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# Potentials and Limitations of Adenovirus-*p53* Gene Therapy for Brain Tumors

We investigated the antineoplastic potentials of recombinant adenovirus containing wild-type p53 cDNA (Ad5CMV-*p53*) for malignant gliomas. In four human glioma cell lines (U-251 and LG expressing endogenous mutant *p53*, and U-87 and EFC-2 expressing wild-type *p53*) and two rat glioma cell lines (9L and C6, each expressing mutant and wild-type *p53*), gene transfer efficiency determined by X-gal staining and Western blotting was varied (10-99% at 10-500 multiplicity of infection, MOI). Growth inhibitory effect was drastic (>90% at 100 MOI) in U-251 cells and only moderate or minimal in other cell lines harboring wild-type p53 or low gene transfer efficiency. Ex vivo transduction of U-251 cells with Ad5CMV-*p53* suppressed the in vivo tumorigenicity of the cells. Histopathologic examination for Ad5CMV-*p53* toxicity to rat brains showed inflammatory reactions in half of the tested brains at $10^8$ MOI. U-251 cells were inoculated intracerebrally in nude mice and injected Ad5CMV-*p53* into the tumor, in which neither the tumor suppression nor the survival benefit was observed. In conclusion, heterogeneity of the cellular subpopulations of malignant glioma in p53 status, variable and insufficient gene delivery to tumor, and adenoviral toxicity to brain at higher doses may be limiting factors to be solved in developing adenovirus-*p53* gene therapy for malignant gliomas.

Key Words: *Genes, p53; Gene Therapy; Glioma; Brain Neoplasms; Adenoviridae*

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## INTRODUCTION

Malignant gliomas, the most common primary brain tumors are very aggressive tumors. The prognosis for patients with malignant gliomas remains grim despite contemporary surgery, radiation therapy and chemotherapy. Considerable efforts have been made to develop new therapeutic strategies for the management of malignant gliomas. One of the promising new cancer therapies is intervention at the molecular level, and gene therapy is a clinical method for such intervention (1).

Among the genes with therapeutic potential for cancer treatment, the *p53* tumor suppressor gene is the most extensively studied (2, 3). Inactivation of the *p53* gene by mutation or deletion is the most common genetic alteration in human malignancies including malignant gliomas, 35-60% of which have alterations in *p53* gene (4). Wild-type p53 has been shown to suppress cell transformation and neoplastic cell growth. The growth suppression mechanism of p53 has not been clearly established, but it has been demonstrated that p53 transcriptionally activates the production of its downstream mediators, WAF1/Cip1 (5,6), which are potent negative cell cycle control factor and bcl-2 associated X-protein, Bax (7), that induce apoptosis.

As a gene transfer vector, adenoviruses are double stranded DNA viruses with an average genome size of 36 kb, which have the potential to carry large or multiple foreign genes. They are also easy to grow and manipulate, and can be obtained in high titers. These vectors have a broad range of hosts and can transfer genes to nonproliferating as well as proliferating cells, which appear preferable for efficient gene transfer to malignant gliomas consisting of heterogeneous cell cycling within the tumor microenvironment (8). Adenoviral vectors in current use have been known to transduce solid tumors without significant toxicity (9-11) and evoke nonspecific inflammation and antivector cellular immunity (8).

As a cancer gene therapy strategy, adenovirus-mediated wild-type *p53* gene transfer has been shown to

suppress the growth of various cancer cell lines from lung (12), colon (13), head and neck (10, 14), prostate (15) and cervical (16) cancers. It has also been shown to be safe, feasible, and biologically effective in patients with head and neck (11) and lung (17) cancer. This approach for brain tumor therapy has been studied by many investigators and provoked debates on the potential of this strategy for malignant gliomas. Köck et al. (18) and Gomez-Manzano et al. (19) have reported that adenovirus-mediated exogenous *p53* gene delivery resulted in apoptotic cell death in human glioma cell lines containing a mutant *p53* gene but had only a minimal effect on the growth of glioma cell lines containing wild-type *p53* gene. Li et al. (20) reported that overexpression of adenovirus-mediated wild-type *p53* gene induced apoptosis of human glioma cells independent of endogenous p53 status. Badie et al. (21) showed that adenovirus-mediated *p53* gene delivery inhibited 9L glioma growth in rats but Dorigo et al. (22) reported that it did not affect 9L cell proliferation. Geng et al. (23) showed that adenovirus-mediated wild-type *p53* gene transfer enhanced radiosensitivity of both glioma cell lines containing wild-type or mutant p53, however, Lang et al. (24) reported that the radiosensitization was detected only in a glioma cell line containing wild-type p53. Here, we investigated the controversial antineoplastic role of adenovirus-mediated *p53* gene transfer in malignant gliomas therapy.

## MATERIALS AND METHODS

### Cell lines and cultures

Human glioma cell lines, EFC-2 and LG, were isolated from human malignant gliomas. Other human glioma cell lines (U-251 and U-87) and rat glioma cell lines (9L and C6) were purchased from American Type Culture Collection (Rockville, MD). Three cell lines (U-251, LG, 9L) express endogenous mutant p53 and the other three cell lines (U-87, EFC-2, C6) express wild-type p53 (19, 25, 26). Cell cultures were routinely maintained in Dulbecco's Modified Eagle's Medium and Ham's F12 Medium (1:1, v:v) supplemented with 5% fetal bovine serum (FBS), and incubated at 37℃ in a humidified 5% $CO_2$ environment.

### Recombinant adenovirus and infection conditions

Construction and properties of the replication-defective type 5 adenovirus in which the E1 region is replaced with a cytomegalovirus (CMV) promoter and wild type *p53* cDNA (Ad5CMV-*p53*) was reported previously (27). Cells were plated in triplicate in 35-mm tissue culture plates (Costar 3505, Cambridge, MA) at $2\times10^4$-$1\times10^5$ cells/well. Twenty-four hr after plating, the cells were treated with 1 mL of fresh medium or serum-free medium containing Ad5CMV-*p53* or Ad5CMV at multiplicity of infection (MOI) of 10-500 plaque forming units (PFU)/cell for cell survival assay, or treated with medium containing Ad5CMV-β-gal at 10-500 MOI for β-galactosidase histochemistry, and incubated at 37℃ for 30 min with brief agitation every 10 min. This was followed by the addition of 1 mL culture medium supplemented with 10% FBS and the return of the infected cells to the 37℃ incubator.

### β-galactosidase histochemistry

The cells infected by Ad5CMV-β-gal for 48 hr were stained with 5-bromo-4-chloro-3-indolyl-β-D-galactoside (X-gal) as described previously (12). Five hundreds cells were counted and the percentage of blue cells were calculated.

### Growth inhibition of glioma cells

The cells treated with fresh medium or medium containing Ad5CMV-*p53* or Ad5CMV were subsequently refed with fresh medium every 3 days. The monolayer cultures were harvested everyday from day 1 to day 5 by brief trypsinization and cell count was done. The cell numbers were calculated and expressed as percentage of the control group.

### Protein extraction and Western blotting

U-251 and LG cells infected with Ad5CMV-*p53* at 25 MOI for 6 hr and 72 hr were lysed with lysis buffer containing 0.02 M Tris-HCl, 0.15 M NaCl, 0.005 M EDTA and 0.5% NP-4027. Twenty μg of the protein extract from the cells were boiled in sample buffer containing 125 mM Tris-HCl, pH 6.8, 1% SDS, 2% β-mercaptoethanol and 0.01% bromophenol blue for 5 min, and loaded onto a 10% SDS polyacrylamide gel. After overnight electrophoresis at 45 V, the protein was transferred to an Immobilon polyvinylidene difuride membrane (Millipore Corp., Bedford, MA), blocked with solution composed of 50 nM Tris-HCl (pH 7.5), 0.9% NaCl, 3% nonfat dry milk and 0.05% Tween 20 for 4 hr, and then incubated overnight with anti-p53 antibody DO-1, anti-actin antibody (Oncogene Science, Uniondale, NY) and anti-p21WAF1/Cip1 antibody (PharMingen, San Diego, CA). The levels of protein were analyzed using the enhanced chemiluminescence system (Amersham, Arlington Heights, IL) according to the

manufacturer's instruction manuals.

### Tumorigenicity in vivo

U-251 cells were treated with mock, Ad5CMV, or Ad5CMV-*p53* at 25 MOI for 24 hr and approximately $1\times10^6$ cells suspended in 10 $\mu$L phosphate buffered saline (PBS) were inoculated subcutaneously at right shoulder of nude mice. Serial caliper measurements of perpendicular diameters were used to calculate the tumor volume in cubic millimeters by using the following formula: (longest diameter)×(shortest diameter)$^2\times0.52$.

### In vivo toxicity

Thirty-six Wistar rats, 4-6 weeks old, were divided into three groups, anesthetized by intraperitoneal injection of xylazine (Cutter Laboratories, Shawnee, KS) 12 mg/kg and ketamine (Parke Davis, Morris Plains, NJ) 30 mg/kg, and held in a stereotactic frame. A burr hole was made in the skull 1 mm anterior and 3 mm lateral to the bregma to expose the dura. Using a Hamilton microliter syringe connected to the manipulating arm of the stereotactic frame, PBS (N=5) or Ad5CMV-*p53* of $10^7$ (N=5), $10^8$ (N=5), $10^9$ (N=5) MOI in 5 $\mu$L were injected over 3 min into the right cerebral hemisphere at a depth of 3.5 mm from the dura. Neurologic signs and body weight were checked every day. The animals were sacrificed 7 days postinjection and fixed by intracardiac perfusion of 4% paraformaldehyde in PBS. The brain, lung, heart, liver, kidney spleen tissues were processed for histological examination.

### Intracerebral tumor model and treatment

Fifteen athymic nude mice (BALB/nu-c Slc, Japan), 6-8 weeks old, were prepared for intracerebral stereotactic injection as described above. $2\times10^5$ U-251 cells in 3 $\mu$L PBS were injected over 3 min into the caudate nucleus at a depth of 3 mm from the dura. The needle was left in place for 2 min and then withdrawn slowly over another 2 min. The scalp wound was closed with Autoclips (Beckon Dickinson, Sparks, MD). Seven days after the tumor implantation, the animals were divided into three groups and intratumorally injected with therapeutic molecules via the same burr hole using the stereotactic system. Each group (N=5) received PBS (mock), Ad5CMV, or Ad5CMV-*p53* of $3\times10^8$ MOI in 3 $\mu$L. Neurologic signs, body weight and survival of the animals were checked. When the animals began to die, they were sacrificed and fixed by an intracardiac perfusion of 4% paraformaldehyde in PBS. The presence of tumor in the brain was evaluated by serial coronal cutting with 1 mm thickness from the tumor implantation site. The midcoronal sections of the whole tumors were processed for histological and immunohistochemical assays. To measure the tumor sizes, the maximal area in coronal section stained with hematoxylin-eosin was selected and transcribed on a paper (×4.0 actual size) by a zoom stereomicroscope (SZH-111, Olympus Optical, Tokyo, Japan) with a drawing attachment (SZH-DA, Olympus Optical). The transcribed area of the tumor was then measured with a planimeter (KP-21, Koizumi, Japan).

## RESULTS

### Gene transfer in vitro

Gene transfer efficiency determined by X-gal staining was varied depending on the cell lines. U-251, U-87, and EFC-2 cells showed higher gene transfer efficiency in a dose-dependent fashion and reached to almost maximum level (80-100%) at 50-100 MOI. LG, 9L, and C6 cells demonstrated lower gene transfer efficiency (10-30%) at 500 MOI (Table 1). Western blotting was done to analyze the exogenous *p53* gene transfer and expression. Both U-251 and LG cells expressed a high level of

**Table 1.** p53 status, gene transfer efficiency, and growth inhibition by Ad5CMV-*p53* in glioma cell lines

| Cell line | *p53* status* | Gene transfer† | Growth inhibition‡ |
|---|---|---|---|
| U-251 | Mutant (c.273:CGT/CAT;Arg/His) | 80-100% at 25-50 MOI | 80-90% at 25 MOI |
| U-87 | Wild (exons 2-11) | 80-100% at 50-100 MOI | 30-40% at 100 MOI |
| EFC-2 | Wild (exons 5-8) | 80-100% at 50-100 MOI | 40-50% at 100 MOI |
| LG | Mutant (c.242:TGC/TTC;Cys/Phe) | 10-30% at 100-500 MOI | 30-40% at 100 MOI |
| C6 | Wild | 10-20% at 500-1000 MOI | <10% at 500 MOI |
| 9L | Mutant | 10-20% at 500-1000 MOI | <10% at 500 MOI |

*p53 status in the cell lines was reported previously (reference 19, 25, 26). MOI, multiplicity of infection
†Gene transfer efficiency was determined by X-galactosidase staining after infection with Ad5CMV-$\beta$-gal at the indicated MOI.
‡Growth inhibitory effect was evaluated by cell count assay after infection with Ad5CMV-p53 at the indicated MOI.

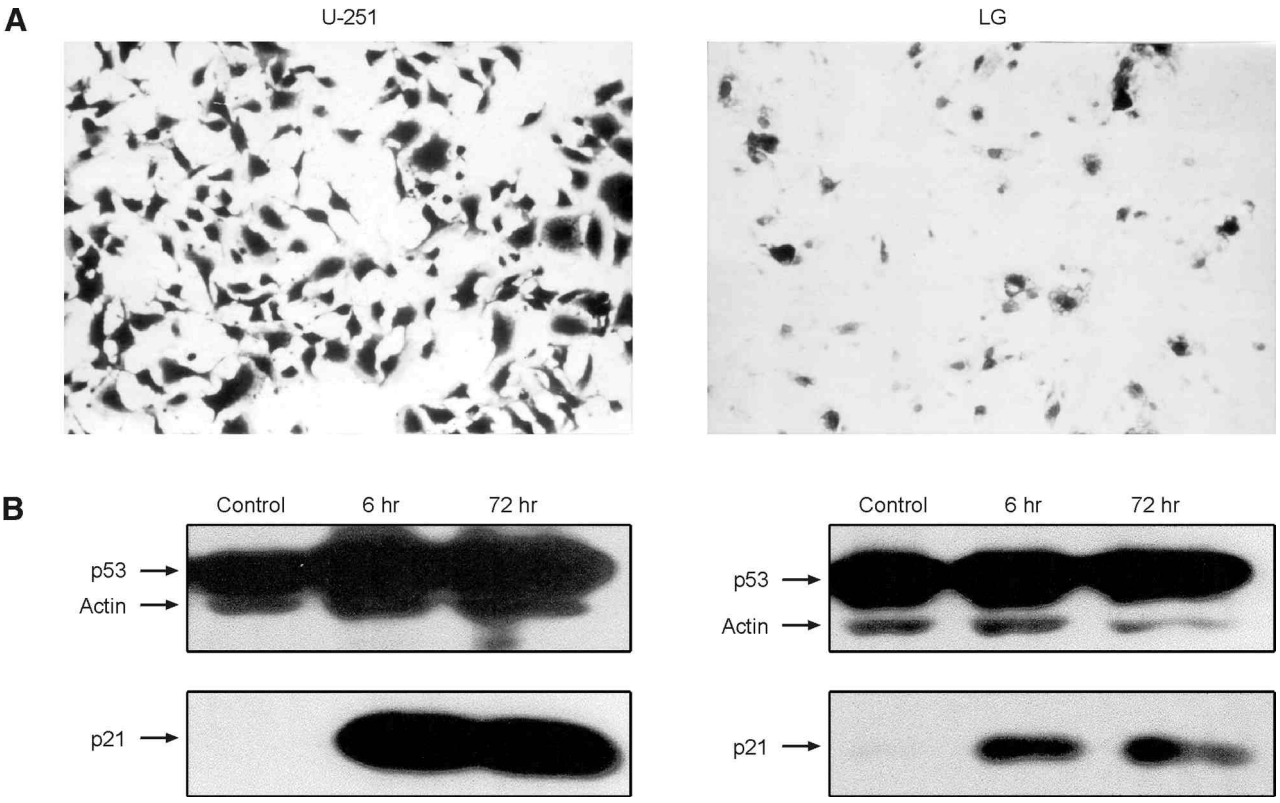


Fig. 1. (A) X-gal assay for gene transfer. U-251 and LG cell cultures were treated with Ad5CMV-β-gal at MOI of 10-500 PFU/cell for 48 hr and X-gal staining was done. Five hundreds cells were counted and the percentage of blue cells were calculated. Pictures of X-gal stained U-251 and LG cells were taken under phase-contrast inverted microscope (×100). (B) Western assay for exogenous *p53* gene transfer and $p21^{WAF1/CIP1}$ induction. U-251 and LG cells were harvested at 6 hr and 72 hr after the treatment with control media or Ad5CMV-*p53* at 25 MOI, and protein extraction and Western analysis was done. The extent of *p53* gene transfer and $p21^{WAF1/CIP1}$ induction determined by X-gal staining and Western blotting is very consistent.

endogenous mutant p53 protein. With Ad5CMV-*p53* treatment, a significant increase of exogenous wild-type p53 protein and induction of $p21^{WAF1/CIP1}$ was found in U-251 cells, and only a minimal increase of exogenous p53 protein and induction of $p21^{WAF1/CIP1}$ was noticed in LG cells. The extent of gene transfer determined by X-gal staining (Fig. 1A) and Western blotting (Fig. 1B) was very consistent.

## Growth inhibition of glioma cells

Ad5CMV-*p53* infection inhibited the growth of U-251 cells in a dose-dependent fashion at the MOI range of 10-500 PFU. The recombinant adenovirus without *p53* gene showed no evidence of cytotoxicity to tumor cells at lower doses but significant cytotoxicity at the MOI range over 100 PFU (Fig. 2A). The growth inhibitory activity of Ad5CMV-*p53* on U-251 cells appeared to reach maximum level on day 3 and cellular growth activity increased again after that time point (Fig. 2B). The degree of growth inhibition was varied depending on the extent of gene transfer and p53 status of the cell lines. LG and 9L cells containing mutant p53 and having lower gene transfer efficiency were less sensitive (5-36% growth inhibition) than U-251 cells with mutant p53 and higher gene transfer efficiency (85% growth inhibition) to Ad5CMV-*p53* treatment (Table 1). Growth inhibitory effect of Ad5CMV-*p53* was moderate in EFC-2 and in U-87 cells with higher gene transfer efficiency and wild-type p53, and minimal in C6 cells with lower gene transfer efficiency and wild-type p53 (Table 1). About 24-72 hr after the treatment, an apparent morphologic change occurred in tumor cells, with portions of the cell population enlarged, rounding up, and flat (data not shown).

## In vivo toxicity

For in vivo toxicity assay, Ad5CMV-*p53* from $10^7$ to $10^9$ MOI in 5 μL PBS was injected into the rat brain and histopathologic examination was performed. No pathologic change was found in the brains of all animals injected with Ad5CMV-*p53* of $10^7$ MOI, but inflammatory reaction was noticed in the three brains of five

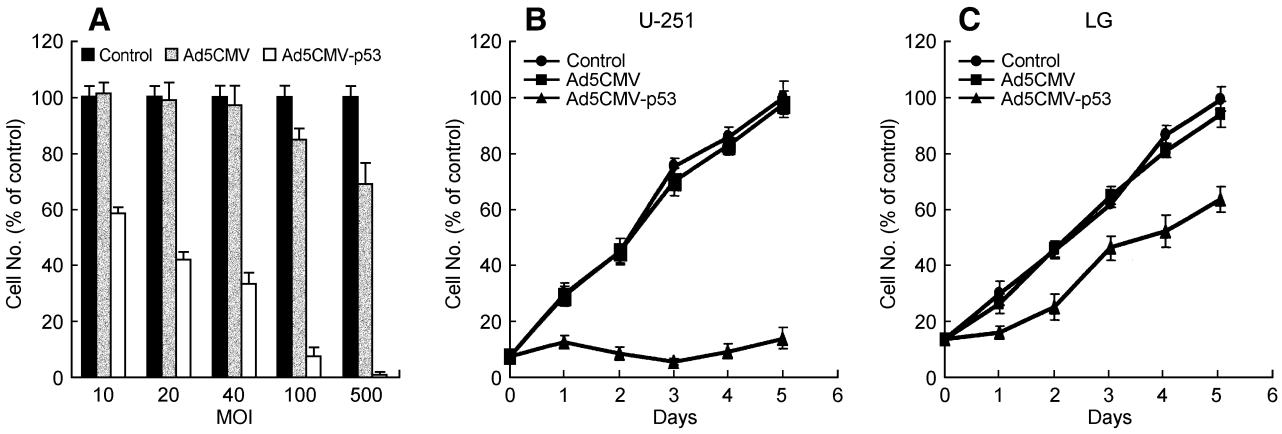


Fig. 2. Growth inhibitory effects of Ad5CMV-*p53* on human glioma cells. U-251 and LG cells were plated on 6-well plates and treated with control media, Ad5CMV, and Ad5CMV-*p53* at 10-500 MOI. Cell count was done from day 1 to day 5 after the treatment. (A) Dose-dependent growth inhibition is observed in U-251 cells. U-251 (B) and LG (C) cells demonstrate different levels of growth inhibition at 25 MOI. Values are the average of triplicate counts with SD.

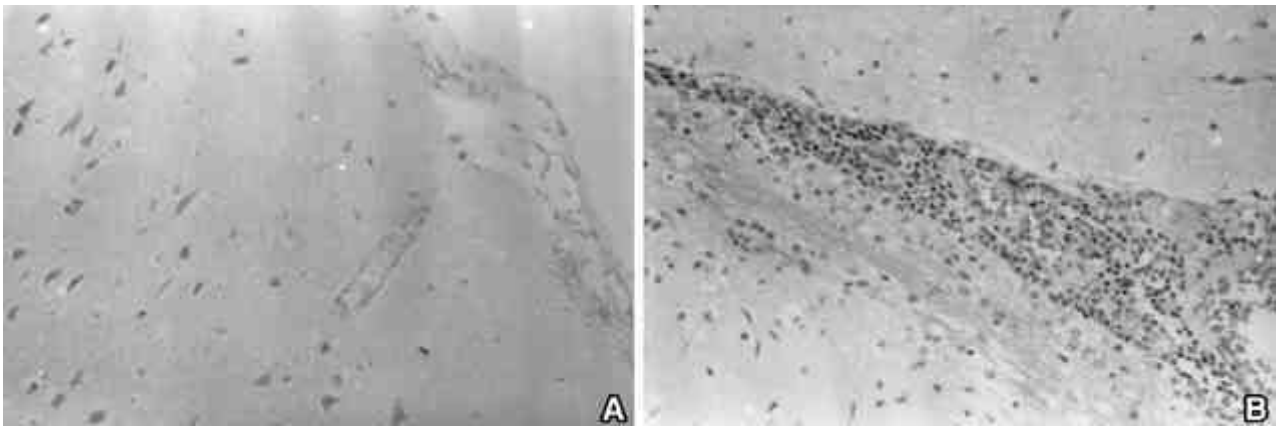


Fig. 3. In vivo toxicity of Ad5CMV-*p53* to the brain. The rat brain injected with Ad5CMV-*p53* of $10^7$ MOI (A) appears normal except for mild edema and capillary congestion in the subarachnoid space. Vascular congestion and many lymphocytic infiltrations mixed with a few neutrophils are present in the subarachnoid space of the brain injected with Ad5CMV-*p53* of $10^8$ MOI (B). A few inflammatory cells infiltrated the brain cortex (H-E, ×100).

animals injected with Ad5CMV-*p53* of $10^8$ MOI and in all brains of the animals injected with $10^9$ MOI. The inflammation appeared to be more prominent at the brain surface and meninges neighboring the cerebrospinal fluid than at the brain parenchyme of injection site (Fig. 3). No pathologic change was found in major organs including the lung, heart, liver, kidney and spleen.

### Effect on in vivo tumor growth

In in vivo tumorigenicity assay, U-251 cells treated ex vivo with control media, Ad5CMV, or Ad5CMV-p53 formed subcutaneous tumors in nude mice of 1,010±350 μL, 928±380 μL, and 27±9 μL at 21 days post-implantation, respectively (Fig. 4). To study in vivo growth inhibitory effect on established tumors, U-251 cells were implanted intracerebrally in nude mice, followed by intratumoral injection of Ad5CMV-*p53* of $10^7$ MOI via the same burr hole seven days post-implantation. Neither the tumor growth suppression nor the systemic toxicity was observed by this local gene therapy (Fig. 5).

## DISCUSSION

One of the requirements for the ideal gene transfer vector would be the capability of efficiently delivering the genes to the target cells (8). Adenoviral vector has been known to have a broad range of hosts and is able to deliver the genes more efficiently than other vectors, such as retrovirus and liposome (8). In our study, however, Ad5CMV-*p53* showed efficient gene delivery in only three of six glioma cell lines. Considering the heterogeneity of the tumor cellular subpopulations, further

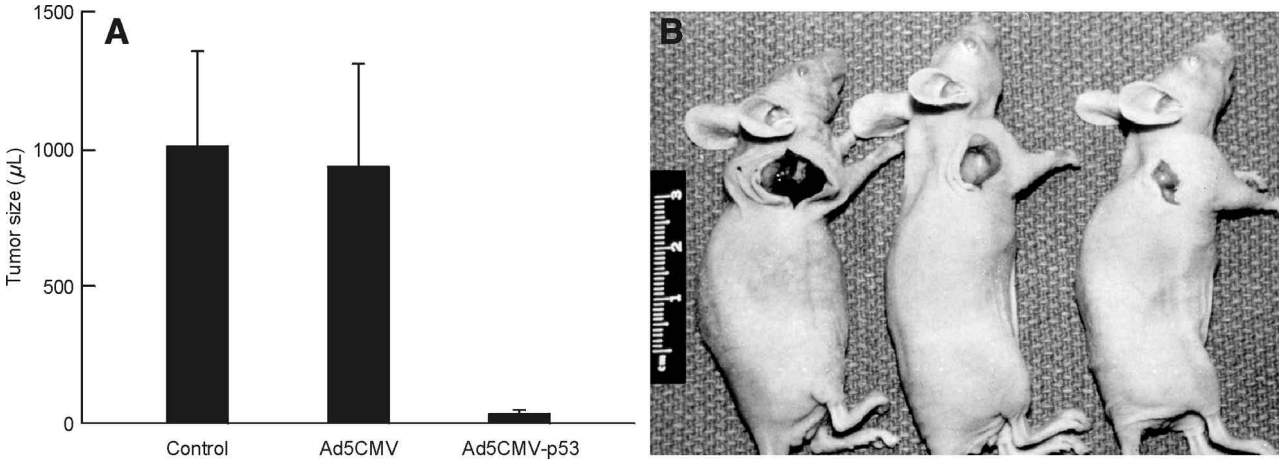


**Fig. 4.** Ad5CMV-*p53* effect on the tumorigenicity of U-251 cells. Cultures of U-251 cells were treated with control media, Ad5CMV, or Ad5CMV-*p53* at 25 MOI for 24 hr and were injected subcutaneously in nude mice. **(A)** The size of the exposed subcutaneous tumors were measured at 21 days post-inoculation. **(B)** Representative figures of the subcutaneous tumors formed from the U-251 cells treated ex vivo with control media (left), Ad5CMV (middle), or Ad5CMV-p53 (right).

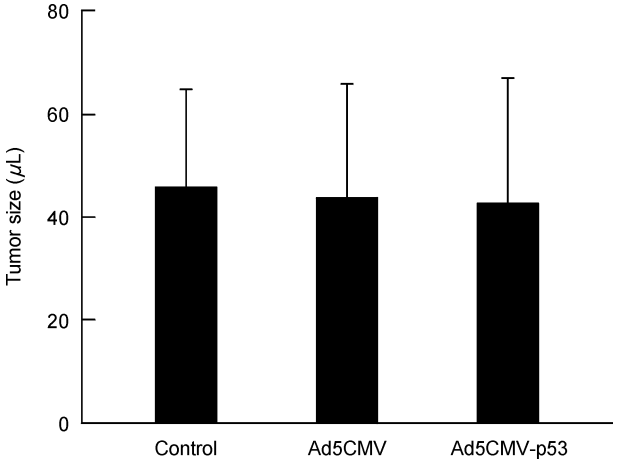


**Fig. 5.** Treatment of the intracerebral glioma by intratumoral injection of Ad5CMV-*p53*. $2\times10^5$ U-251 cells were injected into the caudate nucleus of nude mice. Seven days after tumor implantation, the animals were divided into three groups and each was intratumorally injected with therapeutic molecules (control PBS, Ad5CMV, Ad5CMV-*p53* of $3\times10^8$ MOI in 3 μL) via the same burr hole using the stereotactic system. Fourteen days later, the animals were sacrificed and the tumor sizes were measured in the maximal area in coronal section stained with hematoxylin-eosin.

studies are needed to overcome the variable gene transfer efficiency of Ad5CMV-*p53*. In contrast to the popularity of using adenovirus for cancer gene therapy, relatively little is known of adenovirus-mediated gene transfer mechanisms. Adenoviral vectors are known to enter the cell by means of two receptors (a specific receptor for the adenovirus fiber and $\alpha_v\beta_3$ or $\alpha_v\beta_5$ surface integrins that serve as a receptor for the adenovirus penton), and integrins $\alpha_v\beta_3$ and $\alpha_v\beta_5$ facilitate adenovirus-mediated gene delivery (28, 29). Screening the integrins receptors, level of tumors and up-regulation of the receptors by gene delivery or cytokines would be an alternative approach to improve the gene transfer efficiency of adenoviral vectors (28, 29).

There have been some arguments on the biologic responses to adenovirus-mediated *p53* gene transfer in malignant gliomas. In relation to the p53 status of the cells, Köck et al. (18) and Gomez-Manzano et al. (19) reported that adenovirus-mediated exogenous *p53* gene delivery resulted in apoptotic cell death in human glioma cell lines containing a mutant *p53* gene but had only a minimal effect on the growth of glioma cell lines containing wild-type *p53* gene. However, Li et al. (20) reported that it induced apoptosis of human glioma cells independent of endogenous p53 status. In our study, Ad5CMV-*p53* showed drastic growth inhibition of U-251 cells containing mutant p53 and high-gene transfer efficiency, but it had only a minimal growth inhibitory effect on cell lines with wild-type p53 or lower gene transfer efficiency (Table 1). The growth inhibitory activity of Ad5CMV-*p53* on U-251 cells appeared to reach maximal level on day 3 and the cellular growth activity increased again after that time point (Fig. 2B). This may be related to the peak level of p53 protein expression on day 3 post-infection or regrowth of the incompletely infected cells, indicating that resistance or recurrence to Ad5CMV-*p53* may develop depending on both gene transfer extent and p53 status of the tumor cell subpopulations. Malignant gliomas consist of heterogeneous cellular subpopulations and approximately 35-60% of the gliomas have been reported to contain mutations in *p53* gene or alterations in p53 protein activity (4). Given that gliomas designated "p53 mutant" are also likely to contain wild-type clones, *p53* gene replacement strategies

would have limited success as a single modality for glioma therapy. Thus, combined approaches including other tumor suppressor genes (7), irradiation (23, 24) or chemotherapy (22) could exploit the physiological functions of the p53 protein and improve the efficacy of *p53* gene therapy.

9L and C6 are rat glioma cell lines and have been very frequently used for various brain tumor studies, and they contain mutant and wild type p53, respectively. Badie et al. (21) reported that adenovirus-mediated *p53* gene delivery inhibited 9L glioma growth in rats, but Dorigo et al. (22) showed that wild-type p53 expression using Lipofectamine did not affect 9L cell proliferation. Our study demonstrated that Ad5CMV-*p53* has low gene transfer efficiency and little growth inhibition effect on 9L and C6 cells. It is not clear whether or not such different results of significant (21) versus little (22) growth inhibitory effect were caused by different laboratory conditions and/or different gene expression levels and/or species specificity to the *p53* DNA or not. Careful interpretation may be necessary in adenovirus-*p53* gene therapy study using 9L or C6 cells.

Clayman et al. (10, 11) reported that intratumoral administration of adenovirus-mediated wild-type *p53* gene with doses up to $10^{11}$ MOI by multiple injections reduced the growth of head and neck cancers without serious adverse effect on preclinical and clinical studies. In our study, ex vivo treatment with Ad5CMV-*p53* suppressed in vivo tumorigenic potential of U-251 cells, but administration of Ad5CMV-*p53* into the established intracerebral tumor did not affect the tumor growth in nude mice. Even though many factors may underlie the discrepancy between the in vivo and in vitro or ex vivo treatment effect, insufficient gene transfer to the invasive tumor cells by single injection or low dose ($10^7$ MOI) of Ad5CMV-*p53* may be one of the most important limiting factors for the poor antitumor effect of this in vivo local gene therapy. This can be supported by the previous studies that showed intracerebral injections of adenovirus in a rat (30) and a non-human primate (31) resulted in localized distribution near the injection site. Further investigations into alternative delivery systems for adenoviral vectors targeting a greater percentage of brain tumor cells will be required. Fine et al. (32) could improve gene delivery efficiency to brain tumors by increasing adenoviral inoculum and injecting virus under high pressure. These data are consistent with the hypothesis that adenoviral virions may be carried along the pressure gradients associated with tumor-induced intracerebral edema. Adenoviral vectors may be capable of infecting a significant number of tumor cells at a distance from the site of stereotactic injection.

As a gene transfer vector, adenovirus has been reported to be safe for local gene therapy of brain (9) and head-neck (10, 11) tumors. In our in vitro study, Ad5CMV-*p53* had a significant growth inhibitory effect on U-251 cells at 10 to 500 MOI, and cytotoxicity was also observed at doses higher than 100 MOI. To study in vivo toxicity following intracerebral administration, we injected various doses of Ad5CMV-*p53* into rat brains. No pathologic reaction was noticed with $10^7$ MOI, but inflammatory reactions were observed with higher doses than $10^8$ MOI in half of the tested rat brains, which appeared more prominent in the brain surface neighboring the cerebrospinal fluid (Fig. 5). This was thought to be due to a back-flow of the adenovirus along the injection tract and dissemination into the CSF, suggesting that appropriate doses, careful delivery methods avoiding regurgitation or spill into the CSF should be considered and particular attention should be taken in patients with tumors located close or communicated to the CSF pathways. Further studies will be needed to prevent, decrease or treat adverse reactions of adenoviral vectors to the brain.

Although Ad5CMV-*p53* demonstrated a potential to be developed into an agent for human glioma therapy, several questions still need to be answered. The heterogeneity of the human glioma cells in p53 status and gene transfer efficiency, and adverse reactions to the brain are limiting factors that need to be solved in order to increase its therapeutic potential.

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