**TAp73 Is a Downstream Target of p53 in Controlling the Cellular Defense against Stress**

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TAp73 is a p53 tumor suppressor gene homologue that is known to be mainly involved in apoptosis. We report here that TAp73 is necessary for the cellular response to oxidative stress and that TAp73 functions as a downstream target of p53 in this process. We show that p53 physically interacts with the TAp73 promoter under stress conditions that lead to cell death. Particularly, p53 binds to a palindromic site in the TAp73 promoter, activates the promoter of TAp73, and selectively induces TAp73 transcription. TAp73 expression is highly increased under oxidative stress in a p53-dependent manner. Furthermore, knockdown of TAp73 expression inhibits the cellular apoptotic response to oxidative damage. In contrast, the ectopic expression of TAp73 in p53-/- mouse embryonic fibroblasts induces oxidative cell death. Our findings demonstrate that p53 is a direct transcriptional regulator of TAp73. Our data reveal a new pathway for cellular protection against oxidative damage and provide evidence that TAp73 is a stress-response gene and a downstream effector in the p53 pathway.

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p53 is the most frequently mutated gene in human cancers (1). p53 plays a fundamental role in multiple cellular processes, including cell cycle checkpoint control, cell death induction, DNA repair, and genetic stability (2–5). p53 acts as a sequence-specific DNA-binding protein and activates transcription by binding specific DNA consensus sequences (6). The p53 consensus sequence was initially defined as two copies of the 10-bp motif 5′-PuPuPuC(A/T)(T/A)GPyPyPy-3′, separated by a 0–13-bp spacer (7). As a negative regulator of cell cycle progression, p53 functions to control the transition from G1 to S phase of the cell cycle. p53 does this through transcriptional induction of p21, a universal inhibitor of cyclin/cyclin-dependent kinases, which is required for cell cycle arrest in response to DNA damage caused by γ-irradiation (8).

It is well established that p53 functions as a mediator of cell death (9). p53 is required for irradiation-induced apoptosis in mouse thymocytes and is associated with cellular sensitivity to drug-mediated cell killing in chemotherapy (10, 11). p53 is also required for the apoptotic response to oxidative stress (12). The mechanisms by which p53 regulates this apoptotic pathway have been explored extensively. It is known that p53 transactivation plays an essential role in induction of apoptosis (13). Indeed, many of the known transcriptional targets of p53 are involved in cell death processes, such as Bax (14), Noxa (15), p53AIPI (16), Apaf-1 (17), PUMA (18, 19), Pidd (20), and p53DINPI (21). Almost all of these are functionally related with caspase signaling and mitochondrial apoptosis pathways (22). Although p53 mediates apoptosis in response to oxidative stress, the downstream effectors of p53 function in this pathway are largely unknown. We previously reported that PAC1 and MKP2, protein phosphatases and inhibitors of the mitogen-activated protein kinase (MAPK) cascade, are two transcriptional targets of p53 in signaling the cellular response to nutritional stress and oxidative stress (23, 24). We propose that there are more targets of p53 transactivation involved in cellular defense processes.

p73 was discovered due to its considerable homology to p53, and it was originally considered a potential tumor suppressor because of its localization at chromosome 1p36, a region frequently deleted in neuroblastoma and other tumors (25). The p73 gene is able to encode transcriptionally active TA73, as well as an N-terminally truncated form, ΔNp73 (DNp73), lacking the transactivation domain, which is transcribed from a different promoter within intron 3 of the gene (26). TA73 protein shares structural and functional similarities with p53. The TAp73 gene encodes four polypeptides through alternative splicing of exons 11–13, designated as p73α, -β, -γ, and -δ. TAp73 is expressed in either a full-length form, p73α, or shorter mRNA variants: β (splicing out exon 13), γ (splicing out exon 11), and δ (splicing out exons 11, 12, and 13) (27). TAp73 has significant sequence identity to the transactivation, DNA binding, and oligomerization domains of p53. In addition, TAp73α has a SAM-like domain in its C terminus. TAp73 can bind to a p53 consensus sequence and transactivate some, but not all, p53-regulated genes. A main function of TAp73 is to mediate apoptosis, but unlike p53, it appears to be activated in response to certain types of DNA damage but not others (25, 28). In contrast, DNp73 acts in a dominant-negative manner and blocks transactivation of target genes by p53 and TAp73 (29). The role of p73 in tumorigenesis is controversial. Unlike p53, which is the most commonly mutated gene in human cancer, mutations in the p73 gene are extremely rare (30, 31), and there are no mutations found in hematopoietic cancers (32), breast...
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cancer (30), and prostate cancer (33). p73 mutations are restricted to certain tumors, such as non-Hodgkin lymphomas (34). The expression of p73, however, is frequently altered in human tumors. For example, the expression of TAp73 is reduced in lymphoma and leukemia (35). In contrast, the overexpression of p73 was found in a variety of cancers, including those from breast, ovary, bladder, colon, and lung (36–39). These observations suggest that p73 is not a classic tumor suppressor and even an oncogene. Although p53-null mice develop normally and are susceptible to tumorigenesis (40), p73 knockout mice have neurological, pheromonal, and inflammatory defects but do not develop spontaneous tumors (41). Thus, p53 and TAp73 have overlapping and distinct functions; p53 regulates the stress response to suppress tumorigenesis, and TAp73 might regulate both the stress response and development. Because p53 and TAp73 are linked to different upstream pathways, this family of transcription factors might regulate a common set of genes in response to different extracellular signals and developmental cues. On the other hand, p53 and TAp73 seem to be linked functionally in signaling apoptosis in mouse embryonic fibroblast (MEFs)3 induced by DNA-damaging agents. It is shown that p53-deficient E1A mouse embryonic fibroblasts (MEFs)3 have high levels of cellular p53 (11). However, p53-mediated cell death induced by DNA-damaging agents is inhibited in p73-null E1A mouse embryonic fibroblasts, suggesting that p73 is required for p53-dependent apoptosis in these E1A MEFs (42). This is puzzling because if p73 functions as a transcription factor in parallel with p53, loss of p73 should not affect p53 function. Instead, it is possible that p73 is a downstream target of p53. That could explain why p73 overexpression induces apoptosis in the absence of p53, but p53 needs p73 to mediate apoptosis. Our data lend support to this model.

We have previously demonstrated that p53 is required for the cellular response to oxidative stress (12, 43). In this study, we report that p73α, which is referred to as TAp73 or TAp73α in the text, is transcriptionally regulated by p53 for signaling apoptosis following oxidative stress. We reveal that p53 transactivates the first promoter of the human p73 gene. We further demonstrate that p53 physically and specifically binds to a novel palindrome in the p73 promoter. It has been reported that p53 and TAp73 have overlapping and distinct functions; p53 activates the first promoter of the human p73 gene. We further demonstrate that p53 as a transcription factor for TAp73 and that TAp73 is necessary for the cellular response to oxidative stress.

**EXPERIMENTAL PROCEDURES**

**Cell Culture and DNA Transfection**—EB and EB-1 have been described previously (44). MEFs were described elsewhere (12, 45). Primary p73+/+ and p73−/− MEFs at passages 2–4 were cultured under normal growth conditions (42). Cancer cell lines used were originally purchased from ATCC. These cell lines were maintained in Earle’s minimal essential medium (Invitrogen) supplemented with 10% fetal bovine serum (FBS) at 37 °C in a humidified atmosphere with 5% CO2. Transfection was performed using Lipofectamine reagent (Invitrogen). For transient transfection, 2 × 105 cells were seeded per well of a 6-well culture plate and incubated for 24 h. Plasmid DNA was added into 100 μl of serum-free medium, mixed with 50 μl of Lipofectamine reagent, and incubated for 15 min. The solution was added directly onto the cells in 2 ml of serum-free medium. The cells were incubated in Earle’s minimal essential medium plus 20% FBS overnight. For stable clones, transfected cells were grown for about 2 weeks in a complete growth medium containing 2 μg/ml hygromycin B for hygro resistance. Colonies were picked for further studies.

**Construction of Luciferase Reporters, TAp73 Expression Plasmid, and siRNA Vector**—Human genomic DNA was purified from normal human fibroblasts as template for promoter cloning. The regulatory region of the human TAp73α was amplified by PCR. The primers used to amplify the p73 promoter by PCR are as follows: 5′-GGGGAGCCCTGCTACACCCGAGAGATCGT-3′ (forward); 5′-GGCCCAAGTCCAGGGCGATCCA-3′ (reverse). PCR reactions were performed under the following conditions: seven cycles of 94 °C, 2 s, 72 °C, 3 min, 35 cycles of 94 °C, 2 s, 67 °C, 3 min, and one cycle of 67 °C, 4 min. The promoter was ligated into the pGL3-basic reporter (Promega), resulting in pGL3/p73p-luc. pGL3/p73mt-luc was generated by changing the palindromic sequence from 5′-TGGACGCGGCCCA-3′ to 5′-TGGACGCGGCCCA-3′. As underlined using the QuikChange site-directed mutagenesis kit (Stratagene), according to the manufacturer’s protocol. The mouse TAp73 expression vector was constructed from a selectable constitutive expression vector pcDNA3.1 (Invitrogen). The full coding region of mouse TAp73α (1.9 kb) was amplified from a mouse cDNA library (Clontech) by PCR using the following primers: mp73-20up, 5′-GCAGAATGAGCGGCAGCGTT-3′ (forward); mp73-20dn, 5′-CATCGTTCTCATGGCTCTGCT-3′ (reverse). The resulting PCR product (1928 bp) was ligated into pT-Adv and then subcloned into pcDNA3.1, resulting in pcDNA3/mp73. pSilencer 1.0-U6 siRNA expression vector (Ambion) was used to generate a TAp73/siRNA made against a 19-nucleotide-specific mouse TAp73 sequence, which is in the starting coding region of the mouse TAp73α. The U6/p73 siRNA construct was created by ligating the following annealed oligonucleotides into the designed sites of Apal and EcoRI of the U6 vector as follows: forward, 5′-GGGGAGCCCTGCTACACCCGAGAGATCGT-3′; reverse, 5′-ATTAAAGGATTCCTGGCTCCATGCTCTTCTTGTTC-3′; reverse, 5′-ATTAAAGGATTCCTGGCTCCATGCTCTTCTTGGTTCTGTTGGTTCTGTTGGTTCTGTTGGTTCTGTTGGTTCTGTTGGTTCTGTTGGTTCTGTTGGTTCTGTTGGTTCTGTTGGTTCTGTTGGTTCTGTTGGTTCTGTTGGTTCTGTTGGTTCTGTTGGTTCTGTTGGTTCTGTTGGTTCTGTTGGTTCTGTTGGTTCTGTTGGTTCTGTTGGTTCTGTTGGTTCTGTTGGTTCTGTTGGTTCTGTTGGTTCTGTTGGTTCTGTTGGTTCTGTTGGTTCTGTTGGTTCTGTTGGT
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according to the manufacturer’s instructions. Luciferase activity was measured with a Berthold Autolumat LB953 rack luminometer. Luciferase values were normalized against β-galactosidase activity. Luciferase readout was always obtained from triplicate transfections and averaged.

RNA Isolation, Northern Blot, and Western Blot—Total RNA was isolated from growing cells using TRIzol reagent (Invitrogen). Poly(A)+ RNA was purified using a PolyATtract mRNA isolation system (Promega), according to the manufacturer’s instructions. For Northern analysis, RNA was separated on a 1.2% formaldehyde gel and transferred to an N-Hyb membrane using a Turboblotter system (Schleicher & Schuell). The probe for TAp73 is based on the N-terminal sequence of TAp73, which is different from that of DNp73. The DNp73 probe is a unique sequence corresponding to the exon 3′ of DNp73α. DNA probes were labeled with [α-32P]dCTP (Amersham Biosciences) using the Prime-It RmT random primer labeling Kit (Stratagene). The membrane was hybridized with labeled DNA probes in the QuikHyb hybridization (Stratagene) at 65 °C for 2 h and developed for autoradiography. For Western blotting, growing cells at 60–70% confluence were lysed in cold Nonidet P-40 buffer with protease/phosphatase inhibitors. The samples were resolved by 7.5% SDS-polyacrylamide gels and then transferred onto nitrocellulose filters after protein separation. For the detection of TAp73, a monoclonal antibody specific for TAp73α (Calbiochem, ER-13) was used to recognize the α form of TAp73. We used an anti-DNp73 monoclonal antibody that recognizes the β form of p73 (Calbiochem, 38C674). Immunoblots were incubated with primary antibodies and then incubated with peroxidase-conjugated rabbit anti-mouse IgG as secondary antibody. The signals were detected with enhanced chemiluminescence (Amersham Biosciences).

DNase I Footprinting Analysis—Fragments of the human p73 promoter were end-labeled with [γ-32P]ATP by T4 polynucleotide kinase, digested with BamHI, and purified as the sense strand 5′-end-labeled probe. Recombinant p53 protein was produced in insect cells infected with a baculovirus vector expressing human WT p53 and partially purified through affinity chromatography. p53 protein was added to bind to radiolabeled probe fragment (1–2 × 104 cpm) at 37 °C for 20 min followed by the addition of DNase I from the Core footprinting system (Promega) according to the manufacturer’s protocol. The reaction products were subjected to polyacrylamide gel electrophoresis under denaturing conditions, and the gel was dried and exposed for autoradiography.

Electrophoretic Mobility Shift Assay—Oligonucleotides (pairs of sense and antisense) were synthesized, annealed, and labeled with 32P by using T4 polynucleotide kinase and [γ-32P]ATP as described elsewhere (46). Recombinant p53 protein was produced in insect cells infected with a baculovirus vector expressing human WT p53 and partially purified through affinity chromatography. 32P-labeled probes (2 × 104 cpm) were mixed with 0.5 μg of purified recombinant p53 in a 20-μl DNA binding reaction buffer consisting of 20 mM Tris-HCl (pH 7.5), 4% Ficoll-400, 2 mM EDTA, 0.5 mM dithiothreitol, 0.2 mg of poly(dl-dC). For specificity or competition controls, a labeled random oligonucleotide and excess of unlabeled corresponding oligonucleotide were added together in the reactions.

For supershift, 100 ng of the anti-p53 monoclonal antibodies (pAb 421 or pAb 1801, Oncogene Research Products) was included. The reaction mixtures were incubated at 4 °C for 20 min, resolved by a 4% polyacrylamide gel, and exposed for autoradiography. Oligonucleotides (5′-3′) used in electrophoretic mobility shift assay (EMSA) are: p73-36W, GCGGCACGGCGCATGAGGGCCAGGCG; p73-36M1, CCCGACTTTCAGCGCCGAGCTTGAGGGCCAGGCG; p73-36M2, CCCGACCTTCAGCACGGCCGACGTGAGGGCCAGGCG; p73-36M3, CCCGACCTTTGACCGCCGAGCCAGCGGAGAGGCGGAGCCG; p73-36M3, CCCGACCTTTGACCGCCGAGCCAGCGGAGAGGCGGAGCCG; p21-30W, GGAAGAAGACTGGACATGCTGGAGAGGCGGAGAGGCGGAGCCG; p21-30W, GGAAGAAGACTGGACATGCTGGAGAGGCGGAGCCG; p21-30W, GGAAGAAGACTGGACATGCTGGAGAGGCGGAGCCG; p73-30W, GGAAGAAGACTGGACATGCTGGAGAGGCGGAGCCG; p73-30W, GGAAGAAGACTGGACATGCTGGAGAGGCGGAGCCG; p73-30W, GGAAGAAGACTGGACATGCTGGAGAGGCGGAGCCG; p73-30W, GGAAGAAGACTGGACATGCTGGAGAGGCGGAGCCG.

RESULTS

Transcriptional Regulation of TAp73 by p53 in Signaling Apoptotic Stress Response—To reveal the mechanism of the cellular response to genotoxic stress, we performed microarray analysis of gene expression profiles under stress conditions. We observed that TAp73 expression is increased following oxidative damage (data not shown). Because p53 is a controller of this process, we proposed that p53 is a regulator of TAp73. To determine whether p53 regulates the transcription of TAp73, we examined the expression of TAp73α, the full-length form of TAp73, in the p53-inducible system, EB-1. The EB cell line was originally derived from a human colon cancer with a mutant p53 gene. EB-1 is a stable cell clone of EB that expresses ectopic wild-type p53 (WT p53) under the control of the metallothionein promoter. p53 is induced by ZnCl2 (47). EB-1 cells undergo cell cycle arrest following WT p53 induction alone and typical apoptosis following serum deprivation in the presence of p53 (23, 47). As shown in Fig. 1A, the transcriptional level of p21, a p53 target gene involved in cell cycle regulation (48, 49), is low in uninduced EB-1 cells (middle panel, lane 1) and increases following WT p53 induction by ZnCl2 (middle panel, lanes 2 and 3). TAp73α transcripts are undetectable in EB-1 without p53 (upper panel, lane 1). Interestingly, TAp73α transcription is only slightly induced in EB-1 cells with ZnCl2 (lane 1 versus lane 2), although p21 levels are highly increased (middle panel, lane 2), and cell cycle progression is blocked (8, 23). However, TAp73α expression is greatly increased in EB-1 cells treated with both ZnCl2 and serum starvation (upper panel, lane 3), of which both increase the levels of p53 protein (lanes 3 and 6) and induce apoptosis (23, 47). ZnCl2 has no effect on TAp73α in parental EB cells (data not shown). We further examined the protein levels of TAp73α under these conditions. Although p53 protein is induced in the presence of ZnCl2 (Fig. 1B, lanes 2, 3, 5, and 6), the expression of TAp73α protein is increased only when p53 is induced by ZnCl2 plus serum starvation or oxidative damage (Fig. 1B, upper panel, lanes 3 and 6). Our results suggest that TAp73α is regulated by p53 and that stress conditions are necessary for TAp73α induction.

To determine whether p53 is required for the induction of TAp73α transcription, we tested normal and p53-null mouse embryonic fibroblasts (p53+/− and p53−/− MEFs) for TAp73α expression in response to oxidative damage by H2O2, which
induces apoptosis (43). We used a ribonuclease protection assay (RPA) for precise detection and quantitation of TAp73α mRNA following oxidative stress. As shown in Fig. 1C, the level of TAp73α mRNA is extremely low in p53+/+ MEFs (lane 1). However, TAp73α transcription is increased 11–12-fold following oxidative damage by H2O2 (lanes 2–4). In contrast, the level of TAp73α transcription is only increased by 1–1.5-fold in p53−/− MEFs following oxidative damage (lanes 5–8). Similarly, the expression of TAp73α protein is greatly increased following oxidative stress (Fig. 1D, lane 1 versus lane 3) in p53+/+ MEFs but not in p53−/− MEFs (Fig. 1D, lane 5 versus lane 7). TAp73α protein is not induced during cell cycle arrest caused by either γ-irradiation or serum starvation in these p53+/+ MEFs (Fig. 1D, lanes 2 and 4), although p21 is induced under these conditions. p21 is up-regulated by p53 following γ-irradiation or serum starvation in these MEFs at the translational and transcriptional levels (Fig. 1, D and E, lanes 2 and 4). These observations suggest that TAp73 is specifically inducible by oxidative stress in a p53-dependent manner and that p53 can regulate TAp73 in response to oxidative damage. To determine whether p53 regulates TAp73 in vivo, we monitored the levels of TAp73 protein in various mouse tissues from p53 knock-out mice. As shown in Fig. 1F, TAp73 is expressed in all the normal mouse tissues examined, including brain, lung, thymus, liver, kidney, and bladder. However, TAp73 is low or almost undetectable in thymus (lane 5 versus lane 6), liver (lane 7 versus lane 8), and kidney (lane 9 versus lane 10) in the absence of p53. TAp73 expression is not significantly reduced in brain, lung, and bladder, where p53 is disrupted. These results indicate that p53 regulates TAp73 in a tissue-specific manner.

Transactivation of the Human p73 Gene Promoter by p53—To determine the molecular basis for transcriptional regulation of TAp73 by p53, we cloned the first promoter of the human p73 gene by PCR. Sequence analysis of the entire promoter revealed no known p53 consensus binding site defined as 5′-PuPuPuPu(A/T)PuPyPyPy-3′ (7). However, there is a 12-bp (5′-TGGAGCCGGCCA-3′) from 219 to 230 in the human p73 promoter, as indicated in Fig. 2A in bold and underlined letters. It was reported that p53 physically binds to a palindromic site in the promoter of the target gene PAC1 and activates the transcription of this gene (23). These two palindromes are different in sequence, but both are GC-rich elements. Thus, it is possible that p53 can also transactivate the p73 promoter by binding this GC-rich palindromic site.
To determine whether the activity of the p73 promoter is regulated by p53, the putative human p73 promoter sequence was amplified from -726 to -15. The PCR fragment was ligated into a luciferase reporter vector pGL3-basic (Promega) adjacent to a luciferase reporter gene, resulting in the reporter pGL3/hp73-luc. The p73 luciferase reporter was then transfected into p53-null H1299 cells, along with a wild-type p53 expression vector (pCMV/wtp53) (50) or with a pCMV vector as a control. As shown in Fig. 2B, the pGL3/hp73-luc reporter has a basal activity in the absence of functional p53. However, its activity is greatly induced by a wild-type p53 expression plasmid but not by a mutant p53 plasmid (pC53–248) containing a point mutation at codon 248. To test whether the palindromic sequence is important for p53-mediated transactivity, we used a PCR-based site-directed mutagenesis to create a single point mutation by changing G to T in the palindromic sequence, resulting in a mutant reporter, referred to as pGL3/hp73mt-luc. We observed that luciferase activity was reduced significantly in the luciferase reporter containing the mutated palindromic sequence in the presence of p53. These results suggest that p53 regulates the p73 promoter activity likely through this palindromic site. However, there is still a 2-fold increase of the pGL3/hp73mt-luc in the presence of p53, indicating that p53 may regulate the promoter through other unknown mechanisms.

To determine whether p53 can also regulate the promoter of mouse p73, we cloned the first promoter of mouse p73. We found that there is no consensus binding site for p53. However, there is a 12-bp palindromic sequence (5'-GGACTGCAGGCC-3') located from -485 to -474 in the mouse p73 promoter (supplemental Fig. 1). Although it is different from the palindrome (5'-TGGACGCGGCCA-3') in the human TAp73 promoter, these two palindromes are both GC-rich. We created a luciferase reporter for the mouse p73 promoter (pGL3/mp73-luc) by ligating the mouse p73 promoter sequence (from -758 to -21) into the luciferase reporter vector pGL3-basic (Promega) adjacent to a luciferase reporter gene. To examine luciferase activity, the luciferase reporters were transfected into p53-/- MEFs along with a pCMV vector (pcDNA3) as a control or with a pCMV/wild-type p53 expression vector (pCMV/wtp53). As shown in Fig. 2B, there is a basal activity of the mouse p73 promoter in the absence of p53. The activity of the mouse p73

**FIGURE 2. Transactivation of the human and mouse p73 promoters by p53.** A, sequence of the human p73 promoter. Nucleotide sequence upstream of the transcription start site is annotated from a PCR-amplified regulatory region of human p73 gene. The TATA box-like sequence is bolded. The 12-bp palindromic site is bold and underlined. Numbering is with respect to the transcriptional initiation site. B, activation of the p73 promoters by wild-type p53. Luciferase assays were carried out for induction of promoter activities of p73. H1299 cells (groups 1–6) or p53-/- MEFs (groups 7 and 8) were transiently transfected with respective plasmids described below using Lipofectamine in OPTI-MEM medium (Invitrogen). Cell extracts were assayed for luciferase activity on a Berthold Autolumat LB953 rack luminometer. The luciferase activity readout is expressed as means ± S.D. of triplicate cultures and transfections. The transfection groups are as follows: 1) empty pCMV vector plus pGL3-Basic; 2) pCMV/wtp53 plus pGL3-Basic; 3) pCMV vector plus human p73 promoter reporter, pGL3/hp73-luc; 4) pCMV/wtp53 plus pGL3/hp73-luc; 5) pC53–248 plus pGL3/hp73-luc; 6) pCMV/wtp53 plus mutated reporter, pGL3/hp73mt-luc; 7) pcDNA3 empty vector plus mouse p73 promoter reporter, pGL3/mp73-luc; and 8) pCMV/wtp53 plus pGL3/mp73-luc.

**TABLE 2.**
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FIGURE 3. Physical interaction between p53 and the human p73 promoter. A, the detection of p53 association with the promoter of p73 in vivo. EB-1 cells were grown under the following conditions: 10% FBS (lane 1); 10% FBS plus 100 μM ZnCl₂ (lane 2); 0.1% FBS (serum starvation) plus 100 μM ZnCl₂ (lane 3); 0.1% FBS without ZnCl₂ (lane 4). EB-1 cells were also exposed to γ-irradiation (6 Gy, lane 5), or H2O₂ (140 μM, lane 6). The cells were harvested after 2 h and processed for ChIP by an anti-p53 antibody (pAb 1801). The ChIP was processed using a ChIP assay kit (Upstate Biotechnology), according to the manufacturer’s instructions. The p73 promoter associated with p53 was amplified by PCR using the corresponding primers. Genomic DNA from each group was used as positive control for the PCR reaction (Input). B, footprinting of the p53-binding site in the promoter of human p73. The end-labeled promoter fragment was incubated with DNase I in the absence or presence of recombinant human wild-type p53 protein at the indicated concentrations. The reaction was electrophoresed in sequencing gels. The A/G ladders were prepared by using the Maxam-Gilbert sequencing method as size markers. The region protected from DNase I digestion between −190 and −230 was indicated. C, electrophoretic mobility shift detection for specific binding of p53 protein to a palindromic motif in the p73 promoter. Purified recombinant wild-type p53 protein was incubated with PCR-amplified human p73 promoter fragment containing a point mutation either within the palindromic sequence or in the flanking sequence and tested the interaction of these probes with p53. As shown in Fig. 3A, one prominent shifted band was formed when this radiolabeled probe was incubated with recombinant human wild-type p53 (lane 2). This shifted band was blocked by adding an excess of the same unlabeled p73-36w oligonucleotide (lane 3) but not by an unrelated oligonucleotide NS30w (lane 4).

To further demonstrate that p53 physically binds the palindromic site, we synthesized a 36-bp oligonucleotide encompassing the palindromic sequence (p73-36W; for sequence information, see the oligonucleotide list under “Experimental Procedures”) and used it as a probe to perform an EMSA. As shown in Fig. 3C, one prominent shifted band was formed when this radiolabeled probe was incubated with recombinant human wild-type p53 (lane 2). This shifted band was blocked by adding an excess of the same unlabeled p73-36w oligonucleotide (lane 3) but not by an unrelated oligonucleotide NS30w (lane 4).
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A control, incubation of a labeled 30-bp oligonucleotide encompassing the p53 consensus binding site in the p21 promoter (p21–30W) resulted in a shift band (lane 1), which was supershifted by the anti-p53 antibody pAb 1801 (lane 12). These results clearly demonstrate that p53 can physically bind the palindromic sequence in the p73 promoter.

As shown in Fig. 2B, p53 is also able to transactivate the promoter of mouse p73. Because p53 can interact with the promoter of human p73, we set up the ChIP assay to determine whether endogenous p53 can also interact with the promoter of mouse p73. Because p53 can interact with the promoter of human p73, we set up the ChIP assay to determine whether endogenous p53 can also interact with the promoter of mouse p73. The efficiency of ChIP was confirmed by PCR using the corresponding primers. Genomic DNA from each group was used as input. IP, immunoprecipitation. β, phosphorylation of p53 following oxidative damage. MEFs were either exposed to γ-irradiation (6 Gy) or H2O2 (140 μM) for 2 h and processed for ChIP with an anti-p53 antibody (pAb 421) plus an anti-phospho-p53 (Ser-15). The p53-associated mouse p73 promoter was amplified by PCR using the corresponding primers. The reactions were separated by 4% native polyacrylamide gel electrophoresis and visualized by autoradiography. The oligonucleotide sequences are listed as one strand (5'–3') under “Experimental Procedures.”

As we discussed above, there is no classic consensus site for p53 binding, but there is a 12-bp palindromic sequence (5'-GGACTGCAGGCCG-3') located from −485 to −474 in the first promoter of the mouse p73 gene. This is also a GC-rich element, although it is different from the human p73 palindrome in sequence. To determine whether p53 can physically interact with this palindrome, EMSA was performed using a 32-mer oligonucleotide corresponding to the mouse p73 promoter containing the palindromic sequence (mp73-32W; see the oligonucleotide list under “Experimental Procedures”). As shown in Fig. 4C, there is a shifted band as a complex of p53 and labeled mp73-32W (lane 2). This band can be competed out by excess of cold mp73-32W (lane 3) but not by the unrelated oligonucleotide NS30W (lane 4), indicating that this binding is specific. The shifted band can be further shifted by the addition of an anti-p53 antibody (lane 5). As a control, p53 forms a shift band with p21–30W that contains a conventional p53 consensus binding site (lane 8). As expected, this shifted band can be supershifted by the anti-p53 antibody (lane 9). These results suggest that p53 can directly bind to the palindrome in the promoter of mouse p73 gene.

**Induction of Cell Death by TAp73 in Response to Oxidative Stress**—If TAp73 is a key downstream mediator of p53 function, the ectopic expression of TAp73 should be able to mimic p53 by inducing the cellular response to oxidative stress in the absence of p53. To define the function of TAp73 in the p53 pathway, we constructed a mouse TAp73α expression vector by cloning the 1.9-kb full-length coding sequence of mouse TAp73α cDNA into a mammalian expression vector driven by the cytomegalovirus promoter (pcDNA3/hygro, Invitrogen). The resulting TAp73α expression vector, pcDNA3/mp73, or an empty vector, pcDNA3, was transfected into p53+/− MEFs, which were then selected with hygromycin B to isolate stable clones of p53−/− MEF/p73. In addition to an empty vector-transformed MEFs under stress conditions. As shown in Fig. 4B, lower panel, p53 is phosphorylated at serine 37 and serine 392 under oxidative stress (lane 1 versus lane 3). p53 is also phosphorylated at serine 18 under oxidative stress, whereas it is slightly phosphorylated at this serine 18 following γ-irradiation (lane 1 versus lane 2). These results suggest that p53 is phosphorylated at different sites under different conditions, which may influence the choice of p53 binding to the promoter under these conditions.
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clone (p53\(^{-/-}\) MEF/pcDNA), we obtained two stable clones that have moderate to high levels of ectopic TAp73\(^{\alpha}\) expression, named p53\(^{-/-}\) MEF/p73-c6 and p53\(^{-/-}\) MEF/p73-c11 (Fig. 5A). We then tested the susceptibility of these p53-null MEF cells with or without ectopic TAp73\(^{\alpha}\) expression to oxidative stress. As shown in Fig. 5B, whereas control p53\(^{-/-}\) MEFs are highly resistant to oxidative stress, the same cells expressing ectopic TAp73\(^{\alpha}\) undergo cell death following H\(_2\)O\(_2\) treatment. As control, three clones exhibit similar viability without any treatment. These results suggest that TAp73\(^{\alpha}\) can cooperate with a collateral signal induced by H\(_2\)O\(_2\) for induction of cell death in the absence of p53. Therefore, TAp73 is a downstream effector of p53 function during the cellular response to oxidative stress.

We previously reported that p53 is required for the apoptotic response to oxidative stress (12). As a transcriptional target of p53, TAp73 is specifically up-regulated by p53 in response to oxidative stress. The fact that TAp73 is only responsive to oxidative stress but not to \(\gamma\)-irradiation indicates that TAp73 may be necessary in this former process. To demonstrate the importance of TAp73 in signaling cell death, we used RNA interference technology to reduce TAp73 expression. Using the pSilencer 1.0-U6 vector for siRNA expression (52), we constructed a mouse TAp73/siRNA vector under the control of the U6 RNA polymerase III promoter. The mouse TAp73/siRNA vector and a scrambled control U6 vector containing a random sequence (U6) were then introduced into MEFs, and stable clones selected by hygromycin B were tested for TAp73 expression by Western blotting (Fig. 5C). MEFs with TAp73 siRNA contain either low or undetectable TAp73 protein levels when compared with their normal counterparts under oxidative stress (Fig. 5C, lanes 2 and 3 versus lanes 4–7). This reduction is specific because the expression of DNp73 is still inducible in these cells by oxidative stress (Fig. 5D). As a control, p53 is highly induced by H\(_2\)O\(_2\) in these MEF/p73 siRNA cells (Fig. 5C), suggesting that p53 is still functional in these clones. We then determined the susceptibility of MEF/U6 and MEF/ p73 siRNA to oxidative stress. As shown in Fig. 5E, the MEF/p73 siRNA cells with undetectable or lower levels of TAp73 were highly resistant to cell killing by H\(_2\)O\(_2\). We also performed a TUNEL assay to determine the nature of cell death and to measure the kinetics of cell death. As shown in Fig. 5F, MEFs gradually underwent apoptosis following treatment with H\(_2\)O\(_2\), whereas the majority of MEF/p73 siRNA cells did not become apoptotic by H\(_2\)O\(_2\). These results demonstrate that TAp73 plays an essential role in the p53-mediated apoptotic response to oxidative damage. Our results are consistent with the previous report that loss of p73 inhibits p53-dependent apoptosis induced by E1A following DNA damage (42). It was recently
reported that p73 is not required for p53-dependent apoptosis in T cells (53). These findings indicate that p73 plays different roles in different cell types and that it is differentially responsive to various stresses.

**DISCUSSION**

p53 plays a fundamental role in controlling apoptosis. TAp73 also mediates cell death in a similar manner, and interestingly, it is required for p53-dependent apoptosis. Therefore, it is important to explore the relationship between these two homologous tumor suppressor genes. In this study, we demonstrate that TAp73, or TAp73S, is a transcriptional target for p53 in mediating apoptosis. As a homologue of p53, TAp73 has been widely considered to function in parallel with p53. Indeed, TAp73 can regulate some p53 target genes and mediate apoptosis in response to either DNA damage or chemotherapeutic agents (54). However, it is unclear whether and how TAp73 is regulated at the transcriptional level. Regulation of p53 function occurs mostly at the protein level, whereas p53 transcription is rarely influenced by environmental stress. However, TAp73 transcription is regulated by transcription factor E2F-1, and TAp73 is involved in E2F-1-mediated apoptosis as a downstream effector (55, 56). It has been shown that p73 is required for the p53-mediated apoptotic response to DNA damage, raising the question of whether p73 is a downstream component in the p53 pathway (42). It was previously reported that p73 is transcriptionally regulated by DNA damage, p53, and p73 (57). We found that TAp73 is significantly up-regulated by p53 under conditions of serum starvation or oxidative damage that causes apoptosis but not cell cycle arrest. Our results demonstrate that p53 directly regulates the expression of TAp73 during the apoptotic response to nutritional stress or oxidative damage.

One fundamental question is how p53 regulates TAp73 in response to oxidative stress. The well-characterized p53 consensus binding sequence encompasses two copies of the 10-bp motif 5’-PuPuPuC(A/T)(T/A)-PyPyPyA-3’, separated by a 0–13-bp spacer (7). This consensus site has been found in the regulatory regions of a number of genes controlled by p53, such as p21, Bax, and PIG3 (14, 48, 58). However, there are many cases where no known p53-responsive consensus sequence can be identified in p53-inducible genes. This is true in our study of the regulation of TAp73. Although p53 can transactivate the p73 promoter, there is no known p53 consensus binding site in the first promoter of the human p73 gene. We have previously identified a novel mechanism for p53 to regulate its target gene PAC1 through binding a palindromic motif in the promoter (23). Interestingly, there is also a similar palindromic site in the promoter of the human p73 gene. We speculated that p53 is able to bind to this palindromic motif to activate TAp73 transcription in response to apoptotic stimuli. As shown above, we have generated sufficient data to classify TAp73 as a stress-inducible gene in the p53 pathway.

Systematic analysis of p53 target genes using DNA microarray technology has demonstrated that p53 regulates more than 60 genes with defined functions in many systems, resulting in a variety of biological effects (44). The remaining challenge is to elucidate how p53 regulates so many genes and what is the molecular basis for p53 function in response to various factors and environmental stresses. We propose that p53 utilizes this palindromic binding motif to regulate a distinct class of target genes, which differ functionally from the genes regulated through the conventional p53-binding sequence. Interestingly, endogenous p53 only binds to the p73 promoter in vivo when it is activated under oxidative stress but not following γ-irradiation. Thus, we speculate that p53 may be modified under oxidative stress and that this modified form of p53 may have access to the promoter of p73 containing the palindrome. As supporting evidence, we observed that mouse p53 is phosphorylated after incurring oxidative damage. Because p53 can bind to the p21 promoter containing a perfect p53 consensus site and induces the expression of p21 following γ-irradiation, it would be conceivable that under different conditions, p53 may have options to bind the promoters of a subset of its target genes containing different types of binding sites: conventional consensus sites versus palindromic motifs, leading to distinct transcriptional and biological outcomes. We have searched the human genome data base for other genes with palindromic sequences and found that there are a number of genes containing either identical or similar palindromic motifs in their promoters. Therefore, the identification of the palindromic sequences as a second type of p53-binding site will pave the way for a better understanding of how p53 plays multiple roles and how other factors influence p53 binding and transactivation of select target genes.

The importance of TAp73 in signaling oxidative cell death was evaluated using newly developed RNA interference technology. Because reduction of TAp73 by RNA interference significantly blocks cell death triggered by oxidative stress, we propose that TAp73 is required for the p53-mediated apoptotic response to oxidative stress. Our data reveal a novel mechanism for tumor suppression by p53 and may explain why p53 has broad and powerful effects on genetic defense and why most cancer-associated mutations occur in p53 instead of its p73 homolog. Because TAp73 is an essential downstream effector of p53 and it is not mutated in human cancers, TAp73 may serve as an excellent target for inducing apoptosis in tumor cells using chemical compounds, providing more effective treatments for cancer patients.

Ever since p73 was cloned, it has been speculated that p73 and p53 have similar functions. However, the observations that rare mutations and the overexpression of p73 are found in some human cancers imply that p73 is at least not a classic tumor suppressor and even an oncogene. Consistently, an early report on a p73 knock-out mouse model implicates p73 in epithelial stem cells and development neurogenic responses but not in tumor suppression (41). The complex pattern of p73 transcription resulting in isoforms with a diversity of functions may be an explanation for these contradictory reports. For example, TAp73 seems to function in a manner much like p53, whereas DNp73 is oncogenic. It has been shown that p53 induces the expression of its antagonist DNp73, establishing an autoregulatory feedback loop (59). Since DNp73 is also induced by oxidative stress, the fate of the stressed cells should be determined by the balance between TAp73 and DNp73, both of which are induced by p53. Obviously, these cells are killed following oxi-
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In summary, we have demonstrated that TAp73 is a direct transcription target of p53 and that p53 selectively regulates TAp73 expression by binding to the p73 promoter. We suggest that p53 may be modified following different damage signals, and modified p53 may specifically and selectively regulate its target genes through different binding sites to mediate proper responses to environmental stresses. TAp73 functions to mediate cell death induced by nutritional and oxidative stresses.

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REFERENCES

1. Hollstein, M., Sidransky, D., Vogelstein, B., and Harris, C. C. (1991) Science 253, 49–53
2. Levine, A. J. (1997) Cell 88, 323–331
3. Kastan, M. B., Cannan, C. E., and Leonard, C. J. (1995) Cancer Metastasis Rev. 14, 3–15
4. Hartwell, L. H., and Kastan, M. B. (1994) Science 266, 1821–1828
5. Yin, Y., Tainsky, M. A., Bischoff, F. Z., Strong, L. C., and Wahl, G. M. (1992) Cell 70, 937–948
6. Kern, S. E., Kinzler, K. W., Bruskin, A., Jarosz, D., Friedman, P., Prives, C., and Vogelstein, B. (1991) Science 252, 1708–1711
7. El Deiry, W. S., Kern, S. E., Pietenpol, J. A., Kinzler, K. W., and Vogelstein, B. (1992) Nat. Genet. 1, 45–49
8. Brugarolas, J., Chandrasekaran, C., Gordon, J. I., Beach, D., Jacks, T., and Hannon, G. J. (1995) Nature 377, 552–557
9. Yonish Rouach, E., Resnitzky, D., Lotem, J., Sachs, L., Kimchi, A., and Oren, M. (1991) Nature 352, 345–347
10. Lowe, S. W., Schmitt, E. M., Smith, S. W., Osborne, B. A., and Jacks, T. (1993) Nature 362, 847–849
11. Lowe, S. W., Ruley, H. E., Jacks, T., and Housman, D. E. (1993) Cell 74, 957–967
12. Yin, Y., Terauchi, T., Solomon, G. A., Aizawa, S., Ranganathan, P. N., Yaguchi, Y., Kadokawa, T., and Barrett, J. C. (1998) Nature 391, 707–710
13. Sabbatini, P., Lin, J., Levine, A. J., and White, E. (1995) Genes Dev. 9, 2184–2192
14. Miyashita, T., and Reed, J. C. (1995) Cell 80, 293–299
15. Oda, E., Ohki, R., Murasawa, H., Nemoto, J., Shibue, T., Yamashita, T., Tokino, T., Taniguchi, T., and Tanaka, N. (2000) Science 288, 1053–1058
16. Oda, K., Arakawa, H., Tanaka, T., Matsuda, K., Tanikawa, K., Mori, T., Nishimori, H., Tamai, K., Tokino, T., Nakamura, Y., and Taya, Y. (2000) Cell 102, 849–862
17. Moroni, M. C., Hickman, E. S., Lazzerini Denchi, E., Caprara, G., Collil, E., Cevenini, F., Muller, H., and Heim, K. (2001) Nat. Cell Biol. 3, 552–558
18. Yu, J., Zhang, L., Hwang, P. M., Kinzler, K. W., and Vogelstein, B. (2001) Mol. Cell 7, 673–682
19. Nakano, K., and Vousden, K. H. (2001) Mol. Cell 7, 683–694
20. Lin, Y., Ma, W., and Benchimol, S. (2000) Nat. Genet. 26, 122–127
21. Okamura, S., Arakawa, H., Tanaka, T., Nakano, H., Ng, C. C., Taya, Y., Monden, M., and Nakamura, Y. (2001) Mol. Cell 8, 85–94
22. Michael, D., and Oren, M. (2002) Curr. Opin. Genet. Dev. 12, 53–59
23. Yin, Y., Liu, Y. X., Jin, Y. I., Hall, E. J., and Barrett, J. C. (2003) Nature 422, 527–531
24. Shen, W. H., Wang, J., Wu, J., Zherkin, V. B., and Yin, Y. (2006) Cancer Res. 66, 6033–6039
25. Kaghad, M., Bonnet, H., Yang, A., Creancier, L., Biscan, J. C., Valant, A., Minty, A., Chalon, P., Lejeus, J. M., Dumont, X., Ferrara, P., McKeon, F., and Caput, D. (1997) Cell 90, 809–819
26. Irwin, M. S., and Kaelin, W. G. (2001) Cell Growth & Differ. 12, 337–349
27. De Laurenzi, V., Costanzo, A., Barcaroli, D., Terrinoni, A., Falco, M., Annicchiari-Petruzelli, M., Levrevo, M., and Melino, G. (1998) J. Exp. Med. 1763–1768
28. Catani, M. V., Costanzo, A., Savini, I., Levrevo, M., De Laurenzi, V., Wang, J. Y., Melino, G., and Avigliano, L. (2002) Biochim. J. 364, 441–447
29. Yang, A., Kaghad, M., Caput, D., and McKeon, F. (2002) Trends Genet. 18, 90–95
30. Schwartz, D. I., Lindor, N. M., Walsh-Vockley, C., Roche, P. C., Mai, M., Smith, D. I., Liu, W., and Couch, F. J. (1999) Breast Cancer Res. Treat. 58, 25–29
31. Coates, P. (2006) J. Pathol. 210, 385–389
32. Stirewalt, D. L., Clurman, B., Appelbaum, F. R., Willman, C. L., and Radich, J. P. (1999) Leukemia (Basingstoke) 13, 985–990
33. Yokomizo, A., Mai, M., Bostwick, D. G., Tindall, D. J., Qian, J., Cheng, L., Jenkins, R. B., Smith, D. L., and Liu, W. (1999) Prostate 39, 94–100
34. Stoffel, A., Filippa, D., and Rao, P. F. (2004) Leuk. Res. 28, 1341–1345
35. Corn, P. G., Kuerbitz, S. J., van Noesel, M. M., Esteller, M., Compitello, N., Baylin, S. B., and Herman, J. G. (1999) Cancer Res. 59, 3352–3356
36. Zalka, A. I., Kovalov, S., Marchenko, N. D., and Moll, U. M. (1999) Cancer Res. 59, 3257–3263
37. Ng, S. W., Yu, G. K., Liu, Y., Huang, L. W., Palnati, M., Jun, S. H., Berkowitz, R. S., and Mok, S. C. (2000) Oncogene 19, 1885–1890
38. Yokomizo, A., Mai, M., Tindall, D. J., Cheng, L., Bostwick, D. G., Naito, S., Smith, D. I., and Liu, W. (1999) Oncogene 18, 1629–1633
39. Mai, M., Yokomizo, A., Qian, C., Yang, P., Tindall, D. J., Smith, D. I., and Liu, W. (1998) Cancer Res. 58, 2347–2349
40. Donehower, L. A., Harvey, M., Slagle, B. L., McArthur, M. J., Montgomery, C. A., Jr., Butel, J. S., and Bradley, A. (1992) Nature 356, 215–221
41. Yang, A., Walker, N., Bronson, R., Kaghad, M., Oosterwegel, M., Bonnin, I., Vagner, C. H. B., Dikkes, P., Sharpe, A., McKeon, F., and Caput, D. (2000) Nature 404, 99–103
42. Flores, E. R., Tsai, K. Y., Crowley, D., Sengupta, S., Yang, A., McKeon, F., and Jacks, T. (2002) *Nature* 416, 560–564
43. Yin, Y., Solomon, G., Deng, C., and Barrett, J. C. (1999) *Mol. Carcinog.* 24, 15–24
44. Zhao, R., Gish, K., Murphy, M., Yin, Y., Notterman, D., Hoffman, W. H., Tom, E., Mack, D. H., and Levine, A. J. (2000) *Genes Dev.* 14, 981–993
45. Almasan, A., Yin, Y., Kelly, R. E., Lee, E. Y., Bradley, A., Li, W., Bertino, J. R., and Wahl, G. M. (1995) *Proc. Natl. Acad. Sci. U. S. A.* 92, 5436–5440
46. Maiyar, A. C., Huang, A. J., Phu, P. T., Cha, H. H., and Firestone, G. L. (1996) *J. Biol. Chem.* 271, 12414–12422
47. Shaw, P., Bovey, R., Tardy, S., Sahli, R., Sordat, B., and Costa, J. (1992) *Proc. Natl. Acad. Sci. U. S. A.* 89, 4495–4499
48. El Deiry, W. S., Tokino, T., Velculescu, V. E., Levy, D. B., Parsons, R., Trent, J. M., Lin, D., Mercer, W. E., Kinzler, K. W., and Vogelstein, B. (1993) *Cell* 75, 817–825
49. Xiong, Y., Hannon, G. J., Zhang, H., Casso, D., Kobayashi, R., and Beach, D. (1993) *Nature* 366, 701–704
50. Baker, S. I., Markowitz, S., Fearon, E. R., Willson, J. K., and Vogelstein, B. (1990) *Science* 249, 912–915
51. Kastan, M. B., Zhan, Q., El Deiry, W. S., Carrier, F., Jacks, T., Walsh, W. V., Plunkett, B. S., Vogelstein, B., and Formace, A. J. Jr. (1992) *Cell* 71, 587–597
52. Sui, G., Soohoo, C., Affar el, B., Gay, F., Shi, Y., Forrester, W. C., and Shi, Y. (2002) *Proc. Natl. Acad. Sci. U. S. A.* 99, 5515–5520
53. Senoo, M., Manis, J. P., Alt, F. W., and McKeon, F. (2004) *Cancer Cell* 6, 85–89
54. Yuan, Z. M., Shioya, H., Ishiko, T., Sun, X., Gu, J., Huang, Y. Y., Lu, H., Kharbanda, S., Weichselbaum, R., and Kufe, D. (1999) *Nature* 399, 814–817
55. Irwin, M., Marin, M. C., Philips, A. C., Seelan, R. S., Smith, D. I., Liu, W., Flores, E. R., Tsai, K. Y., Jacks, T., Vousden, K. H., and Kaelin, W. G. J. (2000) *Nature* 407, 645–648
56. Lissy, N. A., Marin, M. C., Philips, A. C., Seelan, R. S., Smith, D. I., Liu, W., Flores, E. R., Tsai, K. Y., Jacks, T., Vousden, K. H., and Kaelin, W. G. J. (2000) *Nature* 407, 642–645
57. Chen, X., Zheng, Y., Zhu, J., Jiang, J., and Wang, J. (2001) *Oncogene* 20, 769–774
58. Polyak, K., Xia, Y., Zweier, J. L., Kinzler, K. W., and Vogelstein, B. (1997) *Nature* 389, 300–305
59. Kartasheva, N. N., Contente, A., Lenz-Stoppler, C., Roth, J., and Dobbelstein, M. (2002) *Oncogene* 21, 4715–4727
60. Flores, E. R., Sengupta, S., Miller, J. B., Newman, J. I., Bronson, R., Crowley, D., Yang, A., McKeon, F., and Jacks, T. (2005) *Cancer Cell* 7, 363–373

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