Cancer Chemother Pharmacol (1999) 44: 143–151

ORIGINAL ARTICLE

Maya Gurnani · Philip Lipari · Janet Dell · Bin Shi
Loretta L. Nielsen

Adenovirus-mediated p53 gene therapy has greater efficacy when combined with chemotherapy against human head and neck, ovarian, prostate, and breast cancer

Received: 5 October 1998 / Accepted: 3 December 1998

Abstract *Purpose*: Adenovirus-mediated p53 gene therapy for cancer is currently undergoing phase I/II clinical trials. The drug used in our clinical trials (p53 Ad; ACN53; SCH58500) consists of a replication-deficient, type 5 adenovirus vector expressing human wildtype p53 tumor suppressor under the control of the cytomegalovirus promoter. In preclinical models, p53 Ad has therapeutic efficacy against a wide range of human tumor types containing nonfunctional p53, both in vitro and in vivo. Results from early clinical trials using p53 gene therapy by itself support optimism for the future of this therapeutic approach. However, it is likely that many phase II/III trials will incorporate an arm comparing traditional chemotherapy against chemotherapy combined with p53 gene therapy. Therefore, it is important to study possible interactions between p53 Ad and chemotherapeutic drugs in preclinical models before starting the clinical trials. *Methods*: Proliferation of tumor cells was quantitated after incubation with various combinations of p53 Ad and chemotherapeutic drugs. Human tumor xenografts in scid mice were dosed with intraperitoneal or intratumoral p53 Ad with or without chemotherapeutic drugs and the tumor burden after therapy monitored. *Results*: p53 Ad combined with cisplatin, doxorubicin, 5-fluorouracil, methotrexate, or etoposide inhibited cell proliferation more effectively than chemotherapy alone in SCC-9 head and neck, SCC-15 head and neck, SCC-25 head and neck, SK-OV-3 ovarian, DU-145 prostate, MDA-MB-468 breast, and MDA-MB-231 breast tumor cells. No obvious dependence on dosing schedule was observed. Greater anticancer efficacy was also demonstrated in four human tumor xenograft models in vivo. Of particular significance, there was enhanced efficacy using the three drug combination of p53 Ad, cisplatin, and paclitaxel in an ovarian cancer model. *Conclusion*: These results support the combination of p53 gene therapy with chemotherapy in clinical trials.

Key words p53 gene therapy · p53 adenovirus · Cisplatin · Paclitaxel · Doxorubicin · 5-Fluorouracil · Methotrexate · Etoposide

M. Gurnani · P. Lipari · J. Dell · Bin Shi · L.L. Nielsen (✉)
Tumor Biology Department, K15-4945
Schering-Plough Research Institute,
2015 Galloping Hill Rd., Kenilworth,
NJ 07033-0539, USA
Tel. +1-908-740-7335; Fax +1-908-740-7115

Introduction

Adenovirus-mediated p53 gene therapy for cancer is currently undergoing phase I/II clinical trials in several countries. The drug used in our clinical trials (p53 Ad; ACN53; SCH58500) consists of a replication-deficient, type 5 adenovirus vector expressing human wildtype p53 tumor suppressor under the control of the cytomegalovirus promoter [39]. In preclinical models, p53 Ad has been shown to have therapeutic efficacy against a wide range of human tumor types containing nonfunctional p53, both in vitro and in vivo [13, 23–26, 39]. Introduction of wildtype p53 into tumors with null or mutant p53 offers a novel strategy for suppressing tumors by inducing apoptotic death in neoplastic cells. Results from early clinical trials using p53 gene therapy by itself support optimism for the future of this therapeutic approach. However, it is likely that many phase II/III trials will incorporate an arm comparing traditional chemotherapy against chemotherapy combined with p53 gene therapy. Therefore, it is important to study the possible interactions between p53 Ad and chemotherapeutic drugs in preclinical models before starting clinical trials.

p53 is a DNA binding protein which acts as a transcription factor to control the expression of proteins involved in the cell cycle [30, 34]. In response to DNA damage, p53 protein accumulates in the cell nucleus causing cells to undergo cell cycle arrest and DNA repair or apoptosis (programmed cell death) [36]. Functional inactivation of p53 can occur by several mechanisms including direct genetic mutation, binding to viral oncoproteins or cellular factors (e.g. mdm2), or

alteration of the subcellular localization of the protein [30, 34]. Although p53 is not essential for normal development, p53 'knock-out' mice are susceptible to tumors early in life [7]. Mutations in p53 have been reported in a majority of clinical cancers, and it has been estimated that p53 function is altered in half of all human malignancies [30, 34]. In general, cancers containing nonfunctional p53 tumor suppressor protein are less sensitive to chemotherapy [18]. Many anticancer agents induce apoptosis via p53-dependent (cisplatin, doxorubicin, 5-fluorouracil) or p53-independent (paclitaxel) pathways [6, 18, 38]. In theory, the introduction of wildtype p53 into cells with nonfunctional p53 protein should enhance their sensitivity to most chemotherapeutic drugs. The reverse situation, where chemotherapy sensitizes tumor cells to p53-induced apoptosis is also quite probable.

Cisplatin, doxorubicin, and etoposide are anticancer drugs that interact with cellular DNA to disrupt DNA synthesis [19]. Cisplatin alkylates genomic DNA, while doxorubicin and etoposide inhibit topoisomerase II activity. 5-Fluorouracil and methotrexate accomplish the same results by disrupting cell metabolism. 5-Fluorouracil is a pyrimidine antagonist, while methotrexate acts as a folate antagonist. Paclitaxel (Taxol) interacts with cellular microtubules to inhibit mitosis by a mechanism quite distinct from that of most chemotherapeutic drugs. Paclitaxel inhibits cell replication by enhancing polymerization of tubulin monomers into stabilized microtubule bundles that are unable to reorganize into the proper structures for mitosis [14, 33]. This results in cell cycle blockage and subsequent activation of an apoptotic pathway which seems to be p53-independent [6, 38].

When considering the possible interactions between a chemotherapy drug and p53 Ad, it is tempting to assume that the drug is interacting only with wildtype p53 protein expressed from the introduced transgene. However, the second drug in this mix is an adenovirus which contains a p53 transgene construct within its genome, not naked p53 DNA. Therefore, the interactions between chemotherapy drugs and p53 Ad must be considered in this much more complicated context. We have demonstrated synergistic interactions between paclitaxel and p53 Ad [25]. These effects are mediated, at least in part, by the unexpected ability of low nanomolar concentrations of paclitaxel to help adenoviruses transduce tumor cells in vitro. In other words, more tumor cells are infected with p53 Ad and exposed to high levels of wildtype p53 protein when paclitaxel "sensitizes" them to transduction by recombinant adenovirus. Other "adenovirus-specific" effects have been documented in vivo. For example, although the nature and extent of the immune response to recombinant adenovirus has not been fully characterized in humans, preclinical models suggest that cytotoxic T lymphocyte and natural killer cell responses lead to elimination of adenovirus-transduced cells and may enhance the antitumor efficacy of p53 Ad in vivo [27, 42].

Materials and methods

Cell lines and adenovirus infections in vitro

All cell lines were obtained from ATCC (Rockville, Md.). SCC-9, SCC-15, and SCC-25 head and neck tumor cells ($p53^{null}$) were cultured in a 1:1 mixture of DMEM and Ham's F-12 (GIBCO/Life Technologies, Grand Island, N.Y.) with 10% fetal calf serum (FCS; Hyclone, Logan, Utah), 0.4 μg/ml hydrocortisone (Sigma Chemical Co., St. Louis, Mo.), and 1% nonessential amino acids (GIBCO) at 37 °C in an atmosphere containing 5% CO_2. SK-OV-3 human ovarian tumor cells ($p53^{null}$) and DU-145 human prostate tumor cells ($p53^{mut}$) were cultured in Eagle's MEM plus 10% FCS at 37 °C in an atmosphere containing 5% CO_2. MDA-MB-231 human mammary tumor cells ($p53^{mut}$) were cultured in DMEM (GIBCO) with 10% FCS at 37 °C in an atmosphere containing 5% CO_2. MDA-MB-468 human mammary tumor cells ($p53^{mut}$) were cultured in Leibovitz's L-15 medium (GIBCO) containing 10% FCS at 37 °C in an atmosphere with no CO_2.

MDA-MB-231 mammary tumor cells carry an Arg-to-Lys mutation in codon 280 of the p53 gene and express mutant p53 [1]. DU-145 prostate tumor cells carry two mutations on different chromosomes, a Pro-to-Leu mutation in codon 223 and a Val-to-Phe mutation in codon 274 [16]. They express mutant p53. SK-OV-3 ovarian tumor cells are p53-null [40]. SCC-9 cells have a deletion between codons 274 and 285 resulting in a frame shift mutation [17]. No immunoreactive p53 protein is detectable in SCC-9 nuclei [4, 17, 20]. SCC-15 cells have an insertion of five base pairs between codons 224 and 225. They produce low levels of p53 mRNA, but no detectable p53 protein [20]. SCC-25 cells have loss of heterozygosity (LOH) at chromosome 17 and a two base pair deletion in codon 209 on the remaining allele [4]. p53 mRNA is not detectable in SCC-25 cells and no immunoreactive p53 protein is observed in their nuclei [4].

Construction and propagation of human wildtype p53 and *E. coli* β-galactosidase adenoviruses (β-gal Ad) have been described previously [39]. The concentration of infectious viral particles was determined by measuring the concentration of viral hexon protein - positive 293 cells after a 48-h infection period [15]. Cellular infectious units (CIU) have been defined previously [22]. Adenoviruses were administered in phosphate buffer (20 m*M* NaH_2PO_4, pH 8.0, 130 m*M* NaCl, 2 m*M* $MgCl_2$, 2% sucrose). For in vitro studies with p53 Ad, cells were plated at a density of 1.5×10^4 cells/well on a 96-well plate and cultured for 4–5 h at 37 °C in an atmosphere containing 5% CO_2. Drug, p53 Ad, or the appropriate vehicle was added to each well and cell culture was continued overnight. Then drug, p53 Ad, or the appropriate vehicle was added to each well. Cell culture was continued for an additional 2 days. Cell proliferation was measured using the MTT assay [21]. Briefly, 25 μl of 5 mg/ml MTT vital dye [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] was added to each well and incubation was continued for 3–4 h at 37 °C in an atmosphere containing 5% CO_2. Then, 100 μl of 10% SDS detergent was added to each well and the incubation was continued overnight. Fluorescence in each well was quantitated using a Molecular Devices microtiter plate reader (n = 3–12 wells per group). Cisplatin, doxorubicin, methotrexate, 5-fluorouracil, and etoposide were purchased from Sigma Chemical Co. (St. Loius, Mo.). Paclitaxel was purchased from CalBiochem (La Jolla, Calif.).

Adenovirus treatment in vivo

C.B.17/ICR-scid mice were purchased from Taconic Farms (Germantown, N.Y.) or Charles River Laboratories (Wilmington, Mass.). Athymic *nu*/*nu* mice were purchased from Charles River Laboratories. All mice were maintained in a virus-antigen free-barrier facility and all animal procedures were performed in accordance with the rules set forth in the NIH Guide for the Care and Use of Laboratory Animals.

SK-OV-3 ovarian tumor model

Cisplatin efficacy Female scid mice were injected with 5×10^6 SK-OV-3 ovarian tumor cells intraperitoneally (IP) on day 0. Mice were dosed with both drugs, IP, on days 6, 8, 10, 13, and 15, followed by p53 Ad alone on day 17. Mice received 0.2 ml total volume (0.1 ml cisplatin vehicle or cisplatin plus 0.1 ml Ad buffer or p53 Ad). The p53 Ad dose was 2.5×10^8 CIU/mouse per day (5.2×10^9 viral particles). The cisplatin dose was 2 mg/kg per day. Tumors were harvested and weighed on day 20.

Cisplatin/paclitaxel efficacy Female scid mice were injected with 2.5×10^6 SK-OV-3 ovarian tumor cells, IP, on day 0. Mice were dosed IP on days 7, 9, 11, 16, and 18. Mice received 0.3 ml total volume (0.1 ml cisplatin vehicle or cisplatin plus 0.1 ml paclitaxel vehicle or paclitaxel plus 0.1 ml Ad buffer or p53 Ad). The p53 Ad dose was 2.5×10^8 CIU/mouse per day (5.2×10^9 viral particles). The cisplatin dose was 0.5 mg/kg per day. The paclitaxel dose was 1 mg/kg per day. Tumors were harvested and weighed on day 30 (seven to ten mice per group).

DU-145 prostate tumor model

Male scid mice were injected with 2.5×10^6 DU-145 cells, IP, on day 0. Mice were dosed IP on days 7, 9, 11, 14, and 16. Mice received 0.2 ml total volume (0.1 ml cisplatin vehicle or cisplatin plus 0.1 ml Ad buffer or p53 Ad). The p53 Ad dose was 8.3×10^8 CIU/mouse per day (2.9×10^{10} viral particles). The cisplatin dose was 1 mg/kg per day. Tumors were harvested and weighed on day 22.

MDA-MB-468 mammary tumor model

Cisplatin efficacy Female scid mice were injected with 1×10^7 MDA-MB-468 cells into the mammary fat pad 11 days before the start of dosing on day 0. The IP cisplatin dose was 1 mg/kg per day. The intratumoral p53 Ad dose was 8.3×10^8 CIU/mouse per day (2.9×10^{10} viral particles). Drugs were dosed on days 0–4.

Doxorubicin efficacy Female nude mice were injected with 1×10^7 MDA-MB-468 cells subcutaneously 12 days prior to the start of dosing on day 0. The intraperitoneal doxorubicin dose was 4 mg/kg per day on days 0, 2, 7, and 9. The intratumoral p53 Ad dose was 5×10^8 CIU/mouse per day (1.03×10^{10} viral particles) on days 0–4 and 7–11.

SCC-15 head and neck tumor model

Scid mice were injected with 5×10^6 SCC-15 cells subcutaneously 7 days prior to the start of dosing on day 0. The 5-fluorouracil dose was 50 mg/kg per day in 40% hydroxypropyl-β-cyclodextran (Cerestar, Hammond, Ind.) given IP on days 0, 7, and 14. The p53 Ad dose was 2×10^8 CIU/mouse per day (4×10^9 viral particles), on days 0, 1, 7, 8, 14, and 15.

Tumor volumes for different treatment groups on each day were compared using Student's *t*-test using Statview II software (Abacus Concepts, Berkeley, Calif.). Tumor growth curves show mean tumor volume ± SEM There were ten mice per group unless otherwise indicated.

Results

Combination therapy in vitro

The antiproliferative efficacy of recombinant adenoviruses was first evaluated in combination with cisplatin or doxorubicin using $p53^{null}$ SK-OV-3 ovarian carcinoma cells as a model system. As shown in Fig. 1, β-gal Ad had no intrinsic antiproliferative activity, even when combined with DNA-damaging drugs ($P \geq 0.05$). By contrast, p53 Ad had enhanced efficacy in combination with cisplatin or doxorubicin ($P \leq 0.001$), even at concentrations where p53 Ad had no activity by itself.

The antiproliferative effects of p53 Ad and cisplatin were next evaluated in a panel of cell lines expressing mutant or no p53 protein. Three treatment regimens were studied: (1) cisplatin 24 h before p53 Ad, (2) p53 Ad 24 h before cisplatin, and (3) simultaneous drug administration. Figure 2 shows representative data, while Table 1 summarizes all results. Under all three treatment regimens, p53 Ad had enhanced efficacy when combined with cisplatin. The antiproliferative effects of p53 Ad and doxorubicin were also evaluated in a panel of tumor cell lines in vitro. Figure 3 shows representative data, while Table 2 summarizes the results from all experiments. Once again, p53 Ad had enhanced efficacy when combined with chemotherapy, with no obvious schedule dependence. Similarly, the combination of 5-fluorouracil and p53 Ad had enhanced efficacy over either drug alone. Figure 4 shows representative data for the SCC-15 squamous cell carcinoma line. The same type of results were obtained using the DU-145 or MDA-MB-231 cell lines ($P \leq 0.0001$).

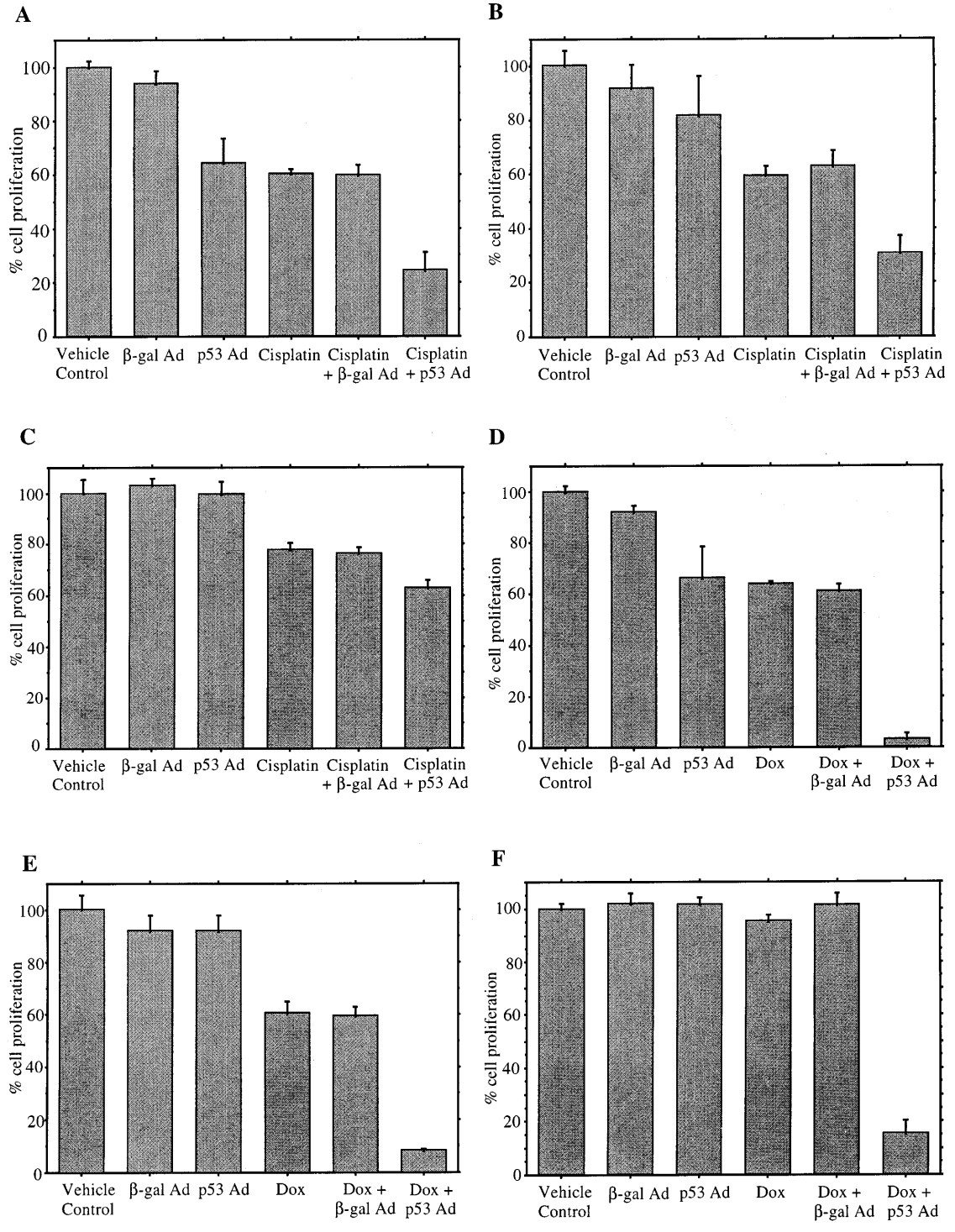
The combination of methotrexate and p53 Ad was tested in one cell line. When SCC-15 cells were treated with 0.7 μM methotrexate 24 h before 5 m.o.i. p53 Ad (ciu/cell), the combined antiproliferative effect of the two drugs was only 5% more than with p53 Ad alone, although this difference was statistically significant ($P \leq 0.003$). Pretreatment of DU-145 cells with 2.6 μM etoposide 24 h before 5 or 10 m.o.i. p53 Ad also resulted in greater combined efficacy over either drug alone ($P \leq 0.0001$). When SCC-15 cells were treated with 0.3 μM etoposide 24 h before 5 m.o.i. p53 Ad, the combined antiproliferative effect of the two drugs was only 5% more than with p53 Ad alone, although this difference was statistically significant ($P \leq 0.003$).

Combination therapy in vivo

It has been well-documented that p53 Ad is a drug with antitumor efficacy attributable to both the p53 tumor suppressor gene and the adenovirus delivery vector [23]. The in vivo pharmacology experiments were designed to mimic the clinical situation in which efficacy of the adenovirus drug (with or without chemotherapy) will be compared to clinical outcome with traditional chemotherapy.

SK-OV-3 ovarian tumor model

Established intraperitoneal SK-OV-3 tumors were treated with IP doses of vehicles, p53 Ad, cisplatin, or



both drugs. Mice were given six injections of p53 Ad over a period of 2 weeks. The total virus dose was 1.5×10^9 CIU (3.1×10^{10} viral particles). Mice in one treatment group received five doses of cisplatin simultaneously with the first five p53 Ad doses. As shown in Fig. 5, this dose of IP p53 Ad reduced mouse tumor burden by only 17% by day 20 ($P \leq 0.01$). However, when combined with cisplatin, p53 Ad caused a 38% decrease in tumor burden as compared to cisplatin alone ($P \leq 0.0008$). Mice treated with drug vehicles or with p53 Ad alone had bloody ascites and invasive tumor nodules in the diaphragm muscle. These symptoms were absent in the mice treated with cisplatin alone or cisplatin with p53 Ad.

In a second study, SK-OV-3 ovarian tumors were treated with IP doses of vehicles, p53 Ad, cisplatin plus paclitaxel, or all three drugs simultaneously. The combination of all three drugs reduced tumor burden 34%

◀

Fig. 1A–F Antiproliferative effects of recombinant adenoviruses against SK-OV-3 ovarian carcinoma cells in combination with cisplatin or doxorubicin in vitro. All drugs were added on day 0 and cell proliferation was quantitated on day 3. **A** Drug concentrations were 200 m.o.i. p53 Ad (5714 PN/cell), 5714 PN/cell β-gal Ad, and 7 μ*M* cisplatin. **B** Drug concentrations were 100 ciu/cell p53 Ad (2857 PN/cell), 2857 PN/cell β-gal Ad, and 7 μ*M* cisplatin. **C** Drug concentrations were 25 m.o.i. p53 Ad (714 PN/cell), 714 PN/cell β-gal Ad, and 7 μ*M* cisplatin. **D** Drug concentrations were 200 m.o.i. p53 Ad (5714 PN/cell), 5714 PN/cell β-gal Ad, and 1.2 μ*M* doxorubicin. **E** Drug concentrations were 100 m.o.i. p53 Ad (2857 viral particles/cell), 2857 PN/cell β-gal Ad, and 1.2 μ*M* doxorubicin. **F** Drug concentrations were 25 m.o.i. p53 Ad (714 PN/cell), 714 PN/cell β-gal Ad, and 0.8 μ*M* doxorubicin

more than the combination of cisplatin plus paclitaxel, demonstrating the enhanced efficacy of the three-drug combination (Fig. 6; $P \leq 0.0006$).

DU-145 prostate tumor model

IP DU-145 tumors were treated with IP doses of vehicles, p53 Ad, cisplatin, or both drugs. As shown in Fig. 7, the combination of p53 Ad and cisplatin had greatly enhanced antitumor efficacy compared to either drug alone, even though the adminstered dose of cisplatin had little efficacy by itself ($P \leq 0.0004$).

MDA-MB-468 mammary tumor model

Established MDA-MB-468 tumors were treated with vehicles, p53 Ad, cisplatin, or both drugs. The cisplatin dose was 1 mg/kg per day, while the p53 Ad dose was 8.3×10^8 CIU/mouse per day (2.9×10^{10} viral particles) given simultaneously on days 0–4. As shown in Fig. 8, p53 Ad had enhanced efficacy when combined with cisplatin (days 8–31, $P \leq 0.0004$).

In a second experiment, MDA-MB-468 tumors were treated with vehicles, p53 Ad, doxorubicin, or both drugs. The doxorubicin dose was 4 mg/kg per day given on days 0, 2, 7, and 9. The p53 Ad dose was 5×10^8 CIU/mouse per day (1.03×10^{10} viral particles) given on days 0–4 and 7–11. As shown in Fig. 9, p53 Ad had greater efficacy when administered in combination with doxorubicin (days 14–24, $P \leq 0.05$).

SCC-15 head and neck tumor model

Subcutaneous SCC-15 tumors were treated with vehicles, p53 Ad, 5-fluorouracil, or both drugs. The p53 Ad dose was 2×10^8 CIU/mouse per day (4×10^9 viral particles) given in six intratumoral injections over a period of 3 weeks. The intraperitoneal 5-fluorouracil dose of 50 mg/kg was given once a week for 3 weeks. The combination of p53 Ad and 5-fluorouracil resulted

Fig. 2A, B Antiproliferative effects of p53 Ad in combination with cisplatin in vitro. **A** SCC-9 head and neck carcinoma cells. Doses were 17, 17, and 17 μ*M* cisplatin with 5, 5, and 2.5 m.o.i. p53 Ad, respectively. **B** SCC-25 head and neck carcinoma cells. Doses were 3, 3, and 3 μ*M* cisplatin with 5, 2.5, and 2.5 m.o.i. p53 Ad, respectively (m.o.i. = CIU/cell). Values are means ± SD

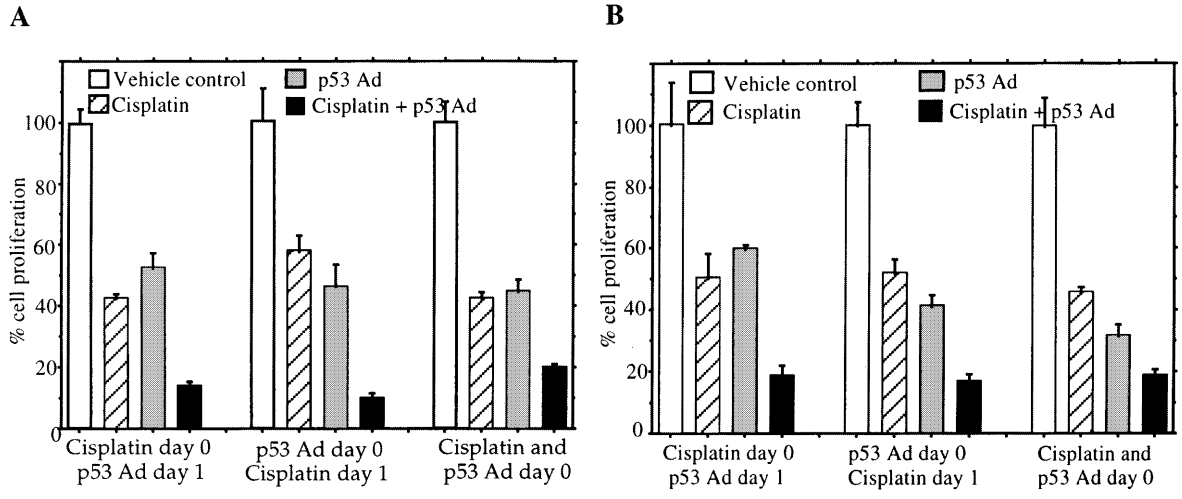


Table 1 Antiproliferative effects of p53 Ad in combination with cisplatin (*ND* not done)

Cell line	p53 protein	Tissue type	Greater combined efficacy?		
			Cisplatin first	p53 Ad first	Simultaneous
SK-OV-3	Null	Ovarian	Yes ($P \leq 0.0001$)	Yes ($P \leq 0.0001$)	Yes ($P \leq 0.0001$)
SCC-9	Null	Head & neck	Yes ($P \leq 0.0001$)	Yes ($P \leq 0.0001$)	Yes ($P \leq 0.0001$)
SCC-15	Null	Head & neck	Yes ($P \leq 0.0001$)	ND	ND
SCC-25	Null	Head & neck	Yes ($P \leq 0.0001$)	Yes ($P \leq 0.0001$)	Yes ($P \leq 0.0001$)
MDA-MB-468	Mutant	Breast	Yes ($P \leq 0.0001$)	ND	Yes ($P \leq 0.0001$)
MDA-MB-231	Mutant	Breast	Yes ($P \leq 0.0001$)	Yes ($P \leq 0.0001$)	Yes ($P \leq 0.0001$)

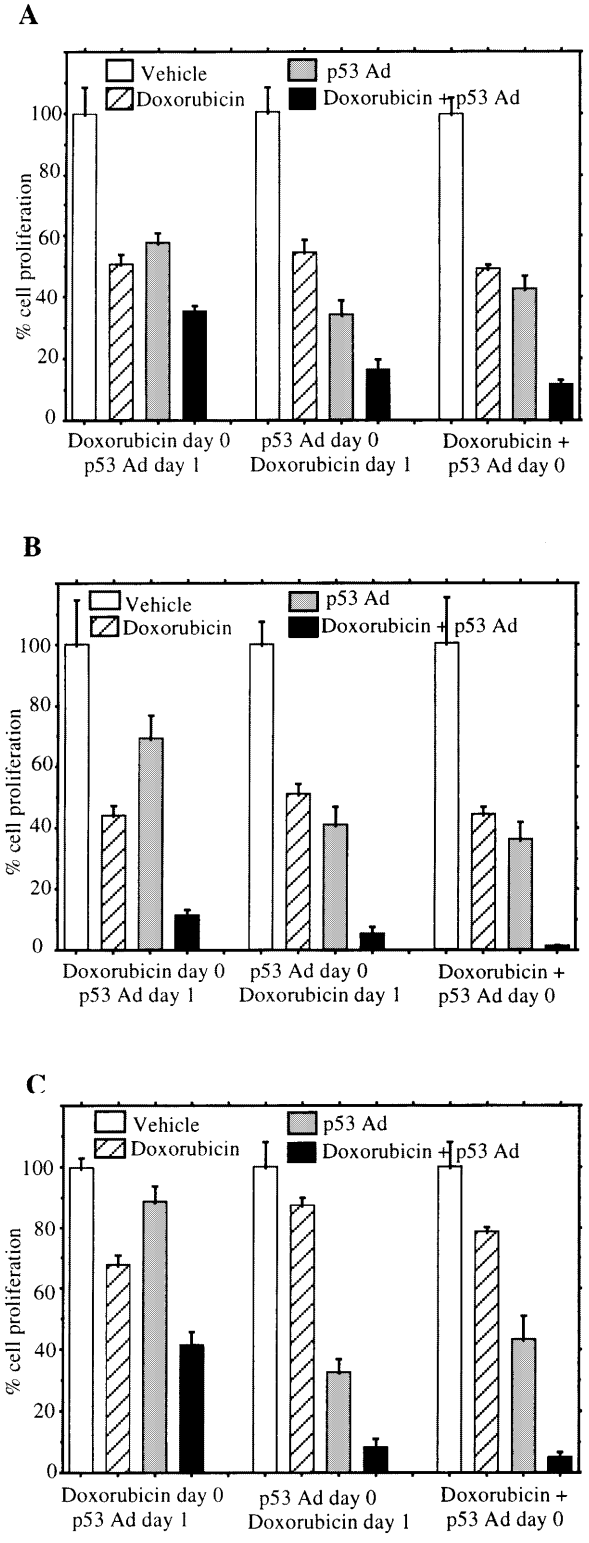


Fig. 3A–C Antiproliferative effects of p53 Ad in combination with doxorubicin in vitro. **A** SCC-9 head and neck carcinoma cells. Doses were 0.3, 0.3, and 0.3 μ*M* doxorubicin with 5, 5, and 2.5 m.o.i. p53 Ad, respectively. **B** SCC-25 head and neck carcinoma cells. Doses were 0.9, 2, and 0.9 μ*M* doxorubicin with 5, 2.5, and 2.5 ciu/cell p53 Ad, respectively. **C** DU-145 head and neck carcinoma cells. Doses were 95, 95, and 95 n*M* doxorubicin with 10, 5, and 5 m.o.i. p53 Ad, respectively. Values are means ± SD

in greater antitumor activity than when either drug was used alone ($P \leq 0.04$; data not shown).

Discussion

In the experiments reported here, treatment of tumor cells in vitro with p53 Ad combined with cisplatin, doxorubicin, 5-fluorouracil, methotrexate, or etoposide was more effective at killing tumor cells than chemotherapy alone. This result was independent of the type of p53 gene mutation in the cells, and cells expressing mutant p53 protein were indistinguisable from $p53^{null}$ cells in regard to response. No obvious dependence on dosing schedule was observed between p53 Ad and chemotherapy in vitro. In other words, it did not matter whether cells were pretreated with p53 Ad 1 day before chemotherapy or pretreated with chemotherapy 1 day before p53 Ad or treated with both drugs simultaneously. A control adenovirus expressing β-galactosidase had no intrinsic antiproliferative effects in vitro, even when combined with chemotherapy. However, it would be premature to conclude that p53 sensitized the tumor cells to chemotherapy based on these results, since the reverse situation, chemotherapy sensitizing tumor cells to the adenoviral p53 drug, would give identical results.

Cisplatin has a broad spectrum of clinical activity against ovarian, breast, head and neck, lung, prostate, and gastric cancers. Clinical trials have demonstrated enhanced efficacy when cisplatin and paclitaxel are combined in patients with advanced solid tumors (ovarian, non-small-cell lung, breast) or melanomas [9]. Sophisticated statistical analyses have been used to prove synergy between p53 Ad and paclitaxel in antiproliferative tumor cell assays in vitro [25]. Vasey et al. [37] have previously shown that while the sensitivity of the A2780 ovarian cell line to cisplatin is reduced by inactivation of p53, there is no effect on paclitaxel sensitivity, suggesting that paclitaxel induces cell death via a p53-independent mechanism. This apparent contradiction between the findings of Nielsen et al. [25] and those of Vasey et al. [37] can be explained, at least in part, by the observation that paclitaxel increases the efficiency of tumor cell transduction by recombinant adenovirus.

It has been well-documented that p53 Ad is a drug with antitumor efficacy attributable to both the p53 tumor suppressor gene and the adenovirus delivery vector [23]. The in vivo pharmacology experiments were designed to mimic the clinical situation in which efficacy of the p53 Ad drug (with or without chemotherapy) will be compared to clinical outcome with traditional chemotherapy. In the clinical setting, the artificial dichotomy of an "adenovirus-effect" and a "p53-effect" is irrelevant. It is unethical and prohibitively expensive to have a clinical study arm where patients receive empty vector. Given this paradigm, the preclinical models clearly demonstrate greater anticancer efficacy when p53 Ad and paclitaxel are combined in the SK-OV-3 ovarian,

Table 2 Antiproliferative effects of p53 Ad in combination with doxorubicin

Cell line	p53 protein	Tissue type	Greater combined efficacy?		
			Dox first	p53 Ad first	Simultaneous
SK-OV-3	Null	Ovarian	Yes ($P \leq 0.0001$)	Yes ($P \leq 0.0001$)	Yes ($P \leq 0.0001$)
SCC-9	Null	Head & neck	Yes ($P \leq 0.0001$)	Yes ($P \leq 0.0001$)	Yes ($P \leq 0.0001$)
SCC-15	Null	Head & neck	Yes ($P \leq 0.0001$)	Yes ($P \leq 0.0001$)	Yes ($P \leq 0.0001$)
SCC-25	Null	Head & neck	Yes ($P \leq 0.0001$)	Yes ($P \leq 0.0001$)	Yes ($P \leq 0.0001$)
DU-145	Mutant	Prostate	Yes ($P \leq 0.0001$)	Yes ($P \leq 0.0001$)	Yes ($P \leq 0.0001$)
MB-231	Mutant	Breast	Yes ($P \leq 0.0001$)	Yes ($P \leq 0.0001$)	Yes ($P \leq 0.0001$)

DU-145 prostate, MDA-MB-468 breast, and MDA-MB-231 breast tumor xenograft models [25]. In the study reported here, we further demonstrated that the combination of p53 Ad and cisplatin also had greater efficacy in the same four models. For ovarian cancer, the combination of cisplatin with paclitaxel has become the standard regimen for first-line therapy in the United States [31]. This makes our observation of greater combined efficacy for the three-drug combination of p53 Ad, paclitaxel, and cisplatin in the SK-OV-3 ovarian model especially relevant to ongoing clinical trials.

A limited number of studies by other investigators into the efficacy of p53 gene therapy in combination with DNA-damaging agents have been reported over the last few years [2, 3, 5, 8, 10–12, 28, 29, 32, 35, 41]. In total, 13 tumor cell lines with nonfunctional p53 have been examined from head and neck, lung, brain, breast, colorectal, ovarian, and prostate lesions. Greater efficacy in combination with p53 gene expression has been reported for cisplatin, 5-fluorouracil, topotecan, doxorubicin, etoposide, actinomycin D, mitomycin C, CPT11, or γ-irradiation. Significantly, no observations of antagonistic interactions between p53 gene therapy and more traditional anticancer therapeutic agents have been reported. Only one other study has been reported on the combination of p53 Ad and paclitaxel [2]. The investigators observed enhanced efficacy in vitro at lower concentrations of paclitaxel which they could not explain.

The conclusion from published studies is that p53 gene therapy combined with DNA-damaging agents has additional efficacy over p53 gene therapy alone. However, in all cases the data were generated by treating cells in vitro or after intratumoral administration of p53 Ad. The study reported here is the first to examine the efficacy of IP administration of p53 Ad and DNA-damaging agents, where there is the possibility of physical interactions between the particulate virus and the che-

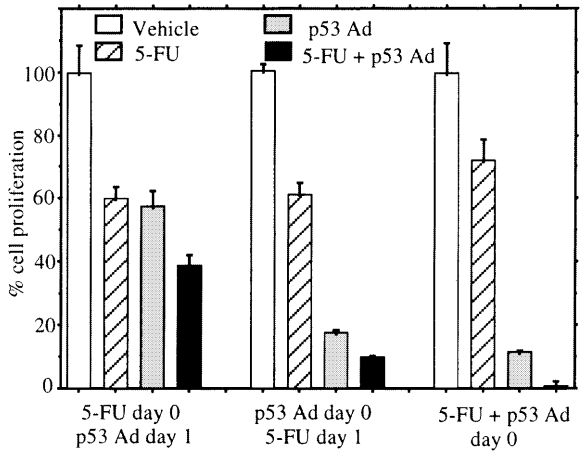


Fig. 4 Antiproliferative effects of p53 Ad combined with 5-fluorouracil (5-FU) against SCC-15 head and neck carcinoma cells in vitro. Doses were 2 μM 5-FU with 5 ciu/cell p53 Ad. Values are means ± SD

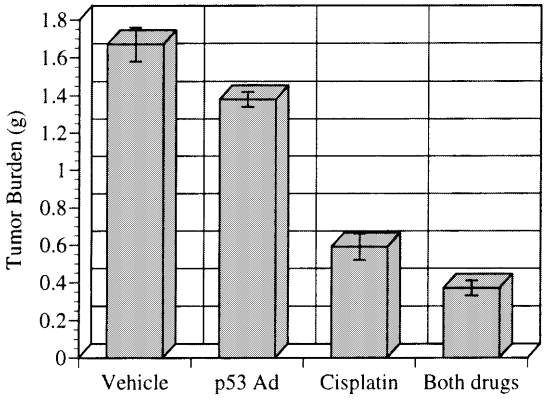


Fig. 5 Efficacy of p53 Ad in combination with cisplatin against SK-OV-3 ovarian carcinoma xenografts in scid mice (n = 9 or 10 mice per group). Values are means ± SEM

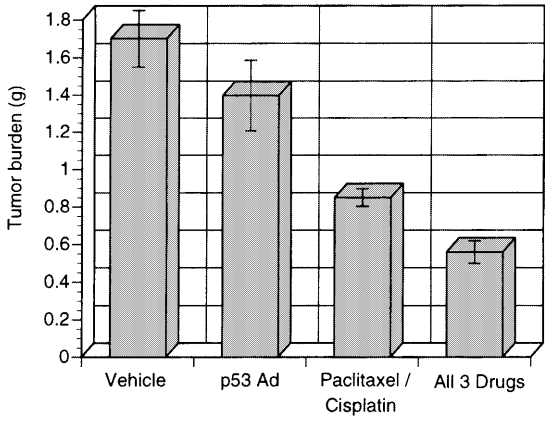


Fig. 6 Efficacy of p53 Ad in combination with cisplatin/paclitaxel against SK-OV-3 ovarian carcinoma xenografts in female scid mice. Values are means ± SEM

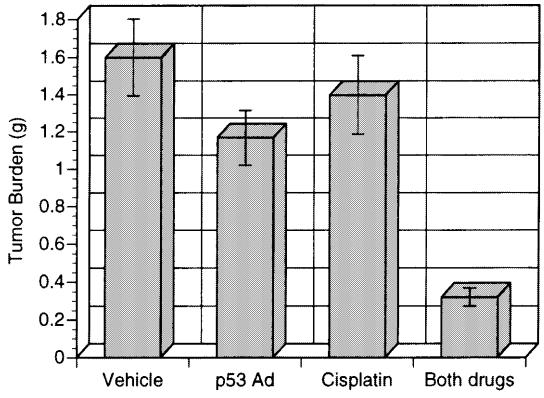


Fig. 7 Efficacy of p53 Ad in combination with cisplatin against DU-145 prostate tumor xenografts in male scid mice. Values are means ± SEM

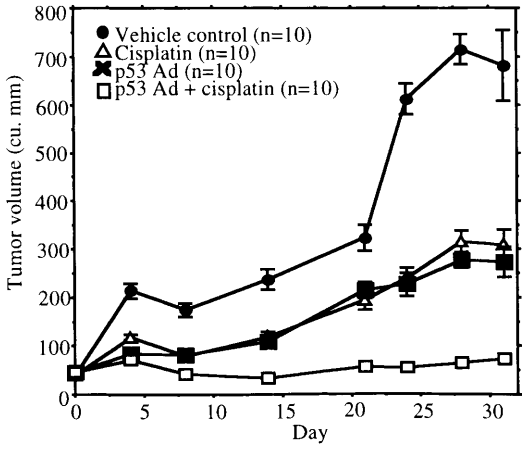


Fig. 8 Efficacy of p53 Ad in combination with cisplatin against MDA-MB-468 mammary tumor xenografts in female scid mice. Values are means ± SEM

motherapeutic agent. We are also the first to show greater efficacy for the three-drug combination of p53 Ad, a platinum DNA-damaging agent, and a microtubule stabilizer against IP ovarian cancer. Taken together, the preclinical data on the combination of p53 gene therapy with DNA-damaging or microtubule-stabilizing agents support the evaluation of these combinations in clinical trials.

Adenovirus-mediated liver toxicity has been reported in mice, when virus is administered intravenously or IP [26]. By contrast, hepatic toxicity has never been observed when adenovirus is administered intratumorally. The adenovirus and chemotherapy drugs used in the in vivo studies reported here were purposely kept below the maximum tolerated dose for each drug, therefore no significant hepatic toxicity was observed. In ongoing phase I clinical trials, various chemotherapy drugs are being tested in combination with p53 Ad by the IP and intrahepatic artery routes of administration. The preliminary results of these clinical trials indicate no significant hepatic toxicity in humans at the maximum achievable

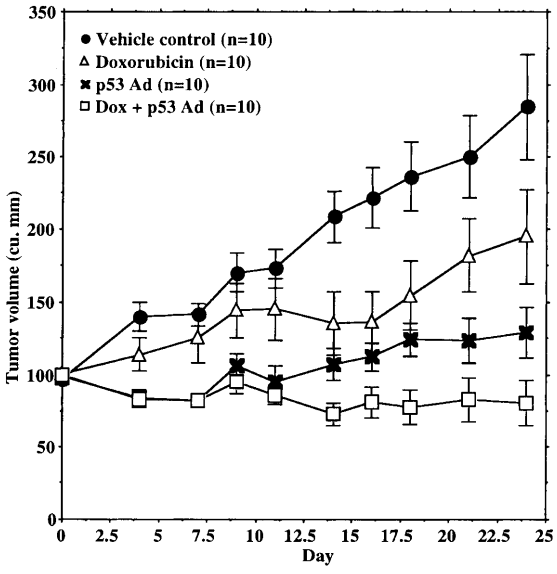


Fig. 9 Efficacy of p53 Ad in combination with doxorubicin against MDA-MB-468 xenografts in nude mice. Values are means ± SEM

doses of p53 Ad (limited by the maximum volume patients can tolerate and the maximum virus concentration that can be manufactured without viral aggregation).

References

1. Bartek J, Iggo R, Gannon J, Lane DP (1990) Genetic and immunochemical analysis of mutant p53 in human breast cancer cell lines. Oncogene 5: 893
2. Blagosklonny M, El-Diery WS (1996) In vitro evaluation of a p53-expressing adenovirus as an anti-cancer drug. Int J Cancer 67: 386
3. Blagosklonny MV, El-Diery WS (1998) Acute overexpression of WT p53 facilitates anticancer drug-induced death of cancer and normal cells. Int J Cancer 75: 933
4. Caamano J, Zhang SY, Rosvold EA, Bauer B, Klein-Szanto AJP (1993) p53 alterations in human squamous cell carcinomas and carcinoma cell lines. Am J Pathol 142: 1131
5. Chang EH, Jang YJ, Hao Z, Murphy G, Rait A, Fee WE, Sussman HH, Ryan P, Chiang Y, Pirollo KF (1997) Restoration of the G1 checkpoint and the apoptotic pathway mediated by wild-type p53 sensitizes squamous cell carcinoma of the head and neck to radiotherapy. Arch Otolaryngol Head Neck Surg 123: 507
6. Donaldson KL, Goolsby GL, Wahl AF (1994) Cytotoxicity of the anticancer agents cisplatin and taxol during cell proliferation and the cell cycle. Int J Cancer 57: 847
7. Donehower LA, Harvey M, Slagle BL, McArthur MJ, Montgomery CA, Butel JS, Bradley A (1992) Mice deficient for p53 are developmentally normal but susceptible to spontaneous tumours. Nature 356: 215
8. Dorigo O, Turla ST, Lebedeva S, Gjerset RA (1998) Sensitization of rat glioblastoma multiforme to cisplatin in vivo following restoration of wild-type p53 function. J Neurosurg 88: 535
9. Dorr RT, Von Hoff DD (1994) Cancer chemotherapy handbook. Appleton & Lange, Norwalk
10. Fujiwara T, Grimm EA, Mukhopadhyay T, Zhang W-W, Owen-Schaub LB, Roth JA (1994) Induction of chemosensitivity in human lung cancer cells in vivo by adenovirus-mediated transfer of the wild-type p53 gene. Cancer Res 54: 2287

11. Gallardo D, Drazan KE, McBride WH (1996) Adenovirus-based transfer of wild-type p53 gene increases ovarian tumor radiosensitivity. Cancer Res 56: 4891
12. Gjerset RA, Turla ST, Sobol RE, Scalise JJ, Mercola D, Collins H, Hopkins PJ (1995) Use of wild-type p53 to achieve complete treatment sensitization of tumor cells expressing endogenous mutant p53. Mol Carcinog 14: 275
13. Harris MP, Sutjipto S, Wills KN, Hancock W, Cornell D, Johnson DE, Gregory RJ, Shepard HM, Maneval DC (1996) Adenovirus-mediated p53 gene transfer inhibits growth of human tumor cells expressing mutant p53 protein. Cancer Gene Ther 3: 121
14. Horwitz SB (1992) Mechanism of action of taxol. Trends Pharmacol Sci 13: 134
15. Huyghe BG, Liu X, Sutjipto S, Sugarman BJ, Horn MT, Shepard H, Scandella CJ, Shabram P (1995) Purification of a type 5 recombinant adenovirus encoding human p53 by column chromatography. Hum Gene Ther 6: 1403
16. Isaacs WB, Carter RS, Ewing CM (1991) Wild-type p53 suppresses growth of human prostate cancer cells containing mutant p53 alleles. Cancer Res 51: 4716
17. Jung M, Notario V, Dritschilo A (1992) Mutations in the p53 gene in radiation-sensitive and -resistant human squamous carcinoma cells. Cancer Res 52: 6390
18. Lowe SW (1995) Cancer therapy and p53. Curr Opin Oncol 7: 547
19. McGovern JP (1994) Pharmacologic principles. In: Dorr RT, Von Hoff DD (eds) Cancer chemotherapy handbook. Appleton & Lange, Norwalk, pp 15–34
20. Min B, Baek J, Shin K, Gujuluva CN, Cherrick HM, Park N (1994) Inactivation of the p53 gene by either mutation or HPV infection is extremely frequent in human oral squamous cell carcinoma cell lines. Oral Oncol Eur J Cancer 30B: 338
21. Mosmann T (1983) Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 64: 55
22. Musco ML, Cui S, Small D, Nodelman M, Sugarman B, Grace M (1998) A comparison of flow cytometry and laser scanning cytometry for the intracellular evaluation of adenoviral infectivity and p53 protein expression in gene therapy. Cytometry 33: 290
23. Nielsen LL, Maneval D (1998) p53 tumor suppressor gene therapy for cancer. Cancer Gene Ther 5: 52
24. Nielsen LL, Dell J, Maxwell E, Armstrong L, Maneval DC, Catino J (1997) Efficacy of p53 adenovirus-mediated gene therapy against human breast cancer xenografts. Cancer Gene Ther 4: 129
25. Nielsen LL, Lipari P, Dell J, Gurnani M, Hajian G (1998) Adenovirus-mediated p53 gene therapy and paclitaxel have synergistic efficacy in models of human head and neck, ovarian, prostate, and breast cancer. Clin Cancer Res 4: 835
26. Nielsen LL, Gurnani M, Syed J, Dell J, Hartman B, Cartwright M, Johnson RC (1998) Recombinant E1-deleted adenovirus-mediated gene therapy for cancer: efficacy studies with p53 tumor suppressor gene and liver histology in mouse tumor xenograft models. Hum Gene Ther 9: 681
27. Nielsen LL, Gurnani M, Dell J, Maneval DC, Zepeda M (1998) Immune modulation of the host response to p53 adenovirus. Proc Am Assoc Cancer Res 39: 514
28. Nguyen DM, Spritz FR, Yen N, Cristiano RJ, Roth JA (1996) Gene therapy for lung cancer: enhancement of tumor suppression by a combination of sequential systemic cisplatin and adenovirus-mediated p53 gene transfer. J Thorac Cardiovasc Surg 112: 1372
29. Ogawa N, Fujiwara T, Kagawa S, Nishizaki M, Morimoto Y, Tanida T, Hizuta A, Yasuda T, Roth JA, Tanaka N (1997) Novel combination therapy for human colon cancer with adenovirus-mediated wild-type p53 gene transfer and DNA-damaging chemotherapeutic agent. Int J Cancer 73: 367
30. Ozbun MA, Butel JS (1995) Tumor suppressor p53 mutations and breast cancer: a critical analysis. Adv Cancer Res 66: 71
31. Ozols RF, Vermorken JB (1997) Chemotherapy of advanced ovarian cancer: current status and future directions. Semin Oncol 24 [Suppl. 2]: S2-1–S2-9
32. Pirollo K-F, Hao Z, Rait A, Jang Y, Fee WE, Ryan P, Chiang Y, Chang E (1997) p53 mediated sensitization of squamous cell carcinoma of the head and neck to radiotherapy. Oncogene 14: 1735
33. Rowinsky EK, Cazenave LA, Donehower RC (1990) Taxol: a novel investigational antimicrotubule agent. J Natl Cancer Inst 82: 1247
34. Selter H, Montenarh M (1994) The emerging picture of p53. Int J Biochem 26: 145
35. Spitz FR, Nguyen D, Skibber JM, Meyn RE, Cristiano RJ, Roth JA (1996) Adenoviral-mediated wildtype *p53* gene expression sensitizes colorectal cancer cells to ionizing radiation. Clin Cancer Res 2: 1665
36. Thompson CB (1995) Apoptosis in the pathogenesis and treatment of disease. Science 267: 1456
37. Vasey PA, Jones NA, Jenkins S, Dive C, Brown R (1996) Cisplatin, camptothecin, and taxol sensitivities of cells with p53-associated multidrug resistance. Mol Pharrnacol 50: 1536
38. Wahl AF, Donaldson KL, Fairchild C, Lee FYF, Foster SA, Demers GW, Galloway DA (1996) Loss of normal p53 function confers sensitization to taxol by increasing G2/M arrest and apoptosis. Nat Med 2: 72
39. Wills KN, Maneval DC, Menzel P, Harris MP, Sutjipto S, Vaillancourt M-T, Huang W-M, Johnson DE, Anderson SC, Wen SF, Bookstein R, Sherpard HM, Gregory RJ (1994) Development and characterization of recombinant adenoviruses encoding human p53 for gene therapy of cancer. Hum Gene Ther 5: 1079
40. Yaginuma Y, Westphal H (1992) Abnormal structure and expression of the p53 gene in human ovarian carcinoma cell lines. Cancer Res 52: 4196
41. Yang B, Eshleman, JR, Berger NA, Markowitz SD (1996) Wild-type p53 protein potentiates cytotoxicity of therapeutic agents in human colon cancer cells. Clin Cancer Res 2: 1649
42. Yang Y, Nunes FA, Berencsi K, Furth EE, Gomczol E, Wilson JM (1994) Cellular immunity to viral antigens limits E1-deleted adenoviruses for gene therapy. Proc Natl Acad Sci USA 91: 4407