Cancer Chemother Pharmacol (2001) 47: 161–169
DOI 10.1007/s002800000196

ORIGINAL ARTICLE

**Renqi Yuan · Qinghui Meng · Hao Hu**
**Itzhak D. Goldberg · Eliot M. Rosen · Saijun Fan**

# P53-independent downregulation of p73 in human cancer cells treated with Adriamycin

Received: 2 March 2000 / Accepted: 4 August 2000 / Published online: 12 January 2001


**Abstract** P73, a new p53 homologue, has been recently identified as a candidate tumor suppressor gene. *Purpose*: We studied the alterations in p73 in a panel of human cancer cell lines treated with the chemotherapeutic agent, Adriamycin (ADR), in comparison with the changes in p53. *Methods*: P73 and p53 mRNA and protein were determined using semiquantitative reverse transcription-polymerase chain reaction (RT-PCR) and Western blotting, respectively. ADR cytotoxicity was examined by a trypan blue dye exclusion assay. *Results*: The cell lines bearing wild-type p53 were more susceptible to ADR than the cell lines bearing mutant p53. ADR treatment resulted in a significant accumulation of p53 protein and mRNA expression in the wild-type p53 cell lines and caused little (slight increase) or no influence on p53 expression in the cell lines with p53 mutation and deletion. However, in striking contrast to the alterations in p53, a decline in p73 at both the protein and mRNA levels was observed in all the cell lines examined following ADR treatment. Further studies indicated that this p53-independent downregulation of p73 was induced by ADR in a dose- and time-dependent manner. Moreover, the p73 protein decline was abrogated by the presence of proteasome inhibitors. *Conclusions*: Our findings revealed that although p73 shares a similar structural and functional composition with p53, there is a significant difference in the mechanisms that govern the responses of p53 and p73 to ADR-induced DNA damage.

R. Yuan · Q. Meng · H. Hu · I. D. Goldberg
E. M. Rosen · S. Fan (✉)
Laboratory of Molecular Oncology,
Department of Radiation Oncology,
Long Island Jewish Medical Center,
The Long Island Campus for the Albert Einstein College
of Medicine, 270-05, 76th Avenue,
New Hyde Park, New York 11042, USA
E-mail: fan@lij.edu
Tel.: +1-718-4707456; Fax: +1-501-4233792

**Key words** Adriamycin · Cancer · p73 · p53 · Proteasome inhibitor

## Introduction

Wild-type p53 is known as a tumor suppressor gene because it activates or suppresses target genes that regulate genome stability, cell cycle progression, DNA damage and apoptosis induction. P73 is a newly identified *p53* family homologue which, at chromosome 1p36.33, encodes a protein of approximately 73 kDa with remarkable sequence similarity to p53, including 29% identity with p53 in the transcription activation domain (TAD, residues 1–43), 63% identity in the DNA binding domain (DBD, residues 113–290), and 38% identity in the oligomerization domain (OD, residues 330–360) [1]. P73 is a new candidate tumor suppressor, since alteration and loss of heterozygosity in a region of chromosome 1p36 at which p73 is located have been found in many types of cancers, such as gastric adenocarcinoma, neuroblastoma, lung cancer, colorectal cancer, prostate cancer, oligodendroglioma and others [1–4]. Moreover, the development of renal cell carcinoma is clearly associated with loss of imprinting or switching of allelic expression of the *p73* gene [5].

However, inactivation of p73 seems not to be essential in tumorigenesis of other types of tumors, such as breast cancer [6], ovarian adenocarcinoma [7], melanoma [8, 9], hepatocellular carcinoma [10], neuroblastoma [11] and esophageal carcinoma [12]. Due to significant homology to p53, p73 has some functions similar to p53, such as binding DNA and MDM2, activating p21 and Gadd45, inhibiting cell proliferation and triggering apoptosis [13–19]. Moreover, p73 transcription has been also found to be affected by expression of mutant p53 protein in some tumor cells [20].

Recently, attention has been paid to significant differences in some functions between p53 and p73. Firstly, p73 contains an additional conserved carboxyterminal region, and the alteration of this region can modulate p73

transactivation ability and other functions [21]. Secondly, in contrast to the degradation of p53 promoted by binding to MDM2, the half-life of p73 is not affected, and is even increased, by MDM2 [15, 22, 23]. Thirdly, p73 shows a degree of specificity for the promoters of target genes that is quantitatively distinct from the response mediated by p53 [16]. Fourthly, p73 is not affected or bound by oncoproteins such as adenovirus E4orf6, JC virus, E1B55K, E434K, SV40 T-antigen and human papillomavirus E6, which are known to bind to p53 and to inactivate the p53 protein [24–28]. Fifthly, there have been reports of a decrease or no alteration in p73 mRNA in response to UV irradiation [13, 29], while exposure to UV results in a significant upregulation of active p53 [29]. In addition, the apoptotic activation of p73 has been recently found to be modulated by tyrosine kinase c-bl1 through its binding to c-bl1 and by phosphorylation caused by c-bl1 [17–19].

In this study, we investigated the effect of a cancer chemotherapeutic agent, Adriamycin (doxorubicin, ADR), on protein and mRNA expression of p73 and p53 using Western blotting and semiquantitative reverse transcription-polymerase chain reaction (RT-PCR), respectively, in eight human cancer cell lines showing different *p53* gene status.

## Materials and methods

### Cell cultures, proteasome inhibitors and Adriamycin treatment

Human cancer cell lines were initially purchased from the American Type Culture Collection (ATCC, Manassas, Va.), and cultured in 10% DMEM supplemented with 2 m*M* glutamine, 100 μg/ml streptomycin, 100 U/ml penicillin and 10% fetal calf serum (Bio-Whittaker, Walkersville, Md.) at 37 °C in an atmosphere containing 5% $CO_2$. ADR was purchased from Sigma (St. Louis, Mo.), dissolved in sterilized $ddH_2O$ and stored at –20°C until use. Lactacystin and carbobenzoxyl-leucinyl-leucinyl-leucinal-D-norleucinal β-lactone (MG-132), were obtained from Calbiochem (La Jolla, Calif.). These proteasome inhibitors were dissolved in DMSO and stored in –20°C prior to use.

### RNA isolation and semiquantitative RT-PCR

Expression of p73 and p53 mRNAs was assayed using a semiquantitative RT-PCR assay as described previously [30]. Briefly, total RNA was isolated from subconfluent proliferating cells untreated or treated with ADR using a TriPure reagent (Boehringer Mannheim, Germany) following the manufacturer's instructions. Total RNA (5 μg) was transcribed using Superscript II reverse transcriptase (GIBCO Life Technologies, Gaithersburg, Md.). Aliquots of cDNA corresponding to 0.5 μg of original RNA were used for PCR amplification that was performed in a Perkin-Elmer DNA thermal cycler. For p53 and β-actin (as a control), the amplification reaction was performed with a "hot start" protocol, starting with 94°C for 3 min, then 94°C for 1 min, 50°C for 1 min, 72°C for 1 min, followed by a final incubation at 72°C for 7 min in a Perkin-Elmer DNA thermal cycler for 30 cycles. For p73, the samples were subjected to 30 cycles of denaturation at 94°C for 3 min, 94°C for 1 min, and 68°C for 5 min. The final extension step at 72°C was lengthened to 10 min.

Three specific primers were designed using a Primer Premier Program (Premier Biosoft International, Calif.) based on human cDNA sequences retrieved from the GenBank database, and synthesized by a DNA synthesizer (Life Technologies, Gathersburg, Md.). The sequences of each primer and expected size of PCR products were as follows: *p53* 5′ TCTGGGACAGCCAAGTCTGT 3′ (forward), 5′ GGAGTCTTCCAGTGTGATGA 3′ (reverse), 435 bp; *p73* 5′ CACCCACTCGCCCTACGCACAA 3′ (forward), 5′ TGCCGGTGACAGGGTCATCCAC 3′ (reverse), 417 bp; *β-actin* 5′ TTGTTACCAACTGGGGACGATA 3′ (forward), 5′ TCGTGGTTCTAGTTCTAG 3′ (reverse), 764 bp. The 435 bp segment amplified from p53 was located from position 560 to 994 in the published cDNA sequence (GenBank accession number K03199). The 417 bp segment amplified from p73 was located from position 392 to 808 in the published cDNA sequence (GenBank accession number Y11416). β-Actin, amplified as a control, resulted in a 764 bp product located from position 265 to 1028 in the published cDNA sequence (GenBank accession number G34615). The specificity of each of the PCR products was confirmed by complete sequencing of the purified product at the DNA sequencing facility at the Albert Einstein College of Medicine (Bronx, N.Y.). The PCR products (10 μl) were electrophoresed on a 2% agarose gel containing ethidium bromide and bands were visualized by UV illumination. A 100–2070 bp DNA molecular weight marker was loaded as a size control.

### Western blot assay

Analysis of protein expression was performed using a Western blot assay as described previously [30]. Briefly, untreated or treated cells were collected and lysed using a mini-modified ice-cold RIPA lysis buffer containing protease inhibitors (Calbiochem, San Diego, Calif.) and 100 m*M* sodium orthovanadate. Cell lysates containing an equal amount of total protein (50 or 75 μg) were electrophoresed on 10% SDS-polyacrylamide gels and transferred to polyvinylidene difluoride membranes (Millipore, Mass.) by electroblotting. The membranes were incubated with primary antibodies for p53 (Ab-2, 1:1000 dilution) and p73 (Ab1, 1:100 dilution) for 1 h, and then incubated with secondary antibodies for another 1 h. All the antibodies were obtained from Oncogene Research Products (Cambridge, Mass.). Ab-2 p53 (Pab1801) monoclonal antibody recognizes either wild-type or mutant p53 protein (around 53 kDa). Ab-1 p73 monoclonal antibody recognizes only p73α protein (around 80 kDa), a major isoform of p73 protein. Antibody reaction was revealed using an enhanced chemiluminescence kit (Amersham Life Science) as instructed by the manufacturer. Equal protein loading was confirmed by immunoblotting against polyclonal actin antibody (I-19, Santa Cruz, Calif.) to determine actin protein expression (around 46 kDa). A colored marker (Bio-Rad Laboratories, Hercules, Calif.) was used as a molecular size standard.

### Analysis of cell viability

Cell viability, an indicator of cytotoxicity, was evaluated using a trypan blue dye exclusion assay as described previously [30]. Briefly, cells untreated or treated with 10 μ*M* ADR were collected and resuspended in an equal volume of 0.4% trypan blue dye and stained for 1–2 min. Trypan blue-positive and -negative cells were recorded using a hemacytometer in at least three independent experiments in each of which at least two fields of over 500 cells were assessed. Wilcoxon's rank sum test was used to compare the cytotoxicity of ADR between the cell lines bearing wild-type p53 and the cell lines bearing mutant p53.

## Results

### ADR-induced alteration in p53 and p73

A panel of eight human cancer cell lines with different *p53* gene status were treated with 10 μ*M* ADR for 2 h

(except PA-1 cells which were treated with 1 μ*M* ADR for 2 h), washed with drug-free medium and then collected to analyze cell viability and expression of p73 and p53 mRNA and protein 24 h following treatment. The *p53* gene status of each cell line studied is presented in Table 1. The trypan blue dye exclusion assay showed that 10 μ*M* ADR (1 μ*M* ADR in PA-1 cells) resulted in a loss of cell viability, indicating cytotoxicity, of different magnitudes in these cell lines (Fig. 1a). However, the cell lines bearing wild-type p53 were significantly more sensitive to ADR than those bearing mutant p53. Approximately 77% of wild-type p53 cell lines, excluding the PA-1 cell line, lost viability, whereas about 26% of mutant p53 cell lines lost viability ($P < 0.05$) following treatment with 10 μ*M* ADR. PA-1 was the cell line most sensitive to ADR among the cell lines examined, losing about 75% cell viability relative to untreated control cells following exposure to 1 μ*M* ADR. These results indicate that ADR shows a strong p53-dependent cytotoxicity in the cancer cell lines examined.

To characterize the expression of p73 mRNA, we designed a pair of oligonucleotide primers specific for p73 mRNA (positions 392 to 808) using a Primer Premier Program (Premier Biosoft International, Calif.) based on the published original cDNA sequence of the *p73* gene (GenBank accession no. Y11416) after careful comparison with the cDNA sequence of the *p53* gene (GenBank accession no. K03199). Single PCR products of 417 bp for p73, 435 bp for p53 and 764 bp for *β*-actin (as a control) were detected as expected. As illustrated in Fig. 1b, a significant downregulation of p73 mRNA was observed in all the cell lines after treatment with 10 μ*M* ADR (1 μ*M* for PA-1 cells). However, in contrast to a decrease in p73 mRNA, ADR caused a significant increase in p53 mRNA in the four cell lines with wild-type p53 (MCF-7, RKO, LNCap and PA-1) and resulted in little (slight increase) or no alteration in p53 mRNA expression in the two cell lines with p53 mutation (MDA-MB-231 and Du-145) and the two cell lines with p53 deletion (Tsu-Pr1 and SK-OV-3).

Consistent results were also obtained for the alterations in p73 and p53 protein expression in these cell lines after ADR treatment, as determined using Western blotting (Fig. 1c). For example, breast cancer MCF-7 cells (wild-type p53) contained a low basal level of p53 protein and showed 5.7-fold and 6.7-fold increase in the expressions of p53 mRNA and protein, while the expression of p73 mRNA and protein decreased to 28% of the untreated control levels and to an undetectable level after treatment with 10 μ*M* ADR, respectively. At the same dose in prostate cancer Du-145 cells (p53 mutation), p73 mRNA and protein expression significantly declined to 27% and 15% of the corresponding untreated control levels, respectively, but neither p53 mRNA expression nor protein expression was affected. These observations demonstrate that ADR-induced p73 downregulation occurred at both the mRNA and protein levels and there was a *p53* gene- and function-independent regulation of p73 in the cancer cells that showed ADR-induced DNA damage. In addition, compared with the cell viability results (Fig. 1a), these studies showed no significant relationship between ADR cytotoxicity and p73 decline.

## Dose- and time-dependence of ADR-induced p73 downregulation

To elucidate the effect of ADR on p73 expression, further studies were performed in two human prostate cancer cell lines, one with wild-type p53 (LNCap) and one with mutant p53 (Du-145). As shown in Fig. 2a, b, exposure to ADR (2 h) caused a marked dose-dependent accumulation of both p53 mRNA and protein levels at a fixed time of 24 h after treatment in LNCap cells, whereas p53 mRNA and protein did not change or slightly increased in Du-145 cells. However, in both LNCap and Du-145 cells, a dose-dependent downregulation of p73 was observed at both the mRNA and protein levels. The decline pattern of p73 was very similar in both cell lines. The dose required for the reduction in mRNA and protein levels to 50% of the corresponding untreated control level was approximately 6–7 μ*M* in LNCap and Du-145 cell lines. Furthermore, following 2 h treatment with a fixed ADR

**Table 1** Alterations in p53 and p73 mRNA and protein expression in human cancer cell lines treated with 10 μ*M* ADR based on the alterations in p53 and p73 mRNA and protein expression shown in Fig. 1b, c (↑ increase, *s*↑ slight increase, – no-change, ↓ decrease)

| Cell line | Cancer type | mRNA alteration[a] | | Protein alteration[a] | |
|---|---|---|---|---|---|
| | | p53 | p73 | p53 | p73 |
| Wild-type p53 cell lines | | | | | |
| MCF-7 | Breast | ↑ | ↓ | ↑ | ↓ |
| PA-1 | Ovary | ↑ | ↓ | ↑ | ↓ |
| RKO | Colon | ↑ | ↓ | ↑ | ↓ |
| LNCap | Prostate | ↑ | ↓ | ↑ | ↓ |
| Mutant p53 cell lines | | | | | |
| MDA-MB-231 | Breast | s↑ | ↓ | s↑ | ↓ |
| SK-OV-3 | Ovary | – | ↓ | – | ↓ |
| Tsu-Pr1 | Prostate | – | ↓ | – | ↓ |
| Du-145 | Prostate | – | ↓ | s↑ | ↓ |

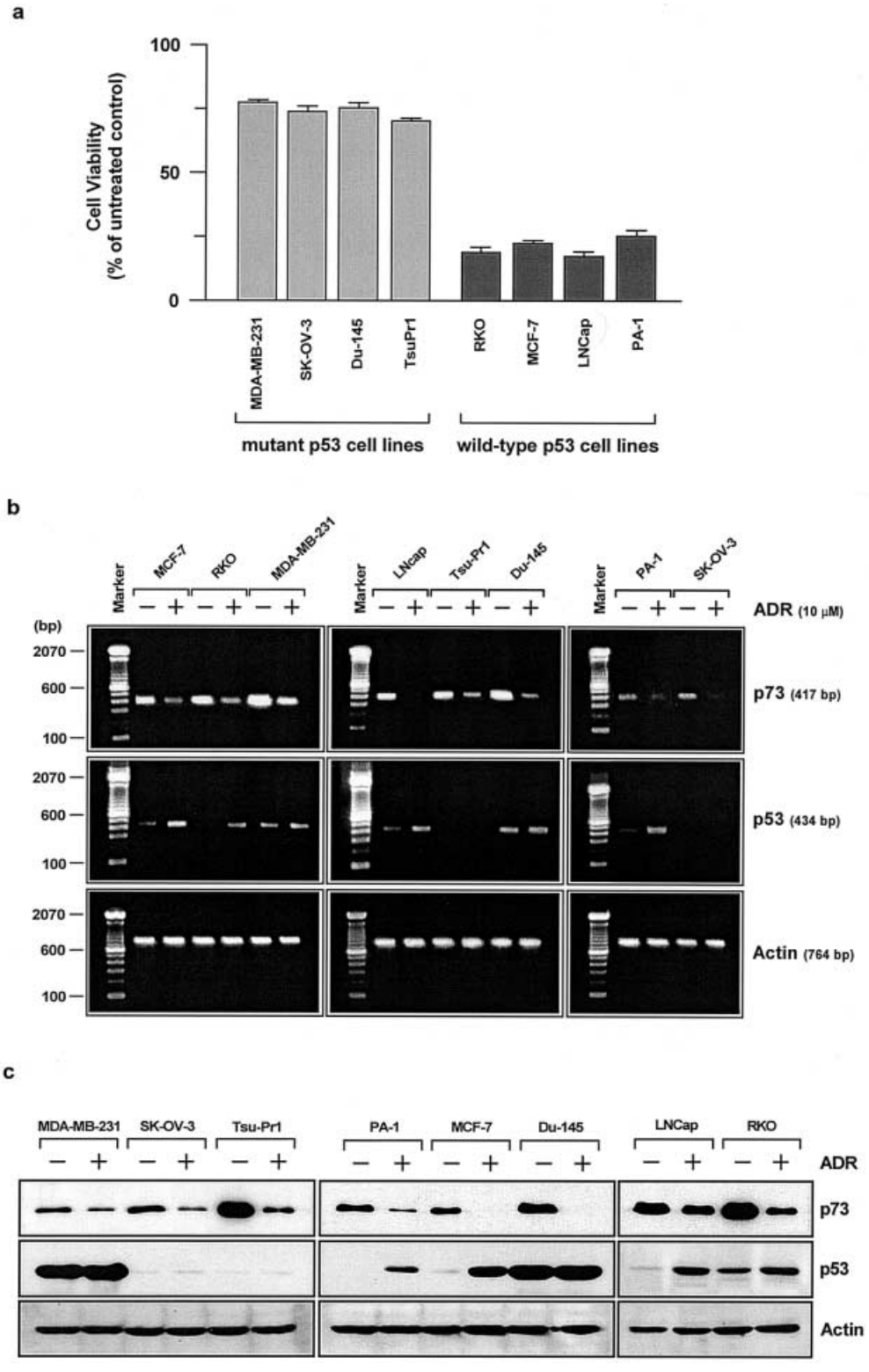


**Fig. 1a–c** Effect of ADR on cell viability and expression of p53 and p73 in human cancer cell lines. Subconfluent proliferating cells were treated with 10 μ*M* ADR for 2 h (except PA-1 cells which were treated with 1 μ*M* ADR for 2 h), washed with drug-free medium incubated for a further 24 h and then harvested for trypan blue dye exclusion assay (**a**), semiquantitative RT-PCR assay (**b**) and Western blotting (**c**). **a** The mean cell viability for wild-type p53 cell lines ($n = 3$, excluding PA-1) is $24 \pm 4\%$, and the mean cell viability for mutant p53 cell lines ($n = 4$) is $74 \pm 2\%$. **c** Equal amounts of protein (50 μg) were loaded in each lane

dose of 10 μ*M*, the decrease in both p73 mRNA and protein levels was first observed as early as 1 h and became undetectable 32 h after treatment (Fig. 3a, b). This suggests that the regulation of p73 is an early event following exposure of tumor cells to ADR.

The time required for the reduction in mRNA and protein levels to 50% of the respective untreated control levels was approximately 7 h in LNCap cells and 6 h in Du-145 cells. In contrast to p73, after exposure to ADR there was a time-dependent increase in p53 mRNA and

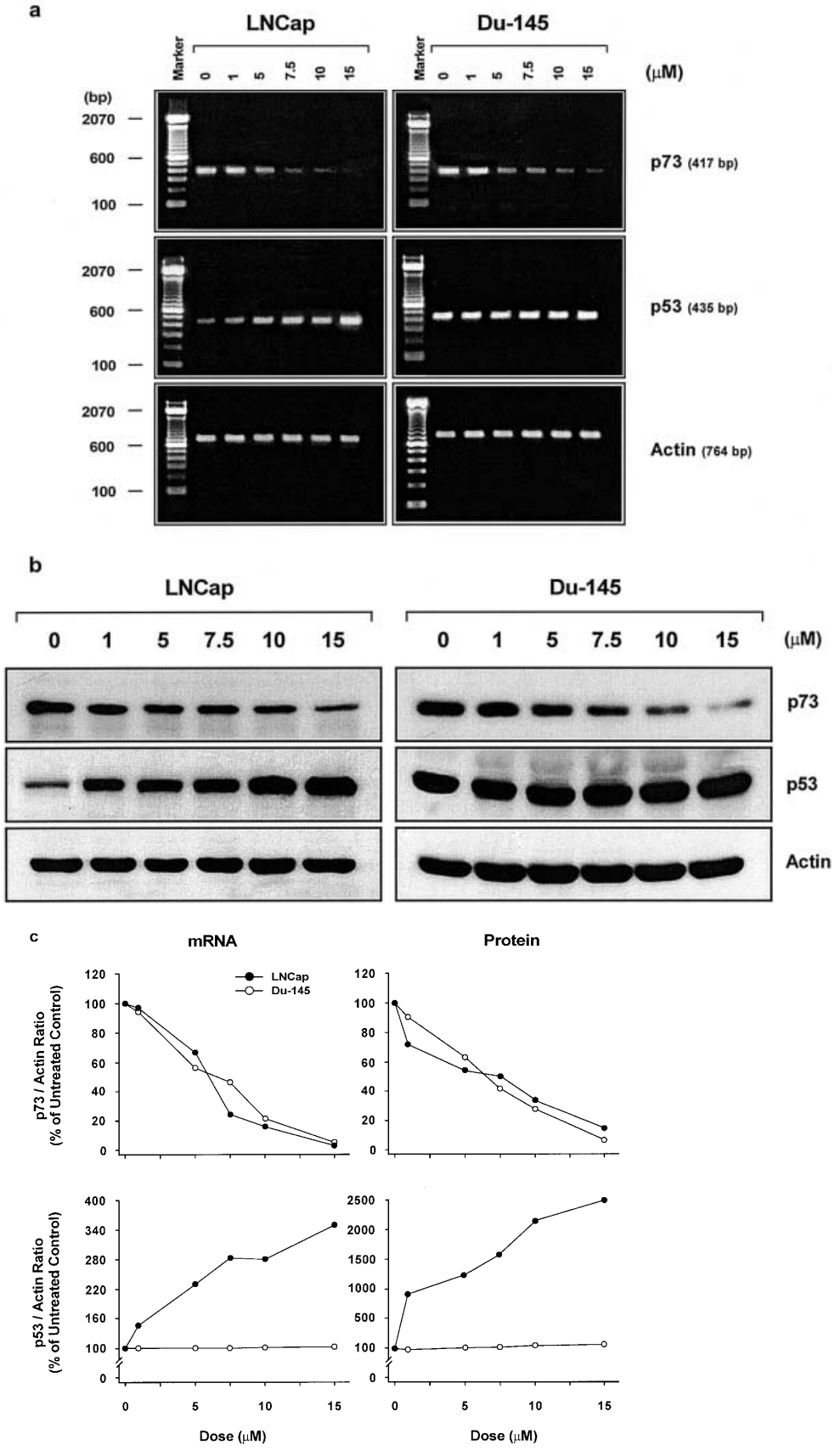


**Fig. 2a–c** Dose-dependent effects of ADR on p73 and p53 expression in human prostate cell lines with a wild-type (LNCap) and mutant (Du-145) *p53* gene status. Subconfluent proliferating cells were exposed to ADR at the indicated doses for 2 h, washed with drug-free medium, incubated for a further 24 h and then harvested for analysis by semiquantitative RT-PCR (**a**) and Western blotting (**b**). **b** Equal amounts of protein (50 μg) were loaded in each lane. **c** The mRNA and protein bands in **a** and **b** were quantitated by densitometry (these values are expressed relative to the respective actin control bands)

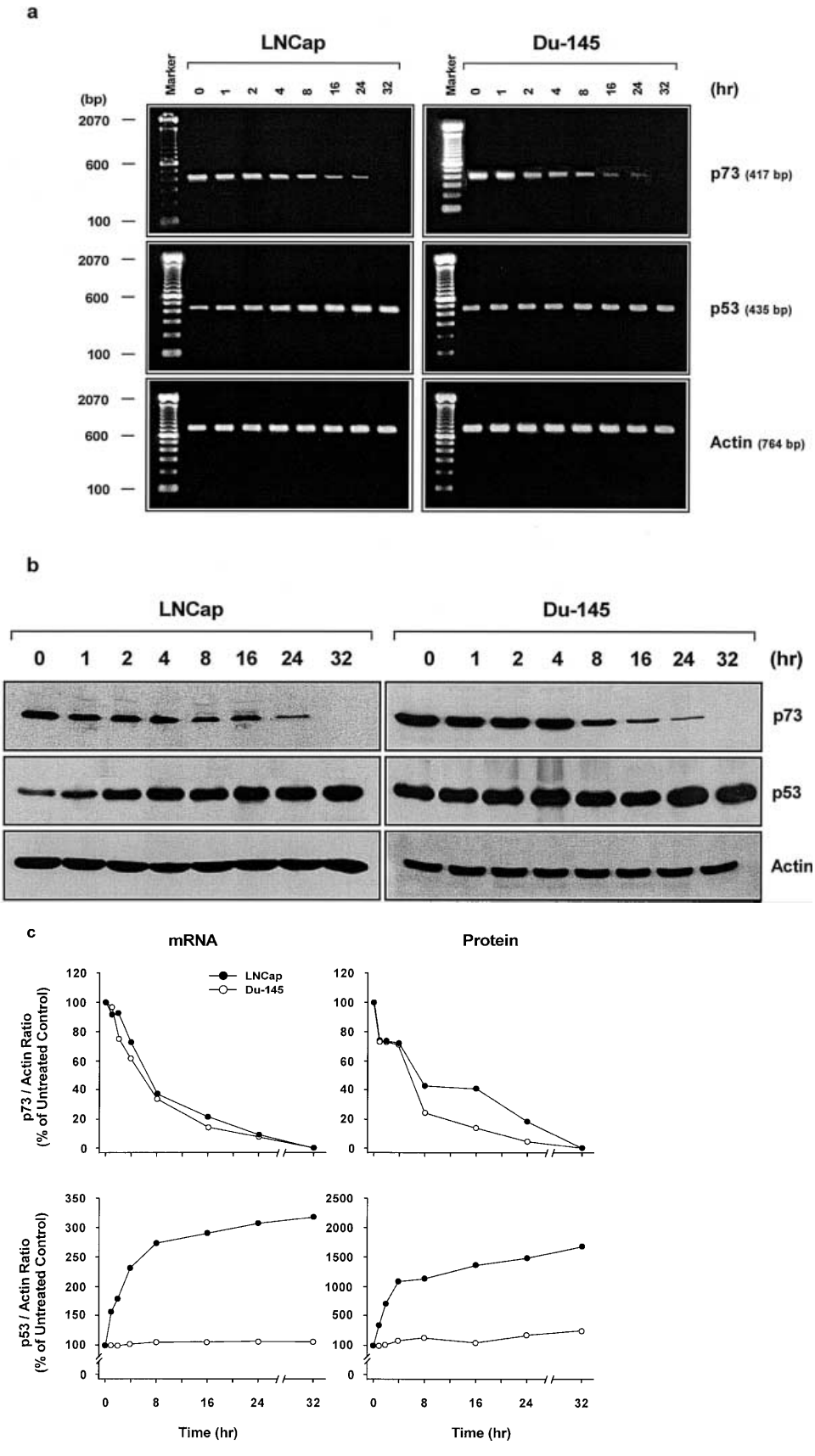


**Fig. 3a–c** Time-dependent effect of ADR on p73 and p53 in human prostate cell lines with wild-type (LNCap) and mutant (Du-145) *p53* gene status. Subconfluent proliferating cells were treated with 10 μ*M* ADR for 2 h, washed with drug-free medium, further incubated and harvested at the indicated times for analysis by semiquantitative RT-PCR (**a**) and Western blotting (**b**). **b** Equal amounts of protein (50 μg) were loaded in each lane. **c** The mRNA and protein bands in **a** and **b** were quantitated by densitometry (these values are expressed relative to the respective actin control bands)

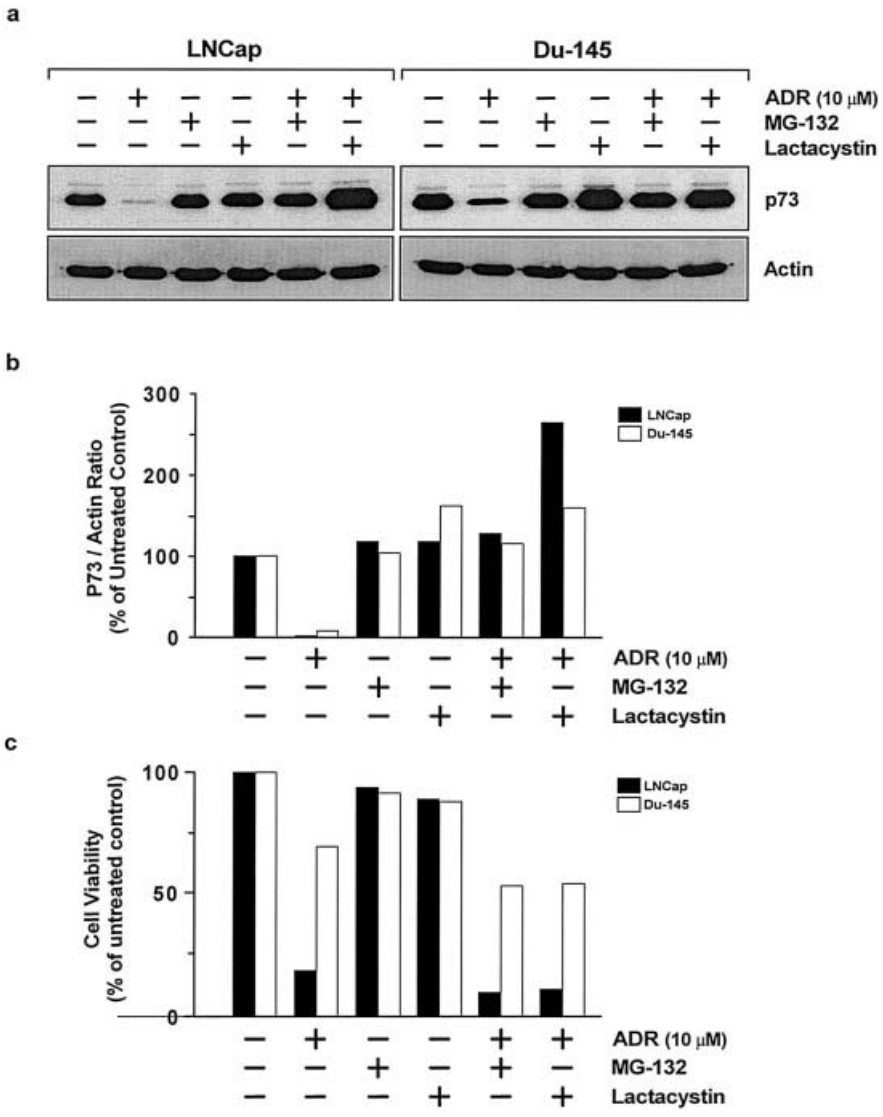


**Fig. 4a–c** Effect of proteasome inhibitors on ADR-mediated regulation of p73 proteins. Subconfluent proliferating cells were treated with 0 or 10 μ*M* ADR for 2 h, washed with drug-free medium and further incubated in medium containing 10 μ*M* lactacystin or 10 μ*M* MG132 for 6 h. The cells were washed again incubated for a further 18 h and finally harvested for Western blotting (**a**) and trypan blue dye exclusion (**c**). Equal amounts of protein (75 μg) were loaded in each lane. **b** Quantitation of p73 protein bands by densitometry relative to actin and untreated bands. **c** Data are means ± SE from three independent experiments

protein expression in LNCap cells, no alteration or a slight increase in p53 mRNA and protein levels with the time course seen in Du-145 cells. These findings indicate a dose- and time-dependence in the ADR-induced decrease in p73, regardless of p53. Bar graphs showing the densitometric quantitation of the p53 and p73 mRNA and protein bands as a function of ADR dose and post-treatment time are shown in Figs. 2c and 3c, respectively.

## Effect of proteasome inhibitors on ADR-mediated regulation of p73 protein

It is known that the ubiquitin-proteasome degradation pathway is involved in the regulation of many proteins, including p53 [31]. To examine the potential role of this regulation pathway in the ADR-induced downregulation of p73 protein, LNCap and Du-145 cells were treated with 0 or 10 μ*M* ADR for 2 h, washed with ADR-free medium and then incubated for another 6 h in the presence or absence of two selective proteasome inhibitors (lactacystin or MG132, 10 μ*M*). The cells were incubated for a further 18 h and collected for Western blot assay. In agreement with the results described above, exposure of LNCap and Du-145 cells to ADR resulted in a dramatic decline in p73 protein expression, by approximately 94% and 82%, respectively, relative to the respective untreated control (Fig. 4a). Under basal conditions, both proteasome inhibitors increased p73 protein expression.

The proteasome inhibitor-induced alterations in p73 protein levels were qualitatively similar in LNCap and Du-145 cell lines. Treatment with ADR plus inhibitors resulted in a significant increase as compared with the effect of ADR alone in the two cell lines, and even higher than that with inhibitors alone. A dramatic increase was found in Du145 cells treated with ADR plus lactacystin. Therefore, the increased p73 levels resulted from stabilization of the protein. These findings indicate that in addition to decreased p73 mRNA expression, the prostate cancer cells treated with ADR also exhibited

downregulation of p73 protein level through increased degradation in proteasomes. There was an increase in the cytotoxicity in both LNCap and Du-145 cell lines treated with ADR in the presence of proteasome inhibitors compared with that in the absence of proteasome inhibitors (Fig. 4c).

## Discussion

We report here for first time that ADR induces a significant decrease in p73 mRNA and protein expression in a variety of human cancer cell lines regardless of the genetic status and function of p53, although it results in p53-dependent cytotoxicity. ADR is a potent DNA topoisomerase II inhibitor and is a commonly used chemotherapeutic agent in the treatment of cancer. Studies in two human prostate cancer cell lines, LNCap (wild-type p53) and Du-145 (mutant p53), revealed a dose- and time-dependent pattern in the ADR-induced downregulation of p73, and the reductions in p73 mRNA and protein were generally similar in these two cell lines. Moreover, the decline in p73 protein was abrogated in the presence of selected proteasome inhibitors (lactacystin and MG132), indicating that the ubiquitin-proteasome degradation pathway also contributes to the decrease in p73 protein levels observed following ADR treatment, in addition to the decreased expression of p73 mRNA.

P73 exhibits four isoforms, $\alpha$, $\beta$, $\gamma$ and $\epsilon$, which differ at their carboxytermini as a result of differential splicing of p73 mRNA and in their ability to form homotypic interactions [1, 21]. All these splicing variants contain the same cDNA sequences present in the PCR products obtained by the primers we used to analyze p73 mRNA expression, so the alteration in these four p73 mRNA isoforms should be similar, i.e. a decrease in response to ADR. However, we do not know whether various p73 protein isoforms show similar decreases after exposure to ADR, since the Ab-1 p73 antibody used in our protein studies only recognized the p73$\alpha$ protein. Therefore, further investigations in our laboratory are under way to determine whether other p73 isoform proteins are also downregulated after ADR treatment.

Although the mechanism involved in the downregulation of p73 by ADR is not yet understood, several lines of evidence suggest that regulation of multiple genes may be involved in the p73's response to ADR. We have previously reported that ADR downregulates BRCA1, BRCA2, Rad51 and p300 mRNAs in human prostate cancer cells [30, 32]. All these genes have been shown to be involved in the regulation and function of p53. BRCA1 and BRCA2 are *br*east and ovarian *ca*ncer susceptibility genes. Germ-line mutations of BRCA1 confer a risk of development of breast, ovarian and prostate cancers [33–35]. BRCA1 knockout embryos exhibit decreased expression of the p53 transcriptional inhibitor Mdm-2, normal levels of p53, and increased levels of p21 [36]. P53 mutation partially rescues developmental arrest in Brca1- and Brca2-null mice [37]. BRCA1 also contains BRCT, a p53-binding protein (53BP1) at the protein C-terminus [38]. In addition, our unpublished data reveals that overexpression of BRCA1 increases p73 mRNA expression. P53 forms a specific protein complex with Rad51 that plays an important role in DNA recombination and DNA strand break repair [39] as well as binding to p300 that functions as a cofactor for many transcription genes. A dominant negative form of p300 prevents transcriptional activation by p53 [40]. Transactivation of both p53 and p73 requires the function of the p300/TBP/TFIIB complex [16, 41]. Therefore, further studies are in progress to investigate whether p73 interacts with these genes and the possible role of these genes in the modulation of p73's response to ADR.

It was found that, as well as conserved transcriptional activation, DNA binding and oligomerization domains shared with p53, p73 has an additional conserved C-terminal region containing a small five-helix bundle with striking similarity to the SAM (sterile alpha motif) domains of two ephrin receptor tyrosine kinases [21, 42, 43]. The SAM domain is a putative protein-protein interaction domain found in a variety of cytoplasmic signaling proteins and has been shown to form both homo- and hetero-oligomers. This domain of p73 can interact with additional, as yet uncharacterized, proteins in a signaling and/or regulatory role [42, 43], moreover, changes in this region can modulate p73 transactivation and other functions [21]. Thus, it would be of interest to explore whether this region is involved in the response of p73 to DNA damage.

In conclusion, regardless of the underlying mechanism(s), our present study for the first time demonstrated that ADR causes downregulation of p73 expression through a p53-independent pathway in human cancer cell lines and provided experimental evidence that p73 may be a new target gene in the therapy of cancers resistant to commonly used chemotherapeutic agents due to dysfunction of the *p53* gene. Together with other members of the p53 family, these studies also provide further information in the unsolved field of p53 function and regulation in cancer chemotherapy.

## References


1. Kaghad M, Bonne H, Yang A, Creabcier L, Biscan JC, Valent S, Minty A, Chalon P, Lelias JM, Dumont X, Ferrara P, McKeon F, Caput D (1997) Monoallelically expressed gene related to p53 at 1p36, a region frequently deleted in neuroblastoma and other human cancers. Cell 90: 809
2. Takahashi H, Ichimiya S, Nimura Y, Watanabe M, Furusato M, Wakui S, Yatani R, Aizawa S, Nakagawara A (1998) Mutation, allelotyping, and transcription analyses of the p73 gene in prostatic carcinoma. Cancer Res 58: 2076
3. Nomoto S, Haruki H, Kondo M, Konishi H, Takahashi T, Takahashi T, Takahashi T (1998) Search for mutations and examination of allelic expression imbalance of the p73 gene at 1p36.33 in human lung cancers. Cancer Res 58: 1380
4. Yokozaki H, Shitara Y, Fujimoto J, Hiyama T, Yasui W, Tahara E (1999) Alterations of p73 preferentially occur in

gastric adenocarcinomas with foveolar epithelial phenotype. Int J Cancer 83: 192
5. Mai M, Qian C, Yokomizo A, Tindall DJ, Bostwick D, Polychronakos C, Smith DI, Liu W (1998) Loss of imprinting and allele switching of p73 in renal cell carcinoma. Oncogene 17: 1734
6. Zaika AI, Kovalev S, Marchenko ND, Moll UM (1999) Overexpression of the wild type p73 gene in breast cancer tissues and cell lines. Cancer Res 59: 3257
7. Imyanitov EN, Birrell GW, Filippovich I, Sorokina N, Arnold J, Mould MA, Wright K, Walsh M, Mok SC, Lavin MF, Chenevix-Trench G, Khanna KK (1999) Frequent loss of heterozygosity at 1p36 in ovarian adenocarcinomas but the gene encoding p73 is unlikely to be the target. Oncogene 18: 4640
8. Kroiss MM, Bosserhoff AK, Vogt T, Buettner R, Bogenrieder T, Landthaler M, Stolz W (1998) Loss of expression or mutations in the p73 tumour suppressor gene are not involved in the pathogenesis of malignant melanomas. Melanoma Res 8: 504
9. Tsao H, Zhang X, Maijewski P, Haluska FG (1999) Mutational and expression analysis of the p73 gene in melanoma cell lines. Cancer Res 59: 172
10. Mihara M, Nimura Y, Ichimiya S, Sakiyama S, Kajikawa S, Adachi W, Amano J, Nakagawara A (1999) Absence of mutation of the p73 gene localized at chromosome 1p36.3 in hepatocellular carcinoma. Br J Cancer 79: 164
11. Kovalev S, Marchenko N, Swendenman S, LaQuaglia M, Moll UM (1998) Expression level, allelic origin, and mutation analysis of the p73 gene in neuroblastoma tumors and cell lines. Cell Growth Differ 9: 897
12. Nimura Y, Mihara M, Ichimiya S, Sakiyama S, Seki N, Ohira M, Nomura N, Fujimori M, Adachi W, Amano J, He M, Ping YM, Nakagaeara A (1998) p73, a gene related to p53, is not mutated in esophageal carcinomas. Int J Cancer 78: 437
13. Jost CA, Marin MC, Kaelin WG Jr (1987) p73 is a human p53-related protein that can induce apoptosis. Nature 389: 191
14. Zhu J, Jiang J, Zhou W, Chen X (1998) The potential tumor suppressor p73 differentially regulates cellular p53 target genes. Cancer Res 58: 5061
15. Balint E, Bates S, Vousden KH (1999) Mdm2 binds p73 alpha without targeting degradation. Oncogene 18: 3923
16. Lee CW, La Thangue NB (1999) Promoter specificity and stability control of the p53-related protein p73. Oncogene 18: 4171
17. Yuan ZM, Shioya H, Ishiko T, Sun X, Gu J, Huang YY, Lu H, Kharbanda S, Weichselbaum R, Kufe D (1999) p73 is regulated by tyrosine kinase c-Abl in the apoptotic response to DNA damage. Nature 399: 814
18. Agami R, Blandino G, Oren M, Shaul Y (1999) Interaction of c-Abl and p73alpha and their collaboration to induce apoptosis. Nature 399: 809
19. Gong JG, Costanzo A, Yang HQ, Melino G, Kaelin WG Jr, Levrero M, Wang JY (1999) Interaction of c-Abl and p73alpha and their collaboration to induce apoptosis. Nature 399: 809
20. Di Como CJ, Gaiddon C, Prives C (1999) p73 function is inhibited by tumor-derived p53 mutants in mammalian cells. Mol Cell Biol 19: 1438
21. Ueda Y, Hijikata M, Takagi S, Chiba T, Shimotohno K (1999) New p73 variants with altered C-terminal structures have varied transcriptional activities. Oncogene 18: 4993
22. Dobbelstein M, Wienzek S, Konig C, Roth J (1999) Inactivation of the p53-homologue p73 by the mdm2-oncoprotein. Oncogene 18: 2101
23. Ongkeko WM, Wang XQ, Siu WY, Lau AW, Yamashita K, Harris AL, Cox LS, Poon RY (1999) MDM 2 and MDMX bind and stabilize the p53-related protein p73. Curr Biol 9: 829
24. Marin MC, Jost CA, Irwin MS, DeCaprio A, Caput D, Kaelin WG Jr (1998) Viral oncoproteins discriminate between p53 and the p53 homolog p73. Mol Cell Biol 18: 6316
25. Roth J, Konig C, Wienzek S, Weigel S, Ristea S, Dobbelstein M (1998) Inactivation of p53 but not p73 by adenovirus type 5 E1B 55-kilodalton and E4 34-kilodalton oncoproteins. J Virol 72: 8501
26. Dobbelstein M, Roth J (1998) The large T antigen of simian virus 40 binds and inactivates p53 but not p73. J Gen Virol 79: 3079
27. Higashino F, Pipas JM, Shenk T (1998) Adenovirus E4orf6 oncoprotein modulates the function of the p53-related protein, p73. Proc Natl Acad Sci USA 95: 15683
28. Reichelt M, Zang KD, Seifert M, Welter C, Ruffing T (1999) The yeast two-hybrid system reveals no interaction between p73 alpha and SV40 large T-antigen. Arch Virol 144: 621
29. Wang J, Fan S, Yuan R, Ma X, Meng Q, Erdos MR, Brody LC, Goldberg ID, Rosen EM (1999) p53-independent decrease of p21 proteins caused by UV radiation in human cancer cells. Int J Radiat Biol 75: 301
30. Fan S, Wang J, Yuan R, Ma YX, Meng Q, Erdos M, Goldberg ID, Rosen EM (1998) BRCA1 as a potential human prostate tumor suppressor: modulation of proliferation damage responses and expression of cell regulatory proteins. Oncogene 16: 3069
31. Maki CG, Huibregtse JM, Howley PM (1996) In vivo ubiquitination and proteasome-mediated degradation of p53. Cancer Res 56: 2649
32. Yuan RQ, Fan S, Wang JA, Ma YX, Meng Q, Erdos M, Goldberg ID, Rosen EM (1999) Co-ordinate alterations in the expression of BRCA1 BRCA2 p300 and Rad51 in response to genotoxic and other stresses in human prostate cancer cells. Prostate 40: 37
33. Miki Y, Swensen J, Shattuck-Eidens D, Futreal PA, Harshman K, Tavtigian S, Liu Q, Cochran C, Bennett LM, Ding W, et al (1994) A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. Science 266: 66
34. Wooster R, Neuhausen SL, Mangion J, Quirk Y, Ford D, et al (1994) Localization of a breast cancer susceptibility gene BRCA2 to chromosome 13q12-13. Science 265: 2088
35. Langston AA, Stanford JL, Wicklund KG, Thompson JD, Blazej RG, Ostrander EA (1996) Germ-line BRCA1 mutations in selected men with prostate cancer. Am J Hum Genet 58: 881
36. Hakem R, de la Pomba JL, Sirard C, Mo R, Woo M, Hakem A, Wakeham A, Potter J, Reitmair A, Billia F, Firpo E, Hui CC, Roberts J, Rossant J, Mak TW (1996) The tumor suppressor gene BRCA1 is required for embryonic cellular proliferation in the mouse. Cell 85: 1009
37. Brugarolas J, Jacks T (1997) Double indemnity: p53 BRCA and cancer. P53 mutation partially rescues developmental arrest in Brca1 and Brca2 null mice suggesting a role for familial breast cancer genes in DNA repair. Nat Med 3: 721
38. Bork P, Hofmann K, Bucher P, Neuwald AF, Altschul SF, Koonin EV (1997) A superfamily of conserved domains in DNA damage-responsive cell cycle checkpoint proteins. FASEB J 11: 68
39. Sturzbecher HW, Donzelmann B, Henning W, Knippschild U, Buchhop S (1996) p53 is linked directly to homologous recombination processes via RAD51/RecA protein interaction. EMBO J 15: 1992
40. Avantaggiati ML, Ogryzko V, Gardner K, Giordano A, Levine AS, Kelly K (1997) Recruitment of p300/CBP in p53-dependent signal pathways. Cell 89: 1175
41. Sang N, Avantaggiati ML, Giordano A (1997) Role of p300 pocket proteins and hTBP in E1A-mediated transcriptional regulation and inhibition of p53 transactivation activity. J Cell Biochem 66: 277
42. Chi SW, Ayed A, Arrowsmith CH (1999) Solution structure of a conserved C-terminal domain of p73 with structural homology to the SAM domain. EMBO J 18: 4438
43. Thanos CD, Bowie JU (1999) p53 family members p63 and p73 are SAM domain-containing proteins. Protein Sci 8: 1708