GYNECOLOGIC ONCOLOGY **60,** 373–379 (1996)
ARTICLE NO. 0057

Growth Inhibition of Human Cervical Cancer Cells with the Recombinant Adenovirus *p53 in Vitro*[1]

KATSUYUKI HAMADA,[2] WEI-WEI ZHANG, RAMON ALEMANY, JUDITH WOLF,*
JACK A. ROTH, AND MICHELE FOLLEN MITCHELL*

*Section of Thoracic Molecular Oncology, Department of Thoracic and Cardiovascular Surgery, and *Department of Gynecologic Oncology, The University of Texas M. D. Anderson Cancer Center, Houston, Texas 77030*

Received May 1, 1995

Human papillomavirus (HPV) has been identified in the majority of invasive cancers of the uterine cervix sampled and has been found to contribute in a significant way to the genesis of human cervical cancer. HPV has two transforming genes that encode the oncoproteins E6 and E7. E6 can form complexes with p53 and promote p53 degradation. We introduced wild-type *p53* into a cervical cancer cell line via a recombinant adenoviral vector, Ad5CMV-*p53.* Human cervical cancer cell line HeLa, which has HPV type 18 and wild-type *p53,* was used in this study. Cells were grown in RPMI medium supplemented with 10% heat-inactivated fetal bovine serum. Ad5CMV-*p53* was created by inserting the cytomegalovirus promoter, wild-type *p53* cDNA, and SV40 polyadenylation signal in a minigene cassette into the E1-deleted region of the modified Ad5 adenovirus. The transduction efficiency was 100% when a dose ensuring a multiplicity of infection of 100 or greater was used. The p53 protein was detected in Ad5CMV-*p53*-infected cells by immunohistochemical and Western blot analyses. The growth of the Ad5CMV-*p53*-infected cells was greatly suppressed as detected by both cell count and [^{3}H]thymidine incorporation assay. These data suggest that transfection of HPV-positive cervical cancer cells with a wild-type *p53* gene in a form such as Ad5CMV-*p53* is a potential novel therapy for cervical cancer.

INTRODUCTION

Cervical cancer is the second most common malignancy in women worldwide, accounting for 15% of all cancers diagnosed in women [1]. In 1989, 500,000 cases were expected worldwide and an overall 5-year survival rate is 40% [1]. Despite aggressive screening with the Papanicolaou smear, this cancer remains an important health problem for women. The most important risk factor is human papillomavirus (HPV) infection, which increases the relative risk of developing cervical intraepithelial neoplasia and cervical cancer by 11- to 60-fold [1].

HPV types 16 and 18 have been identified in anywhere from 50–90% of cervical cancers [2–4]. The E6 and E7 genes of HPV 16 and 18 are frequently coexpressed and are the most abundant viral transcripts in biopsies from HPV-positive cervical cancers [5, 6]. Virus-encoded oncoproteins are important cellular regulatory proteins by which viruses can transform cells. The E6 and E7 proteins of HPV 16 and 18 have been demonstrated to be necessary and sufficient for malignant transformation of mammalian cells [7, 8]. Furthermore, the continued expression of the E6 and E7 region of the viral genome appears to be necessary for the maintenance of the malignant phenotype [9]. E7 forms complexes with the retinoblastoma protein (pRB) and E6 forms complexes with the p53 protein.

E7 shares functional and structural features with the adenovirus E1A proteins. The E7 proteins of the ''high-risk'' HPVs for the pRB have an approximately 10-fold higher affinity than do E7 proteins of the ''low-risk'' HPVs; this is thought to be due to the inclusion of the pRB-binding domain in the amino acid terminal sequences of these proteins [9]. The E6 oncoprotein shares functional and structural features with SV40 large T and adenovirus 5 E1B. *In vitro*, E6 promotes degradation of p53 via the ubiquitin-dependent protease system. This selective degradation of negative regulatory proteins provides a novel mechanism for dominant acting oncoproteins [10–12]. Howley and others have demonstrated that the inactivation of RB and p53 are important steps in cervical carcinogenesis [13]. HPV-positive cell lines expressed normal pRB and low levels of wild-type p53 [13].

[1] This study was partially supported by grants from the National Cancer Institutes and the National Institute of Health (RO1 CA45187) (J.A.R.); by gifts to the Division of Surgery from Tenneco and Exxon for the Core Laboratory Facility; by the M. D. Anderson Cancer Center Support Core Grant (CA16672); and by a grant from the Mathers Foundation, the University Cancer Foundation, and the Business and Professional Women's Fund.

[2] To whom reprint requests should be addressed at Department of Thoracic and Cardiovascular Surgery, 1515 Holcombe, Box 109, Houston, TX 77030. Fax: 713-794-4901.

0090-8258/96 $18.00

The wild-type *p53* gene may have important roles not only in cell cycle but in apoptosis.

Growth of human non-small cell lung cancer cells and squamous cell carcinomas of the head and neck was significantly inhibited *in vitro* and *in vivo* by the introduction of wild-type *p53* via a recombinant adenoviral vector, Ad5CMV-*p53* [14, 15]. The *p53* adenovirus was directly injected into non-small cell lung cancer cells with a homozygous deletion of *p53;* subcutaneous implantation of these cells in nu/nu mice, followed by intraperitoneal administration of cisplatin, induced massive apoptotic destruction of the tumors [16]. HPV-immortalized human cervical epithelial cell lines cannot tolerate large amounts of exogenous wild-type p53 transfected by Lipofectin [17]. However, there are no reports on the efficacy of the *p53* adenoviral vector on cervical cancer cells.

We introduced the wild-type *p53* gene into cervical carcinoma cells via the recombinant adenoviral vector, Ad5CMV-*p53,* to determine its effects on growth of the HPV-18-positive human cervical cancer HeLa cell line.

MATERIALS AND METHODS

Cell Line

Human cervical carcinoma cell line HeLa was obtained from the American Type Culture Collection (Bethesda, MD). HeLa contains either HPV-18 or HPV-related sequences and expresses *p53* mRNA which has a wild-type sequence [18]. Cells were grown in RPMI medium supplemented with 10% heat-inactivated fetal bovine serum.

Recombinant Adenovirus and Infection

The recombinant p53 adenovirus (Ad5CMV-*p53*) contains the cytomegalovirus promoter, wild-type *p53* cDNA, and SV40 polyadenylation signal in a minigene cassette which is inserted into the E1-deleted region of modified adenovirus Ad5 [14]. The adenoviral vector containing the same cassette but without *p53* cDNA was used as control (Ad5CMV-*poly A*). The adenovirus containing the β-galactosidase (β-gal) gene, Ad5CMV-*LacZ,* was used to determine transduction efficiencies. Viral stocks were propagated in 293 cells, which are derived from primary embryonal kidney cells transformed by introducing sheared fragments of Ad5 DNA. This cell line contains E1A and is thus highly permissive of the replication of the E1 replication-deficient adenovirus. Cells were harvested 36–40 hr after infection, pelleted, resuspended in phosphate-buffered saline, and lysed; cell debris was removed by subjecting the cells to CsCl gradient purification. Concentrated virus was dialyzed and stored in aliquots at −80°C. Infection was carried out by adding the virus to high-glucose Dulbecco's minimal essential medium and to the cell monolayers. The cells were incubated at 37°C for 60 min with constant agitation. Medium was added, and the cells were incubated at 37°C for the desired length of time.

Northern Blot Analysis

Total RNA was isolated by the RNAzol B method (TEL-TEST Inc., Friendswood, TX). Northern analysis was performed on 20 μg of total RNA. The membrane was hybridized with a *p53* cDNA probe labeled by the random primer method in 5 × SSC-5 × Denhardt' solution–0.5% SDS–denatured salmon sperm DNA (20 μg/ml).

Western Blot Analysis

Total cell lysates were prepared by lysing monolayer cells in dishes with SDS–PAGE sample buffer. Five micrograms of protein from samples was subjected to 10% SDS–polyacrylamide gel electrophoresis and transferred to Hybond-ECL membrane (Amersham Corp., Arlington Heights, IL). The membrane was blocked with 1% nonfat dry milk and 0.1% Tween 20 (Sigma Chemical Co., St. Louis, MO) and probed with the primary antibodies, mouse anti-human p53 monoclonal antibody DO7 (DAKO, Carpinteria, CA), and mouse anti-human actin monoclonal antibody (Amersham), and the secondary antibody, horseradish peroxidase-conjugated sheep anti-mouse IgG (Amersham).

Immunohistochemical Analysis

The infected cell monolayers were fixed with 3.8% formalin and treated with 3% H_2O_2 in methanol for 5 min. Immunohistochemical staining was performed by using the Vectastain Elite kit (Vector Labs, Burlingame, CA). The primary antibody was the anti-p53 antibody DO7 (DAKO), and the secondary antibody was an avidin–antibody complex. Biotinylated horseradish peroxidase ABC complex reagent was used to detect the antigen–antibody complex.

Cell Count Assay

Cells were plated at a density of 5×10^4/3 ml/well in 12-well plates in triplicate. RPMI supplemented with 10% heat-inactivated fetal bovine serum was used as growth medium. Cells were infected with either Ad5CMV-*p53* or the Ad5CMV-*poly A* control. Cells were harvested and counted at each point of the dose and the date. Cell viability was determined by trypan blue exclusion.

[^{3}*H*]*Thymidine Incorporation Assay*

Cells were also cultured at 2000 cells/well in 96-well flat-bottomed plates. Cells were infected with either Ad5CMV-*p53* or Ad5CMV-*poly A* and cultured in RPMI medium containing 10% fetal bovine serum for 5 days. Each well was pulse-treated with 1 μCi [^{3}H]thymidine (sp

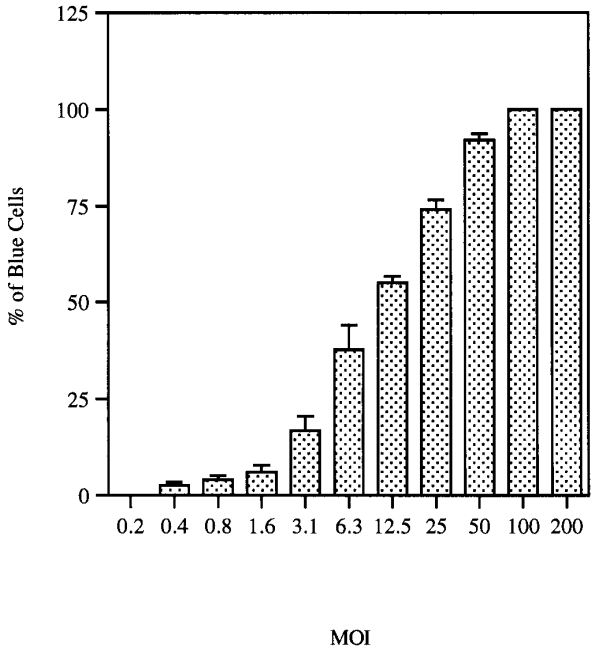

FIG. 1. Transduction efficiency in the HeLa cell line. A recombinant β-gal adenovirus, Ad5CMV-*LacZ*, was used to infect the cells at different m.o.i.s ranging from 0.2 to 200. The percentages of β-gal-positive cells were obtained from scoring 500 cells each on replicate dishes.

act 6.7 Ci/mmol; Amersham) for an additional 24 hr, after which the cells were harvested with a Ph.D. cell harvester (Cambridge Technology, Cambridge, MA). Individual filter disks were then processed for liquid scintillation counting. The data were presented as the means of triplicate samples.

RESULTS

The conditions for optimal adenoviral transduction were determined by infecting cells with an adenovirus, Ad5CMV-*LacZ*, that expresses the β-gal gene. The transduction efficiency was assessed by counting the percentage of blue cells after X-gal staining. The percentage of β-gal-positive cells was obtained by scoring 500 cells each on replicate dishes. There appeared to be a linear relationship between the number of infected cells and the number of adenovirus particles used in the infection. Cells inoculated with a single dose of Ad5CMV-*LacZ* at a multiplicity of infection (m.o.i.) of 100 exhibited 100% blue cells (Fig. 1). The transduction efficiency of this vector in HeLa cells was similar to those in other cell lines examined previously, HepG2, LM2, and human non-small cell lung cancer cell lines [14].

The recombinant wild-type *p53* adenovirus, Ad5CMV-*p53*, was used to infect the cervical cancer cell line HeLa. Twenty-four hours after infection, total RNA was isolated,

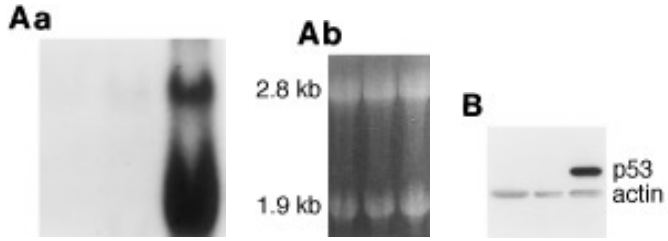

FIG. 2. (A) Northern blot analysis. (a) Total RNA was isolated to detect expression of exogenous *p53* mRNA 24 hr after Ad5CMV-*p53* infection. (b) The ethidium bromide-stained gel scanned at the 18S and 28S rRNA band served as a standard for loading. Left lane is mock infection, middle lane is Ad5CMV-*poly A* infection, and right lane is Ad5CMV-*p53* infection. (B) Western blot analysis. Cellular extracts isolated from cells 24 hr after infection were subjected to 10% SDS–polyacrylamide gel electrophoresis. Left lane is mock infection, middle lane is Ad5CMV-*poly A* infection, and right lane is Ad5CMV-*p53* infection.

and Northern blot analysis was performed. The levels of the 2.8-kilobase endogenous *p53* mRNA detected in the samples isolated from mock-infected cells and from the cells infected with a control vector Ad5CMV-*poly A* were similar (Fig. 2A). The level of exogenous 1.9-kilobase *p53* mRNA was increased in the cells infected with Ad5CMV-*p53* (Fig. 2A), indicating that the exogenous *p53* cDNA was successfully transduced into cells and efficiently transcribed. The level of endogenous 2.8-kb p53 mRNA was also increased in the cell infected with Ad5CMV-*p53*. Western blot analysis was performed to compare the amount of p53 protein produced. A p53 band, recognized by the monospecific anti-p53 antibody DO7, was observed in cellular extracts isolated from infected cells. Samples isolated from mock-infected (medium only) and Ad5CMV-*poly A*-infected cells did not exhibit the p53 protein (Fig. 2B). Immunohistochemical analysis of cells infected with Ad5CMV-*p53* revealed the characteristic nuclear staining of the p53 protein (Fig. 3), whereas mock-infected cells failed to show the p53 protein.

The growth of the Ad5CMV-*p53*-infected cells was

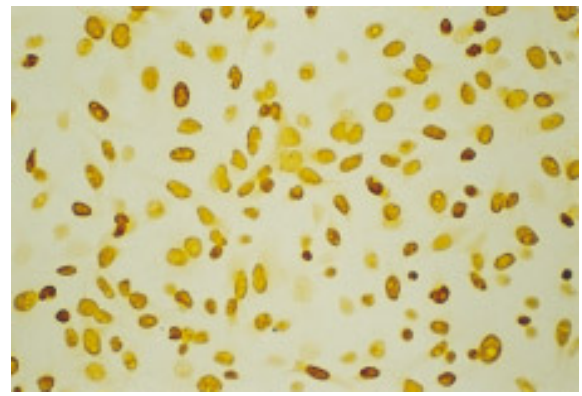

FIG. 3. Immunohistochemical staining of HeLa cells 24 hr after infection of Ad5CMV-*p53* at 100 m.o.i.

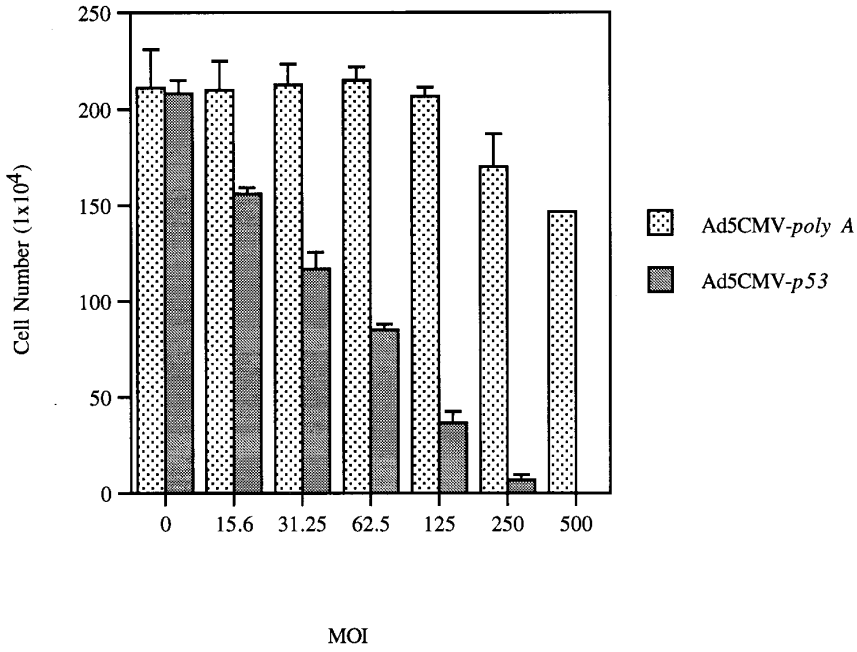

FIG. 4. Inhibition of the growth of HeLa cells by cell count assay. At each indicated point, cells in three wells on each well plate were trypsinized and counted. The means of cell counts for triplicate wells 6 days after infection were plotted. Bars indicate SD.

greatly suppressed when estimated by cell count and [^{3}H]-thymidine incorporation assay (Figs. 4–6). Cell growth assays were reproducible in five repeated experiments. Twenty-four hours after infection, an apparent morphological change occurred, with portions of the cell population rounding up and their outer membranes forming blebs. These are part of a series of histologically predictable events that suggest programmed cell death. Cells infected with the con-

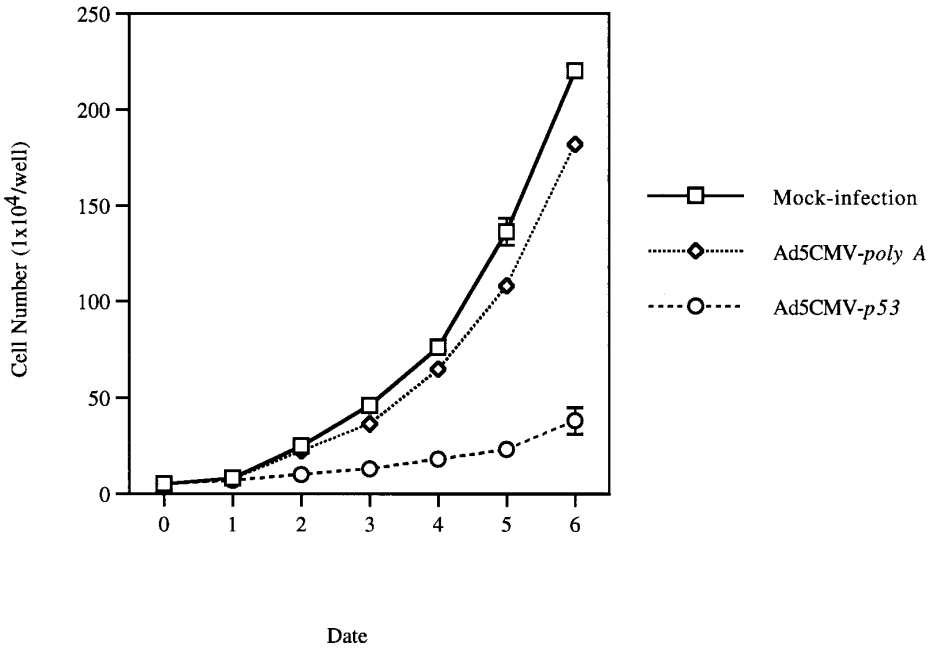

FIG. 5. Time course of the growth inhibition of HeLa cells by cell count assay. A growth curve of mock-infected cells, Ad5CMV-*poly A*-infected cells, and Ad5CMV-*p53*-infected cells is shown. At each indicated time point, cells in three wells on each plate were trypsinized and counted. The mean cell counts for triplicate wells were plotted against the number of days since infection. Bars indicate SD.

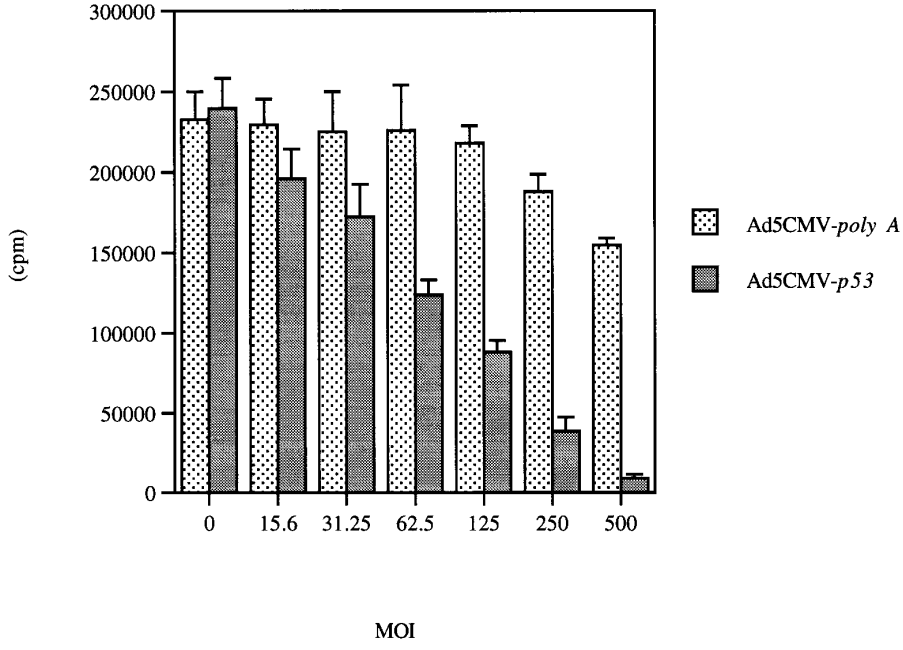

FIG. 6. Inhibition of the growth of HeLa cells by [^{3}H]thymidine incorporation assay. At each indicated point, cells in three wells on each plate were trypsinized, harvested, and counted on Day 6 after infection The means of cpm for triplicate wells were plotted. Bars indicate SD.

trol adenovirus, Ad5CMV-*poly A,* demonstrated normal growth characteristics with no histomorphological abnormalities.

DISCUSSION

HPV has been identified in the majority of invasive cancers of the uterine cervix and has been found to contribute in a significant way to the genesis of human cervical cancer. Inactivation of *p53* gene by allelic loss or by point mutation is infrequent in primary cervical cancer and the overall incidence in these cancers of point mutations in the *p53* gene is 1–6% [18]. HPV has two transforming genes that encode the oncoproteins E6 and E7. E6 can form complexes with p53 and promote p53 degradation. HPV-positive cell lines express low levels of wild-type p53 [13, 19]. Since the wild-type *p53* gene is believed to be involved primarily in delivering antiproliferative signals that may be capable of antagonizing the growth-stimulatory signals propagated by oncogene products, the potential molecular therapeutic effect of this gene in cervical cancer deserves attention.

The rapid advancement in the field of gene therapy, including the creation of adenoviral vectors, has created an opportunity for progress toward novel gene therapy of cervical cancer. Because of their natural tropism for epithelial cells, adenoviruses may be uniquely suitable for the transient delivery of genes to cancers in epithelial tissues. The recombinant, replication-defective adenoviruses that have been developed for gene therapy are missing the entire E1A and part of the E1B regions and are, therefore, capable of propagating only in cells that can provide the E1 proteins in trans, such as the 293 cell line. In the past few years, recombinant adenoviruses have been extensively investigated as vectors for *in vivo* gene therapy. The high transfer efficiency of adenoviral vectors over a broad range of hosts both *in vitro* and *in vivo* make them attractive vehicles for molecular therapy.

The recent creation of a recombinant wild-type *p53* adenoviral vector (Ad5CMV-*p53*) provided us with an excellent candidate for investigation of the biological effects of the wild-type *p53* gene product on the cervical cancer cell line HeLa, in which the wild-type *p53* gene is inactivated by HPV-18 infection. A β-gal recombinant adenovirus allowed us to establish the gene transfer efficiency in cervical cancer cells. At more than 100 m.o.i., all cervical cancer cells were positive for X-gal staining. There appeared to be a linear correlation between the number of cells expressing the gene and the amount of viral particles used in the experiment. This result coincided with the efficiency obtained in cells infected with Ad5CMV-*p53,* which was assessed by immunostaining using a monoclonal anti-p53 antibody. The transduction efficiency we observed was almost the same as these achieved in other cell lines tested, including HepG2, LM2, and the human non-small cell lung cancer cell lines.

The *p53* gene is wild-type in HeLa cells. The *p53* mRNA is detected in untransfected HeLa cells, but p53 protein was barely detected in untransfected cells. Western blot and immunohistochemical analyses demonstrated that production of p53 protein was significantly higher in Ad5CMV-*p53*-

infected cells than in control cells, suggesting that the exogenous *p53* mRNA was efficiently translated. Time-course protein expression studies in human non-small cell lung cancer cells have shown protein expression to peak 3 days after infection and progressively decline to levels still detectable by Western blotting on Day 15, but nondetectable levels 3 weeks after infection [14]. In most systems of adenoviruses, expression is transient.

The cervical cancer cells transduced with the wild-type *p53* gene exhibited significantly more inhibition of growth *in vitro* then mock-infected and control adenovirus-infected cells, clearly illustrating that these results were not mediated by the virus itself. The mechanism by which wild-type p53 protein inhibits growth *in vitro* may be related to arrest of the cell cycle at G_1 [20], apoptosis [21], or induction of another tumor suppressor gene such as WAF1/CIP1 [22]. The induction of apoptosis is one of the several documented functions of wild-type p53. When HeLa cells were infected with Ad5CMV-*p53* at 100 m.o.i., the characteristic apoptotic histomorphology, such as rounded-up cells and the formation of blebs, was apparent 24 hr after infection and was followed by cell death. We plan further studies on other cervical cancer cell lines *in vitro* and *in vivo*. However, the mechanism of growth suppression and cell death induced by Ad5CMV-*p53* requires further investigation including DNA ladder, morphological demonstration of nuclear condensation, and DNA fragmentation.

Adenoviral vectors have many advantages over other viral vector systems and other *in vitro* techniques for introducing DNA into eukaryotic cells. Human adenoviruses are double-stranded DNA viruses with an average genome size of approximately 36 kb, and thus have the potential to carry large or multiple foreign genes. Adenoviruses are easy to grow and manipulate, and they have a broad range of hosts *in vitro* and *in vivo*. Because the life cycle of adenovirus does not require integration into the host genome, the foreign genes delivered by adenoviral vectors are expressed episomally and therefore have low genotoxicity to host cells. Adenoviruses appear to be linked only to mild diseases, since there is no known association of human malignancies with adenoviral infection. Moreover, no side effects have been reported following vaccination with wild-type adenoviruses, demonstrating their safety and therapeutic potential as *in vivo* gene transfer vectors [23]. Recombinant adenoviruses have been successfully administered to a variety of tissues through different routes, including intratracheal instillation [24], muscle injection [25], peripheral intravenous injection [26], and stereotaxic inoculation to the brain [27].

Several studies with different gene products have revealed that following infection in a wide variety of target tissues, only transient expression is observed [28–30]. Since the cells infected with the recombinant adenovirus are rapidly eliminated, it is likely that the host immune system plays a major role in preventing sustained expression of the foreign genes. Recent results where a temperature-sensitive E2A gene was used to construct recombinant adenovirus, suggests that cytotoxic T lymphocyte response to viral antigens can be reduced at nonpermissive temperature [31]. Thus it is hopeful that a second generation of adenoviral vectors where E1A, E2A, and E4 gene have been deleted or rendered ineffective will be able to reduced the cell-mediated immunity problem.

Gene replacement with the wild-type *p53* gene has reversed features of the malignant phenotype, including soft agar colony formation, rapid cell proliferation, and tumorigenicity, in human tumor cells with homozygous *p53* deletions or mutant *p53* genes [14, 16, 21]. Human cervical cancer cells have a functional genotype equivalent to cells with an inactivated *p53* gene product due to the complexing of the E6 protein with the p53 protein. Restoration of p53 function in these cells is effective in reducing proliferation. The adenoviral vector is capable of mediating high levels of p53 expression which apparently are sufficient to overcome the capabilities of the endogenous E6 to bind the protein.

Our data indicate that *p53* has an important role as a determinant of malignant cell growth in human cervical cancer cells. A variety of treatment protocols, including surgery, chemotherapy, and radiotherapy, have been tried for human cervical cancer, but the long-term survival rate remains unsatisfactory. A treatment strategy based on restoration of tumor suppressor gene function might be effective as an adjuvant treatment to prevent local recurrence following primary tumor resection or as a treatment that could be given by intralesional injections in drug-resistant primary, metastatic, or locally recurrent cancers. Protocols are being developed to explore these clinical applications.

ACKNOWLEDGMENTS

We sincerely thank Ms. Ronetta Fairchild and Ms. Carol Torrence for their help in preparing the manuscript. We also sincerely thank Ms. Yoko Akimaru for her help in preparing the immunohistochemical slides.

REFERENCES

1. Brinton, L. A. The epidemiology of human papillomavirus and cervical cancer, in *Epidemiology of cervical cancer—Overview* (N. Munoz, F. Bosch, K. V. Shah, and A. Meheus, Eds.), Lyon, Vol. 119, pp. 3–23 (1992).
2. zur Hausen, H. Papillomaviruses in anogenital cancer as a model to understand the role of viruses in human cancer, *Cancer Res.* **49,** 4677–4681 (1989).
3. Galloway, D. A., and McDougall, J. K. Human papillomaviruses and carcinoma, *Adv. Virus Res.* **37,** 125–171 (1989).
4. Durst, M., Gissmann, L., Ikenberg, H., *et al.* A papillomavirus DNA from a cervical carcinomas and its prevalence in cancer biopsy samples from different geographic regions, *Proc. Natl. Acad. Sci. USA* **80,** 3812–3815 (1983).

5. Wettstein, F. O. Papillomaviruses and human cancer. in *State of viral DNA and gene expression in benign vs. malignant tumors* (H. Pfister, Ed), CRC Press, Boca Raton, FL, p. 145 (1990).
6. Seedorf, K., Krammer, G., Durst, M., *et al.* Identification of early proteins of the papillomaviruses type 16 (HPV 16) and type 18 (HPV 18) in cervical carcinoma cells, *EMBO J.* **6,** 139–144 (1987).
7. Kaur, P., McDougall, J. K., and Cone, R. Immortalization of primary human epithelial cells by cloned cervical carcinoma DNA containing human papillomavirus type 16 E6/E7 opening reading frames, *J. Gen. Virol.* **70,** 1261–1266 (1989).
8. Münger, K., Phelps, W. C., Bubb, V., *et al.* The E6 and E7 genes of the human papillomavirus type 16 together are necessary and sufficient for transformation of primary human keratinocytes, *J. Virol.* **63,** 4417–4421 (1989).
9. Heck, D. V., Yee, C. L., Howley, P. M., *et al.* Efficiency of binding the retinoblastoma protein correlates with the transforming capacity of the E7 oncoproteins of the human papillomaviruses, *Proc. Natl. Acad. Sci. USA* **89,** 4442–4446 (1992).
10. Kessis, T. D., Slebos, R. J., Nelson, W. G., *et al.* Human papillomavirus 16 E6 expression disrupts the p53-mediated cellular response to DNA damage, *Proc. Natl. Acad. Sci. USA* **90,** 2988–2992 (1993).
11. Crook, T., and Vousden, K. H. Properties of p53 mutations detected in primary and secondary cervical cancers suggest mechanisms of metastasis and involvement of environment carcinogens, *EMBO J.* **11,** 3935–3940 (1992).
12. Scheffner, M., Takahashi, T., Huibregtse, J. M., *et al.* Interaction of the human papillomavirus type 16 E6 oncoprotein with wild-type and mutant human p53 proteins, *J. Virol.* **66,** 5100–5105 (1992).
13. Howley, P. M., Scheffner, M., and Munger, K. Oncoproteins encoded by the cancer-associated human papillomavirus target the products of the retinoblastoma and p53 tumor suppressor genes, *Cold Spring Harbor Symp.* **56,** 149–155 (1991).
14. Zhang, W. W., Fang, X., Mazur, W., French, B. A., Georges, R. N., and Roth, J. A. High-efficiency gene transfer and high-level expression of wild-type p53 in human lung cancer cells mediated by recombinant adenovirus, *Cancer Gene Ther.* **1,** 4–15 (1994).
15. Liu, T. J., Zhang, W. W., Taylor, D. L., *et al.* Growth suppression of human head and neck cancer cells by the introduction of a wild-type p53 gene via a recombinant adenovirus, *Cancer Res.* **54,** 3662–3667 (1994).
16. Fujiwara, T., Grimm, E. A., Mukhopadhyay, T., *et al.* Induction of chemosensitivity in human lung cancer cells in vivo by adenovirus-mediated transfer of the wild-type p53 gene, *Cancer Res.* **54,** 2287–2291 (1994).
17. Chen, T. M., Chen, C. A., Hsieh, C. Y., *et al.* The state of p53 in primary human cervical carcinomas and its effects in human papillomavirus-immortalized human cervical cells, *Oncogene* **8,** 1511–1518 (1993).
18. Yee, C., Krishnan-Hewlett, I., Baker, C. C., *et al.* Presence and expression of human papillomavirus sequences in human cervical carcinoma cell lines, *Am. J. Pathol.* **119,** 361–366 (1985).
19. Scheffner, M., Münger, K., Byrne, J. C., and Howley, P. M. The state of the p53 and retinoblastoma genes in human cerivcal carcinoma cell lines, *Proc. Natl. Acad. Sci. USA* **88,** 5523–5527 (1991).
20. Diller, L., Kassel, J., Nelson, C. E., *et al.* p53 functions as a cell cycle control protein in osteosarcomas, *Mol. Cell. Biol.* **10,** 5772–5781 (1990).
21. Fujiwara, T., Grimm, E. A., Mukhopadhyay, T., *et al.* A retroviral wild-type p53 expression vector penetrates human lung cancer spheroids and inhibits growth by inducing apoptosis, *Cancer Res.* **53,** 4129–4133 (1993).
22. El-Deiry, W. S., Harper, J. W., O'Connor, P. M., *et al.* WAF1/CIP1 is induced in p53-mediated G1 arrest and apoptosis, *Cancer Res.* **54,** 1159–1174 (1994).
23. Couch, R. B., Chanock, R. M., Cate, T. R., *et al.* Immunization with types 4 and 7 adenovirus by selective infection of the intestinal tract, *Am. Rev. Respir. Dis.* **88,** 394–403 (1963).
24. Rosenfeld, M. A., Siegfried, W., and Yoshimura, K. Development and analysis of recombinant adenoviruses for gene therapy of cystic fibrosis, *Hum. Gene Ther.* **252,** 431–434 (1991).
25. Ragot, T., Vincent, N., Chafey, P., *et al.* Efficient adenovirus-mediated transfer of a human minidystrophin gene to skeletal muscle of mdx mice, *Nature* **361,** 647–650 (1993).
26. Herz, J., and Gerald, R. D. Adenovirus-mediated transfer of low density lipoprotein receptor gene acutely accelerates cholesterol clearance in normal mice, *Proc. Natl. Acad. Sci. USA* **90,** 2812–2816 (1993).
27. Le Gal La Salle, G., Robert, J. J., Bernard, S., *et al.* An adenovirus vector for gene transfer into neurons and glia in the brain, *Science* **259,** 988–990 (1993).
28. Jaffe, H. A., Danel, C., Longenecker, G., *et al.* Adenovirus-mediated in vivo gene transfer and expression in normal rat liver, *Nat. Genet.* **1,** 372–378 (1992).
29. Engelhardt, J. F., Simon, R., Yang, Y., *et al.* Adenovirus-mediated transfer of the CFTR gene to lung of nonhuman primates: Biological efficacy study, *Hum. Gene Ther.* **4,** 759–769 (1993).
30. Dai, Y., Schwarz, E. M., Gu, D., Zhang, W. W., Sarvetnick, N., and Verma, I. M. Cellular and humoral immune responses to adenoviral vectors containing factor IX gene: Tolerization of factor IX and vector antigens allows for long-term expression, *Proc. Natl. Acad. Sci. USA* **92,** 1401–1405 (1995).
31. Engelhardt, J. F., Xuehai, Y., Doranz, B., and Wilson, J. M. Ablation of E2A in recombinant adenoviruses improves transgene persistence and decreases inflammatory response in mouse liver, *Proc. Natl. Acad. Sci. USA* **91,** 6196–6200 (1994).