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Wild-type p53 Expression Overcomes p21-mediated G1 Arrest and Induces Apoptosis in Cancer Cells Expressing Bax

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ABSTRACT

Tumor suppression by p53 is deficient in the majority of human cancers. Previous studies have suggested that expression of p53 in human cancer cells can result in either growth arrest or apoptosis. The biological and genetic determinants that dictate which of these two pathway - apoptosis or arrest - will be chosen by a particular cell following p53 expression are largely unknown. To investigate the basis of this difference, we evaluated the role of p21, a mediator of p53-induced growth arrest. We generated a replication-deficient adenoviral recombinant which expresses p21 and compared its tumor suppressive abilities with Ad-p53. Infection with Ad-p21 resulted in high levels of p21 expression and suppressed the growth of human cancer cells, through the G1 arrest of the cell cycle. We then examined the effects of combined infection with Ad-p21 and Ad-p53 to investigate which of these molecules had the dominant function. Introduction of exogenous p53 in RERF-LC-OK, BT549 and ZR-75-1 cells overcame p21-mediated cell cycle arrest at G1 and induced apoptosis, suggesting that this affect is a general event among human cancer cell lines. We then evaluated the role of Bax/Bcl-2 in the response to p53. A Significantly greater amount of Bax protein was present in cell lines undergoing apoptosis than in cells with arrested growth,

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suggesting that Bax might be an important component of the p53-mediated apoptosis of cancer cells.

Key words : p53, p21Waf1/Cip1, Recombinant adenovirus, Apoptosis, G1 arrest

INTRODUCTION

It has been demonstrated previously that the growth of some cells is arrested in response to p53 expression, whereas others undergo apoptosis[1,2]. p53 can arrest the growth of some cells by inhibiting progress directly through the cell cycle[3]. This inhibition is in part mediated by transcriptional activation of p21 gene, which encodes a cyclin-dependent kinase inhibitor. Homozygous deletion of p21 was compatible with normal mouse development, that is, was not required for apparently normal development, while fibroblasts isolated from these mice had a deficiency in their G1 checkpoint following exposure to DNA damage. Additionally, targeted disruption of both p21 alleles in human cancer cells abrogated the radiation-induced G1 arrest mediated by p53[4,5]. Although p21 was induced during p53-mediated apoptosis[3], its expression did not appear to be required for p53-mediated apoptosis of mouse thymocytes[6]. Alternatively, p53 expression can stimulate apoptosis, which is consistent with the results showing that the apoptotic response to radiation and other DNA-damaging agents is diminished in the absence of p53[6-9]. However, the biochemical and genetic determinants that dictate which of these two pathways, apoptosis or arrest, will be chosen by a particular cell following p53 expression remain largely unknown[10].

In this paper, we prepared a p21-expressing, replication-deficient adenovirus (Ad-p21) and studied its biochemical and biological effects on human cancer cells in vitro. We found that overexpression of p53 but not p21 in certain cell types resulted in apoptosis. We have also began to attempt to investigate the cellular mechanism whereby human cancer cell lines either arrest or die following an identical introduction of p53.

MATERIALS AND METHODS

Cells and culture conditions

The cell lines used in this study are shown in Table 1. they were, HSC-3, a human squamous cell tongue carcinoma cell line[11]; ZR-75-1, a human breast carcinoma cell line[12]; BT549, a human breast carcinoma cell line[12]; MCF-7, a human breast carcinoma cell line[12]; and RERF-LC-OK, a human

Table 1 p53 status and adenovirus gene transfer efficiency of the human cancer cell lines

Cell lines	origin	p53 status	β-gal (%)[b]	Ref.
HSC-3	Tongue	nonsense mutation (exon 8)	83.2	(12)
MCF-7	Breast	wt	31.0	(13)
ZR-75-1	Breast	mut	75.0	(13)
BT549	Breast	mut (249 Ser)	77.5	(13)
RERF-LC-OK	Lung	mut (113 cys)[a]	100	

[a]100% red colony in yeast functional assay
[b]β-gal is percentage of X-gal positive cells 48 hours after Ad-LacZ infection at a multiplicity of infection (moi) of 20.

lung carcinoma cell line. A human transformed embryonic kidney cell line, 293 was used in the construction and plaque formation of recombinant adenovirus. Cells were cultured at 37℃ in Dulbecco's modified Eagle's medium (DMEM) supplemented with 5% fetal bovine serum (FBS).

Recombinant adenovirus

The recombinant adenoviral vector expressing human p21 cDNA was constructed as follows. p21 cDNA was obtained as a 2.1 kb EcoRI fragment from pcD2SRαΔ-sdi1[13] and subcloned into the HindIII site of the pAd-BglII vector[14] containing a cytomegalovirus promoter/enhancer and a bovine growth hormone polyadenylation signal which are flanked by Ad5 E1 sequences (1-356 nucleotide position (n.p.) and 3329-5788 n.p.) to construct the vector pAd-p21. Both the plasmids pAd-p21 and pJM17, a plasmid containing the genomic sequence of Ad5 (Microbix biosystems Inc., Toronto, Canada), were cotransfected into 293 cells using Lipofectin reagent (GibcoBRL), and cultured in RPMI1640 medium supplemented with 3% FBS for two to three weeks to generate recombinant adenoviruses. Recombinants were purified from single plaques and tested for production of p21 protein by immunoblot assay (see below), and named Ad-p21. Culture supernatants of the viral stocks were quantified by a plaque forming assay using 293 cells. The generation of recombinant adenovirus Ad-p53 containing p53 gene has been described previously[15]. Recombinant adenovirus Ad-LacZ was kindly provided by Dr. M. J. Imperiale of Michigan University.

β-galactosidase activity

The relative efficiency of adenovirus infection was determined by X-gal staining of cells infected with Ad-LacZ, a control adenovirus vector containing the bacterial lacZ gene. After cells had been seeded in 6-well culture

plates and cultured for 24h, the cells were infected with Ad-LacZ and cultured for 48h. To assess β-galactosidase activity following infection, cells were washed with PBS, fixed with 2% formaldehyde and 0.2% glutaraldehyde for 5 min at 4℃, then washed with phosphate buffered saline (PBS). Cells expressing β-galactosidase were visualized after incubation at 37℃ in a 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal). Blue staining in the cells indicated expression of β-galactosidase; infection efficiency was evaluated by the ratio of the number of positively stained cells to those negatively stained (Table 1).

G418 colony assay

To test the effect of p53 on the growth of human cancer cells, the colony forming efficiency of G418-resistant cells was determined by transfecting the neomycin (G418) resistant gene-carrying plasmids pCMV-NeoBam and pCMV-p53[16] and culturing them in the G418-containing media. Transfection was performed according to the methods previously described[17]. Transfected cells were exposed to 15% glycerol in HEPES buffer for one minute, washed with PBS and DME and refed with cultured media. At 2 to 3 h after transfection, cells were detached, split and cultured in the DMEM with 5% FBS and G418 (250 μg/ml for HSC-3; 300 μg/ml for ZR-75-1 and BT549; 350 μg/ml for RERF-LC-OK; 550 μg/ml for MCF-7 cells) and cultured for 10 to 14 days. Cells were fixed by methanol and stained with Giemsa to count the G418 resistant colonies.

Flow cytometry

For FACS analysis, adherent and floating cells were collected together and washed in ice-cold PBS. Cells were fixed in 1.0 ml of 70% cold ethanol (-20℃) and stored on ice for 2 h. Then cells were rehydrated in cold PBS and treated with RNaseA (50 μg/ml) at 37℃ for 30 min. After incubation, cells were rinsed twice in ice-cold PBS and resuspended in 2.0 ml PBS with 50 μg/ml propidium iodine (Sigma) at 4℃ for 2h. The cells were analyzed in a FACScan cell sorter (Becton Dickinson). Cell debris and fixation artifacts were gated out, and G1, S, and G2/M populations were quantified using the CELL QUEST program.

Western blot analysis

Cells (2×10^6) were plated in a 100-mm dish and infected with Ad-LacZ, Ad-p53 or Ad-p21 at the m.o.i of 20 pfu/cell as described above. At 12, 24, and 48h after infection, cells were harvested on ice, washed three times

with PBS, and lysed in a lysis buffer (10 mM KCl, 1.5 mM $MgCl_2$, 10 mM Tris (pH 7.4), 0.5% SDS, 1 mM PMSF). The cell lysate was disrupted with sonication for 30 sec and centrifuged at 15000 rpm for 3 min. The protein concentration in the supernatant was determined using a BCA protein assay kit (Pierce, Rockford, IL). Samples containing 5 μg of protein were denatured by boiling (for 4 min) in loading buffer (20% glycerol, 5% β-mercaptoethanol, 4% SDS, 125 mM Tris (pH 6.8), and 0.1 μg/ml of bromphenol blue) and then electrophoresed on a 12% SDS-PAGE. The separated protein was transferred onto a nitrocellulose membrane (Schleicher and Schuell). The membrane was blocked overnight at 4℃ using PBS-T (0.1% Tween 20 in PBS) containing 5% low-fat milk and probed with the primary antibodies as follows: 1 μg/ml mAb for p53 (DO-7; NOVOCASTRA); 1 μg/ml mAb for p53 (pAb1801; Oncogene Science); 1 μg/ml mAb for Waf1 (Waf1; NOVOCASTRA); 1 μg/ml mAb for Bcl-2 (Santa Cruz); 1 μg/ml pAb for Bax (Phar Mingen) in PBS-T. The blot was then washed with PBS-T and incubated with horseradish peroxidase conjugated to a secondary antibody. The specific complexes were detected by the ECL chemiluminescence reagent (Amersham).

DNA fragmentation analysis

Cells were seeded at 4×10^5/6cm dish and cultured for 24h. The cells were infected with 20 pfu/cell of the recombinant adenoviruses, incubated for one h and refed with DMEM with 1% FBS. DNA fragmentation assay was performed as follows. After incubation for 48 h, adherent and floating cells were collected and resuspended in 400 μl of 5mM Tris-HCl (pH8.0), 10mM EDTA and 0.5% Triton X-100. After centrifugation at 16,000g for 20 min, the supernatant was incubated with 100 μg/ml of RNaseA for one h at 37℃ and then with 200 μg/ml proteinase K and 1.0% SDS for 2h at 50℃. The solution was extracted with phenol followed by precipitation with ethanol. The precipitant was resuspended with TE buffer, electrophoresed in a 1.0% agarose gel and visualized by ethidium bromide staining.

Analysis of BrdU incorporation

The cells were infected with 20 pfu/cell of the recombinant adenovirus Ad-p21, incubated for one h and refed with DMEM with 1% FBS. At 12 h after transfection, BrdU (Becton Deckinson) was added at a final concentration of 20uM and the cells were incubated for 48 h at 37℃. Cells were treated with a mouse anti-BrdU monoclonal antibody (Dako) and biotin-conjugated goat anti-mouse IgG (Dako) and the ABC horseradish peroxidase method

(Dako).

RESULTS

Effect of p53 on human cancer cell proliferation

The effect of wild-type p53 on the growth suppression of human cancer cell lines was examined by the G418 colony formation assay. Table 2 shows that introduction of a p53 expression plasmid, pCMV-p53 resulted in substantial growth suppression. The growth suppression observed in MCF-7 containing wild-type p53 gene was less compared with that seen in HSC-3, ZR-75-1, BT549, and RE-RF-LC-OK having mutated p53 gene (Table 2).

Table 2 Effect of wt p53 on colony formation of human cancer cell lines[a]

	Numbers of G418-resistant colonies[b]				
DNA	HSC-3	MCF-7	ZR-75-1	BT549	RERF-LC-OK
pCMV-NeoBam	496(1.0)	136(1.0)	482(1.0)	710(1.0)	77(1.0)
pCMV-p53	119(0.2)	58(0.4)	92(0.2)	114(0.2)	8(0.1)

[a]Cells were transfected with pCMV-NeoBam and pCMV-p53 plasmids, split and cultured in the G418 media for 10 to 14 days.
[b]Total numbers of G418-resistant colonies obtained from three separate experiments are shown.

To evaluate the effect of exogenous wild-type p53 overexpression on the proliferation of human cancer cells, cell lines were infected with a replication defective adenovirus having a wild-type p53 cDNA expression cassette (Ad-p53). The relative efficiency of adenovirus infection was determined by X-gal staining of cells infected with a control adenovirus vector having the bacterial lacZ gene in place of the p53 gene (Ad-lacZ). In four of the five cell lines examined, >70% of the cells could be infected at a multiplicity of 20:1 (Table 1). The effect of wild-type p53 expression on these five lines was assessed by DNA fragmentation assay and flow cytometry analysis. Ad-p53 infection resulted in apoptosis in three lines (ZR-75-1, BT549 and RERF-LC-OK) (Fig.1), and in growth arrest (>50% decrease of cells in S phase in the absence of significant p53-dependent apoptosis) in one line (HSC-3) (Fig.2). In the other line, MCF-7 having endogenous wild-type p53, p53 overexpression had little effect on the flow cytometric patterns (Fig.2). We found that the exogenous p53 protein levels were comparable in all the cell lines examined (examples in Fig.3).

The role of p21

For further study, we selected three cell lines that exhibited representa-

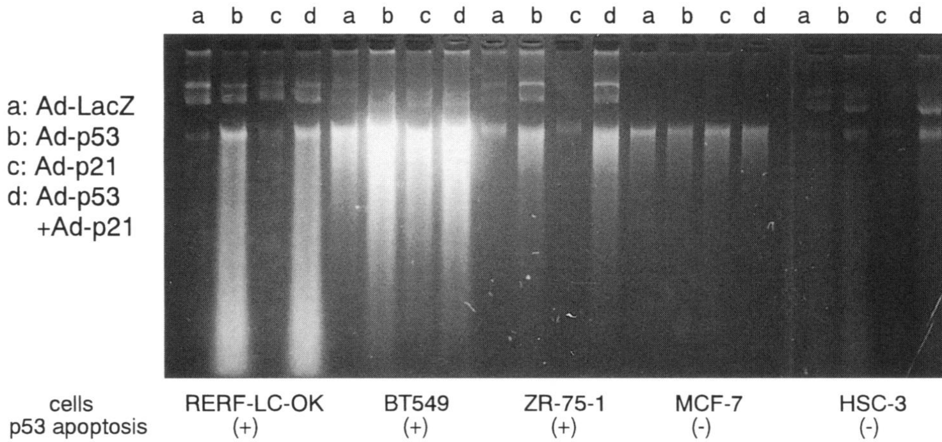

Fig. 1 *In vitro* chromosomal DNA fragmentation analysis. Cells were infected with Ad-p53 or Ad-LacZ at the MOI of 20 pfu/cell and then incubated for 48 h. Whole cells were extracted and fractionated by 1.5% agalose gel electrophoresis and then stained with 0.5 μg/ml of ethidium bromide.

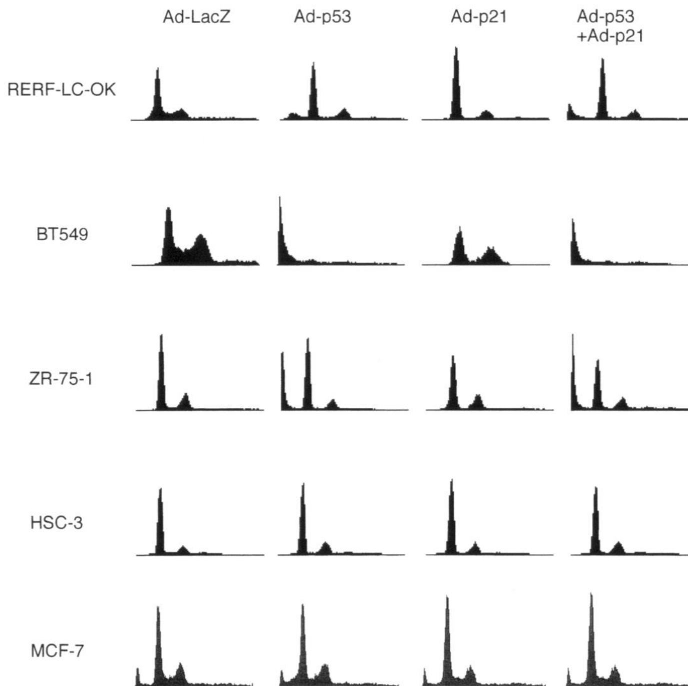

Fig. 2 Cell-cycle analysis of ZR-75-1, BT549, RERF-LC-OK, HSC-3, and MCF-7 cell lines infected with Ad-LacZ, p53, p21, p53+p21. Cells ($2X10^6$) were infected with each virus at the MOI of 20 pfu/cell for 48 h and subjected to flow cytometry.

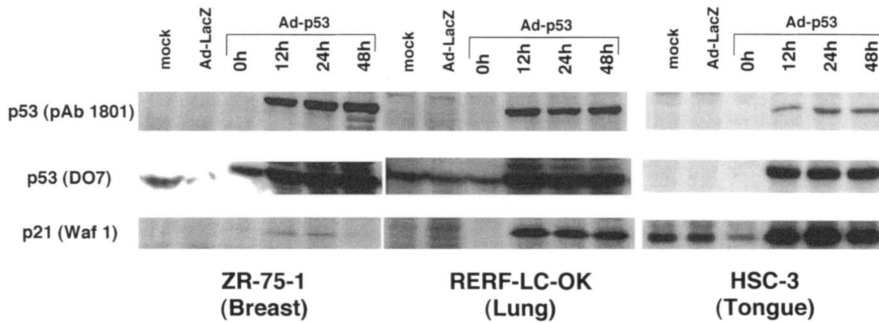

Fig. 3 Western blot analysis of Ad-p53-mediated protein expression in ZR-75-1, RERF-LC-OK and HSC-3 cells.
Cells were infected with Ad-p53 at the MOI of 20 pfu/cell for 0, 12, 24 and 48 h. For each group, 5 μg of cell lysate was separated on a 12% SDS-PAGE, electroblotted onto nitrocellulose membrane, and probed with the respective mAbs. Expression was detected using the enhanced chemiluminescence method.

tive apoptotic (ZR-75-1 and RERF-LC-OK) or growth arrest responses (HSC-3). Ad-p53 infected cells showed high levels of both p53 and p21 proteins 12h to 48h after infection (Fig.3), suggesting that exogenous p53 overexpression induced endogenous p21 in the cancer cell lines examined. p21 is a known mediator of p53-dependent growth arrest. To further study the properties of p21-induced growth arrest compared to p53-mediated growth apoptosis, we analyzed the effect of exogenous p21 overexpression on human cancer cells. Cancer cells were infected with Ad-p21, harvested at 48h post-infection, and then assayed for DNA content by flow cytometry. As expected, only p21 protein was increased in Ad-p21 infected cells; p21 expression peaked at 24h after infection in these three cell lines and had declined by 48h (data not shown).

Flow cytometric analysis revealed that cells showed a decreased fraction of S-phase cells resulting from an accumulation of most cells at the G0/G1 phase after Ad-p21 infection (Fig.2).

However, in contrast to Ad-p53 infected cells, Ad-p21 infected cells remained stably arrested for up to 48 hr after infection, with no cytometric signs of apoptosis (Fig.2). Furthermore, to confirm that p21 overexpression inhibited cell-cycle progression into the S-phase following its transfection into these cancer cells, DNA synthesis was assessed by BrdU incorporation. p21 inhibited new DNA synthesis in both ZR-75-1 and RERF-LC-OK cells (Fig.4). The extent of inhibition of BrdU incorporation following Ad-p21 infection was similar to Ad-p53 infection, although p21 did not appear to induce apoptosis in these cells (data not shown). Our results suggested that p21 inhibited

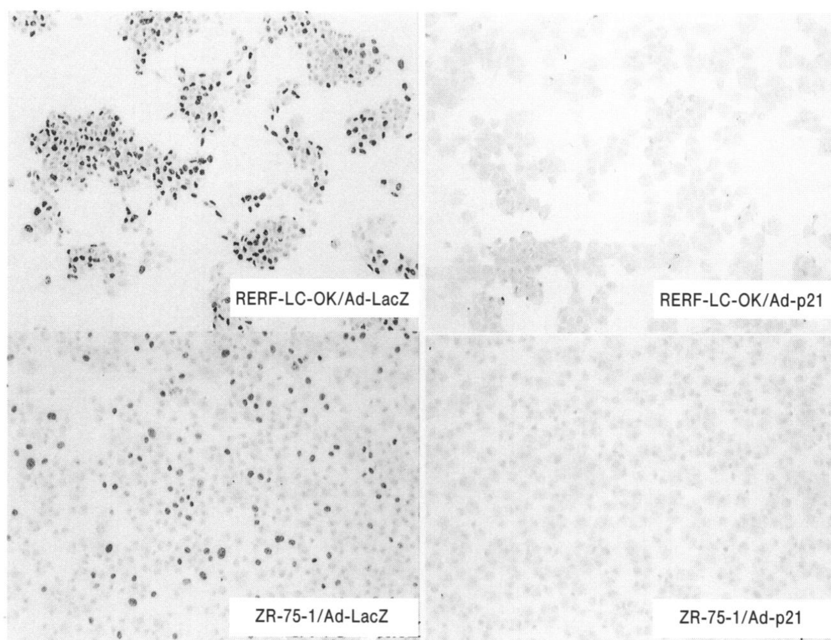

Fig. 4 BrdU incorporation of cancer cell lines after infection of Ad-p21
Immunohistochemical staining of RERF-LC-OK and ZR-75-1 cell with anti-BrdU antibody. Ad-LacZ infected (control) cells show strong nuclear BrdU incorporation (left), whereas Ad-p21 infected cells no reaction product (right).

cell-cycle progression into the S-phase in human cancer cells.

Apoptosis mediated by p53 can overcome p21 induced G1 arrest

To investigate the effect of p21 overexpression on p53-mediated apoptosis cell death in human cancer cell lines, double infection with both Ad-p53 and Ad-p21 was carried out at 20 MOI for 48 h and subjected to flow cytometry. As shown in Fig.2 (Ad-p53 + Ad-p21), introduction of exogenous p53 in RERF-LC-OK, BT549 and ZR-75-1 cells overcame p21-mediated cell cycle arrest at G1 and induced apoptosis, suggesting that this affect is a general event among human cancer cell lines.

The role of BAX/Bcl-2 in the response to p53

In an attempt to understand the mechanism of cytotoxicity of Ad-p53 infection on the five cell lines, expression of Bcl-2 and Bax gene products that may mediate apoptosis were analyzed by Western blotting (Fig.5). Bax protein was found in cell lines that underwent apoptosis in response to p53 overexpression (ZR-75-1 and RERF-LC-OK), whereas a small amout of Bax protein that did nor enter the apoptotic pathway even 48 hr after Ad-p53 infec-

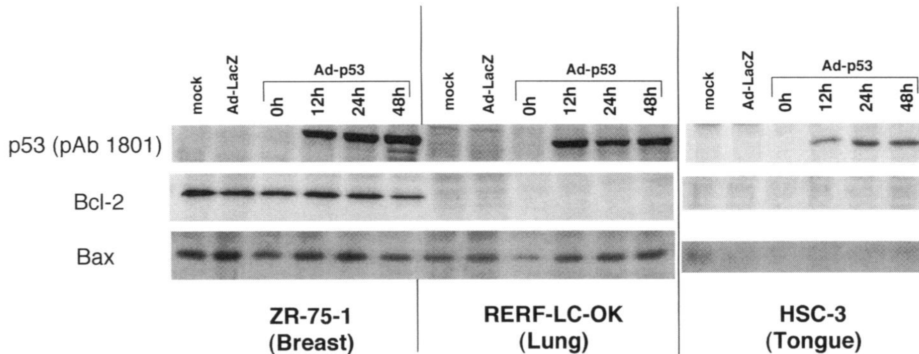

Fig. 5 Western blot analysis of Bcl-2 and Bax expression in ZR-75-1, RERF-LC-OK and HSC-3 cells.
Cells were infected with Ad-p53 at MOI of 20 pfu/cell for 0, 12, 24 and 48 h. For each group, 5 μg of cell lysate was separated on a 12% SDS-PAGE, electroblotted onto nitrocellulose membrane, and probed with the respective mAbs. Expression was detected using the enhanced chemiluminescence method.

tion was detected in HSC-3. However, Bcl-2 protein was detected only in ZR-75-1, suggesting that the Bcl-2 expression level had no correlation with the apoptosis in response to p53 overexpression. Therefore, Bax might be an important component of the p53-mediated apoptosis of human cancer cells.

DISCUSSION

We assessed the effect of exogenous wild-type p53 on the growth suppression of human cancer cell lines. Our results suggest that the status of endogenous p53 (wild-type or mutant) had a correlation with the cancer cell line's response to p53 overexpression. p53 overexpression had little effect on MCF-7 cells having endogenous wild-type p53. This study as well as previous ones has suggested that expression of wild-type p53 in cancer cells can result in either growth arrest or apoptosis[1,2]. To investigate the basis of this difference, we evaluated the role of p21, a mediator of p53-induced growth arrest. We found that overexpression of p21 itself induced a cell cycle arrest of several cancer cells at G1 phase and that p21 expression alone was not sufficient to trigger the apoptotic process. Furthermore, we demonstrated that combined introduction of p53 and p21 resulted in cell death as did p53 alone. p53-mediated apoptosis was not inhibited by p21-induced G1 arrest, suggesting that induction of p21 could not account for the differential response to p53. These observations were consistent with those reported previously[19]. In contrast, in the process of apoptosis induced by a DNA damaging

agents, caspase-3-mediated cleavage and inactivation of p21 protein may convert cancer cells from the condition of growth arrest to apoptosis[20).

Previous studies reported that two death regulator proteins, Bcl-2 and Bax, were affected oppositely by p53 and an increased Bax/Bcl-2 ratio made cells more susceptible to apoptosis under certain conditions[21,22]. We evaluated the role of Bax/Bcl-2 in the response to p53. Western blot analysis revealed no significant differences in the expression of Bax in the cell lines examined, before or after Ad-p53 infection. However, significantly more Bax protein was expressed in cell lines undergoing apoptosis than in cells displaying growth arrest. This result suggests that Bax might be an important component of the p53-mediated apoptosis of cancer cells. Further studies are required to evaluate whether biological and genetic determinants of p53-induced apoptosis and growth arrest.

Initial descriptions of oncogene and tumor suppressor gene action concentrated on relatively simple linear pathways. As more has become known about signal transduction, it has become clear that important cellular pathways are much more complex than originally believed. These complexities involve downstream and upstream branching as well as positive and negative feedback controls. Investigators have postulated that several genes are responsible for mediating p53-dependent apoptosis[23,24]. However, these studies have employed different cell types and have used a large and varied number of agents to elevate p53 expression. Therefore, it has been difficult to put mediators of p53-induced apoptosis into perspective or to interpret and compare the induction patterns observed. Viewed in the above context, the heterogeneity in apoptosis induction was not surprising. The presence or absence of p53-induced apoptosis is in turn likely to depend on other genetic alterations that have accumulated during the long history of neoplastic cells.

To examine the molecular events following wild-type p53 expression on a global level, cDNA microarrays will be helpful to define the biological and genetic determinants that dictate p53-induced apoptosis.

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REFERENCES

1. Symonds H, Krall L, Remington L, Saenz-Robles M, Lowe S, Jacks T,

Van Dyke T. p53-dependent apoptosis suppresses tumor growth and progression in vivo. Cell 1994, 78: 703-711.

2. Bates S, Vousden KH. p53 in signaling checkpoint arrest or apoptosis. Curr Opin Genet Dev 1996, 6: 12-18.
3. El-Deiry WS, Harper JW, O' Connor PM, Velculescu VE, Canman CE, Jackman J, Pietenpol JA, Burrell M, Hill DE, Wang Y, Wiman KG, Mercer WE, Kastan MB, Kohn KW, Elledge SJ, Kinzler KW, Vogelstein B. WAF1/CIP1 is induced in p53-mediated G1 arrest and apoptosis. Cancer Res 1994, 54: 1169-1174.
4. Deng C, Zhang P, Harper JW, Elledge SJ, Leder P. Mice lacking p21CIP1/WAF1 undergo normal development, but are defective in G1 checkpoint control. Cell 1995, 82: 675-684.
5. Brugarolas J, Chandrasekaran C, Gordon JI, Beach D, Jacks T, Hannon GJ. Radiation-induced cell cycle arrest compromised by p21 deficiency. Nature 1995, 377: 552-557.
6. Clarke AR, Purdie CA, Harrison DJ, Morris RG, Bird CC, Hooper ML, Wyllie AH. Thymocyte apoptosis induced by p53-dependent and independent pathways. Nature 1993, 362: 849-852.
7. Clarke AR, Gledhill S, Hooper ML, Bird CC, Wyllie AH. p53 dependence of early apoptotic and proliferative responses within the mouse intestinal epithelium following gamma-irradiation. Oncogene 1994, 9: 1767-1773.
8. Lowe SW, Schmitt EM, Smith SW, Osborne BA, Jacks T. p53 is required for radiation-induced apoptosis in mouse thymocytes. Nature 1993, 362: 847-849
9. Merritt AJ, Potten CS, Kemp CJ, Hickman JA, Balmain A, Lane DP, Hall PA. The role of p53 in spontaneous and radiation-induced apoptosis in the gastrointestinal tract of normal and p53-deficient mice. Cancer Res 1994, 54: 614-617.
10. Polyak K, Waldman T, He TC, Kinzler KW, Vogelstein B. Genetic determinants of p53-induced apoptosis and growth arrest. Genes Dev 1996, 10: 1945-1952.
11. Sakai E, Tsuchida N. Most human squamous cell carcinomas in the oral cavity contain mutated p53 tumor-suppressor genes. Oncogene 1992, 7: 927-933.
12. Bartek J, Iggo R, Gannon J, Lane DP. Genetic and immunochemical analysis of mutant p53 in human breast cancer cell lines. Oncogene 1990, 5: 893-899.
13. Noda A, Ning Y, Venable SF, Pereira-Smith OM, Smith JR. Cloning of senescent cell-derived inhibitors of DNA synthesis using an expression

screen. Exp Cell Res 1994, 211: 90-98.

14. Takahashi M, Ilan Y, Chowdhury NR, Guida J, Horwitz M, Chowdhury JR. Long term correction of bilirubin-UDP-glucuronosyltransferase deficiency in Gunn rats by administration of a recombinant adenovirus during the neonatal period. J Biol Chem 1996, 271: 26536-26542.
15. Ishida S, Yamashita T, Nakaya U, Tokino T. Adenovirus-mediated transfer of p53-related genes induces apoptosis of human cancer cells. Jpn J Cancer Res 2000, 91: 174-180.
16. Yamano S, Tokino T, Yasuda M, Kaneuchi M, Takahashi M, Niitsu Y, Fujinaga K, Yamashita T. Induction of transformation and p53-dependent apoptosis by adenovirus type 5 E4orf6/7 cDNA. J Virol 1999, 73: 10095-10103.
17. Baker SJ, Markowitz S, Fearon ER, Willson JK, Vogelstein B. Suppression of human colorectal carcinoma cell growth by wild-type p53. Science 1990, 249: 912-915.
18. van der Eb AJ, Graham FL. Assay of transforming activity of tumor virus DNA. Methods Enzymol 1980, 65: 826-839.
19. Kagawa S, Fujiwara T, Hizuta A, Yasuda T, Zhang WW, Roth JA, Tanaka N. p53 expression overcomes p21WAF1/CIP1-mediated G1 arrest and induces apoptosis in human cancer cells. Oncogene 1997, 15: 1903-1909.
20. Zhang Y, Fujita N, Tsuruo T. Caspase-mediated cleavage of p21Waf1/Cip1 converts cancer cells from growth arrest to undergoing apoptosis. Oncogene 1999, 18: 1131-1138.
21. Miyashita T, Reed JC. Tumor suppressor p53 is a direct transcriptional activator of the human bax gene. Cell 1995, 80: 293-299.
22. Oltvai ZN, Korsmeyer SJ. Checkpoints of dueling dimers foil death wishes. Cell 1994, 79:189-192.
23. Polyak K, Xia Y, Zweier JL, Kinzler KW, Vogelstein B. A model for p53-induced apoptosis. Nature 1997, 389: 300-305.
24. Buckbinder L, Talbott R, Velasco-Miguel S, Takenaka I, Faha B, Seizinger BR, Kley N. Induction of the growth inhibitor IGF-binding protein 3 by p53. Nature 1995, 377: 646-649.