Quantitative analysis of p53-targeted gene expression and visualization of p53 transcriptional activity following intratumoral administration of adenoviral p53 in vivo

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Abstract
To analyze the mechanism of the antitumor effect of an adenoviral vector expressing the p53 tumor suppressor (Ad-p53) in vivo, we quantitatively assessed p53-targeted gene expression and visualized transcriptional activity of p53 in tumors in nude mice treated with Ad-p53. Human lung cancer (H1299) xenografts established in nude mice were treated by intratumoral administration of Ad-p53. The levels of expression of exogenous p53 and p53-targeted genes p21, MDM2, Noxa, and p53AIP1 were quantified by real-time reverse transcription-PCR (RT-PCR) and induction of apoptosis was observed histochemically on days 1–3, 7, and 14 after treatment. Expression of mRNA of exogenous p53 and p53AIP1-targeted genes (except p53AIP1) was at its maximum 1 day after Ad-p53 treatment and then decreased rapidly; apoptosis was evident in situ 2–3 days after treatment. We developed a noninvasive and simple method for monitoring the transcriptional activity of exogenous p53 following intratumoral administration of Ad-p53 in nude mice. We established H1299 cells that express the green fluorescent protein (GFP) reporter gene under the control of p53-responsive p21 promoter (i.e., the p53R-GFP reporter system). Xenografts of these cells in nude mice were treated by intratumoral administration of Ad-p53, and the transcriptional activity of exogenous p53 could be visualized as intratumoral GFP expression in real time by 3-CCD camera. Expression of GFP was maximal 3 days after treatment and decreased remarkably by 7 days after treatment. We demonstrated that Ad-p53 treatment rapidly induced p53-targeted genes and apoptosis in tumors and succeeded in visualizing p53 transcriptional activity in vivo. We also found that Ad-p53 infection induced phosphorylation of p53 at Ser46 in p53-sensitive H1299 cells in vitro but not in p53-resistant H226Br cells, suggesting that phosphorylation of Ser46 is involved in p53-dependent apoptosis. Our data indicate that quantitative analysis of p53-targeted gene expression by real-time quantitative RT-PCR and visualization of p53 transcriptional activity in fresh xenografts by using the p53R-GFP reporter system may be useful in assessing the mechanisms of the antitumor effects of Ad-p53 and novel therapeutic approaches.

Introduction
p53 gene therapy is currently in clinical trials as a cancer therapeutic (1–3). Ad-p53 consists of a replication-deficient type 5 adenoviral vector expressing human wild-type p53 tumor suppressor gene. p53 is the most commonly mutated gene in human cancer (4, 5). It is activated in response to several stressful stimuli, including exposure to DNA-damaging agents, hypoxia, nucleotide depletion, or oncogene activation (6, 7). Activated p53 carries out its function as a tumor suppressor through several growth-controlling end points, including cell cycle arrest, apoptosis, senescence, differentiation, and antiangiogenesis (8). The p53 protein is a transcriptional factor capable of inducing the expression of genes containing a p53-responsive element in their promoter.

In preclinical models, Ad-p53 has therapeutic efficacy against a wide range of human tumor types containing nonfunctional p53 both in vitro and in vivo (9–16). It is remains unclear, however, how and when exogenous p53 and p53-targeted genes actually work in tumors after Ad-p53 treatment in vivo because its effectiveness is usually assessed simply by measuring tumor volume or survival duration. Molecular biological analysis of p53-targeted gene expression over time following intratumoral administration of Ad-p53 has never been reported. Therefore, we tried to assess p53-targeted gene expression quantitatively and visualize p53 transcriptional activity following intratumoral administration of Ad-p53 in nude mice. A noninvasive and simple method for molecular visualization of the activities of different signal transduction pathways and the expression of different genes in vivo would be of considerable value. It would aid in understanding the roles of specific genes and signal transduction pathways.

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and elucidate temporal dynamics and regulation during various therapeutic interventions. The green fluorescent protein (GFP), the gene cloned from the genome of the jellyfish *Aequorea victoria*, yields a bright, stable green fluorescence with no other substrates or cofactors in live cells (17). Because the GFP gene integrates into chromosomes and is passed to subsequent generations of cells, in which it is newly synthesized, it is well suited for real-time analysis. We sought, therefore, to develop a reporter system that enabled measurement of p53 transcriptional activity in live mice using GFP.

In this report, we demonstrate that, after Ad-p53 treatment *in vivo*, up-regulation of p53-targeted genes, such as p21, MDM2, Noxa, and p53AIP1, can be quantitatively analyzed by real-time reverse transcription-PCR (RT-PCR) and that up-regulation of p53 transcriptional activity can be visualized by the p53R-GFP reporter system.

**Materials and Methods**

**Cell Lines and Culture Conditions**

The human non-small-cell lung cancer (NSCLC) cell lines H1299, which has homozygously deleted p53 (10, 12), H226Br, which has homozygously mutated p53 gene at codon 254 (18), and A549, which contains wild-type p53 (19), were propagated in RPMI 1640 supplemented with 10% FCS, 25 mM HEPES, 100 units/ml penicillin, and 100 mg/ml streptomycin. The recombinant, replication-deficient adenoviral vector capable of expressing human wild-type p53 cDNA was previously constructed and characterized (9, 12). The resultant virus was named Ad-p53, Ad-LacZ, which expresses nuclear-localized β-galactosidase, and the E1A-deleted adenovirus vector d1312 were used as a control vector.

**Quantitative Real-Time RT-PCR**

Total RNA from the tumor samples and cultured cells was obtained by using the RNasey Mini Kit (Qiagen, Inc., Chatsworth, CA). Approximately 0.1 μg of total RNA was used for reverse transcription. Reverse transcription was performed at 22°C for 10 min and then at 42°C for 20 min. mRNA copy numbers of exogenous p53, p21, MDM2, Noxa, p53AIP1, and β-globin, the housekeeping gene (HKG), were determined by real-time quantitative RT-PCR using a LightCycler instrument, a LightCycler DNA Master SYBR Green I kit, and LightCycler Control Kit DNA (Roche Diagnostics GmbH, Mannheim, Germany). Typical amplification mixes (20 μl) contained 3 mM MgCl₂ (exogenous p53, p21, Noxa, and p53AIP1) or 4 mM MgCl₂ (MDM2 and β-globin), 0.5 μM of each primer, and 2 μl of 10× LightCycler FastStart DNA Master SYBR Green I. PCR amplifications were done in glass capillary tubes. Amplification began with a 600 s denaturation step at 95°C followed by 40 cycles of denaturation at 95°C for 15 s annealing at 62°C for 10 s (exogenous p53, p21, Noxa, and p53AIP1), 40 cycles of denaturation at 95°C for 15 s and annealing at 60°C for 10 s (MDM2), or 45 cycles of denaturation at 95°C for 15 s and annealing at 55°C for 5 s (β-globin), and extension at 72°C for 4 s (p53AIP1), 5 s (p21), 7 s (exogenous p53), 9 s (MDM2 and Noxa), or 10 s (β-globin). For exogenous p53, a sense primer in the cytomegalovirus promoter and an antisense primer within p53 exon 3 were used. The oligonucleotides used as specific primers for each gene were as follows: p53: 5′-AGA GTC TAT ACG CCC ACC CC-3′ and 5′-GCT CGA CGC TAG TAT CTC AC-3′; p21: 5′-AAG ACC ATG TGG ACC TCT-3′ and 5′-GGT AGA GTC TAT CTC TCA TGC-3′; MDM2: 5′-TGT AAG TGA ACA TTC AGG TG-3′ and 5′-TTCT CAA TAG TCA GCT AAG GA-3′; Noxa: 5′-AGA GCT GGA AGT CGA GTG T-3′ and 5′-GCA CCT TCA CAT TCC TCT C-3′, and p53AIP1: 5′-TCT CTC TCT GAG CCG AGC T-3′ and 5′-AGG TGT GTG TGT AGC CC-3′. For β-globin, LightCycler Control Kit DNA (Roche Diagnostics, Indianapolis, IN) was used. Copy numbers of mRNA were calculated from serially diluted standard curves generated from a cDNA template, which represented *in vitro* samples and confirmed bands with conventional PCR. Data were analyzed by using LightCycler Software (Roche Molecular Biochemicals). All expression levels were normalized to β-globin (HKG) in each well. Fold induction was defined as the fold increases for each sample relative to MOCK (MOCK = 1).

**Western Blot Analysis**

Cells were collected by trypsinization and washed twice in cold PBS. Cells were then lysed in SDS solubilization buffer [62.5 mM Tris-HCl (pH 6.8), 2% SDS, and 10% glycerol]. Equal amounts of proteins containing 5% β-mercaptoethanol were boiled for 5 min and electrophoresed under reducing conditions on 12.5% (w/v) polyacrylamide gels. Proteins were then electrophoretically transferred to a Hybond polyvinylidenefluoride transfer membranes (Amersham, Arlington Heights, IL). They were incubated with primary mouse anti-human p53 monoclonal antibody (Ab-2; Oncogene Research Products, San Diego, CA), anti-p53-Ser46 polyclonal antibody (20), or mouse anti-human actin monoclonal antibody (AC-40; Sigma Chemical Co., St. Louis, MO) followed by peroxidase-linked secondary antibody. An enhanced chemiluminescence Western system (Amersham, Tokyo, Japan) was used to detect secondary probes.

**Terminal Deoxynucleotidyl Transferase-Mediated Nick End Labeling Assay**

Apoptotic cells in tumor tissues were detected by the terminal deoxynucleotidyl transferase-mediated nick end labeling (TUNEL) method using MEBSTAIN Apoptosis Kit II (MBL, Purchase, Nagoya, Japan) according to the manufacturer’s instructions. Specimens were examined and photographed (×200 magnification) under a fluorescence microscope (Zeiss, Oberkochen, Germany).

**Generation of p53R-GFP Reporter System**

The p53R-GFP plasmid contains the p53-responsive GFP gene expression cassette, in which the GFP gene is under the transcriptional control of p53-activated promoter consisting of the p21 gene regulatory sequence (2.4 kb). We used the pWWP-GL3 plasmid (kindly provided by Dr. S. Nakamura) containing the p21 promoter linked to the luciferase gene.
The p21 promoter fragment was removed with HindIII restriction enzyme and inserted into the multiple cloning site of the pEGF-1 vector (Clontech Laboratories, Inc., Palo Alto, CA). The resulting plasmid, termed the p53R-GFP plasmid, contained the GFP gene under the control of the p21 promoter. Constitutive expression of the neomycin resistance gene (neo3), driven by the SV40 early immediate promoter, allowed for selection of stably transduced cells by G418.

**DNA Transfection and Selection of Stable p53R-GFP-Expressing Cells**

H1299 cells were transfected with the p53R-GFP plasmid using LipofectAMINE 2000 Reagent (Invitrogen, Rockville, MD). The resulting cell line was termed H1299/p53R-GFP. Transient transfectants were then sparsely cultured (100 cells/dish) under selection with 0.5 mg/ml G418 (WAKO, Osaka, Japan). GFP was induced by infection with Ad-p53 at 50 multiplicities of infection (MOI), and the brightest fluorescing clones were selected and propagated further under selection with 0.5 mg/ml G418. A clone with optimal characteristics was used in further experiments.

**Fluorescent Microscopy and Fluorescence-Activated Cell Sorting Analysis**

Expression of the GFP reporter gene was assessed and photographed (>200 magnification) by an Eclipse TS-100 fluorescent microscope (Nikon, Tokyo, Japan) and quantified by fluorescence-activated cell sorting (FACS; FACScan, Becton Dickinson, Mountain View, CA) using the ModFit LT program for Macintosh (version 1.01; Verity Software House, Inc., Topsham, ME).

**Animal Experiments**

The experimental protocol was approved by the Ethics Review Committee for Animal Experimentation of our institution. H1299 and A549 xenografts were produced on both sides of the back in female BALB/c nu/nu mice by s.c. injection of 5 × 10⁶ H1299 or A549 cells in 100 μl of HBSS. When the tumors were 10 mm in diameter, both tumors were injected with Ad-p53 [1 × 10⁹ plaque-forming units (pfu)/50 μl] or Ad-LacZ (1 × 10⁹ pfu/50 μl). One of the two tumors in each mouse was subjected to quantitative real-time RT-PCR, and the other was used for TUNEL assay at each time point. H1299/p53R-GFP tumors were also produced on both sides of the back in female BALB/c nu/nu mice by s.c. injection of 5 × 10⁶ H1299/p53R-GFP cells in 100 μl of HBSS. When the tumors were 10 mm in diameter, the right tumor in each mouse was injected with Ad-p53 (1 × 10⁹ pfu/50 μl), and the left tumor was injected with Ad-LacZ (1 × 10⁹ pfu/50 μl) as a control. Mice were anesthetized by i.p. injection of pentobarbital (50 mg/kg) on days 1–3 and 7 after injection, and color images were obtained using a Hamamatsu color chilled 3-CCD camera (Hamamatsu Photonics, Hamamatsu, Japan).

**Results**

**Quantitative Real-Time RT-PCR after Ad-p53 Infection in Vitro**

We used real-time RT-PCR to quantitatively analyze the expression of exogenous p53 and p53-targeted genes, such as p21, MDM2, Noxa, and p53AIP1, after Ad-p53 infection in vitro. Monolayer cultures of p53-deficient H1299 human NSCLC cells were infected with Ad-p53 at 0, 10, 50, and 100 MOI and 24 h later were subjected to real-time RT-PCR assay. Exogenous p53 mRNA expression increased in a dose-dependent fashion with increased MOI, and expression of p21, MDM2, Noxa, and p53AIP1 mRNA increased in an almost dose-dependent fashion. The maximal level of p21 mRNA expression was the highest among the p53-targeted genes tested (Fig. 1A). We next assessed expression of those genes over time after Ad-p53 infection. H1299 cells were infected with Ad-p53 at 50 MOI and harvested at 6, 12, 24, and 30 h after infection. The assay showed that maximal expression of exogenous p53, p21, MDM2, Noxa, and p53AIP1 mRNA was achieved 24 h after Ad-p53 infection followed by a rapid decrease. The maximal level of p21 mRNA expression was the highest among the p53-targeted genes tested. Maximal induced p53-targeted gene expression levels 24 h after infection were p53AIP1: 17.7-fold against MOCK, Noxa: 36.7-fold, MDM2: 1210-fold, and p21: 2710-fold (Fig. 1B).

**Phosphorylation of p53 Protein at Ser46 after Ad-p53 Infection in p53-Sensitive and p53-Resistant Cell Lines**

To examine whether different levels of p53 expression could affect induction of apoptosis, we conducted Western blot analysis using antibody against p53. As it has been reported that Ser46 is a phosphorylation site that regulates the apoptosis-inducing ability of p53, we also used polyclonal antibody that recognize p53 phosphorylation at Ser46 (20). Readily detectable p53 protein expression was achieved in a dose-dependent manner in p53-sensitive H1299 cells and p53-resistant H226Br cells 24 h after Ad-p53 infection. Although equivalent amounts of p53 protein were detected in both cell lines, phosphorylation at Ser46 was apparent only in p53-sensitive H1299 cells but not in p53-resistant H226Br cells (Fig. 1C). These results suggest that the levels of p53 expression alone are not closely correlated with apoptosis induction in human cancer cells.

**Quantitative Real-Time RT-PCR following Intratumoral Administration of Ad-p53 in Vivo**

The in vivo assay, applied to tumors treated with Ad-p53 was the same as the in vitro assay already described. Tumors were produced by s.c. injection of H1299 or A549 NSCLC cells bilaterally in nude mice and analyzed by quantitative real-time RT-PCR or TUNEL assay on days 1–3, 7, and 14 after treatment. Expression of exogenous p53, p21, MDM2, and Noxa mRNA was maximal at 1 day (24 h) after Ad-p53 treatment in H1299 tumors followed by rapid decreases, which is consistent with in vitro results (Fig. 2A). Among p53-targeted genes tested, p21 mRNA expression was the highest. Maximal induced p53-targeted gene expression levels were p53AIP1: 2.46-fold against MOCK, Noxa: 4.33-fold, MDM2: 16.2-fold, and p21: 63.2-fold, which also were consistent with in vitro results. The expression patterns of p21, MDM2, and Noxa mRNA almost paralleled that of exogenous p53. However, p53AIP1 mRNA expression was maximal 2 days after treatment, later than the other genes. In the control group, expression
of exogenous p53, p21, Noxa, and MDM2 mRNA was not altered by Ad-LacZ treatment (Fig. 2A). In contrast, p53-targeted gene expression was not induced in A549 tumors despite maximal induction of p53 mRNA expression on day 2 after treatment (Fig. 2B).

Detection of Apoptotic Cells in Tumor Tissue after Infection with Ad-p53 in Vivo

We assessed induction of apoptosis in H1299 and A549 tumors in situ by the TUNEL method. In H1299 tumors, many apoptotic cells could be detected 2–3 days after Ad-p53 treatment but not at later time points (Fig. 2C). A549 tumors exhibited no apoptotic cells after Ad-p53 injection in vivo, although exogenous p53 gene expression was confirmed (data not shown). No apoptotic cells could be observed at any time after Ad-LacZ control treatment (Fig. 2D).

Characterization of Selected H1299/p53R-GFP Clone

We developed H1299/p53R-GFP cell, which can express GFP in response to Ad-p53 treatment. H1299/p53R-GFP cells were infected with Ad-p53 at 0–100 MOI for 36 h, and the transcriptional activity of exogenous p53 was assessed by monitoring GFP expression with fluorescence microscopy and FACS. Although there was some background expression of GFP fluorescence in untreated cells, the cells treated with Ad-p53 expressed higher levels of GFP; the intensity of fluorescence was highest at 50 MOI (Fig. 3A). We next assessed transcriptional activity over time after Ad-p53 infection (50 MOI). The highest level of GFP expression was observed 48 h after Ad-p53 infection (Fig. 3B), suggesting that p53 transcriptional activity was the highest around this time point. These results confirmed that the p53R-GFP reporter system is sufficiently sensitive to allow visualization of exogenous p53 transcriptional activation in vitro.

Visualization of p53 Transcriptional Activity in Vivo

We next assessed whether this p53R-GFP reporter system was applicable in vivo. H1299/p53R-GFP tumors established on the backs of nude mice were treated with Ad-p53 (1 × 10^9 pfu/50 µl) or Ad-LacZ (1 × 10^9 pfu/50 µl). GFP in tumors could be observed with 3-CCD camera on days 0–7 after treatment. Intratumoral GFP fluorescence intensity increased gradually after injection of Ad-p53; it reached its maximum at day 3 and decreased through day 7. The tumor-suppressive effect of Ad-p53 was also observed during this period of time (Fig. 4). No GFP fluorescence could be observed at any time in control tumors (data not shown), so GFP expression in these tumors was solely due to p53 transcriptional activity. These results indicate that activation of the p53 signal transduction pathway in H1299/p53R-GFP tumors can be visualized and that the p53R-GFP reporter system was sufficiently sensitive to allow visualization of exogenous p53 transcriptional activation in vivo.

Discussion

Ad-p53 gene therapy is currently being studied in clinical trials for various cancers (1–3). In preclinical models, Ad-p53 has been shown to have therapeutic efficacy against a wide range of human tumor types containing...
nonfunctional p53 both in vitro and in vivo (9–16). Little is known, however, about the biological behavior of exogenous p53 once it becomes active in tumors. Thus far, the antitumor effect of Ad-p53 in vivo has usually been assessed by simply measuring tumor volume or survival duration. In this study, we tried to analyze the mechanism of this antitumor effect by molecular biological assay, which may provide a key to identifying the mechanism of gene therapy. Our experiments included quantitative assessment of p53-targeted gene expression following intratumoral administration of Ad-p53 in nude mice.

Several groups of genes are transcriptionally regulated by p53, including p21 for cell cycle arrest; MDM2, GADD45, and GoS8 for G0/G1 switch (7); 14-3-3 protein α1 for G2 arrest and repair (7); Noxa, p53AIP1, bax, Fas/APO1, KILLER/DR5, IGF-BP3, PIG3, and PAG608 for apoptosis (7, 20, 22, 23); and GD-AIF and TSP1 for antiangiogenesis (7, 22). p21 is one of the most important proteins in the p53 pathway, acting immediately downstream and mediating many of the actions of p53 (7, 24, 25). Several publications have reported that surgical specimens were examined for p53-targeted gene expression by real-time quantitative PCR.
but such analysis over time following intratumoral administration of Ad-p53 has never been reported. Our quantitative analysis demonstrated that in p53-sensitive H1299 human NSCLC tumors (10, 12) expression of p21 mRNA paralleled that of exogenous p53 mRNA over time both in vitro and in vivo and that the maximal level of p21 mRNA expression was the highest among the p53-targeted genes tested both in vitro and in vivo (Figs. 1 and 2A). p53AIP1 was isolated as a novel gene that is thought to play an important role in mediating p53-dependent apoptosis (20). In quantitative analysis, maximal induced p53AIP1 mRNA expression was the lowest among the p53-targeted genes tested. This may have been due to the very short half-life and extreme instability of p53AIP1.

We demonstrated that both in vitro and in vivo maximal expression of mRNA of exogenous p53 and

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**Figure 3.** A, H1299/p53R-GFP cells were infected with Ad-p53 at 0 (a), 10 (b), 50 (c), and 100 (d) MOI, and p53-responsive GFP expression was assessed with fluorescence microscopy and FACS 36 h after infection. B, H1299/p53R-GFP cells were infected with Ad-p53 at 50 MOI, and p53-responsive GFP expression was assessed with fluorescence microscopy at different time points after infection. a, 0 h; b, 12 h; c, 24 h; d, 36 h; e, 48 h; f, 72 h.

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**Figure 4.** Exogenous p53 transcriptional activity as intratumoral GFP expression was imaged in real time by 3-CCD camera. The right tumor (arrow) on the mouse’s back was injected with Ad-p53 (1 × 10⁸ pfu/50 μl). The left tumor was injected with Ad-LacZ (1 × 10⁸ pfu/50 μl; data not shown). A representative mouse was observed with 3-CCD camera on days 0 (naked (A) and 3-CCD (B)), 1 (C), 2 (D), 3 (E), and 7 (F) after injection.
p53-targeted genes (except p53AIP1) was achieved 1 day (24 h) after Ad-p53 treatment followed by a rapid decrease in H1299 tumors. Notably, Ad-p53 injection in vivo, like that in vitro, induced rapid up-regulation of p53-targeted gene mRNA, while maximal induction of apoptosis in situ was seen 2–3 days after Ad-p53 treatment. The time discrepancy between maximal mRNA expression and apoptosis induction was probably due to time lag of synthesis from the mRNA of the protein that executes apoptosis. Interestingly, maximal induced p53-targeted gene expression increased in the order p53AIP1, Noxa, MDM2, p21 both in vitro and in vivo 24 h after Ad-p53 treatment. Of the examined genes, p21 yielded the most predictable response 24 h after treatment; therefore, p21 expression may be useful for confirming p53 activity and predicting antitumor effect of Ad-p53 treatment in p53-sensitive tumors. As somatic mutations of p21 gene were not observed, this gene is not considered to be a frequent site of point mutation in human cancers (26, 27). Thus, p21 function is not impaired in most human malignancies, indicating that this rule could be applicable to many types of human cancers. We previously reported Ad-p53-mediated transient up-regulation of CD95 ligand, which triggers the rapid induction of apoptosis, in human cancer cells (28). Although CD95 ligand is one of the target genes that predict Ad-p53 sensitivity in vitro, cellular infiltrates expressing CD95 ligand such as activated T-cells and natural killer cells into the tumors (29) may disturb the accurate prediction in vivo.

In contrast to H1299 tumors, A549 human NSCLC tumors, which are relatively resistant to p53-mediated apoptosis (19), showed no increase in p53-targeted gene expression (Fig. 2B) as well as apoptosis (data not shown) despite induction of exogenous p53 mRNA expression. These findings suggest that antitumor efficacy is associated with the cell type rather than p53 expression levels, although little is known about the molecular machinery underlying these interactions. Oda et al. (20) have reported that phosphorylation of Ser65 in p53 regulates the transcriptional activation of the p53AIP1 gene. Our data demonstrated that Ad-p53 infection readily induced phosphorylation of p53 at Ser65 in p53-sensitive H1299 cells, whereas Ser46 phosphorylation could not be detected in p53-resistant H226Br NSCLC cells even at the same dose of Ad-p53 (Fig. 1C). Thus, the cell type-dependent transcriptional activity might be due to the differential modification of p53 such as phosphorylation and/or acetylation at the specific sites. Further studies will be necessary to confirm these possibilities.

Based on these results, it is rational to conclude that the p53R-GFP reporter system contains the GFP gene under the control of the p21 promoter to adequately reflect the activity of the p53 signal transduction pathway. Because the major biochemical activity of p53 is its ability to act as a transcriptional factor, reporter genes have been used to measure p53 activity including chloramphenical acetyltransferase (30) and luciferase (31). The major drawback of these reporter systems, however, is that they require that a cell lysate be prepared to measure their respective enzyme activities, precluding the possibility of further analysis on the treated cells. We therefore sought to develop a reporter system that would measure p53 transcriptional activity in live mice using GFP. We succeeded in visualizing exogenous p53 transcriptional activity following intratumoral administration of Ad-p53 in nude mice. Expression of GFP was maximal 2 days in vitro and 3 days in vivo after Ad-p53 treatment followed by gradual decreases (Figs. 3 and 4). The time discrepancy of maximal expression in vivo and in vitro may have been due to the diffusion of viral particles into tumor and the lower transfection efficacy in vivo. Although Zhang et al. (32) used GFP to monitor both endogenous and plasmid-derived p53 biochemical and biological activity, they have described only the in vitro results. Doubrovin et al. (33) have developed and assessed a method for monitoring the transcriptional activation of endogenous p53 by positron emission tomography, but our p53R-GFP system is a simpler and easier method of visualizing p53 transcriptional activity.

In summary, we have demonstrated that intratumoral injection of Ad-p53 actually up-regulated p53-targeted genes and p53 transcriptional activity, which coincided with induction of apoptosis in p53-sensitive tumors. We also demonstrated that the novel p53R-GFP reporter system was sufficiently sensitive to allow visualization of p53 transcriptional activity in vitro and in vivo. Quantitative analysis of p53-targeted gene expression by real-time quantitative RT-PCR and visualization of p53 transcriptional activity in tumor xenografts by using the p53R-GFP reporter system may be useful in assessing the mechanism of the antitumor effect of Ad-p53 and in evaluating new drugs or novel therapeutic approaches.

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References


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