Mitogen-Activated Protein Kinase Phosphatase 2: A Novel Transcription Target of p53 in Apoptosis

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Abstract

The p53 tumor suppressor plays critical roles in diverse cellular responses such as cell cycle arrest, senescence, and apoptosis through transcriptional control of its target genes. Identification and characterization of new p53 target genes will advance our understanding of how p53 exerts its multiple regulatory functions. In this article, we show that mitogen-activated protein kinase phosphatase 2 (MKP2) is a novel transcription target of p53 in mediating apoptosis. Moreover, we identify a 10-bp perfect palindromic motif (CTGCGGCAGCAG) in the MKP2 promoter as a new binding site for p53 to activate the MKP2 gene. This GC-rich palindrome is completely different from the consensus p53 binding sequence. Induction of MKP2 is highly responsive to oxidative stress in a p53-dependent manner. Interestingly, the p53-dependent induction of MKP2 is prominent only in the cellular response to stimuli leading to apoptosis but not to cell cycle arrest. In response to oxidative stress, MKP2 is not only required for p53-mediated apoptosis, but ectopic MKP2 expression can also enhance apoptotic responses even independent of p53. These data suggest that p53 regulates distinct genes via different binding mechanisms and that MKP2 is an essential target of p53 in signaling apoptosis. (Cancer Res 2006; 66(12): 6033-9)

Introduction

The p53 tumor suppressor is a gatekeeper of the genome, playing a central role in balancing life and death, as well as mitosis and cytostasis, by integrating various stress signals into different biological processes, such as apoptosis or cell cycle arrest. Acting as a transcription factor, p53 regulates its target genes that fall into different categories and carry out different p53-dependent cellular tasks. Identification of new p53 downstream genes, particularly the classification of these genes in accord with distinct p53 functions, would therefore illustrate how p53 elicits its tumor-suppressing effects under different cellular stress conditions.

Mitogen-activated protein (MAP) kinase phosphatase 2 (MKP2) is a dual-specificity phosphatase known to inactivate MAP kinases (MAPK; ref. 5). Despite the important role of MKP2 in regulating MAPKs and its potential tumor-suppressive efficacy (6, 7), direct evidence is limited as for its role in apoptosis. A functional link between the MAPK signaling pathway and p53 has been suggested by their mutual regulation (8). p53 can either act downstream of the MAPKs (9, 10) or regulate the MAPK pathway by suppressing its downstream effectors (11). Our recent study has identified PAC1, another MAPK phosphatase, as a direct transcriptional target of p53, suggesting that p53 can suppress MAPK signaling by inducing negative regulators of this mitotic and survival pathway.

The activity of p53 as a transcription factor is critical for its role as a tumor suppressor, and mutant p53-mediated defects in DNA binding offer an important mechanism for development of various tumors (3). Discovery of the consensus binding site for p53 (12) has greatly contributed to a growing list of p53 transcriptional target genes that mediate the wide range of its biological effects. However, failure to locate this consensus site in promoter regions often precludes intense exploration of regulatory and functional mechanisms for many possible p53 target genes or leads to deliberate search for the consensus site beyond the proper regulatory regions. A microarray-based study indicates that only a small proportion of p53-responsive genes contains this consensus site in their regulatory region (13), suggesting the existence of potential alternative binding sites for p53.

We have previously identified a 12-bp palindromic site for p53 to induce transcription of the PACI gene (14). In search of more potential p53 target genes that mediate its distinct biological functions, we found a new 10-bp palindromic sequence, 5'-CTGCGGCAGCAG-3', in the promoter region of MKP2. In this report, we provide evidence to show that this novel palindromic motif mediates p53 binding and activation of the MKP2 promoter. Acting as a direct p53 transcriptional target, MKP2 is induced prominently only when apoptosis occurs and particularly under oxidative stress. These observations suggest that p53 induces different categories of downstream genes in response to distinct cellular stresses, leading to diverse biological reactions. When MKP2 is knocked down with small interfering RNA (siRNA), cells exhibit substantial resistance and tend to survive oxidative stress, suggesting the indispensability of MKP2 in the p53 cell death pathway. Interestingly, MKP2 can induce apoptosis even in the absence of p53, which highlights the independent role of MKP2 in signaling apoptosis.

Materials and Methods

Cell culture, DNA constructs, and transfection. The EB cell line is derived from a p53-mutated human colon cancer (15). EB-1 cells were generated from EB cells stably transfected with a wild-type p53 gene under control of the metallothionein promoter inducible by zinc chloride (16). Mouse embryob fibroblasts (MEF) with wild-type p53 (p53+/+ MEFs) and p53-null MEFs (p53−/− MEFs) have been described elsewhere (14). Human H1299 lung cancer cell line was obtained from American Type Culture Collection (Manassas, VA). Hp53ER-1 is an H1299 cell clone with an inducible p53 fusion protein with the estrogen receptor (p53ER) where p53 can be activated on binding of p53ER to 17β-estradiol and apoptosis can be induced by additional oxidative stress (17). All cells were maintained at 37°C in Earle’s MEM and supplemented with 10% fetal bovine serum (FBS).
The promoter of the human MKP2 gene was cloned by PCR from normal human genomic DNA and subcloned into the pGL3-basic reporter (Promega, Madison, WI), resulting in a luciferase reporter pGL3-MKP2-luc. A mutant form of the MKP2 reporter with a single mutation (T→G) at the palindromic site, pGL3-MKP2mut-luc, was generated using a site-directed mutagenesis kit (Stratagene, La Jolla, CA) according to the protocol of the manufacturer. The primer for PCR was 5′-GGTCCGGGAGCTGTTCTACGG-3′ (forward) and 5′-AGCTCTGGCTGCTGTTCACTTG-3′ (reverse). The mutant was sequenced to confirm that only the designed point mutation was introduced. A mouse MKP2 siRNA vector, U6/MKP2 siRNA, was generated by ligating the following annealed oligos into pSilencer 1.0-U6 siRNA expression vector (Ambion, Austin, TX): forward, 5′-ACAGCTACGCCGGACCTCTCCTAGGTTCTCGG-3′ (reverse, 5′-ATGCTTTTCTGATGTTGTTTTTT-3′; reverse, 5′-AATTAAAAACACGACTACCCCCACTCTTCTTGAAGGTCGGGTTATGGTGCTGCTG-3′). The mouse MKP2 expression vector was constructed using a selectable mammalian expression vector, pcDNA3.1 (Invitrogen, Carlsbad, CA). Primers used for PCR to amplify the full coding region of MKP2 were forward, 5′-CTAGCTCCCCAGCTTACTG-3′, and reverse, 5′-TGCTGTTTCA-CAGGTGGCTGG-3′. The resulting PCR products were ligated into pcDNA3, resulting in the pcDNA3-MKP2 expression construct. Transfection was conducted by using LipofectAMINE (Life Technologies, Inc., Gaithersburg, MD) according to the protocol of the manufacturer. Stable clones resistant to hygromycin B were selected by culturing transfected cells in medium containing 2 μg/ml hygromycin.

**Analysis of MKP2 promoter activity.** p53-deficient H1299 cells were transiently cotransfected with the luciferase reporters, pGL3-MKP2-luc and pGL3-MKP2mut-luc, or empty vector pGL3, and wild-type p53 expression vectors, pCMV-wtp53, or the empty vector pR8. A pCMVβ-galactosidase internal control plasmid was also introduced to each transfectant to normalize the transfection efficiency. Cells were harvested in triplicates were processed using the Dual-Light kit (Tropix, Bedford, MA) according to the instructions of the manufacturer. Luciferase activity was measured with a Berthold Autolumat LB953 Rack Luminometer and luciferase values were normalized against β-galactosidase activity.

**Electrophoretic mobility shift assay.** Pairs of sense and antisense 34-bp wild-type MKP2 oligonucleotides (34W) were synthesized and labeled with [γ-32P]ATP using T4 polynucleotide kinase. Recombinant p53 protein was produced in SF9 insect cells infected with baculovirus vectors expressing human wild-type p53 and purified by affinity chromatography.32P-labeled p53 proteins were used as a positive control for the PCR reaction (input).

**Chromatin immunoprecipitation.** p53+/+ and p53−/− MEFs were subjected to γ-irradiation (6 Gy) or H2O2 (100 μM/l) treatment for 5 hours and processed for chromatin immunoprecipitation using a chromatin immunoprecipitation assay kit (UBI, Charlottesville, VA) according to the protocol of the manufacturer. Briefly, protein-DNA complexes in these cells were cross-linked in the presence of 1% formaldehyde and cross-linked DNA in cell lysates was sheared by sonication to 500- to 2,000-bp fragments. Precleared lysates were incubated with a monoclonal p53 antibody, PAb421, and a site-specific (Ser15) phospho-p53 polyclonal antibody at 4°C overnight. The p53 immunocomplex was precipitated with protein A-agarose and eluted from the antibody. A no-antibody immunoprecipitation was included as a negative control. After reversing DNA-protein cross-links at 65°C for 4 hours, DNA was purified by phenol extraction and ethanol precipitation. The p53-immunoprecipitable MKP2 promoter was amplified by PCR using the corresponding primers. Genomic DNA isolated from the same panel of cells was used as a positive control for the PCR reaction (input).

**Northern and Western blot analyses.** For Northern blot analysis, total RNA was prepared using TRIzol reagent (Life Technologies) and mRNA was purified using a PolyATtract mRNA isolation system (Promega) according to the protocol of the manufacturer. RNA was separated on a 1.2% formaldehyde gel and transferred to a Nybond-N membrane using a Turboblotter system (Schleicher & Schuell, Keene, NH). DNA probes were labeled with [α-32P]dCTP (Amersham, Piscataway, NJ) using the Prime-It RmT Random Primer Labeling Kit (Stratagene). The membrane was hybridized with labeled DNA probes in the QuickHyb hybridization (Stratagene) at 65°C for 2 hours and exposed to X-ray films. For Western blotting, cells were lysed in cold NP40 buffer. Equal amounts of total protein were resolved in SDS-polyacrylamide gels and transferred onto nitrocellulose membranes. Immunoblots were incubated with indicated primary antibodies and then with peroxidase-conjugated secondary antibodies. Enhanced chemiluminescence (Amersham) was used for detection of specific protein signals.

**Measurement of cell viability and apoptosis.** Cells were harvested following H2O2 treatment as indicated. Cell viability was measured by trypan blue exclusion. Apoptosis was analyzed by the terminal deoxyribonucleotidyl transferase–mediated dUTP nick end labeling (TUNEL) assay using an ApopTag Fluorescin In Situ Apoposis Detection Kit (Chemicon, Temecula, CA) following the instructions of the manufacturer.

**Results**

**Identification of a perfect palindromic sequence as a novel p53 binding site for transactivation of the MKP2 promoter.** We have recently discovered a palindromic site in the promoter of PAC1, a p53-targeted gene, as a p53 binding motif to induce transcription of the PAC1 gene (14). To identify more p53 target genes with palindromes in their regulatory regions, we conducted a genome-wide search for potential p53 targets using palindromic information. A new 10-bp perfect palindromic sequence (5′-CTGGCCGCCG-3′; as highlighted in Fig. 1A) was found to reside in the promoter region of MKP2, a homologue of PAC1. Although this sequence is different from the p53 recognition site in the PAC1 promoter, the perfect palindromic feature implies its potential role as a new site for p53 to bind and activate MKP2 transcription. To test this possibility, we constructed a luciferase reporter driven by the MKP2 promoter containing this palindrome motif and transfected this pGL3-MKP2 reporter into p53-deficient H1299 cells. In the presence of a cotransfected expression plasmid of wild-type p53 (pCMV-wtp53), compared with the control pCMV vector, the MKP2 promoter activity was increased by 4.5-fold (Fig. 1B). The dramatic activation of the palindromic-containing MKP2 promoter by p53 suggests that p53 may induce MKP2 transcription at the transcriptional level and that the 10-bp palindromic sequence may serve as a novel p53 binding site in mediating the transactivation.

To examine the essential role of the palindromic motif in mediating p53-induced MKP2 promoter activity, we created a mutant form of the MKP2 reporter, pGL3-MKP2mut-luc, with a single mutation (T→G) at the palindromic site. Compared with the wild-type MKP2 reporter, this single point mutation caused a 50% reduction in p53-induced luciferase activity (Fig. 1B). These results highlight the critical role of the novel palindromic motif in mediating p53-activated MKP2 transcription. Despite the distinct nucleotide sequences in the promoter regions of PAC1 and MKP2, the palindromic feature categorizes both phosphatases as direct transcriptional targets for p53. The stringency of the palindromic mode is verified by the significant reduction of luciferase activity when the perfect palindromic pattern in the MKP2 promoter was interrupted even by a single point mutation (Fig. 1B). Because this 10-bp palindromic sequence is necessary for p53 to transactivate the MKP2 promoter, our next question is whether p53 directly binds to it and how its integrity is critical for p53 binding.
The integrity of the new palindromic sequence is indispensable for p53 physical binding. We did an electrophoretic mobility shift assay to detect the physical association of p53 protein and the palindrome. A 34-bp oligonucleotide, including the 10-bp palindromic sequence, was synthesized and labeled with \([\gamma-32P]\)ATP as a probe for electrophoretic mobility shift assay. Incubation of this 34-bp radiolabeled oligonucleotide (34W) with purified recombinant human wild-type p53 resulted in an obvious mobility shift (Fig. 2, lane 2). In contrast, a mutant form (34M) of the 34-bp probe containing a single T→G replacement, as seen in the mutant MKP2 reporter, failed to form a shifted band (lane 7), showing the importance of integrity of the palindromic sequence. The p53-associated 34W-MKP2 was eliminated by addition of excess unlabeled 34W (lane 3) but not by adding a nonspecific oligonucleotide (lane 4), indicating binding specificity. To confirm the specific binding of p53 to the 34-bp promoter element of MKP2, a p53-specific antibody, PAb1801, was included in the reaction, which generated a prominent supershift band (lane 5). This supershifted band disappeared under a competition by excess unlabeled 34W (lane 6), illustrating the specificity of p53 binding to the palindromic-containing MKP2 promoter element. The reliability of our electrophoretic mobility shift assay system is shown by a mobility shift of p53 association with a consensus element (30W) from the p21 promoter (lane 9) and a supershift of this 30W (p21)-oligo-p53 complex in the presence of PAb1801 (lane 10). It is important to note that the intensity of p53 binding to the new palindromic sequence on the MKP2 promoter is equivalent to its binding to the consensus site in the p21 promoter. These data establish that p53 physically binds to the new palindromic motif, resulting in the activation the MKP2 promoter.

MKP2 is induced by p53 in response to cellular stresses leading to apoptosis. The increase of MKP2 promoter activity in the presence of p53 leads to the prediction that MKP2 can be induced by p53. To test the ability of p53 to induce MKP2, and to investigate whether MKP2 participates in p53-dependent apoptotic cell death, we measured both mRNA and protein levels of MKP2 in a...
p53-inducible system (Fig. 3A and B). In this system, EB-1 cells were generated from stable transfection of a p53-mutated human colon tumor-derived cell line (EB) with a wild-type p53 gene under control of the metallothionein promoter inducible by zinc chloride (16). Although wild-type p53 is induced in the presence of 100 μmol/L of zinc chloride (ZnCl₂), apoptosis is detectable only when EB-1 cells are exposed to additional stress of serum starvation (16). As shown in both Northern and Western blots (lane 1 in Fig. 3A and B, respectively), the basal expression levels of MKP2 were very low in EB-1 cells. Although a slight induction of MKP2 was detected when p53 was induced by ZnCl₂ alone (lane 2), a significant induction was observed only when cells underwent apoptosis in response to additional serum deprivation (lane 3). In the absence of ZnCl₂-induced p53, serum starvation itself did not elevate MKP2 expression (lane 4), consistent with the idea that MKP2 is involved in p53-dependent apoptotic cell death. Because EB-1 contains an endogenous mutant p53, which may interfere with normal function of p53, we used another inducible system, Hp53ER-1. In this system, a p53ER fusion protein, human p53 protein fused to the hormone binding domain of the human estrogen receptor, is inactive unless estrogen is present and binds to it. The p53ER inducible vector is expressed in the p53-null human lung cancer cell line H1299, which allows us to show the role of p53 in the transcriptional regulation of MKP2. In Hp53ER-1 cells, p53ER fusion protein can be activated on binding of p53ER to 17β-estradiol and apoptosis can be induced by additional oxidative stress (17). As shown in Fig. 3C, MKP2 expression is slightly induced with addition of 17β-estradiol to this Hp53ER system and is further increased on additional H₂O₂ treatment. Data from these two individual p53-inducible systems indicate that p53 is a transcriptional regulator of MKP2 and that substantial induction of MKP2 requires an additional stress signal leading to apoptosis.

To further examine how MKP2 is regulated during distinct biological processes, a MEF cell system was employed (Fig. 4A and B). The ultimate cell fate of MEFS is dependent on different extracellular stimuli: γ-irradiation leads to cell cycle arrest (18) whereas oxidative stress causes apoptosis (19). In normal MEFS with wild-type p53, MKP2 expression at both mRNA and protein levels was significantly increased by oxidative damage (Fig. 4A and B, lane 3, H₂O₂) but not by γ-irradiation (Fig. 4A and B, lane 2, IR). These observations suggest that, in the presence of p53, MKP2 selectively responds to stimuli leading to apoptosis but not to cell cycle arrest. To further determine whether p53 is required for the MKP2 response to different cellular stresses, p53-null MEFS (p53−/− MEFS) were treated with both γ-irradiation and H₂O₂. Consistent with p53+/+ MEFS, γ-irradiation failed to stimulate MKP2 expression. Different from the MKP2 response to oxidative stress in p53−/− MEFS, the induction of MKP2 in response to H₂O₂ was undetectable in p53−/− MEFS. These data provide clear evidence that MKP2 responds to oxidative stress in a p53-dependent fashion, which places MKP2 in the p53 pathway specifically leading to apoptotic cell death.

**Oxidative stress induces in vivo physical association between p53 and the MKP2 promoter.** Our data in Fig. 2 have shown that p53 can directly bind to the palindromic sequence in the MKP2 promoter. To confirm the physical interaction between p53 and the MKP2 promoter in vivo, we did a chromatin immunoprecipitation assay with MEFS. As shown in Fig. 4C, the MKP2 promoter is present in MEFS genomic DNA regardless of treatments (lanes 1-3). Significantly, in p53 immunoprecipitates from p53+/+ MEFS, however, the MKP2 promoter is detectable only when these cells are subjected to oxidative stress (lane 6), but not in untreated cells (lane 4) or irradiated cells (lane 5). As a negative control, there is no detectable MKP2 promoter in the p53 immunoprecipitates from p53−/− MEFS, even under oxidative stress (data not shown). These results are consistent with the selective induction of MKP2 under oxidative stress in p53+/+ MEFS (Fig. 4A and B), suggesting that the in vivo binding of p53 with the MKP2 promoter is also selectively triggered by specific types of cellular stress leading to apoptosis.

**MKP2 is required for p53-induced apoptosis.** Data in Figs. 3 and 4 have shown that MKP2 specifically resides in the p53-mediated apoptotic pathway. To assess the essential role of MKP2 in p53-dependent apoptosis, we examined the change of cell viability of p53−/− MEFS in response to oxidative stress when MKP2 is knocked down with siRNA. We constructed a mouse MKP2 siRNA vector, U6/MKP2 siRNA, and obtained a set of stable transfected clones (c2, c5, and c6) from p53−/− MEFS. Northern blotting (Fig. 5A) confirmed the induction of MKP2, but not of β-actin, by H₂O₂ in parental p53+/+ MEFS. In contrast, the same
oxidative stimulus failed to increase MKP2 mRNA in p53+/+ MEFs containing MKP2 siRNA, indicating that siRNA specifically blocked MKP2 accumulation in response to oxidative stress. As a consequence, these stable p53+/+ MEFs clones with U6/MKP2 siRNA became resistant to H2O2 and resulted in an increased number of viable cells (>50%) compared with the low cell viability (<10%) found in control p53+/+ MEFs transfected with the empty pcDNA3 vector. Ectopic expression of MKP2 reduces the phosphorylation of MAPKs, extracellular signal–regulated kinase (ERK) 1/2 (Fig. 6B, lane 2 versus lane 1), which greatly augmented the sensitivity of these p53−/− MEFs to oxidative stimulation and resulted in almost elimination of ERK1/2 phosphorylation (Fig. 6B, lane 3) and dramatic reduction of cell viability (Fig. 6C). The apoptotic feature of the oxidation-induced cell death in MKP2-expressing p53−/− MEFs is shown by the TUNEL assay (Fig. 6D). These findings provide a link between MKP2 and apoptosis through inhibition of MAPKs and show the capability of MKP2 in responding to oxidative stress and provoking apoptotic cell death through a pathway similar to but independent of p53.

MKP2 mimics p53 in mediating oxidation-induced apoptosis. Given its essential role in the p53 apoptotic pathway, MKP2 may elicit an apoptotic response to oxidative stress even in the absence of p53. To test this idea, we transfected p53−/− MEFs with a mouse MKP2 expression plasmid, pcDNA3-mMKP2, and isolated stable clones (c4, c7, and c17) expressing ectopic MKP2. As shown in Fig. 6A, increased expression of MKP2 was observed in p53−/− MEFs transfected with pcDNA3-mMKP2 compared with p53−/− MEFs transfected with the empty pcDNA3 vector. Ectopic expression of MKP2 reduces the phosphorylation of MAPKs, extracellular signal–regulated kinase (ERK) 1/2 (Fig. 6B, lane 2 versus lane 1), which greatly augmented the sensitivity of these p53−/− MEFs to oxidative stress and resulted in almost elimination of ERK1/2 phosphorylation (Fig. 6B, lane 3) and dramatic reduction of cell viability (Fig. 6C). The apoptotic feature of the oxidation-induced cell death in MKP2-expressing p53−/− MEFs is shown by the TUNEL assay (Fig. 6D). These findings provide a link between MKP2 and apoptosis through inhibition of MAPKs and show the capability of MKP2 in responding to oxidative stress and provoking apoptotic cell death through a pathway similar to but independent of p53.

Figure 4. Selective induction of MKP2 expression and p53 binding to the MKP2 promoter in vivo under oxidative stress. An MEF cell system was employed in which γ-irradiation causes cell cycle arrest whereas oxidative stress causes apoptosis. A, MEFs with either p53+/+ or p53−/− genotype were exposed to either 6 Gy of γ-irradiation (IR) or 100 μmol/L of H2O2 for 3 hours. The level of MKP2 mRNA was detected by Northern blotting. B, MKP2 protein abundance in MEFs stimulated as in (A) was examined by immunoblotting using the anti-MKP2 antibody. Actin was used as a loading control. C, chromatin immunoprecipitation analysis of physical interaction between p53 and the MKP2 promoter in vivo. MEFs were treated as indicated and subjected to chromatin immunoprecipitation analysis. A p53-specific monoclonal antibody (PAb421) and a site-specific (Ser15) phospho-p53 antibody were used to precipitate p53 bound to chromatin. The p53-associated MKP2 promoter was amplified by PCR using corresponding primers. The input DNA from cell lysates before immunoprecipitation was used as positive control.

Figure 5. MKP2 is required for p53-mediated apoptotic responses to oxidative stress. A, MKP2 siRNA eliminated p53-dependent MKP2 expression in MEFs under oxidative stress. p53+/+ MEFs were transfected with either pSilencer 1.0 U6 vector or U6/MKP2 siRNA, and stable clones were selected. Northern blotting was done to detect MKP2 expression in these cell clones exposed to 3-hour treatment with 100 μmol/L H2O2. Equal mRNA levels of actin were shown in each lane. B, knocking down MKP2 endowed resistance to H2O2-induced cell death in p53+/+ MEFs. Exponentially growing MEFs containing either pSilencer U6 vector or U6/MKP2 siRNA were stimulated with 100 μmol/L H2O2 for 24 hours, followed by trypan blue exclusion to measure cell viability. Columns, means of three independent experiments; bars, SE.
Discussion

Genotoxic stress triggers various biological responses that result in either DNA repair coupled with cell cycle arrest (20) or apoptosis (21). Given its critical roles in both cell cycle control and apoptosis, p53 must distinguish various cellular stresses by inducing distinct target genes (22). In this report, we show that p53 responds to oxidative damage through transcriptional induction of an important MAPK phosphatase, MKP2 (Figs. 1, 3, and 4), leading to apoptotic cell death (Figs. 3-6). Residing in the p53 apoptotic pathway, MKP2 can initiate a cellular apoptotic response to oxidative stress (Fig. 6) even in the absence of p53. The recognition of a novel palindromic sequence (Fig. 1A) in the MKP2 promoter and its indispensable integrity in mediating p53 binding (Fig. 2) for transactivation (Fig. 1B) will lead to the identification of more potential p53 target genes.

More than 50% of human cancers contain p53 mutations, 90% of which reside in its sequence-specific DNA binding domain (1). These statistical data point to the importance of DNA binding for p53 to regulate its signaling targets in tumor suppression. Substantial studies have detailed the structural and functional mechanism for p53 binding to its consensus recognition site. However, this consensus site can be found only in regulatory regions of a small proportion of p53-responsive genes (13). Uncovering alternative binding sites in p53-responsive genes, such as the novel 10-bp palindromic sequence (Fig. 1A), may offer a better understanding of p53 regulatory mechanisms. To provide a basis for further exploration of new p53 targets, we screened the human genome database for the 10-bp palindromic sequence. We found >30 genes that contain this motif in their promoters, some of which are known to be apoptosis associated (data not shown). These findings form an informational basis for consolidating the important role of this motif in mediating p53 transactivity and revealing a more complete p53 regulatory network.

The ability of p53 to induce apoptosis is important for its role as a tumor suppressor. Manipulation of p53 apoptotic function constitutes an attractive strategy for cancer therapy. Data shown in this report and those in our recent study (14) begin to elucidate how p53 elicits apoptotic effects through induction of a group of MKPs. Although the involvement of MAPKs in many facets of cellular regulation, such as proliferation and survival, has been intensively documented (23), the roles of MKPs in regulating these important cellular processes are only presumed by their natural biochemical link to MAPKs. There is very limited evidence about the role of MKP2 in cell survival or death. Our data have placed MKP2 specifically in the p53-triggered apoptotic pathway. We establish that MKP2 is not only required for p53 to induce cell death in response to oxidative stress but also exhibits apoptotic potency by itself. Given the tumor-suppressing action of p53 and the role of MAPKs as a convergent point for cell proliferation signaling pathways (24), MKP2 may contribute to tumor suppression. These findings imply that MKP2 may induce apoptosis in response to cellular damages when p53 is genetically or functionally absent. These data also begin to explain how p53 initiates distinct biological processes in response to different types of damage by selectively regulating its target genes in different categories.

Discovery of the palindrome motif as a potential new p53-binding site in the promoter of MKP2 ignites a series of inspiring experimental exploration leading not only to identification of MKP2 as a transcriptional target of p53 but also to significant insights into the mechanism for p53 to decide cell fate in response to different cellular stresses. Recognition of the novel p53 binding...
site in the MKP2 promoter offers an interpretation for the failure to locate the p53 consensus site in many p53-responsive genes (13) and opens the possibility of identifying more potential p53 target genes. Coupled with another p53 binding palindromic motif in the PAC1 promoter (14), an emphasis should be given on their palindromic mode as a structural feature recognized by p53. Moreover, the integrity of the palindromic sequence in the MKP2 promoter is obligatory for p53 binding and subsequent induction of MKP2. Although p53 is well known to signal various cellular responses to stresses and damages (3), MKP2 is induced by p53 only under stimuli that lead to apoptotic cell death. Our results shed light on how p53 responds to different stresses by selectively inducing distinct categories of target genes, directing diverse cellular outcomes. The important role of MKP2 as a critical mediator of p53 function in signaling apoptosis is shown by its essentiality and sufficiency in provoking p53-dependent cell death under oxidative damage. This work may stimulate subsequent studies that seek to identify more potential p53 target genes and elucidate the molecular mechanism for how they are selectively regulated by p53 in carrying out the mission of suppressing cancer.

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References

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