EFFECT ON BIOLOGICAL BEHAVIOR OF CHEMOTHERAPY-RESISTANT TUMOR CELLS BY HUMAN WILD-TYPE p53, GM-CSF AND B7-1 GENES VIA RECOMBINANT ADENOVIRUS

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ABSTRACT

Objective: To explore the effect on biological behavior of chemotherapy-resistant tumor cells by human wild-type p53, GM-CSF and B7-1 genes mediated via recombinant adenovirus. Methods: p53-abnormal KB-v200 (VCR resistant) and KB-s (VCR sensitive) cell lines were used as model tumor cells, which are resistant and sensitive to chemotherapeutic drugs respectively. After infected with recombinant adenovirus carrying human wild-type p53, GM-CSF and B7-1 genes, changes in biological behavior (including drug sensitivity) of these two kinds of gene-transduced cancer cells were observed. Results: Both of the cell lines were susceptible to adenovirus, all of three exogenous genes (p53, GM-CSF and B7-1) could be effectively expressed in these cell lines, their growth was suppressed, and apoptosis was induced. The drug-pumping-out function of Pgp glycoprotein on the cytomembrane of drug-resistant KB-v200 cells was markedly affected 48h after transfection of the recombinant adenovirus, revealed by increase of the amount of rhodamine 123 accumulation in the cells. The MTT assay also indicated the reversal of their sensitivity to VCR drugs. *In vivo* experiment in nude mice it was demonstrated reduction of tumorigenicity of the KB-v200 cells or KB-s cells infected with the recombinant adenovirus, and increase of their sensitivity to VCR. Conclusion: The clinical application of this recombinant adenovirus carrying agents might be more effective in treatment of tumors with multidrug resistance (*MDR*).

Key words: Tumor gene therapy, multidrug resistance, adenoviral vector

In the course of clinical treatment of tumors with chemotherapy, the production of drug-resistance of tumor cells usually leads to failure of chemotherapy[1]. At present a series of drug-resistance mechanisms have been elucidated. One of them is the overexpression of *mdr* 1 mRNA and P-gp (P-glycoprotein) of 170 KDa [2,3]. The expression of *mdr*1 gene is regulated by chemotherapeutic agents, ultraviolet, heat shock, arsenite and other factors [4-8]. Studies in recent years have indicated p53 gene plays a very important role in the induction of apoptosis by various anti-cancer drugs[9]. The expression of p53 gene is closely related to that of *mdr*1 and *mrp* gene expression in drug-resistant tumor cells [10-15]. Gene therapy mediated by adenovirus expressing the gene of interest provides a new treatment measure for clinical control of genesis, development, resolution of tumor [16,17]. In our previous work, a recombinant adenovirus (called BB-102) expressing human wild-type p53, GM-CSF and B7-1 genes has been constructed and was used to infect Primarily cultured laryngocarcinoma cells, resulted in inhibition of proliferation , induction of apoptosis, and elevation of the immunogenecity of the transduced cancer cells. In particular, the elevation of immunogenecity in turn stimulated proliferation of autologous TIL and induced the formation of tumor killing CTL from autologous peripheral blood lymphocytes *in vitro*[18], suggesting the recombinant adenovirus has a very good prospect in anti-tumor gene therapy.

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In this study, p53 abnormal KB-s (sensitive to VCR) and KB-v200 (resistant to VCR) cell lines were employed as the model cells which are sensitive and resistant to chemotherapeutic drugs. The infection efficiencies of the adenoviral vector into these two kinds of cells in which the expressions of human wild-type p53, GM-CSF and B7-1 genes mediated by recombinant adenovirus were evaluted. Besides the reversal of drug resistance, the tumorigenicity in nude mice of KB-v 200 cells infected with the recombinant adenovirus were identified in order to provide an experimental basis in this aspect for clinical application of gene therapy.

MATERIALS AND METHODS

Cell Lines

293 cells, Human embrymo kidney cell line with integration of E1 domain of type 5 adenovirus [19] was provided as a gift by Gene Therapy Unit, Baxter Healthcare Company (Chicago, USA), which was maintained in glucose-enriched DMEM containing 10% FBS at 37℃, 5% CO_2.

p53-abnormal human KB-s (sensitive to VCR) and KB-v200 (resistant to VCR) cell lines were given as gifts by Institute of Tumor (Beijing), Chinese Academy of Medical Sciences, which were cultured in RPMI-1640 containing 10% FBS, at 37 ℃, 5% CO_2.

Recombinant Adenovirus

Recombinant adenovirus carrying green fluorescence protein gene (Ad-GFP) was provided as a gift by Gene Therapy Unit , Baxter Healthcare Company, USA; E1, E3 domain-deleted replication-defective Ad5 recombinant adenoviral vector —Ad-polygenes (called BB-102) containing CMV initiator-driven human wild-type p53 cDNA, human GM-CSF cDNA (These two genes were connected by the internal ribosome entry site of cephalic myocarditis virus ,EMCIRES), and human B7-1 cDNA driven by SV40 initiator, was constructed by this laboratory.

Amplification, Purification, Tittering of the Recombinant Adenovirus

Carried out according to the methods described in the literature [20]. Briefly, 293 cells were inoculated on 150mm culture plates, and grew to a state of 90% confluence, a suitable titer of the virus (around 10 MOI) was added. and the cells were collected 36-48h later when complete pathogenic effect (CPE) appeared. The cells were harvested, then frozen at -80℃. All of the 293 cell collected cumulatively from 50-60 plates were subjected to repeat freezing at -80 ℃ and thawing in 37 ℃ water bath for three times. Following the centrifugation at 3000 rpm for 10 min, the suspernatant was ultracentrifuged in CsCl density gradient solution (1.5g/ml, 1.35g/ml, 1.25g/ml) at 35000rpm, 10℃ for 2h. A white cloudy viral band appeared between 1.25g/ml and 1.35g/ml specific gravity of CsCl solution was collected and mixed with 1.35g/ml CsCl solution. Ultraceutrifugation at 35000rpm, 10℃ for 10h was performed again. The viral band was aspirated out and was diluted with Hanks solution in 1 to 2-fold volumes. The virus stock solution was dialyzed at 4℃ in Hanks solutions three times. After that the purified viral solution was taken out, then mixed with 10% sterile glycerin, and stored at -80℃.

The viral titer of the virus was determined by combinative use of rapid CPE method and plaque analysis [20].

Expression of p53, B7-1 and GM-CSF Genes in KB-s and KB-v200 Cells Transduced by the Recombinant Adenovirus BB-102

Infection Rate of the Adenovirus in KB-s and KB-v200 Cells

Human KB-s and KB-v200 cells were inoculated separately on six-well plates at 5×10^5cells/well. The culture medium was aspirated out 12h later and Ad-GFP (diluted in 0.8 ml culture medium) was used to infected the cells at 0,12.5,25,50 and 100 MOI, respectively, The viral liquid was aspirated out 1-2h later and 2ml fresh medium were added into each well. After 12h, the number of positively GFP-expressing cells were counted under a fluorescence microscope. The count was repeated three times in order to estimate the efficacy of gene transfer.

Immunohistochemical Detection of Human wild-Type p53 Expressed by BB-102 [21]

KB-s and KB-v200 cells were inoculated separately on 12-well plates at 5×10^4cells/well and a small piece of cover glass was placed on the bottom of each well for spontaneous growth of the cells on it. After 24h, the cells were infected separated with BB-102 and Ad-GFP at 50 MOI. The cover glasses were taken out 48h later and the

cells were fixed with acetone. The expression of p53 gene in these cells was detected using anti-human p53 monoclonal antibody Pab240 (PharMingen, San Diego,CA), immunohisto-chemistry following the protocol supplied in Histostain ™-SP kit (Zymed), 3,3'-Diaminoberzidine Tetrehydrochloride Substrate kit (Zymed).

Flow cytometric analysis of B7-1 gene mediated by BB-102 [22]

KB-s and KB-v200 cells were inoculated separately on 60 mm dish at 1×10^6cells. After 24h, the cells were infected with 50 MOI of BB-102. The cells were digested down 48h later, washed twice with Hanks solution ,and resuspended in 100-200 μ l Hanks solution. 20 μ l FITC-labeled murine anti-human B7-1 monoclonal antibody(PharMingen) were added into the suspension and incubated at 4 ℃ for 30 min. Then the cells were washed twice with Hanks solution and resuspended in 1ml Hanks solution. Expression of B7-1 gene on cellular surface of these two kinds of cells transduced by BB-102 was evaluated by flow cytometric (FACS 440, Becton, Dickinson) analysis.

Detection of expression of GM-CSF in KB-s and KB-v200 cells mediated by BB-102 [23]

KB-s and KB-v200 cells were inoculated separately on 60 mm dish at 1×10^6 cells. After 24h, the cells were infected with 50 MOI of BB-102. After that the supernatant was collected and fresh culture medium was added every 24h for consecutive six days. The expression of GM-CSF gene in BB-102-transduced cells was detected with ELISA(Zymed kit) according to the manufactruer's instructions.

The growth and proliferation of BB-102-transduced KB-s and KB-v200 cells [24]

These two kinds of cells were inoculated separately in triplicate on 24-well plates at 1×10^4cells/well. After 24h the cells were infected separately with BB-102 and Ad-GFP at 50 MOI each. Thereafter the growth state was observed every day, and the cells were digested down , counted after trypan blue staining on each day and their growth curves were plotted.

Induction of Apoptosis in KB-s and KB-v200 Cells Transduced by BB-102

These two kinds of cells were inoculated separately on 12-well plates at 5×10^4 cells/well and the cells grow on the surface of cover glass placed on the bottom of each well. After 24h incubation, the cells were infected with 50 MOI of BB-102 or Ad-GFP. The cover glass were taken out and the cells were fixed with neutral formalin solution. Apoptosis was in situ detected by 3'terminal labeling with TdT FragEL™ DNA Fragmentation Detection Kit (Oncogene) according to the manufacturer's instruction.

Detection of Multidrug Resistance

MTT test [15]

KB-v200 cells 24h after infection with Ad-GFP or BB-102 were inoculate in triplicate on 96-well plates separately at 3200 cells/well. And incubated at 37℃, 5% CO_2 for 8h, then a series of concentrations of VCR were added to each well. After 48h incubation, 20 μ l MTT(10 μ g/ μ l) (Sigma) was added into each well and the cells were maintained 37 ℃, 5% CO_2 for 4h.. The reversal of the MDR phenotype was determined based on the measurement of O.D. 570nm wavelength using an enzyme-labeling instrument. The IC_{50} was calculated.

Determination of accumulated drugs in the cells by flow cytometry [25]

Rhodamine 123, a fluorescent dye, can easily be expelled to outside of the cell by the P-gp glycoprotein (product of *mdr* 1 gene). 12h, 24h, 48h after infection with BB-102, the KB-v200 cells were digested with 0.25% trypsin, harvested and washed with serum-free RPMI 1640, the cells were adjusted to concentration of 1×10^6/ml, and then passed through a 1ml syringe to avoid their aggregation. Rhodamine 123 was added to the cells at a final concentration of 2.5 μ g/ml and the mixture incubated at 37℃ for 30min . The cells were washed twice with serum-free RPMI 1640, incubated at 37℃for 10min, and washed with culture medium, isoptin was added to a final concentration of 5 μ g/ml .Finally the fluorescence intensity was measured at 488nm by flow cytometry (FACS 440, Becton, Dickinson).

Tumorigenicity of BB-102 Transduced Tumor Cells in Nude Mice

Female nude mice with 4-6 weeks of age were purchased from Experimental Animal Center, Chinese Academy of Medical Sciences. They were divided randomly into groups, ten each. KB-v200 cells were maintained in 150mm^2 culture flasks and were infected 12h later with BB-102 and Ad-GFP of 25MOI, separately. At 24h post-incubation at 30℃, 5% CO_2, the cells were digested with 0.25% trypsin, washed twice with PBS, and adjusted with serum-free RPMI 1640 to the concentration of 1.5×10^7/ml. Each 0.2 ml of the cell suspension was inoculated subcutaneously into the scapular region of the nude mice. Tumors appeared obviously 4 days later, then mice of each group were subdivided into 2 subgroups, 5 mice each. Mice of one subgroup were ip injected with 0.2ml VCR(5 μ g/ml) every day, while the other subgroup were injected with PBS. .In the meantime, the sizes of tumors were measured with calipers once every 3 days. The method of calculation: volume of tumor(mm3)=(length × width^2)/2. The weights of the tumors were weighted on the 13th day.

Statistical Analysis

The results were analyzed using POMS statistic software, taking $p<0.01$ as very significant difference.

RESULTS

Preparation of Recombinant Adenovirus with High Titer

By CsCl density gradient ultracentrifugation highly concentrated recombinant adenovirus was obtained. According to 1O.D.260=1.1×10^{12} particles, the concentration of the obtained adenovirus was higher than 10^{12} particles / μ l. The purity of the virus was very high (O.D.260/O.D.280>1.3). By plaque assay, the titers were : BB-102, 3×10^{10}pfu/ml.

The Adenovirus Has Relatively High Infection Efficiency for Both Drug-sensitive and Drug-Resistant Cells

Both of drug-sensitive and drug-resistant cells were susceptible to the adenovirus. The adenovirus has higher infective ability to drug-sensitive cells, but its toxic effect was also larger. Along with increase of viral MOI , the transfection rates elevated in both kinds of cells. When the amount of the virus was 50MOI, the transfection rate reached more than 90% (Figure 1). As the viral amount increased further, inhibition of cell growth to a certain extent appeared and the number of dead cells also increased.

Expressions of the p53, GM-CSF and B7-1 genes in transduced drug-sensitive and drug-resistant cells

The results of immunohistochemistry (Figure 2) showed that p53 gene mediated by BB-102 could be expressed in both drug-sensitive and drug-resistant cells.

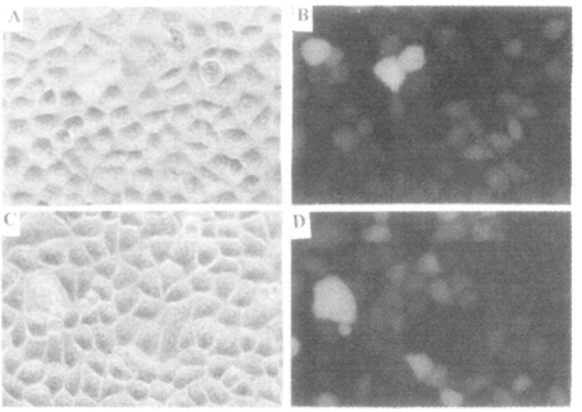

Fig 1 KB-v200 and KB-s cells infected with 50 MOI Ad-GFP.KB-v200 cells (A) same field as in (B),KB-s cells (C) same fieldas in (D) under fluorscence detection using a fluorescein filter set.

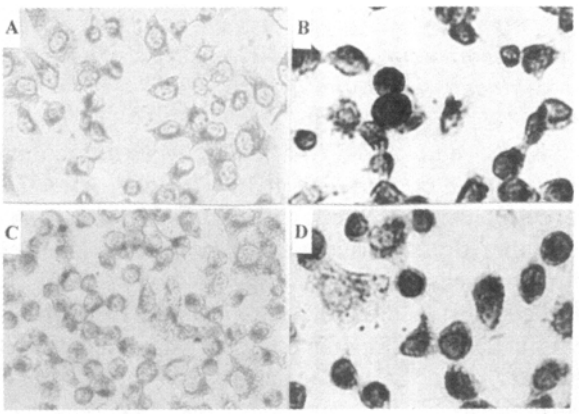

Fig 2 Immunocytochemical staining of the P53 proteinin KB-v200 and KB-s cells 48h after infection with (A,C)Ad-GFP;(B,D)BB-102, (A) and (B)KB-v200 cells ; (C)and (D) KB-s cells.

The ELISA results (Figure 3) showed that one day after infection with 50 MOI BB-102, a relatively high level of GM-CSF expression could be detected in the culture medium of both KB-s and KB-v200 cells. The expression peak appeared on the 2nd to 3nd day and then the expression was trending to decrease. Even on .the 6th day a relatively high level of GM-CSF could still be detected.

The results of flow cytometry (Figure 4) indicated that there was no expression of B7-1 in KB-

s nor in KB-v200 cells without BB-102 infection, whereas a relatively high expression level of B7-1 was noted on the surface of more than 80% of cells 2 days after infection with 50 MOI BB-102.

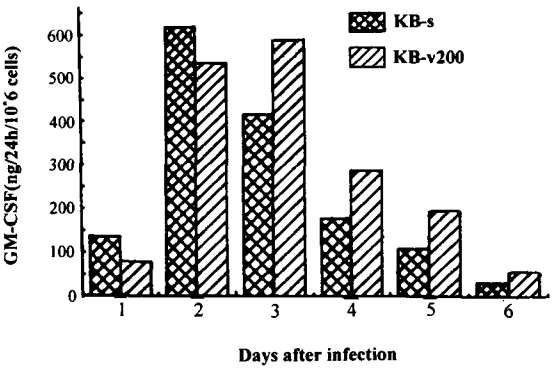

Fig 3. The expression of GM-CSF in KB-s and Kv200 cellsinfected with BB-102,values shown are mean of triplicate wells

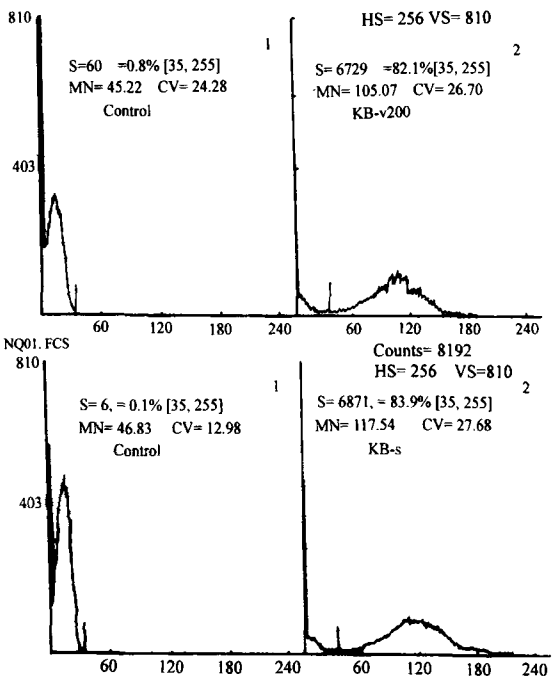

Fig 4 Immunofluorescent flow cytometry analysis for the expression of B7-1in KB-v200 and KB-s cells (infected and uninfected with BB-102)

BB-102 Mediated Gene Transfer Inhibited the Growth of Both Drug-Sensitive and Drug-Resistant Cells and Exerted Killing Effect on Them

As compared with cells without infection with the adenovirus, growth of both KB-s and KB-v200 cells infected with 50 MOI BB-102 was markedly inhibited (Figure 5), especially it was more evident in KB-s cells than in KB-v200 cells. By light microscopy, gradual enlargement of intercellular space and rounding of KB-s cells were seen on the 4th day, whereas these pictures were not fount in KB-v200 cells until the 6th day (Figure 6). There was no considerable effect on cell growth after infection with the same amount of the control virus, Ad-GFP.

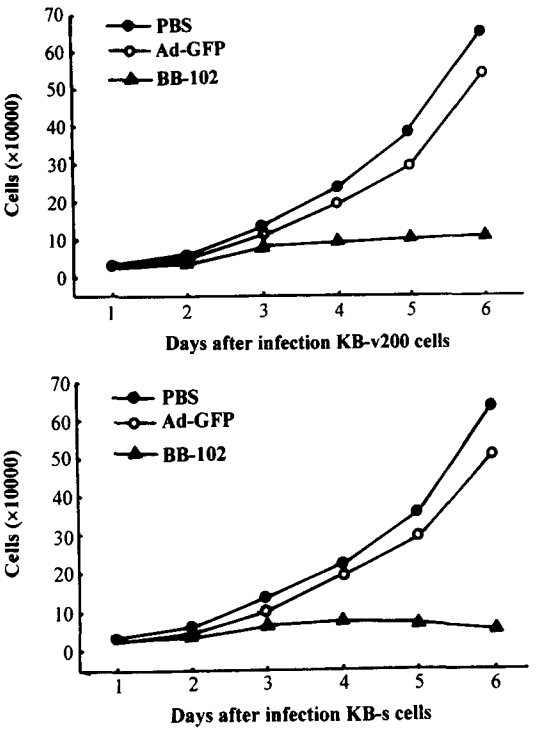

Fig 5. Effect of BB-102 and Ad-GFP on the growth of KB-v200 and KB-s cells. Cells (1×10^4) were plated in triplicate on 24-well plates, exposed to BB-102(50MOI) or Ad-GFP and cell number counted on each day($\bar{x}\pm s$).

Effect of Chemosensitivity for VCR on KB-v200 Cells Mediated by BB-102

The result of MTT assay (Table 1) showed that the sensitivity to VCR of BB-102-infected KB-v200 cells increased, the same as manifested by increase of the amount of rhodamine 123 accumulated in the cells (Figure 8). Our data presented here suggest the wild-type p53 gene could induce elevation of drug-sensitivity in originally drug-resistant cells by regulating the activity of the cytomembrane glycoprotein having out-pumping function for drugs.

BB-102 Mediated Gene Transfer Induce

Apoptosis in Both Drug-Sensitivity and Drug-Resistant Cells

KB-s and KB-v200 cells were infected with 50 MOI BB-102 or KB-v200 and the cells were fixed 72h later. Apoptosis was in situ detected by 3' terminal labeling method and the results (Figure 7) showed that apoptosis occurred in a large number of BB-102 infected cells. Whereas it did not appear in the control cells and Ad-GFP-infected cells.

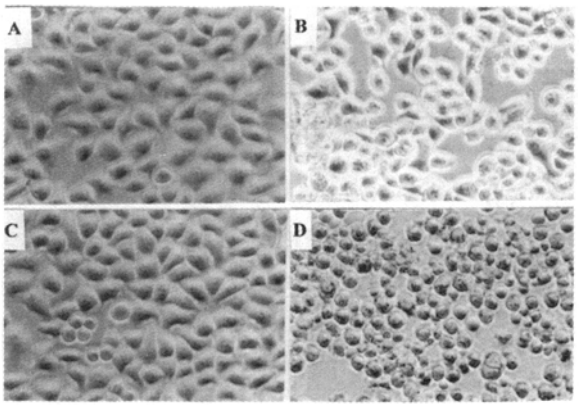

Fig. 6. Effect of recombinant adenovirus on the growth of KB-v200 and KB-s cells on day 6 after infection with (A,C)Ad-GFP; (B,D)BB-102, (A) and (B)KB-v200 cells; (C) and (D) KB-s cells.

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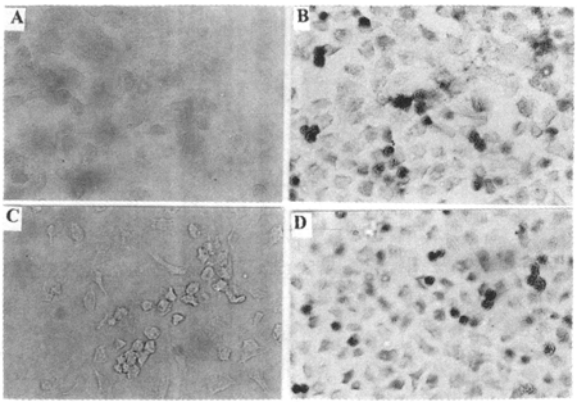

Fig 7 In situ detection of DNA fragmentation by TdT assay in KB-v200 and KB-s cells 72 h after and (B)KB-v200 cells ; (C)and (D) KB-s cells. infection with (A,C)Ad-GFP; (B,D)BB-102, (A)

Tumorigenicity of BB-102 Transduced Tumor Cells in Nude Mice

As shown in Figure 9, Table 2 and Figure 10, the tumorigenicity of KB-v200 cells infected with the recombinant adenovirus BB-102 lowered in the nude mice. The size(Figure 9) and weight(Table2) of tumors in the nude mice inoculated with BB-102-infected KB-v200 cells were significantly different ($p<0.01$) from those of the nude mice inoculated with normal or Ad-GFP-infected-KB-v200 cells, and the sensitivity of the tumors to chemotherapeutic drug (VCR) was elevated, too.

Table 1 Chemosensitivity for VCR in KB-v200, KB-v200 +Ad-GFP and KB-v200 + BB-102 cells($\bar{x}\pm s$)

Cell lines	50%* inhibitory concentration(IC_{50}) (vincristine, μg)
KB-v200	4.65 ± 0.22
KB-v200 + Ad-GFP	3.80 ± 0.28
KB-v200 + BB-102	0.78 ± 0.35

*IC_{50} (μg) was obtained from dose-response curves to the vincristine as measured by MTT assay.

DISCUSSION

Succeeding to retroviral vector, adenoviral vector is an early developed gene transfer system in gene therapy. Owing to its simply preparation, wide host range, high infection efficiency , stable physicochemical properties, without integration property, and other advantages, it has been extensively used in clinical trials of gene therapy[16,17], especially it is considered the viral

Table 2 weight of tumor after 13 days inoculation of tumor cells($\bar{x} \pm s$)

Tumor cells of inoculation	Control	I.P. injection vincristine (1 μg/day/mouse)
KB-s	0.573g ± 0.092	ND
KB-s+BB-102	0.337g ± 0.034 p_0* = 0.048 < 0.05	ND
KB-v200	0.615g ± 0.105	0.582g ± 0.093, p_1*= 0.839 > 0.05
KB-v200 + BB-102	0.363μg ± 0.055, p_1*= 0.049 < 0.05	0.108μg ± 0.014, p_1= 0.005* < 0.01, p_2= 0.007** < 0.01

p_0*, compared to KB-s cells; p_1* , compared to KB-v200 cells control;
p_2** , compared to KB-v200 + BB-102 cells control.

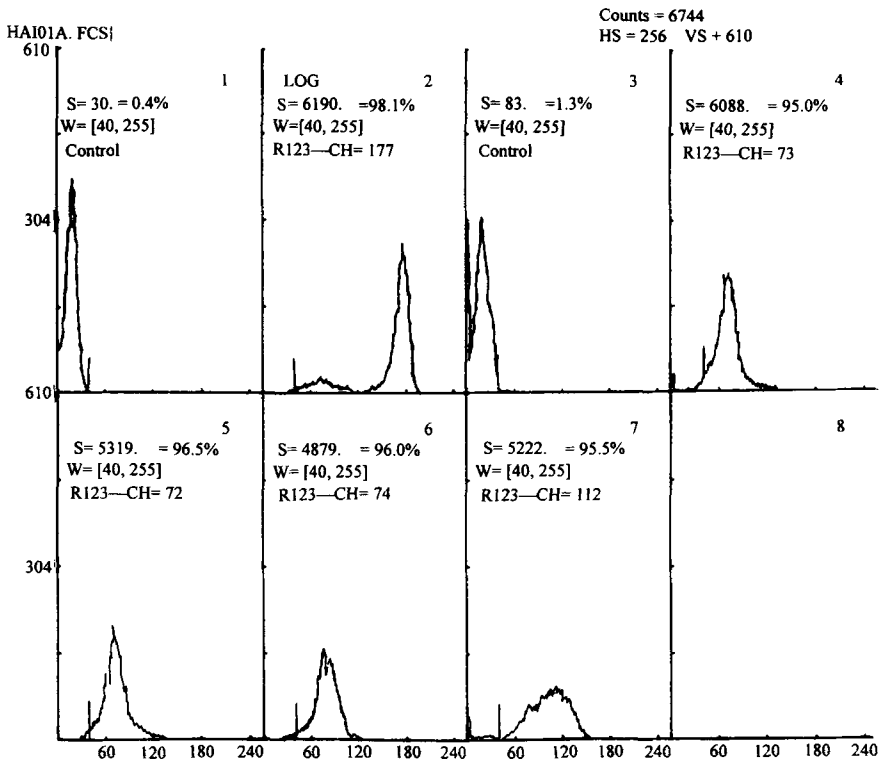

Fig. 8. Intracellular accumulation of rhodamine 123 in KB-s(2), KB-v200(4), BB-102-infected KB-v200 cells (5,6,7; 12h, 24h, 48). The accumulation of rhodamine 123 in the KB-v200 cells was very low, and in the KB-s cells was high, 48h after infected with BB-102, KB-v200 cells indicated theintracellular accumulation of rhodamine 123 was significantly increased. (1) KB-s cells control; (2) KB-v200 cells control

vector of choice in transduction of genes by in vivo route [20].

Chemo- and radio therapy are extensively used in treatment of cancers and it is considered their killing effect on tumor cells are achieved by inducing apoptosis via a p53-dependent way [21]. When cells are responding to injuries caused by environmental stimuli (including chemo- and radio therapy), the expression level of wild-type p53 can be induced to increase rapidly ,resulting in entering of the cells into G1 phase, in which cell growth is inhibited. The latter event facilitates repair of cellular injury or induction of the cells into apoptotic state when the injury is so severe that can

not be repaired. Experiments show that inactivation or deletion of p53 gene can promote formation of various kinds of tumors and induce appearance of drug-resistance or reduction of drug-sensitivity of the tumor cells. In human cancers, lose of p53 function is one of the changes detected frequently at present. [30]

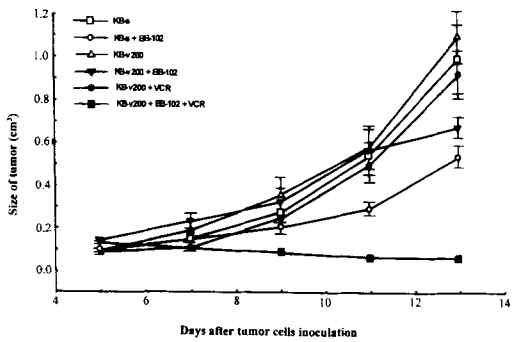

Fig 9 Effect of tumorigenesis on the nude mice when I.P. injected 3×10^6 (■), KB-s cells; (●),BB- infected KB-v200 ; 102-infected KB-s; (▲), KB-v200; (▼),BB-102-(◆)KB-v200+VCR(1 μg/day /mouse); (✚)BB-102-infected KB-v200 + VCR(1μg/day/mouse).

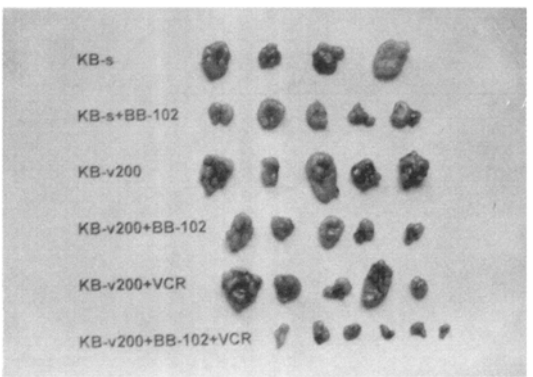

Fig. 10. The size of tumor after 13 days inoculation of tumor cells

In about 200-odd kinds of tumors, 50% of them having abnormal p53 gene. Tumor-suppressing gene therapy taking p53 as the object gene is aimed at resuming the function of p53 gene via transduction of wild-type p53 gene into tumors [27-30].

Tumor-immune gene therapy is transduction of gene encoding tumor immune-associated molecules for the purpose of enhancing immunogenicity of tumor cells and tumor antigen-presenting ability of antigen-presenting cells, resulting in inducting of immunologic response of the organism against the tumor. Currently the subjects mostly studied include cytokines, MHC molecules, and costimulation signal molecules of B7 family members [32]. At the same time when receiving antigen components from antigen-presenting cells, T cells should receive co-stimulation by co-stimulation signal molecules from the B7 family members in order to be activated, to proliferate, and to exert their anti-tumor action.

That is the double-signal theory of tumor immune reaction. Researches indicate that expression of B7 molecules are always lacking on the cytomembrane of tumor cells, leading to T cells being in a state of clonal nonresponsiveness due to lacking co-stimulation for them. Using B7-1 gene transduced tumor cells as the immunogene, systemic antitumor immune response can be induced, including enhancement of specific killing activity on tumor cells by $CD8^+$ cytotoxic T lymphocyte (CTL) and helper activity of $CD4^+$ T cells [22, 32].

GM-CSF is a cytokine very much concerned in tumor immune in recent years [31-33]. It plays important roles in maturation and exerting function of antigen-presenting cells. Local high concentration of GM-CSF elevates specifically the tumor antigen -presenting ability of antigen-presenting cells of the host, leading to induction of specific anti-tumor immune response of the host. The study of Dranoff et al [33] indicates among a large number of known cytokines , GM-CSF is the factor having great potential on long-term activation of specific immunity of the organism against tumor.

Through above-mentioned experiment we have evaluated the therapeutic effect of genes mediated by the recombinant adenovirus on sensitive cells and resistant cells to chemotherapeatic drug and the results indicate that both of them are susceptible to the adenovirus. The three exogenous gene (p53, GM-CSF, B7-1 gene) carried by the recombinant adenovirus can be efficiently expressed carried in these two kinds of cells, the growth of which can be inhibited and apoptosis in which can be induced. These results suggest the recombinant adenovirus could be used in treatment of both sensitive tumor cells and resistant tumor cells to chemotherapeatic drugs.

In drug-resistant KB-v200 cells 48h after transfection with the recombinant adenovirus out - pumping function for drugs of the Pgp glycoprotein on cytomembrane is influenced remarkably, as manifested by increase of accumulated amount of rhodamine 123 within the cells. The result of MTT test also show the increase of sensitivity to VCR. These data suggested that transduction of wild-type p53 gene could increase of sensitivity to

chemotherapeutic drugs of p53-abnormal tumor cells originally resistant to drugs, which is associated with reduction of functional activity of Pgp glycoprotein on cytomembrance.

Our study suggests that the recombinant adenovirus carrying human wild-type p53 gene and tumor immun -associated genes GM-CSF and B7-1 would be similarly effective in treatment of both sensitive and resistant cells to chemotherapeutic drugs. The *in vivo* experiment in nude mice confirms the tumorigenicity of KB-v200 cells infected with the above-mentioned recombinant adenovirus(BB102) lowered, and their sensitivity to VCR is also elevated simultaneously, suggesting clinical application of recombinant adenovirus carrying multiple genes in combination with chemotherapeutic agent could be more effective in treatment of multidrug-resistant tumors.

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EVALUATION OF FREE-TO-TOTAL PROSTATE SPECIFIC ANTIGEN RATIO IN THE DIAGNOSIS OF PROSTATE CANCER

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It's reported that free to total prostate specific antigen ration (f/tPSA) can provide more benefit than the single use of prostate specific antigen (PSA) in the diagnosis of prostate cancer (PCa). We measured serum PSA and fPSA levels in 62 cases of benign prostatic hyperplasia (BPH) and 40 cases of PCa using radioimmunoassay, with patients' age range 59y~89y.

RESULTS

PSA, fPSA and f/tPSA are shown in Table 1.

Table 1. PSA, fPSA and f/tPSA of BPHs and prostate cancer

	PSA (ng/ml)	TPSA (ng/ml)	f/tPSA ratio
BPH	8.14±7.45	1.45±2.35	0.22±0.19
PCa	54.0±63.7	7.94±7.98	0.16±0.09
P	<0.001	<0.001	0.07

Both these two groups shows linear correlation between PSA and fPSA, correlation coefficient of BPH is 0.55 ($P<0.01$), of PCa is 0.44 ($P<0.01$). Two slopes have no difference (0.17 vs 0.054, $P>0.05$).

DISCUSSION

Murphy et al reported that fPSA level could increase corresponding to the increase of total PSA. We suggest that BPH and PCa tissue secrete PSA with similar percentage of free PSA, the mechanism still remains unknown. So free-to-total PSA ratio does not provide additional diagnostic benefit compared with total PSA in differentiating BPH and PCa, further researches are required.

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