Histone deacetylase inhibitor FK228 enhances adenovirus-mediated p53 family gene therapy in cancer models

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Abstract
Therapeutic replacement of the wild-type p53 gene has been pursued as a potential gene therapy strategy in a variety of cancer types; however, some cancer models are resistant to p53 in vivo and in vitro. Therefore, to improve p53 gene therapy, it is important to overcome the resistance to p53-mediated apoptosis. Histone deacetylase inhibitors are a novel class of chemotherapeutic agents that are able to reverse the malignant phenotype of transformed cells. A natural histone deacetylase inhibitor, FK228, is reported to enhance adenovirus infection due in part to the up-regulation of coxsackievirus adenovirus receptor expression. In this study, preclinical experiments were done to establish a mechanistic rationale for the combination of adenovirus-mediated p53 family gene transfer and FK228 pretreatment in future clinical trials. Pretreatment with FK228 enhanced apoptosis in human cancer cells through enhanced transduction of Ad-p53. FK228 also induced hyperacetylation of the p53 protein and specifically enhanced p53-mediated Noxa expression. Additionally, the combination of FK228 and Ad-p53 induced Bax translocation to the mitochondria. The double knockdown of Bax and Noxa expression by small interfering RNA antagonized the synergistic effect of Ad-p53 and FK228 on apoptosis induction. In human cancer xenograft models, FK228 significantly increased the therapeutic effectiveness of p53 as well as p63 gene therapy. These results provide a strong rationale for combining p53 gene therapy and FK228 pretreatment in cancer therapy. [Mol Cancer Ther 2008;7(4):779–87]

Introduction
Inactivation of the p53 pathway not only is involved in carcinogenesis but also confers chemoresistance (1, 2). Therefore, therapeutic replacement of the wild-type p53 gene using adenoviral vectors has been pursued as a potential gene therapy strategy in several types of cancer (3). This strategy potentially relies on p53-mediated apoptosis, which in turn depends on induction of a distinct class of transcriptional target genes, including BAX, PUMA, NOXA, p53AIP1, etc. (4). However, in clinical trials, restoration of wild-type p53 gene function does not always lead to tumor regression or tumor growth inhibition, suggesting that some tumors are resistant to exogenous p53 (5, 6). This resistance may result from the influence of negative regulators of p53, such as MDM2 (7, 8).

The two p53 relatives, p73 and p63, encode proteins that share considerable structural homology with p53 (9–11). p73 and p63 are expressed in different isoforms as a result of differential promoter usage and alternative splicing. The isoforms containing a transactivation domain at their NH2-terminal end can also bind to p33-responsive elements to transactivate the p53 target genes and are termed TApl3 and TApl63. When overexpressed, exogenous TApl3 and TApl63 can induce apoptosis of cancer cells, suggesting that the p53 family members have a potential functional overlap with p53 itself. MDM2 can also bind the NH2-terminal of p73 and p63. On the other hand, MDM2 binding does not promote p63 or p73 degradation (12, 13). Thus, to overcome resistance to p53-mediated apoptosis, p73 and p63 may be useful. For example, a p73-expressing recombinant adenovirus inhibited the growth of human papillomavirus-positive cervical cancer cell lines more efficiently than p53 (14). Additionally, osteosarcoma cell lines having MDM2 amplification undergo apoptosis in response to p63 but not p53 (15).

Histone deacetylase inhibitors (HDACI) are novel chemotherapeutic agents that are potentially effective against a broad range of human cancers. Generally, HDACIs induce accumulation of hyperacetylated nucleosome core histones and results in transcriptional activation of genes, the expression of which potentially causes inhibition of tumor cell growth (16). In addition, HDACIs induce acetylation of nonhistone proteins, raising the possibility of a histone-independent effect of HDACIs is also important for their antitumor activity (17). FK228, also known as depsipeptide (FR901228), is one of the most promising HDACIs for...
cancer treatment because of its effectiveness at low concentrations (18, 19). Clinical trials using FK228 are currently ongoing for patients with acute myelogenous leukemia, chronic lymphocytic leukemia, T-cell lymphoma, and refractory solid-tumor malignancies (20). Importantly, FK228 increases adenovirus-mediated transduction in cancer cell lines as a result of enhancing the levels of coxsackievirus adenovirus receptor (CAR) on the cell surface (21–23).

One of the major weaknesses of adenovirus-mediated gene therapy is the less-than-optimal delivery; thus, high multiplicity of infection (MOI) of adenoviral vectors may be required to achieve effective killing of tumor cells. In the present study, we investigated the combined effect of FK228 and an adenoviral vector containing the p53 family genes against human cancer cells. We found that FK228 significantly increased the therapeutic effectiveness of the p53 family adenovirus using in vitro and in vivo models. The results highlight the importance of combining FK228 with p53 family gene therapy for the treatment of human cancer.

Materials and Methods

Cell Lines, Recombinant Adenovirus, and Drugs

Human cancer cell lines SW480 and MKN45 were purchased from the American Type Culture Collection and the Japanese Collection of Research Bioresources, respectively. MKN45 cells express wild-type p53, whereas SW480 cells express mutated p53 proteins. The recombinant adenoviral vectors expressing human p53 (Ad-p53), TAp63α (Ad-p73α), and TAp63γ (Ad-p63γ) and the bacterial lacZ gene (Ad-lacZ) were constructed as described previously (24, 25). Adenovirus titer in plaque-forming units was determined by plaque formation assays following infection of 293 cells. The MOI was defined as the ratio of the total number of plaque-forming units used in a particular infection to the total number of cells to be infected. FK228 was kindly provided by Fujisawa Pharmaceutical.

Immunoblot Analysis

The primary antibodies used for immunoblotting in this study are as follows: mouse anti-human p53 monoclonal antibody (mAb; DO-1; Santa Cruz Biotechnology), rabbit anti-human p53 polyclonal antibody (acetyl K373/382; Abcam), mouse anti-human p73 mAb (ER-15; Oncogene Research), mouse anti-human p63 mAb (4A4; Oncogene Research), mouse anti-human p21 mAb (EA10; Oncogene Research), mouse anti-human MDM2 mAb (SMP14; Santa Cruz Biotechnology), rabbit anti-human BAX polyclonal antibody (P19; Santa Cruz Biotechnology), mouse anti-human Noxa mAb (114C307; Oncogene Research), rabbit anti-human PUMA polyclonal antibody (BD Transduction Laboratory), goat anti-human histone H3 polyclonal antibody (Santa Cruz Biotechnology), rabbit anti-acetylated human histone H3 polyclonal antibody (Santa Cruz Biotechnology), mouse anti-human poly(ADP-ribose) polymerase mAb (BD Pharmingen), mouse anti-human caspase-9 mAb (BD Transduction Laboratory), mouse anti-human actin mAb (Chemicon), mouse anti-human mitochondrial heat shock protein 70 mAb (JG1; Affinity Bioreagents), and mouse anti-β-galactosidase mAb (Promega). Proteins were transferred to Immobilon P membranes (Millipore) by electroblotting, and immunoblot analysis was done as described previously (15, 25). Subcellular fractionation was done using the Mitochrondria Cytosol Fractionation kit (BioVision).

Detection of Apoptosis

Apoptosis was analyzed by flow cytometry and terminal deoxynucleotidyl transferase–mediated dUTP nick end labeling (TUNEL) analyses. For flow cytometry, both adherent and detached cells were combined, fixed in 90% cold ethanol, treated with RNase A, and stained with propidium iodide. Samples were analyzed on a FACSCalibur as described previously (15). The percentage of apoptotic cells was determined to be the percentage of the area of fluorescence smaller than the G1 peak, out of the total area of fluorescence. For the TUNEL assay, cells were plated at 5 × 10^4 per well in a four-well chamber slide. TUNEL reactions were done using the DeadEnd Fluorometric TUNEL system (Promega) according to the manufacturer’s instructions.

Immunofluorescence Microscopy

Cells were fixed in 4% paraformaldehyde and incubated for 2 h with rabbit anti-Bax polyclonal antibody. The slides were then stained with Alexa 488–conjugated goat anti-rabbit IgG (Invitrogen) for 1 h and counterstained with MitoTracker Red 580 (Invitrogen) for mitochondrial staining. The specimens were examined using a laser-scanning confocal microscope (Fluoview; Olympus).

RNA Interference

Human Bax small interfering RNAs (siRNA), si-BAX (5’-GGUGCCGGAACUGAUACAG-3’), human Noxa siRNA, si-Noxa (5’-CUCCGGCCGAAACUUUCUG-3’), and nonspecific control siRNA (target sequence 5’-NNACTCTATCGCCAGCTGAC-3’) were purchased from Qiagen. Cells (1 × 10^6) were plated per well in six-well plates with 2 mL medium in each well at day 0. Double-stranded siRNAs were transfected every 24 h for 3 consecutive days using Oligofectamine (Invitrogen). The final concentration of siRNA was 50 nmol/L. FK228 was added to the cells 8 h after the last transfection. After 24 h, the cells were incubated with a recombinant adenovirus for 48 h and subjected to flow cytometry and immunoblotting. Down-regulation of the target genes by specific siRNA but not negative controls was confirmed with by semiquantitative reverse transcription-PCR. Primers are listed in Supplementary Table 1.3

Animal Models

All animals were maintained under a specific pathogen-free condition and treated in accordance with guidelines by the Animal Care and Use Committee of Sapporo Medical University. The efficiency of the adenovirus-mediated gene transfer in vivo was determined by β-galactosidase activity

3 Supplementary material for this article is available at Molecular Cancer Therapeutics Online (http://mct.aacrjournals.org/).
and immunoblot analysis. For determination of β-galactosidase activity, each tumor specimen was homogenized in 100 μL per 50 mm³ sample of lysis buffer (Roche). Protein concentration was determined and β-galactosidase activity was assayed chemiluminescently using a β-Gal Reporter Gene Assay (Roche). Activity was expressed as relative light units/mg protein. Five mice were used for each group. For lacZ immunoblotting, nude mice were injected s.c. into right and left flanks with 2 × 10⁶ MKN45 cells. When tumor size reached 400 mm³, mice were treated with 0 or 0.2 μg/g FK228. Ad-lacZ was injected directly into one tumor on days 3 and 5. The other tumor was used as control. Both tumors were resected on day 7 and processed for immunoblotting.

To evaluate the potential to treat established tumors, MKN45 cells were injected s.c. into the right flank of nude mice as described above. When tumor size reached 100 mm³ ± 10%, treatment was started. FK228 (0.2 μg/g) was injected i.p. three times at days 1, 3, and 5. Mice also received direct intratumoral injection of 5 × 10⁹ plaque-forming units of the adenoviruses at days 3 to 5. Seven mice were used for each treatment group. The tumor volume was calculated using the equation: \( V (\text{mm}^3) = \frac{a \times b^2}{2} \), where \( a \) is the largest dimension and \( b \) is the perpendicular diameter.

**Statistical Analysis**

All data were evaluated using Student’s \( t \) test with probability values less than 0.05 considered significant.

**Results**

FK228 Enhances Adenoviral Infection in Human Cancer Cells

We first examined the proapoptotic effect of FK228 in human cancer cells using flow cytometry. Human stomach

![Figure 1](image_url)

**Figure 1.** Combined treatment of human cancer cells with adenovirus-mediated transfer of p53 family genes and FK228. A, MKN45 cells were incubated with or without 5 nmol/L FK228. After 24 h, the indicated adenovirus was added to the medium at a MOI of 50 or 200. Apoptosis was examined by flow cytometry 24 h after infection. Percentage of cells in the sub-G₁ population. Experiments were repeated three times. Representative results. Synergistic effects on apoptosis were also examined in SW480 cells (bottom). B, detection of caspase-9 protein cleavage by immunoblotting. MKN45 cells were treated as described above. Immunoblot of caspase-9 and control actin proteins. Arrow, bands of cleaved products. C, TUNEL assay of MKN45 cells after the combined treatment as described in A. Nuclear DNA was stained with 4',6-diamidino-2-phenylindole. Bottom, percentages of TUNEL-positive cells. Experiments were repeated three times. Mean percentages.
cancer cells MKN45 and colorectal cancer cells SW480 were chosen because they are relatively resistant to p53-induced apoptosis (24). Treatment of MKN45 and SW480 cells with FK228 for 48 h induced apoptosis in a dose-dependent manner (Supplementary Fig. S1A). To analyze the augmentation of antitumor effect of adenovirus-mediated transfer of p53 family by combination with FK228, the optimum condition of FK228 showing no or minimal cytotoxicity by itself was determined to be 5 and 1 nmol/L for MKN45 and SW480, respectively. We also found that expression of the transgene lacZ was increased in MKN45 cells following FK228 pretreatment most likely by enhancing adenoviral infection (Supplementary Fig. S1B). Similar results were observed for SW480 cells (data not shown). Efficient adenovirus infection requires CAR and integrins for attachment and internalization to enter host cells (26, 27). After incubation in FK228, increased expression of CAR as well as integrin aV and b3 mRNA were observed in MKN45 cells (Supplementary Fig. S1C). Additionally, the cell surface expression of CAR and aV integrin apparently increased by exposure to FK228 (Supplementary Fig. S1D), suggesting that FK228 at a noncytotoxic concentration was able to significantly increase the expression of the CAR and integrins and enhance adenovirus-mediated gene transfer.

**Combined Treatment with Adenovirus-Mediated Transfer of p53 Family and FK228**

We then determined whether the combination of FK228 and adenovirus-mediated transfer of the p53 family could enhance apoptosis. Cells were pretreated with or without FK228 at a noncytotoxic concentration for 24 h, and recombinant adenovirus was added to medium. Apoptosis was analyzed by flow cytometry 24 h later. In the absence of FK228, transduction of Ad-p53, Ad-p73beta, and Ad-p63gamma at a MOI of 200 induced a significant cell cycle arrest but no apoptosis in MKN45 cells (Fig. 1A, row 1). Ad-lacZ-infected MKN45 cells pretreated with FK228 also displayed no apoptosis. In contrast, either Ad-p53-, Ad-p73beta-, or Ad-p63gamma-infected MKN45 cells underwent apoptosis when pretreated with FK228 (Fig. 1A, row 2). Importantly, apoptosis was seen even when infected virus was decreased to a MOI of 50 (Fig. 1A, row 3). This synergic effect of FK228 on apoptosis in combination with adenovirus-mediated transfer of p53 family genes was independently confirmed by caspase-9 activation (Fig. 1B) and TUNEL assay (Fig. 1C). Furthermore, this synergic effect was observed in SW480 cells (Fig. 1A, compare row 4 with row 5). These results suggest that FK228 pretreatment increases the sensitivity of cancer cells to adenovirus-mediated p53 family gene therapy in vitro.

**Accumulation of Acetylated p53 and p63 Proteins following FK228 Treatment**

Next, we measured the expression level of p53 protein in Ad-p53 infected cells following the FK228 pretreatment. Following exposure to 5 nmol/L FK228 for 24 h, MKN45 cells were infected with Ad-p53 at various MOIs. An additional 24 h later, p53 protein was detected by immunoblot analysis. In control cells, the endogenous p53 protein was detected at a low level. FK228 pretreatment alone did not alter the expression level of endogenous p53 protein (Fig. 2A, compare lane 1 with lane 5). In contrast, the exogenous p53 protein following Ad-p53 infection was increased in MKN45 cells pretreated with FK228 (Fig. 2A, lanes 6-8) compared with untreated cells (lanes 2-4). The p53 protein level in the FK228-treated cells after infection with Ad-p53 at a MOI of 50 is similar to that cells without FK228 treatment after infection with Ad-p53 at a MOI of 200 (Fig. 2A, compare lane 4 with lane 7). This result is consistent with that of β-galactosidase gene transfer (Supplementary Fig. S1B) in which adenoviral transduction...
FK228 Enhances p53-Induced Noxa Expression
p53-dependent apoptosis is regulated, in large part, by transcriptional activation of its target genes. Thus, we measured the expression level of proapoptotic p53 targets after the combined treatment. MKN45 cells were infected with Ad-p53 or Ad-lacZ in the presence or absence of FK228, and the protein levels of p53 and endogenous MDM2, Bax, Noxa, and PUMA were examined by immunoblot analysis. The exogenous p53 protein was induced by Ad-p53 alone, whereas the FK228 pretreatment further enhanced MDM2 induction (Fig. 3, row 2, compare lane 2 with lane 5). Interestingly, the combined treatment with Ad-p53 and FK228 specifically induced Noxa in MKN45 cells, but the expression levels of Bax and Puma were not significantly enhanced by the combined treatment.

FK228 Promotes the Translocation of Bax to Mitochondria
Bax protein resides normally in the cytosol and translocates to mitochondria in response to a variety of apoptotic stimuli (29, 30). Thus, the mitochondrial localization of Bax protein was examined using subcellular fractionation and immunocytochemical analysis. Under a control condition, a slight effect on p21 induction, but the combination greatly increased p21 expression (Fig. 2A-C).

Acetylation of p53 protein was reported to be important for activation of p53-targeted gene expression and apoptosis induction (28). Thus, we examined whether the FK228 pretreatment resulted in an increase in acetylation of p53 and p63 proteins by immunoblot analysis. The level of acetylated p63 as well as p53 proteins increased after the administration of FK228 (Fig. 2D; Supplementary Fig. S2). These results revealed that the concentrations of FK228 used in this study can also result in accumulation of the acetylated p53 and p63 proteins.

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antagonizes the proapoptotic effect of Ad-p53 and FK228 (Fig. 5A, row 4, si-Bax + si-Noxa). We also showed that the double knockdown of Bax and Noxa almost completely blocked poly(ADP-ribose) polymerase and caspase-9 cleavage after the combined treatment (Fig. 5C, lanes 7 and 9). These results suggest that the synergic effect on apoptosis is at least in part mediated through Bax and Noxa activities.

**Combined Therapy of Ad-p53 Family Gene and FK228**

**In vivo**

We examined whether the FK228 pretreatment could enhance the therapeutic effect of Ad-p53 in vivo xenograft model. We first found that FK228 improved the efficiency of adenovirus-mediated gene transfer in vivo. MKN45 cells were injected s.c. into nude mice. When tumor size reached 400 mm³, FK228 was injected i.p. three times at days 1, 3, and 5. To examine the efficiency of the adenovirus-mediated gene transfer in vivo, each mouse received direct intratumoral injection of $5 \times 10^9$ plaque-forming units of Ad-lacZ at days 3 and 5. We then sacrificed the mice at day 7 and measured β-galactosidase activity in tumor cells. We observed that β-galactosidase activity in cell extracts was increased after the FK228 pretreatment in a dose-dependent manner (Fig. 6A). In a second approach, the efficiency of the adenovirus-mediated gene transfer in vivo was determined by immunoblot using cell extracts from control and FK228-treated tumors after Ad-lacZ infection. Consistent with the results of β-galactosidase activity assay, the increased expression of β-galactosidase protein was detected in tumors pretreated with FK228 (Fig. 6A, compare mice 1, 4, and 5 with mice 2, 3, and 6).

Subsequently, we tested the effect of the combined therapy on the progression of established MKN45 tumors. As shown in Fig. 6C, whereas treatment with Ad-lacZ plus FK228 had a marginal antitumor effect, single treatment with Ad-p53 or Ad-p63γ retarded tumor growth in MKN45 tumor-bearing mice compared with Ad-lacZ treatment alone. Remarkably, Ad-p53 in combination with FK228 synergistically suppressed tumor growth in tumor-bearing mice compared with either Ad-p53 alone ($P = 0.0002$ for tumor volume) or FK228 alone ($P = 0.0001$ for tumor volume). A similar synergistic effect was seen with the FK228 pretreatment in tumors with Ad-p63γ infection (Fig. 6C). Furthermore, there was no significant difference on tumor volume or survival between mice receiving Ad-p53 plus FK228 and those receiving Ad-p63γ plus FK228. Notably, Ad-p53 and Ad-p63γ in combination with FK228 induced complete tumor regression in 50% (4 of 8) and 25% (2 of 8) of tumor-bearing mice, respectively. This in vivo result correlates with the specificity observed in vitro.

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**Figure 4.** Combined treatment of adenovirus-mediated transfer of p53 family genes and FK228 promotes the translocation of Bax to mitochondria. **A,** equal amounts of the mitochondrial proteins isolated from control and FK228-treated MKN45 cells were separated on SDS-polyacrylamide gels and then subjected to immunoblot analysis for Bax. Mitochondrial HSP70 was used as loading control for the mitochondrial protein. The Bax protein bands were quantified and normalized against the corresponding HSP70 bands to obtain relative Bax expression levels. **B** and **C,** immunostaining of Bax subcellular distribution following FK228 treatment. Control and FK228-treated MKN45 cells were incubated with the indicated adenoviruses in a four-well slide chamber. After 16 h, cells were fixed, immunostained for Bax (green), and double stained with MitoTracker Red for mitochondria (red). Mitochondrial and Bax localization was imaged by fluorescence microscopy. Colocalization of Bax in mitochondria was shown (yellow) in merged images. A minimum of 200 cells were assessed for each sample. **C,** percentages of cells having Bax localized to mitochondria. Three independent experiments were done. Mean ± SD.
Discussion

The combination of HDACIs and Ad-p53 has been tested previously. In vitro studies have shown synergy between p53 gene transduction and FK228 in thyroid carcinoma cell lines (31); however, these studies did not determine the mechanisms for synergy with Ad-p53 and FK228. In stomach and liver cancer cell lines, a synergistic effect of p53 gene transduction and another HDACI, sodium butyrate, was observed by Takimoto et al. (32). In the present study, we show that FK228 in combination with adenovirus-mediated transfer of p53 family significantly enhanced the transgene expression as well as therapeutic effect against human cancer cells, which are resistant to apoptosis by p53 gene transfer alone. It has been reported that adenoviral transduction was enhanced in some human carcinoma cells after FK228 treatment, which was associated with increased expression of CAR and integrin (21–23). Our results in vitro are consistent with previous reports. Moreover, when a recombinant adenovirus carrying the lacZ gene was injected into tumors in nude mice, β-galactosidase activity was significantly higher than in tumors of untreated mice (Fig. 6). Recent studies showed that FK228 can increase CAR mRNA and protein in xenograft models (33). Thus, enhancement of CAR expression may be one of the mechanisms contributing to the higher cytotoxic and tumoricidal effects of Ad-p53 on FK228-treated MKN45 cells in vitro and in vivo. In the clinical setting, administration of FK228 at a clinically acceptable dosage may improve the low efficiency of adenovirus-mediated gene transfer into cancer cells.

Lysine residues in the p53 protein can be acetylated by histone acetyltransferases such as p300/CREB-binding protein and p300/CREB-binding protein–associated factor (17, 34). FK228 may also have inhibitory activity against non-histone deacetylases (19). We showed that FK228 treatment can lead to acetylation of p53 and p63 protein. Other potential mechanisms to explain the synergistic effect of the combined treatment presented here are involved in Noxa induction and Bax translocation. Several p53 targets have been reported as candidates for the p53-mediated apoptotic pathway (4). We found that Noxa expression was considerably and specifically increased in MKN45 cells following the combined treatment (Fig. 3). Pretreatment of MKN45 cells with FK228 could enhance p53-dependent transactivation of a subset of target genes probably due to increased p53-DNA binding activity through the modulation of histone acetylation and chromatin composition or through acetylation of lysine residues in the p53 protein. The Bax gene product is known to translocate from the cytosol to mitochondria in the process of apoptosis (29, 30). As shown in Fig. 5, the combined treatment did not induce Bax expression but promoted mitochondrial translocation.
of the Bax protein. The double knockdown of Bax and Noxa expression by siRNA antagonizes the synergistic effect of Ad-p53 and FK228 on apoptosis induction (Fig. 5), suggesting that the two molecules play a central role in the apoptotic pathway in MNK45 cells.

In this study, we showed that FK228 enhances the proapoptotic effect of Ad-p73 and Ad-p63 in addition to Ad-p53. Exogenous expression of p53 inhibits tumor growth by inducing apoptosis. However, p53-based gene therapy is not effective in certain conditions, suggesting many tumors could be resistant to exogenous p53 (5, 6). For example, amplification of MDM2, mutational inactivation of p14ARF, or presence of the HPV E6 oncogene renders the p53 pathway of growth suppression inactive by degrading of p53 protein through ubiquitination (7, 8, 35–37). Thus, inhibition of several alternate candidate genes or other methodologies has been attempted for treatment of these types of cancer. We showed here that Ad-p73β and Ad-p63y induced extensive apoptosis similar to Ad-p53 when combined with FK228 pretreatment in MNK45 and SW480 cells (Fig. 1). Moreover, Ad-p63y in combination with FK228 synergistically suppressed tumor growth in vivo (Fig. 6). Earlier work by our laboratory and others has shown that some of the cancer cells lineage resistant to p53-mediated apoptosis undergo apoptosis after transduction of p73β or p63y (14, 15, 24, 38). Thus, p73 and p63 could be a suitable alternate candidate for gene therapy of human cancers, particularly under conditions where p53 gene therapy is not effective.

In summary, we have shown that a noncytotoxic dose of FK228 can enhance transgene expression as well as therapeutic effect of adenosovirus-mediated p53 family gene transfer. These studies suggest a simple, clinically practical method for increasing the sensitivity of cancer cells to adenoviral gene therapy vectors. By avoiding the need for high adenoviral titers, the likelihood of viral toxicity is reduced. Hence, this study should be considered in planning future clinical trials combining adenovirus-mediated p53 family gene transfer and FK228.

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References


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