

Endogenous *p53* gene status predicts the response of human squamous cell carcinomas to wild-type *p53*

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Prior reports suggest that *p53* protein status may influence the response to gene transduction with wild-type (wt) *p53*. Adenoviral vectors containing the *p53* gene were administered to normal keratinocytes, to squamous cell carcinoma (SCC) lines with varied *p53* protein status (absent, mutant, wt, or degraded by papillomavirus), as well as to tumors formed in severe combined immunodeficient mice. The percentage of cells undergoing apoptosis, G₁ growth arrest, WAF1/p21 induction, and *in vivo* tumor progression were studied after wt *p53* gene transduction. Apoptosis developed first in normal keratinocytes, next in SCCs lacking *p53* protein, and last in SCCs with mutant or degraded *p53* protein. All of the cell lines studied demonstrated an increase in WAF1/p21 protein, but only those lacking *p53* protein showed G₁ arrest. Tumors lacking *p53* protein were more susceptible to *p53* overexpression than those containing mutant or degraded *p53* protein. The endogenous *p53* protein status of SCCs appears to influence the outcome of *p53* gene transduction. **Cancer Gene Therapy (2000) 7, 749–756**

Key words: *p53*; squamous cell carcinoma; growth arrest; apoptosis; WAF1; WAF1; p21.

One of the most frequently mutated genes in human cancer is *p53*.¹ *p53* point mutations can lead to either overexpression of mutant *p53* protein or a non-functional protein, while deletion frequently results in a truncated, nonfunctional protein or in no *p53* protein at all. Mutated *p53* can also act in a dominant negative fashion to suppress wild-type (wt) protein function.² The human papilloma virus (HPV) E6 protein targets *p53* for degradation *via* the ubiquitin-dependent protease system.³ Under normal conditions, wt *p53* protein prevents tumor formation and produces growth suppression when introduced into cancer cells,^{4–8} whereas mutant *p53* protein does not. Gene transduction using adenoviral vectors leads to efficient delivery of DNA.^{9–13} The virus grows to high titers and is easy to manipulate. Wt *p53* gene transduction has been used to treat ovarian,⁹ prostate,¹⁰ lung,¹¹ and cervical cancer,¹² as well as squamous cell carcinoma (SCC) of the head and neck (SCCHN)⁷ and glioblastoma.¹³

Considerable evidence suggests that SCCHN and cervical SCCs develop, at least in part, due to the absence of wt *p53* protein, either as a result of gene

mutation or protein inactivation. There is a high frequency of *p53* mutations in SCCHN^{14,15} and a high incidence of HPV infection leading to *p53* protein inactivation in cervical SCCs.^{16,17} The E6 protein of oncogenic HPVs, including types 16, 18, and 31, has been shown to bind to wt *p53*, shortening its half-life and inactivating its function.^{18,19}

Several studies have evaluated SCCHN to determine whether there is an association between *p53* gene or protein function and tumor stage or nodal status.^{2,3,20} Although it is clear that alterations in both the *p53* gene and its protein product are early events, before the development of invasive cancer, most reports do not indicate an association between *p53* gene or protein status and disease progression once the tumor has become invasive.

However, most studies^{21,22} of cervical SCC have demonstrated that patients with HPV-positive tumors have a better prognosis than those who are HPV-negative but have a *p53* mutation. The importance of loss of wt *p53* protein function is demonstrated by the fact that it is rare to find a cervical SCC that does not have either HPV, in which wt *p53* protein has been inactivated by the HPV E6 protein, or a *p53* mutation, whereby the mutant protein acts in a dominant negative fashion to inhibit wt *p53* protein function.

Two reports have suggested that treatment of cervical SCC¹³ and SCCHN²³ with wt *p53* induces apoptosis and slows their growth. However, neither report took a

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Table 1. Cell Lines Used and Their P53 Gene Status, Protein Expression, and HPV Status

Cell lines	p53 gene status	p53 protein*	HPV status
NKs	Wt	Normal level	Negative
SCC9	Mutant (32-bp deletion; codon 274-285)	Absent	Negative
SCC40	Mutant (deletion)	Absent	Negative
A253	Mutant (deletion)	Absent	Negative
1986LN	Wt	Low	Positive
HeLa	Wt	Low	Positive
SiHa	Wt	Low	Positive
HaCaT	Mutant (codon 174)	Overexpressed	Negative
SCC13	Mutant (codon 179)	Overexpressed	Negative
SCC4	Mutant (codon 151)	Overexpressed	Negative
Det 562	Mutant (codon 248)	Overexpressed	Negative
FaDu	Mutant (codon 258)	Overexpressed	Negative
A431	Mutant (codon 273)	Overexpressed	Negative

*As detected by immunoblotting.

comprehensive look at the relationship between *p53* status and the response to treatment of SCCs with wt *p53*. Our hypothesis was that the presence of either HPV or mutant *p53* would inhibit the response of SCC cells to wt *p53* overexpression. We present the results of the evaluation of a panel of normal and malignant cells, representing a variety of *p53* statuses, for their response to overexpression of wt *p53* introduced *via* an adenoviral vector.

MATERIALS AND METHODS

Cell lines

A total of 11 SCC lines, including 7 SCCHN cell lines (SCC9, SCC4, SCC40, A253, Det 562, FaDu, and 1986LN), 1 facial skin cell line (SCC13), 1 vulvar skin cell line (A431), 2 cervical SCC cell lines (HeLa and SiHa), and one spontaneously transformed but nontumorigenic keratinocyte cell line (HaCaT) were used in this study (Table 1). They were maintained in Dulbecco's modified Eagle's medium (DMEM)/F12 with 10% fetal bovine sera (FBS) (1986LN), in DMEM with 10% FBS (SCC40, A253, Det 562, SCC13, A431, HeLa, SiHa, and HaCaT), or in DMEM/F12 with 10% FBS and 0.4 $\mu\text{g}/\text{mL}$ hydrocortisone (SCC9 and SCC4). The SCC40 line was kindly provided by Dr. James Rheinwald (Brigham and Women's Hospital, Boston, Mass). The 1986LN line was established at the University of Texas MD Anderson Cancer Center (Houston, Tex). The remaining lines were obtained from the American Type Culture Collection (Manassas, Va). The SCC40, A253, Det 562, SCC13, A431, HaCaT, HeLa, SiHa and SCC9 lines were weaned off sera and grown in MCDB 201/L15 (Sigma, St. Louis, Mo) in a 4:1 ratio supplemented with 5 $\mu\text{g}/\text{mL}$ insulin (SCC medium) as noted in the figure legends.

Normal foreskin keratinocytes

The methods described by Southgate et al,²⁴ with minor modifications, were used to grow normal keratinocytes (NKs) in culture. After the removal of excess dermis with fine scissors and mincing of the epithelium, the cell suspension was plated in keratinocyte growth medium (Life Technologies, Gaithersburg, Md). Growth medium was changed twice weekly. At

70–80% confluence, the medium was removed; the cells were incubated in calcium and magnesium free phosphate-buffered saline (PBS) containing 5 mM ethylenediaminetetraacetic acid and 0.25% trypsin for 2 minutes, or until epithelial cells detached. The cells were split 1:2 and replated.

Viral vectors

Two *p53* adenoviral vectors were used in this study. In a series of studies, the effects of each virus were compared. The first vector, p53FAd, is an E1-deleted adenovirus²⁵ that contains the sequence of the FLAG octapeptide (IBI/Eastman Kodak, New Haven, Conn) at the 3' end of the *p53* gene. The FLAG epitope provides a convenient way to identify exogenous *p53*. This vector produces a response similar to the parent *p53* adenovirus without the FLAG epitope.²⁵ Adenovirus dl312, which also lacks the E1 region but does not contain the p53FLAG gene, was used as a control vector for the p53FAd experiments.

The second vector, p53/Ad5, which is also an E1-deleted adenovirus containing wt *p53*,²⁶ was a gift from Dr. Wafik El-Deiry (University of Pennsylvania, Philadelphia, Penn). The LZ/Ad5 control vector for p53/Ad5 is an E1/E3-deleted Ad5 containing the β -galactosidase gene (University of Pennsylvania Vector Core, Philadelphia, Penn).

All SCC cells were infected at a multiplicity of infection (MOI) of 100, whereas NKs were infected at MOIs of both 10 and 100. The MOI of 100 was based on results obtained from infection of the various cell lines with LZ/Ad5 and determination of the percentage of infected cells by staining with 5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside. An MOI of 100 resulted in 60–100% of SCC cells exhibiting positive staining.^{7,13} MOIs of 10 and 100 resulted in 80 and 100% of keratinocytes exhibiting positive staining, respectively.

Growth studies

Cells were seeded at $\sim 5 \times 10^3$ cells/well in triplicate in 96-well dishes. Cells were infected with p53FAd or dl312 at an MOI of 100 or mock-infected. The MTS assay (Promega, Madison, Wis) was done at 0, 1, 2, 3, 4, 5, 7, and 10 days postinfection to measure viable cells. The plates were analyzed on a Titertek Multiscan Plate reader (Fisher, Pittsburgh, Penn) at a wavelength of 492 nm. The three absorbance readings were averaged for triplicate wells at each timepoint.

Immunoprecipitation and immunoblotting

For detection of transduced *p53*, total protein was extracted from the cells at various intervals after transduction. A total of 100 μg from each sample was subjected to immunoprecipitation using antibody (Ab) to *p53* (p53 Ab-2, Oncogene Research Products, Cambridge, Mass) or Ab to the FLAG peptide (M2 Ab, IBI/Kodak), enabling discrimination between exogenous and endogenous *p53* expression. The immunoprecipitated protein was then subjected to electrophoresis in a 10% denaturing polyacrylamide gel and subsequently transferred to a nitrocellulose membrane. The membranes were blocked and probed with primary and secondary Abs (primary Ab, p53 Ab-7, Oncogene Research Products; secondary Ab, biotinylated goat anti-sheep immunoglobulin G, Oncogene Research Products), and bound Abs were detected colorimetrically using an avidin-biotin-peroxidase complex (Vectastain ABC Reagent, Vector Laboratories, Burlingame, Calif).

For detection of WAF1/p21, 20 μg of total protein from each sample was loaded onto a 15% denaturing polyacryl-

Table 2. Onset and Maximum Expression of Exogenous P53 Protein After Infection with P53FAd

	Onset of p53 expression	Time of maximum p53 expression	Onset of change in cell growth*	Time of maximum change in cell growth
SCC9	4 hours	24 hours	24 hours	5 days
SCC4	12 hours	36 hours	48 hours	3 days
1986LN	12 hours	≥36 hours	72 hours	5 days

*Either decline in cell number (SCC9 and SCC4) or lack of cell growth (1986LN) after infection with p53FAd.

amide gel, subjected to electrophoresis, and transferred to a nitrocellulose membrane. Filters were probed with an anti-WAF1/p21 (WAF1/CIP1/SDI1) monoclonal Ab (mAb) (Ab-1; Oncogene Science, Cambridge, Mass), and binding was detected on Hyperfilm by enhanced chemiluminescence (Amersham Life Science, Arlington Heights, Ill). The membranes were then stripped and reprobed with an anti-actin mAb (clone AC-40; Sigma). The films were scanned on a densitometer to obtain a ratio of WAF1/p21 protein to total loaded protein.

Flow cytometry and cell cycle analysis

All cells were examined using both terminal deoxynucleotidyl transferase (TdT)²⁷ and propidium iodide (PI)-based assays to evaluate cell cycle alterations and apoptosis, with the exception of the SCC4, 1986LN, HeLa, and SiHa cell lines, which were only analyzed using PI. If the PI and TdT results were discordant, the higher apoptotic fraction result was used. After removal of the culture medium and release of the attached cells with ethylenediaminetetraacetic acid/trypsin, 1×10^6 cells were pelleted, the supernatant was removed, and the cells were fixed with 1% paraformaldehyde in PBS (pH 7.4) for 10 minutes on ice. After removal of the paraformaldehyde, the cells were resuspended in 80% ice-cold ethanol and refrigerated overnight. The cells were then washed with 1 mL of 1% bovine serum albumin and a 50- μ L reaction mixture consisting of 1/10 $5\times$ terminal transferase buffer, 1/25 cobalt chloride, 1/100 fluorescein-biotin-16-deoxyuridine triphosphate, 1/37.5 TdT enzyme (all from Boehringer Mannheim Biochemicals, Indianapolis, Ind), and distilled water was added. After a 1-hour incubation at 37°C, the cells were resuspended in 100 μ L of staining buffer containing 2.5 μ g/mL fluorescein-labeled avidin, 4 \times saline sodium citrate buffer (0.6 M NaCl, 0.06 M sodium citrate), 0.1% Triton X-100, and 5% (wt/vol) nonfat dry milk and left for 30 minutes in the dark. After removal of the supernatant, the cells were resuspended in 100 μ L of 1% bovine serum albumin with 0.1% Triton X-100. The PI studies on the cell lines were a continuation of the TdT assay. A total of 500 μ L of a solution containing 200 μ g/mL PI and 5 μ g/mL ribonuclease was added, followed by a 20-minute incubation in the dark and analysis using an EPICS XL Flow Cytometer (Coulter, Hialeah, Fla).

For PI studies of SCC9, SCC4, and 1986LN cells, cells were harvested at various timepoints postinfection, fixed in 70% ice-cold ethanol, and washed twice with PBS; next, 500 μ L of PI working solution (50 μ g/mL PI and 20 μ g/mL ribonuclease in PBS) was added. The cells were incubated at room temperature before analysis on an EPICS Profile Analyzer (Coulter). Experiments were performed twice and an average was taken.

Tumor formation in severe combined immunodeficient (SCID) mice

Four cell lines (SiHa, HeLa, A253, and SCC13) were grown in SCC medium to 70–80% confluence. Cells were detached with

0.06% trypsin and counted, and 10^7 cells were injected subcutaneously in the dorsum of SCID mice just medial to the right hind limb. A total of 50 mice (10 for each line) received injections. After tumors had grown to a minimum volume of 100 mm³, 5×10^8 plaque-forming units (PFU) of LZ/Ad5 (five mice for each tumor type) or p53/Ad5 (five mice for each tumor type) in 50 μ L of SCC medium were injected into each tumor using a tuberculin syringe.

To determine whether a dose response was present, 1×10^9 PFU of LZ/Ad5 or p53/Ad5 was administered to 10 mice (5 mice per group) containing a tumor of minimum volume of 100 mm³ formed from SCC13 cells. The response was compared with treatment of SCC13 tumors treated with 5×10^8 PFU virus.

For statistical analysis, each mouse was observed at baseline and 4, 7, 10, 14, and 17 days later. Tumor volumes at these six times were fitted to the following exponential curve: $y = A \exp(\beta t)$, where β is the tumor regression rate estimated for each mouse from the observations. β values in each group were rank ordered and analyzed by the Wilcoxon two-sample (one-sided) procedure to test the hypothesis of equal rates of tumor regression among groups versus the alternative that treated tumors regress faster.

RESULTS

p53 expression and cell growth

A cell line from each category of p53 protein status (SCC9, p53 deletion with no p53 protein expression; SCC4, p53 point mutation with overexpression of mutant p53; 1986LN, wt p53, HPV-positive) was infected with p53FAd to document the time course of wt p53 expression and to determine the effect of wt p53 on cell growth. For each of the three cell lines transduced with p53FAd, maximum p53 expression was soon followed by a decline in cell number or a slowing of cell growth compared with mock- or control-treated cells (Table 2). The cells with mutant p53 protein as a result of point mutation and the cells infected with HPV were delayed in expression of the transduced p53 compared with the cells with no endogenous p53. The effects on cell growth were delayed as well. The growth suppression persisted while the levels of wt p53 remained high.

WAF1/p21 expression and G₁ arrest

WAF1/p21 is induced by wt but not by mutant p53 protein and has been shown to suppress cell growth in a wide variety of tumor types.²⁶ WAF1/p21 expression in SCC9, SCC4, and 1986LN cells was evaluated at various timepoints after p53 transduction. Increased WAF1/p21 expression was first noted at 2 hours posttransduction in

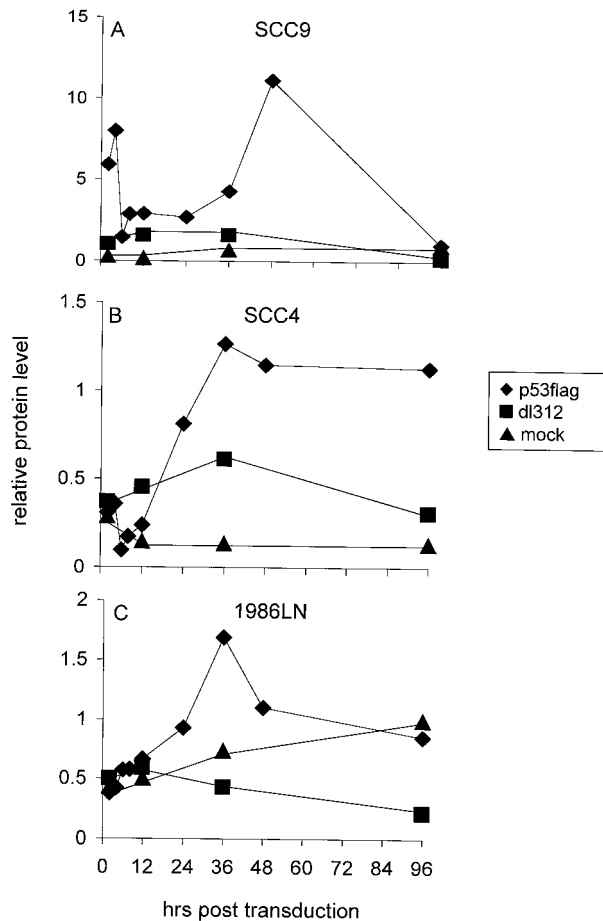


Figure 1. Relative WAF1/p21 protein expression in *p53*-transduced SCC lines (SCC9 (A), SCC4 (B), and 1986LN (C)) compared with control virus and mock transduction. WAF1/p21 protein was detected using 20 μ g of total protein per lane, probed with an anti-p21(WAF1/CIP1/SDI1) mAb. The ratio of loaded protein to WAF1/p21 protein was determined on a densitometer.

SCC9 cells, compared with 12 and 24 hours for SCC4 and 1986LN cells, respectively (Fig 1). Peak levels were reached 36–48 hours after *p53* transduction into the SCCs, and returned to control levels at 48 and 96 hours posttransduction (1986LN and SCC9), or levels remained above control through 96 hours (SCC4).

A higher fraction of SCC9 cells were in G_1 arrest at 24, 48, and 96 hours after *p53*FAd transduction (55–65%) compared with dl312-transduced (38–46%) and mock-transduced (38–50%). No differences in the percentage of cells in G_1 after *p53*FAd transduction were observed in the SCC4 and 1986LN cells (Fig 2).

In vitro apoptosis

One of the mechanisms by which overexpression of wt *p53* suppresses cell growth is through the induction of programmed cell death (apoptosis). Our hypothesis was that the status of *p53* protein (wt, as in NKs, degraded, after HPV infection, deleted, or mutated) was an important factor in predicting response to overexpression of wt

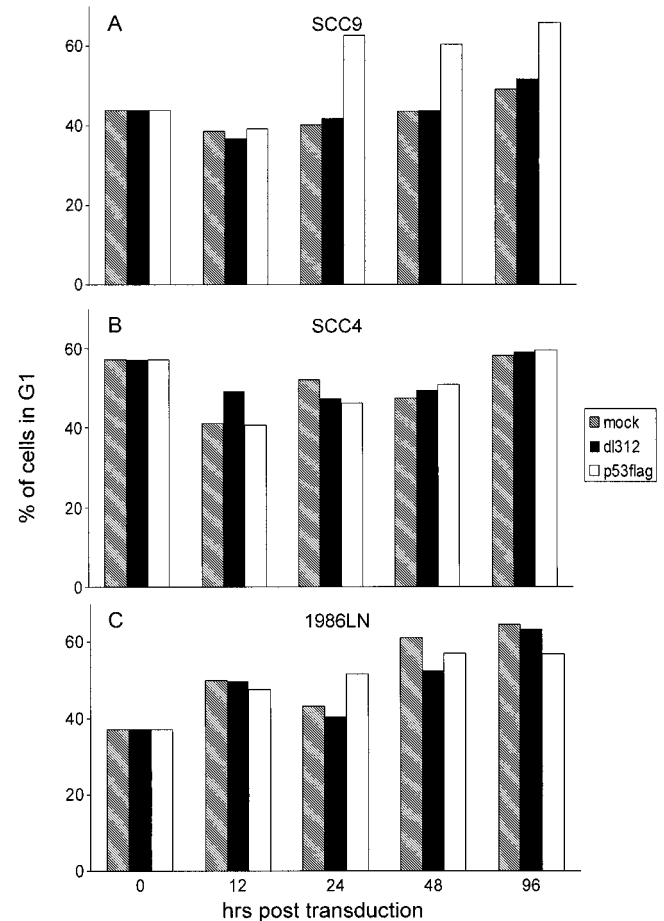


Figure 2. The percentage of cells in G_1 arrest after transduction with 100 PFU of *p53* adenovirus. Cells were harvested at various timepoints postinfection, fixed in ethanol, and washed with PBS; next, PI was added and cells were incubated at room temperature before analysis on a flow cytometer.

p53. Figure 3 illustrates the percentage of cells undergoing apoptosis at various timepoints after *p53* transduction using an MOI of 100. In Figure 3A, cell lines expressing wt *p53* protein are shown. Each of the SCC lines (SiHa, 1986LN, and HeLa) contains HPV, whereas NKs do not. Over 90% of the normal cells underwent apoptosis at 24 hours posttransduction, with the cells gradually undergoing necrosis thereafter. *p53* transduction at an MOI of 10 of NKs led to apoptosis in 56% of cells by 24 hours and in >90% of cells by 48 hours (data not shown). However, overexpression of wt *p53* did not result in a significant apoptotic response ($\geq 30\%$ of cells in apoptosis) until 72 and 96 hours posttransduction in SiHa and HeLa cells, respectively. Through 96 hours, a significant apoptotic response was not observed in 1986LN cells.

Figure 3B illustrates cell lines that have deletions in the *p53* gene and do not express wt *p53*. At 48 hours after gene transduction, two of three (SCC9 and SCC40) cell lines developed a significant apoptotic response; by

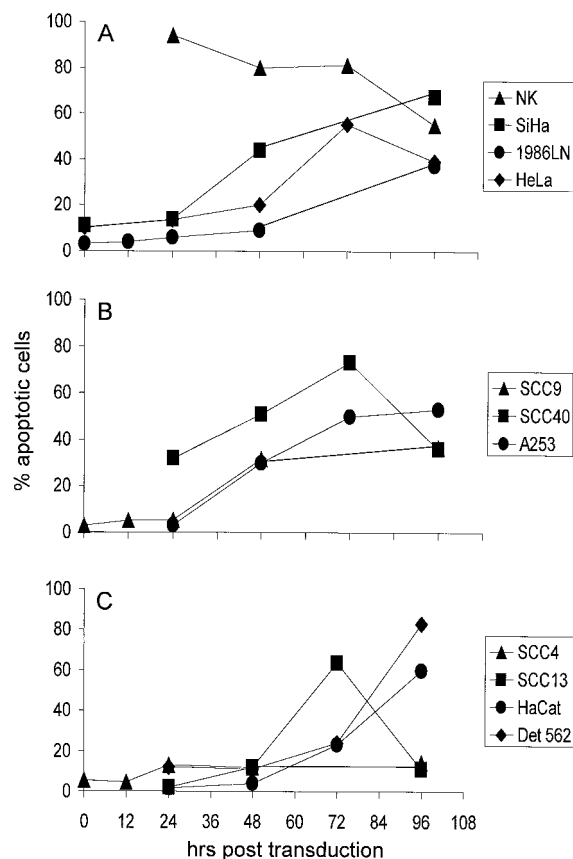


Figure 3. Percentage of cells undergoing apoptosis. All cells were examined using both TdT²⁷ and PI-based assays to evaluate cell cycle alterations and apoptosis, with the exception of the SCC4, 1986LN, HeLa, and SiHa cell lines, which were only analyzed using PI. If the PI and TdT results were discordant, the higher apoptotic fraction result was used. **A:** NKs containing wt *p53*. **B:** Cells containing a *p53* deletion resulting in no *p53* protein expression. **C:** Cells containing a *p53* point mutation resulting in overexpression of mutant *p53* protein.

72 hours posttransduction, the third line (A253) showed significant apoptosis.

Figure 3C shows cell lines that express mutant *p53* protein. None of the four cell lines (SCC4, SCC13, HaCaT, and Det 562) had a significant apoptotic response by 48 hours after *p53* transduction. Only one (SCC13) of these four had a significant response by 72 hours. Taken together, these data suggest that *p53* protein status is predictive of the rate at which cells undergo apoptosis. NKs undergo apoptosis first (wt *p53*, no HPV), followed by lines lacking *p53* protein due to *p53* gene deletion. Lines with mutant *p53* protein and those with a decreased level of wt *p53* due to HPV infection were most resistant to wt *p53*-induced apoptosis. For the LZ/Ad5-transduced and dl312-transduced cells, the percentage of apoptotic cells was always less than in the cells transduced with *p53*.

Tumor formation in SCID mice

Preformed tumors were transduced with *p53*/Ad5 or LZ/Ad5 or were mock-transduced (Fig 4). SCC13 tumors were treated with two different viral concentrations, 5×10^8 and 1×10^9 PFU. With the standard dose of 5×10^8 PFU, SCC13 tumors, which contain a *p53* point mutation and mutant *p53* protein, initially shrank; however, by 14 days postinfection these tumors were larger than controls ($P = .27$). With a doubling of the viral dose, SCC13 tumors shrank significantly by day 7 and remained smaller than baseline throughout the study period ($P = .004$ compared with LZ/Ad5; $P = .0079$ compared with low-dose *p53*/Ad5) (Fig 4, A and B). *p53*/Ad5 did not significantly alter the growth of SiHa tumors ($P = .075$) and HeLa tumors ($P = .16$), both of which contain the HPV E6 protein and therefore inactivated wt *p53* protein (Fig 4, C and D). Treatment of A253 tumors, which contain a *p53* deletion and therefore no *p53* protein, with *p53*/Ad5 significantly inhibited tumor growth ($P = .0040$) compared with LZ/Ad5 treatment (Fig 4E).

DISCUSSION

p53 is the most commonly mutated gene in human cancer.¹ As such, it is potentially an ideal target for cancer gene therapy. When designing gene therapy, it is generally easier to increase the production of a given protein than it is to suppress it. Unfortunately, an increase in the production of most proteins leads to tumor growth. However, because *p53* is a tumor suppressor gene, more of the protein is desirable when one is attempting to suppress growth. Transduction of wt *p53* has already been evaluated in tissue culture and/or in animal studies in a wide variety of tumor types.^{7,9-13}

Overexpression of wt *p53* in SCCHN via *p53* transduction induces apoptosis.²³ Additional preclinical studies^{13,26} evaluating the effect of *p53* gene transduction on SCCHN suggested that tumors expressing mutant *p53* protein or lacking *p53* might more readily undergo apoptosis after *p53* transduction than SCC cells with wt *p53*. To evaluate the effect of wt *p53* on normal tissue, the gene was transduced into normal human fibroblasts.²⁸ Although apoptosis was not seen, NKs, arguably a better control, were not evaluated. In a subsequent phase I clinical trial in which an adenoviral vector containing wt *p53* was used to treat advanced SCCHN, patients with *p53* overexpression fared worse.²⁹ Higher levels of *p53* expression are generally seen with mutant rather than with wt *p53* protein, because the mutant protein has a longer half-life.¹ A total of 6 of 11 (55%) subjects whose tumors expressed *p53* had disease progression compared with only 2 of 6 (33%) subjects lacking *p53*. Mutation analyses confirmed the protein findings. A total of 60% (3 of 5) of subjects harboring tumors with a *p53* mutation had disease progression compared with only 41% (5 of 12) of subjects whose tumor contained a normal *p53* gene.

These clinical findings, in which patients with *p53*



overexpression, most often due to a *p53* mutation resulting in mutant p53 protein, fared worse than subjects with wt p53 protein, appear to be at odds with the preclinical findings indicating that tumors with mutant p53 protein more readily underwent apoptosis than those containing wt p53 protein. To help clarify this apparent discrepancy, we transduced wt *p53* into SCCs with mutant p53 protein, in SCCs with wt p53 protein plus HPV, or into SCCs lacking p53 protein, as well as into NKs possessing wt p53. NKs were analyzed to determine the toxicity of viral transduction and because it is very difficult to find a SCC with a normal level of wt p53 protein.

Our results confirm what was observed in the phase I trial. After *p53* transduction, apoptosis developed first in NKs, next in SCCs lacking p53, and last in SCCs with mutant p53 protein or wt p53 protein with HPV. This is not surprising, given that that mutant p53 protein can function in a dominant negative fashion, inhibiting wt p53-induced apoptosis.^{30,31} HPV E6 protein can inhibit transduced p53, negating its potential effect. Overexpression of wt p53 protein after gene transduction is not inhibited in cell lines containing a *p53* gene deletion, in which there is no p53 protein, either wt or mutant, or in NKs. As a result, there is an earlier onset of apoptosis.

p53 protein status was also predictive of the ability of *p53* transduction to induce WAF1/p21 expression, G₁ arrest, and tumor shrinkage. SCC9, which expresses no endogenous p53, showed the greatest induction of WAF1/p21; SCC4, which contains mutant p53 protein, and 1986LN, which contains HPV, showed lower levels of WAF1/p21 induction. G₁ growth arrest was observed in the SCC9 cells. The increases in WAF1/p21 expression did not correlate with G₁ arrest in the SCC4 and 1986LN cells. This result is consistent with the results obtained by Liu et al²³ regarding other SCCHN cell lines. Increases in WAF1/p21 expression are likely the result of transcriptional activation of the WAF1/p21 gene by the transduced wt p53, and not the result of DNA damage-mediated G₁ growth arrest.

Tumors formed from SCC13 cells, which contain a mutated *p53* gene (and overexpression of mutant p53 protein), only transiently responded to *p53* viral transduction unless a high dose was administered (1×10^9 PFU). At the higher dose, significant tumor shrinkage was observed. Tumors containing HPV (HeLa and SiHa) showed little, if any, response to the treatment. In contrast, A253, which has a *p53* deletion and lacks p53 protein, displayed steady tumor growth in the controls but no growth in the *p53*-treated tumors.

This study provides a possible explanation for some of the failures associated with *p53* treatment of tumors and is supported by data from studies using lung cancer cells. Transfection of the homozygously deleted, p53 protein-negative human lung cancer cell line H1299 with an adenovirus/DNA complex carrying a plasmid expressing the *p53* gene resulted in high levels of p53 protein and induction of apoptosis. Injection of the complex carrying the *p53* gene into subcutaneous tumor sites 5 days after tumor cell implantation resulted in a significant inhibition of tumorigenicity as measured by the number and

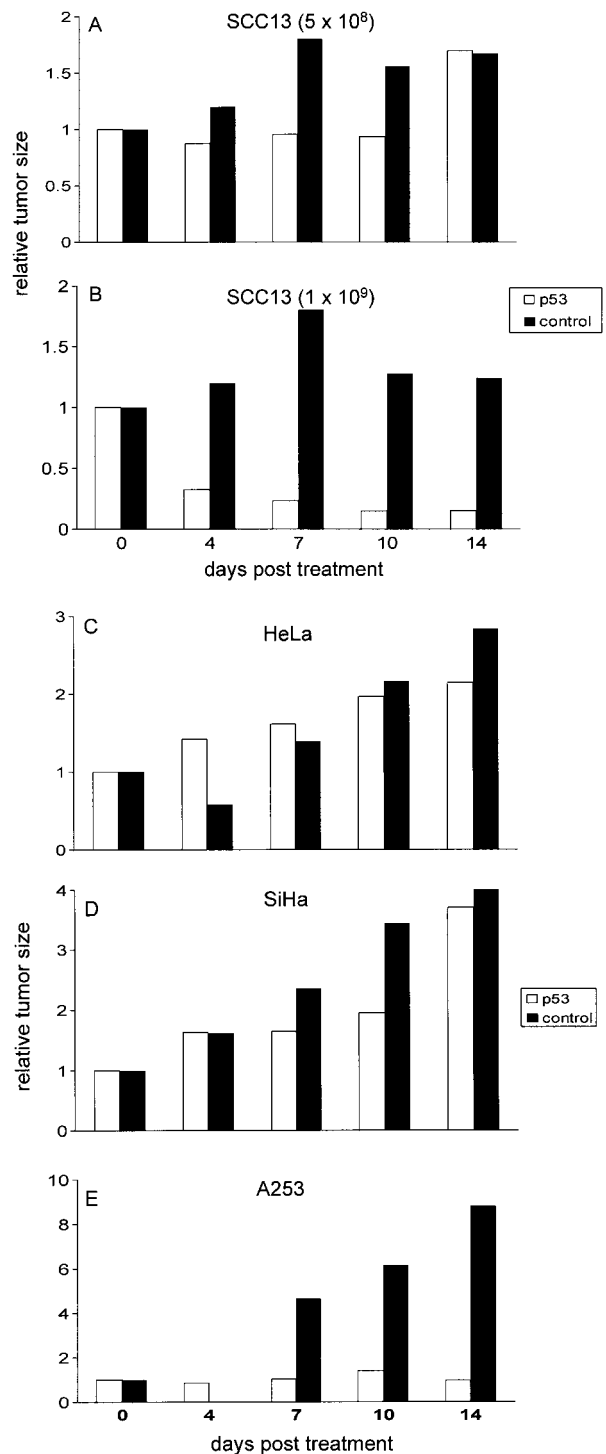


Figure 4. Relative tumor growth in SCID mice after intratumoral injection of p53 adenovirus or control (LZ/Ad5) adenovirus. A total of 50 tumors formed from four cell lines (SCC13 (A); SCC13, double dose p53 (B); HeLa (C); SiHa (D); and A253 (E)) were grown to a minimum volume of 100 mm³; next, 5×10^8 PFU of LZ/Ad5 (five mice for each tumor type) or p53/Ad5 (five mice for each tumor type) in 50 μ L of SCC medium were injected within each tumor using a tuberculin syringe. Tumor volume was then measured (length \times width \times height) at the indicated timepoints. For SCC13, 1×10^9 LZ/Ad5 or p53/Ad5 was administered to a separate group of mice.

size of tumors.³² Transfer of the wt *p53* gene into cisplatin-treated H1299 cells resulted in up to a 60% further inhibition of cell proliferation *in vitro* than did *p53* transfer into untreated H1299 cells.³³ The cisplatin plus *p53* gene transfer strategy yielded significantly greater apoptosis and tumor growth suppression in an animal model of subcutaneous H1299 tumor nodules than did wt *p53* gene transfer alone. This finding of a favorable response to *p53* gene transduction in a cell line lacking *p53* supports our *in vitro* and *in vivo* findings.

This study also demonstrates that NKs are susceptible to the effects of the *p53* adenovirus, undergoing apoptosis after treatment. This is in contrast to the response of normal fibroblasts.²⁸ Thus, it is possible that nonmalignant squamous epithelium could be affected by *p53* adenovirus treatment.

This study investigated the response of SCC cells to *p53* adenovirus treatment. The response was influenced by the *p53* status and/or HPV infection of the cells. These are important considerations when establishing a treatment protocol that includes wt *p53* gene therapy.

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