
Control (N=5)	Intratumoral injection of 0.1ml normal saline on alternate day for 6 injections
Ad-p53 (N=5)	Intratumoral injection of Ad-p53 at a dose of 5×10^{10} viral particles (VP) on alternate day for 6 times
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 four

Ad-p53 plus Adriamycin (N=5)	days for 4 times Ad-p53 group treatments + Adriamycin group treatments
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Table 1 Treatment Groups and Treatment Schedule

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

Cell Groups	IC50 ($\mu\text{g/ml}$)
MCF-7	0.04 \pm 0.01
MCF-7/ADR	4.54 \pm 0.91
MCF-7/ADR + Ad-p53	0.26 \pm 0.11

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 \pm 0.02
MCF-7/ADR	7.35 \pm 0.30 ^a
MCF-7/ADR + Ad-p53	4.66 \pm 0.22 ^b

Note: comparing with MCF-7, ^a $p < 0.05$; ^b $p < 0.001$

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

Treatment Groups	P-gp/ β -actin
Control	1.24 \pm 0.18
Ad-p53	1.01 \pm 0.04 ^a
Adriamycin	0.86 \pm 0.09
Ad-p53 plus Adriamycin	0.63 \pm 0.11 ^b

Note: ^a comparing with Control group $p < 0.05$

^b comparing with Adriamycin group $p < 0.05$

Figures

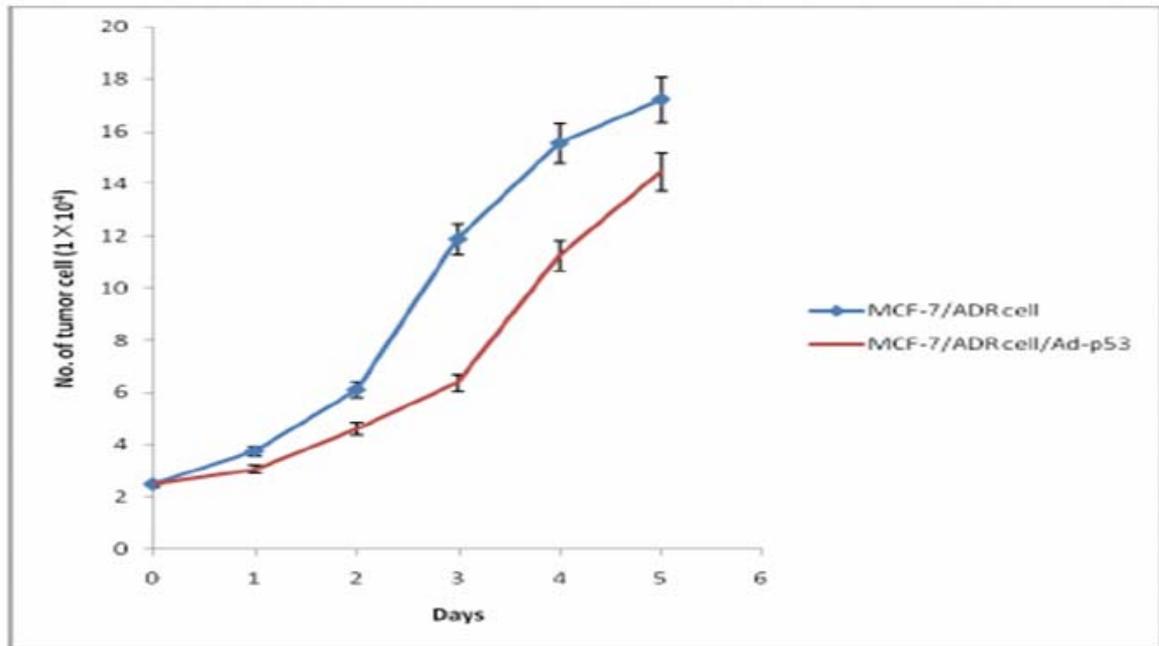


Fig.1 Effect of Ad-p53 transfection on the growth of MCF-7/ADR tumor cells. The MCF-7/ADR tumor cells were plated at density of 2×10^4 cells/well and were infected with Ad-p53 at a MOI of 50. Cell growth in each treatment group was measured by counting cells daily in quadruplicate. The growth of MCF-7/ADR cells was significantly inhibited by Ad-p53 transfection ($P < 0.05$).

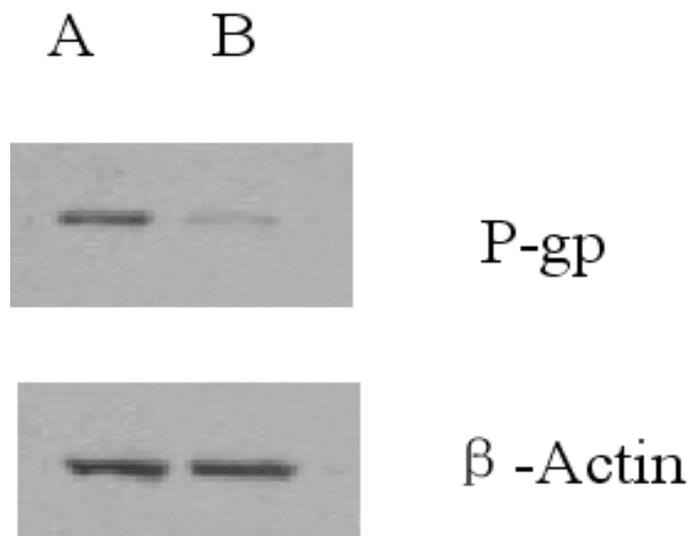


Fig.2. Effect of Ad-p53 transfection on P-gp expression in MCF-7/ADR cells. After infection of Ad-p53 for 48 hours, there was a decrease in P-gp expression.

A: untransfected MCF-7/ADR cells; B: Ad-p53 transfected MCF-7/ADR cells.

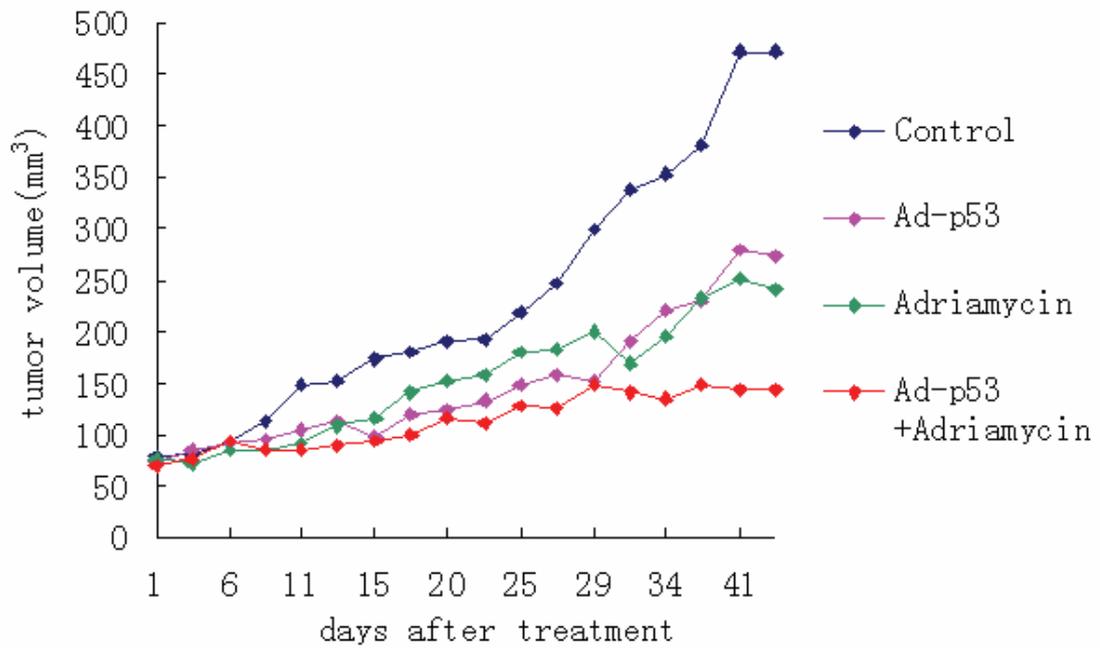


Fig.3 The effects of normal saline, Ad-p53 alone, adriamycin alone, or Ad-p53 plus adriamycin on the xenograft tumor growth in mice. Mice with implanted tumor were randomly assigned to treatment of normal saline, Ad-p53, adriamycin, or Ad-p53 plus adriamycin, 5 mice in each treatment group. Tumor growth was significantly inhibited by Ad-p53, adriamycin, and Ad-p53 plus adriamycin compared to the normal saline group ($P < 0.05$). Ad-p53 plus adriamycin showed most significant inhibitory effect ($P < 0.05$).

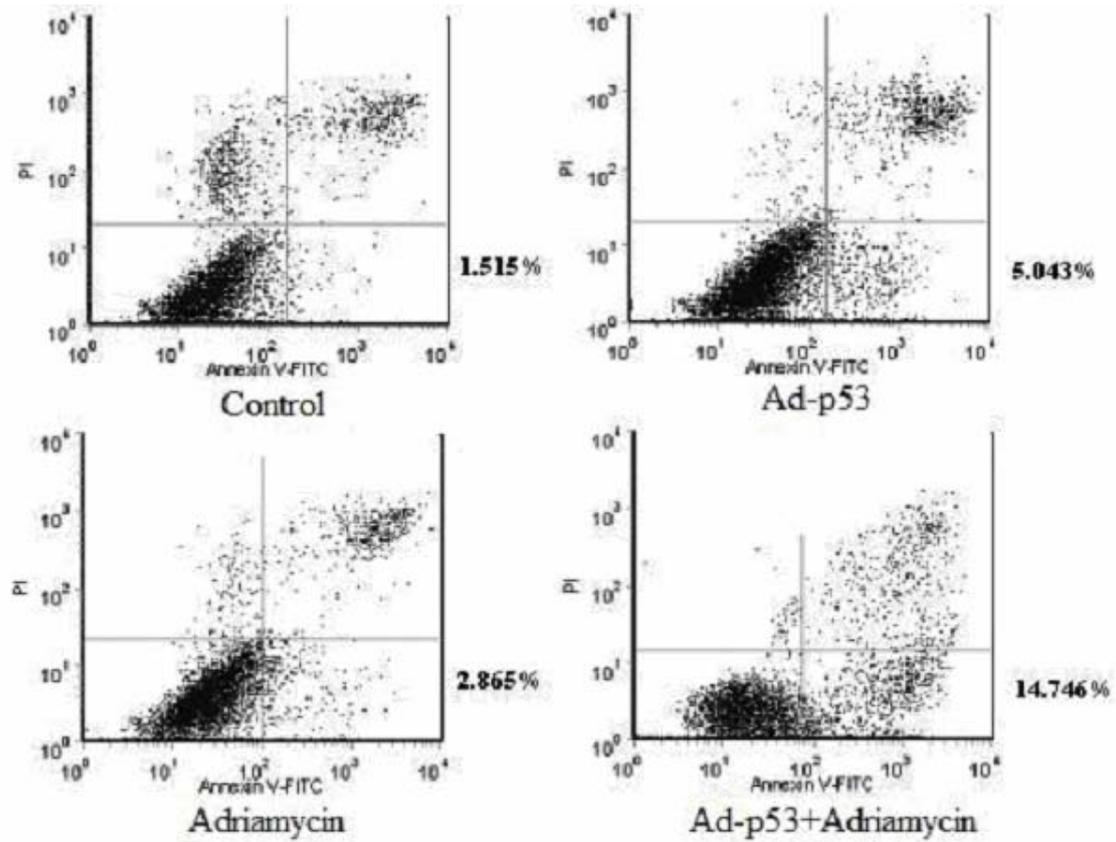


Fig.4. The effects of normal saline, Ad-p53 alone, adriamycin alone, or Ad-p53 plus adriamycin on induction of apoptosis in the implanted tumor. Ad-p53 alone and Ad-p53 plus adriamycin induced a higher proportion of apoptotic cells ($P < 0.05$) compared to normal saline treatment. The Ad-p53 plus adriamycin significant increased number of apoptotic cells in comparison to Ad-p53 alone ($P < 0.05$).

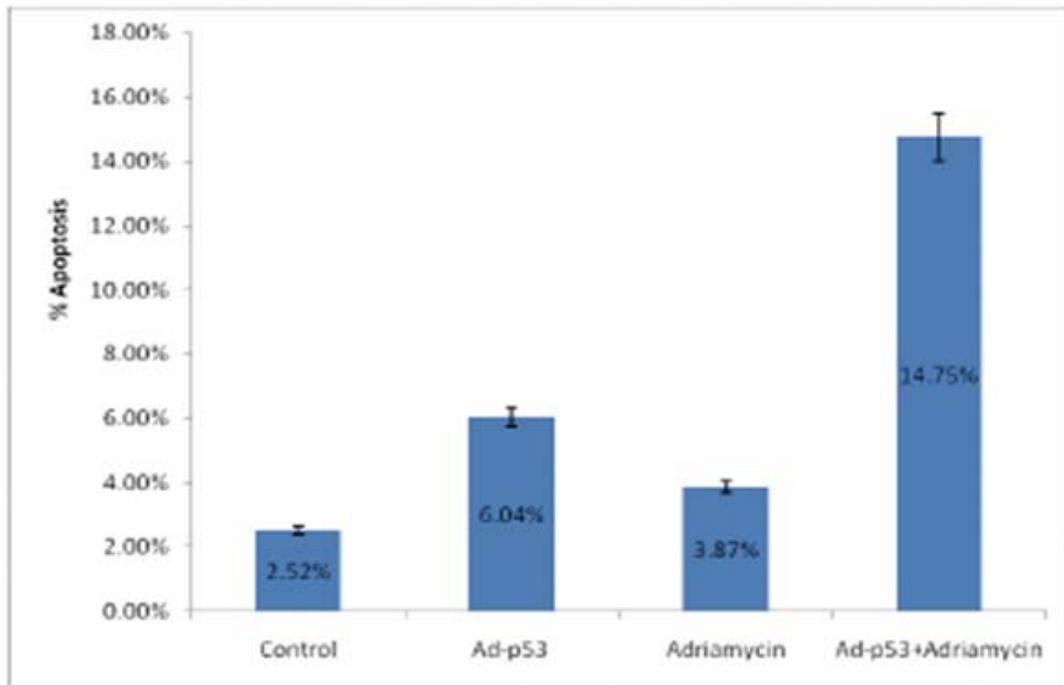


Fig.5. Using column graph to show the effects of normal saline, Ad-p53 alone, adriamycin alone, or Ad-p53 plus adriamycin on induction of apoptosis in the implanted tumor.

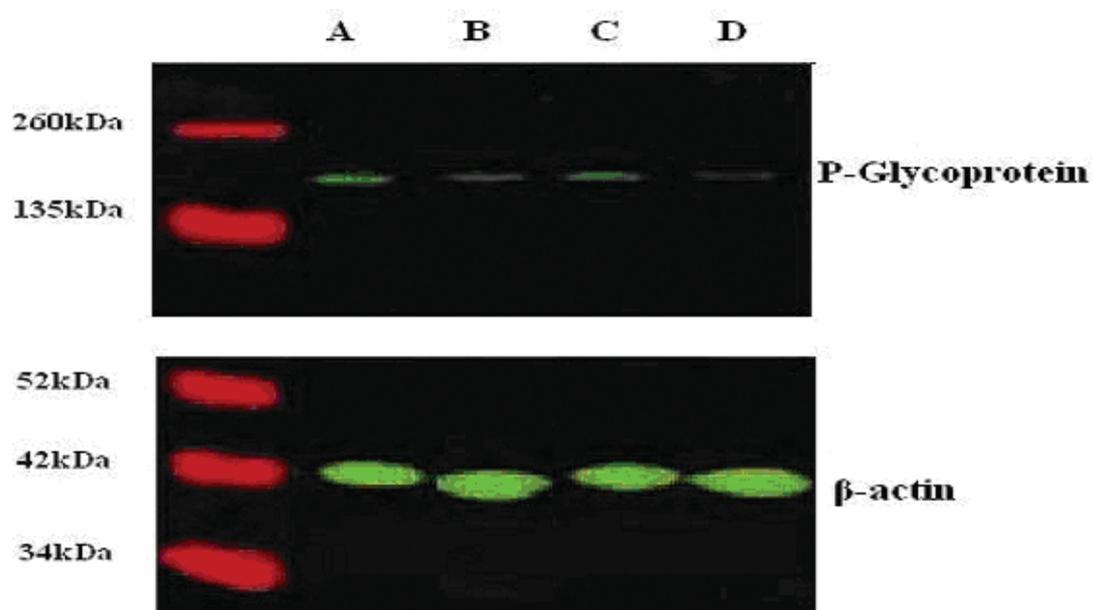


Fig.6. The effects of normal saline, Ad-p53 alone, adriamycin alone, or Ad-p53 plus adriamycin on P-gp expression using WB analysis. Treatment with Ad-p53 plus adriamycin showed a significant decrease of P-gp expression. A: Normal saline; B: Ad-p53; C: Adriamycin; D: Ad-p53+ Adriamycin.