Differential Regulation of MDR1 Transcription by the p53 Family Members

ROLE OF THE DNA BINDING DOMAIN*

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Robert A. Johnson‡§, Eric M. Shepard¶, and Kathleen W. Scotto***

From the ‡Cancer Institute of New Jersey, Robert Wood Johnson School of Medicine, University of Medicine and Dentistry of New Jersey, New Brunswick, New Jersey 08901, §Memorial Sloan-Kettering Cancer Center and the Weill Graduate School of Medical Sciences, Cornell University, New York, New York 10021, and the ¶Fox Chase Cancer Center, Philadelphia, Pennsylvania 19101

Although the p53 family members share a similar structure and function, it has become clear that they differ with respect to their role in development and tumor progression. Because of the high degree of homology in their DNA binding domains (DBDs), it is not surprising that both p63 and p73 activate the majority of p53 target genes. However, recent studies have revealed some differences in a subset of the target genes affected, and the mechanism underlying this diversity has only recently come under investigation. Our laboratory has demonstrated previously that p53 represses transcription of the P-glycoprotein-encoding MDR1 gene via direct DNA binding through a novel p53 DNA-binding site (the HT site). By transient transfection analyses, we now show that p63 and p73 activate rather than repress MDR1 transcription, and they do so through an upstream promoter element (the alternative p63/p73 element (APE)) independent of the HT site. This activation is dependent on an intact DNA binding domain, because mutations within the p63DBD or p73DBD are sufficient to prevent APE-mediated activation. However, neither p63 nor p73 directly interact with the APE, suggesting an indirect mechanism of activation through this site. Most interestingly, when the p53DBD is replaced by the p63DBD, p53 is converted from a repressor working through the HT site to an activator working through the APE. Taken together, these data indicate that, despite considerable homology, the DBD of the p53 family members have unique properties and can differentially regulate gene targeting and transcriptional output by both DNA binding-dependent and -independent mechanisms.

By virtue of its function as a transcriptional regulator, p53 plays an integral role in cell cycle arrest and apoptosis. It is mutated or inactivated in the vast majority of human cancers (1, 2), and engineered loss of p53 in animal models results in a tumor-prone phenotype (3, 4), confirming the tumor suppressor function of this protein. Considerable excitement was generated by the more recent discovery of two p53 family members, p63 and p73, because their similarity to p53 with respect to protein structure (5, 6) (Fig. 1) and certain cellular functions suggested an analogous role for these proteins as transcriptional regulators and tumor suppressors. The central DNA binding domain (DBD)1 shares the highest degree of homology among family members (~60%) and facilitates their interaction with the p53 DNA consensus element in target genes. Given this high degree of homology, it is not surprising that p63 and p73 bind to the p53 consensus sequence and activate a large subset of p53 target genes, albeit with somewhat different efficiencies (7–9). Moreover, both p63 and p73 have been shown to cause cell cycle arrest and apoptosis when overexpressed (9–11).

Despite these structural and functional similarities, several observations underscore fundamental differences among these family members with respect to their roles in oncogenesis and tissue development. First, although p63 and p73 were initially described as tumor suppressor proteins because of their localization to regions of the chromosome either deleted or altered in several cancer types (12–14), few mutations of these homologues have been identified in human tumors, and animals null for either protein do not demonstrate an increase in tumor formation (15–17). Second, unlike p53, both homologues exist as an array of isoforms differing at both their amino and carboxyl termini due to alternative splicing/differential promoter utilization (Fig. 1) (6, 11, 18–20). Alternative splicing introduces novel domains, such as the sterile α-motif, into certain isoforms. Differential promoter utilization results in the loss of the amino-terminal transcriptional activation domain (TAD) (the ΔN forms) and the creation of dominant negative isoforms that can inhibit transcriptional activation by other family members on at least a subset of target genes (21–23). Most interestingly, high levels of certain p63 and p73 isoforms have been observed in some tumors, suggesting that these proteins may act as oncogenes rather than classic tumor suppressor proteins (24–28).

Another notable difference among the homologues lies in their apparent roles in development, with p63 playing a critical role in epithelial stem cell renewal and epithelial homeostasis, and p73 contributing to neurogenesis (1). Among the first evidence for these roles was the observation that p63+/− and p73−/− mice each exhibit a distinct pattern of developmental

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** To whom correspondence should be addressed: Cancer Institute of New Jersey, Robert Wood Johnson School of Medicine, University of Medicine and Dentistry of New Jersey, 195 Little Albany St., New Brunswick, NJ 08901. Tel.: 732-235-4266; Fax: 732-235-3510; E-mail: scottoka@umdnj.edu.

1 The abbreviations used are: DBD, DNA binding domain; APE, alternative p63/p73 element; RT, reverse transcription; PBS, phosphate-buffered saline; EMSA, electrophoretic mobility shift assay; TAD, transcriptional activation domain; Pgp, P-glycoprotein; G3PDH, glyceraldehyde-3-phosphate dehydrogenase.
defects that had not been observed in p53−/− animals. The p63−/− animals were born with severe craniofacial, limb, and epithelial cell defects (16, 17), whereas p73−/− animals have congenital hydrocephalus, hippocampal dysgenesis, and defects in pheromone detection (15). These phenotypic differences demonstrate a subset of unique roles for each family member in both development (p63 and p73) and oncogenesis (p53).

Wild-type p53 is best known as a transcriptional activator, an activity that is dependent on its interaction with a DNA consensus element consisting of two half-sites, each composed of two copies of the sequence PuPuPuC(A/T) (where Pu indicates purine) separated by 0–13 nucleotides. The role of p53 in transcriptional repression, although less well studied, has also been shown to be critical to its function in the apoptotic pathway (29, 30). Yet there have been very few queries of a similar role for p63 and p73. Nevertheless, due to clearly demonstrated functional differences among the homologues with respect to development and tumorigenesis, it seems important to identify and investigate any fundamental differences at the level of transcriptional regulation, including the effect of these homologues on p53-repressed genes.

Our laboratory has a long standing interest in the transcriptional regulation of the P-glycoprotein (Pgp)-encoding MDR1 gene, which was first identified by virtue of its overexpression in multidrug-resistant tumor cells (31). More recently, it has been shown that Pgp plays a general anti-apoptotic role by conferring resistance to a variety of caspase-mediated apoptotic inducers in addition to chemotherapeutics (32–35). Moreover, expression of Pgp in critical organs such as the blood-brain barrier and colon epithelium impacts on drug pharmacokinetics and biodistribution (36, 37). Given the multiple potential roles of MDR1/Pgp in tumor progression and drug response, an understanding of its regulation in normal and tumor cells is essential.

MDR1 was one of the first promoters to be shown to be repressed by p53 (38). More recently, we have dissected the mechanistic basis for this repression, and we identified a region within the MDR1 promoter consisting of an atypical p53 consensus sequence (the HT site) that mediates p53 repression by direct DNA binding (39). This site differs from the p53 consensus (activator) motif in that the quarter sites, which are arranged in a head-to-head (HH) configuration in the consensus motif, are arranged in a head-to-tail (HT) orientation within the MDR1 promoter. When p53 binds to this HT site, MDR1 transcription is repressed; when the HT site is converted to a consensus HH site within the context of the MDR1 promoter, transcription is activated. Thus, the orientation of the quarter sites within this promoter dictates the effect of the promoter-bound p53 on MDR1 transcription.

In light of the high degree of homology within the DBDs of the p53 family members, and the numerous studies that have shown that the p53 homologues are capable of activating genes that contain the p53 consensus (HH) site, we sought to investigate the role of p63 and p73 on the p53-repressed MDR1 promoter. Most surprisingly, we found that both p63 and p73 activate, rather than repress, the MDR1 promoter. Moreover, this activation occurs independent of the HT site and is indirectly mediated by a novel upstream element. Finally, the difference in transcriptional effects of the p53 family members on MDR1 transcription is due, at least in part, to differences among their DNA binding domains.

MATERIALS AND METHODS

Cell Culture and Transfections—The human neuroblastoma cell line SK-N-AS (ATCC CRL-2137), the hepatocellular carcinoma HepG2 cell line (ATCC HB-8065), and the lung adenocarcinoma cell line H1299 (ATCC CRL-8903) were maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum and 100 units of penicillin/streptomycin per ml. Human osteosarcoma Saos-2 cells (ATCC HTB-85) and the colorectal adenocarcinoma cell line SW620 (ATCC CCL-227) were maintained in RPMI 1640 with 10% fetal bovine serum and 100 units/ml penicillin/streptomycin. All cells were grown at 37 °C in a 5% CO2 humidified atmosphere. Cells were transfected with FuGENE 6 (Roche Applied Science) at a ratio of 1:3 (DNA:FuGENE 6). For all transfections, DNA was added at a concentration of 2 μg/well (salmon sperm DNA was used as a carrier when needed). Luciferase activity was measured as described previously (39), using the Luminoskan Ascent plate reader (ThermoLabSystems, Finland). Luciferase activity was normalized to protein concentration, determined using the Coomassie Plus Protein Assay Reagent (Pierce). The luciferase activity of reporter constructs in the absence of exogenous p53, p63, or p73 was arbitrarily set at 1. Experiments were performed a minimum of three times in triplicate.

Plasmids and Site-directed Mutagenesis—All mutant constructs were created using the QuickChange site-directed mutagenesis kit (Stratagene, CA) according to the manufacturer’s suggested protocol. The reporter constructs included pMDR1-HT (containing sequences 1–190 construct was engineered by inserting a SmaI site at GAGAT TCTTCCTGCGTCCG (the HT site) that mediates p53 repression by a heterologous DBD, and the NheI sites were converted to the p63, p53, and p73DBD via site-directed mutagenesis. Mutant plasmids were ligated to a heterologous DBD, and the NheI sites were converted to the p53 promoter (pMDR1-HT promoter mutated within the p53 HT site) (39). The pMDR1/−/− construct was engineered by inserting a Smal site at position −190 of pMDR1-HT using the following oligonucleotide: 5'-GAGGAA GATGCTGGGCTC CGG-3' (the Smal site is shown in boldface and mutated bases are in lowercase).

The resulting construct was digested with Smal and recircularized to generate pMDR1/−/−.

The p63 and p73 isoform expression vectors have been previously described (23, 41–43). pRc-P63y (R304H) and pCDNA-p73a (R293H) were created using the following oligonucleotides: R304H, 5'-GCAAGCG TCGTCTTGGAGCCACATCTGTCGCTCCAGAGAA-3'; R293H, 5'-GAGGGCAGCCTTGGAGCAGCCACATCTGTCGCTCCAGAGAA-3'.

The p53/63DBD and p53/73DBD chimeric constructs were engineered as follows. Nhel sites were placed at the 5' and 3' boundaries of the p63, p53, and p73DBD via site-directed mutagenesis. Mutant plasmids were digested with NheI, and the DBD fragments and vectors lacking the respective DBDs were isolated using the QIAquick gel extraction kit (Qiagen) following gel electrophoresis. Relevant vectors were ligated to a heterologous DBD, and the Nhel sites were converted to wild-type sequences.

The plasmids p53/63DBD and p53/73DBD include amino acids 141–320 of p63 or 131–310 of p73, respectively, in place of amino acids 112–290 of the p53DBD.

Electromobility Shift Assays—p63y and p73a proteins were prepared in vitro using the TNT Quick-coupled Transcription/Translation System as described by the manufacturer (Promega, WI). Gel shift assays were performed as described previously with a few modi-
FIG. 2. p63 and p73 activate the MDR1 promoter. A, SK-N-AS neuroblastoma cells were co-transfected with 1.0 µg of pMDR1-HT and 0.5 µg of either pRC/CMV or pCDNA.3 (empty vectors), or one of the following expression vectors: p63α or p63γ (in pRC/CMV backbone), or p73α, p73β, or p73γ (in pCDNA.3 backbone). Data are expressed as luciferase units normalized to protein content. Expression in the presence of pRC/CMV was arbitrarily set at 1. Experiments were performed a minimum of three times in triplicate. B, Western blot of SK-N-AS cells co-transfected with 10 µg of either p63α, p63γ (upper), or ΔNp63 (lower) expression constructs. β-Actin was used as a control for protein loading. C, Western blot of SK-N-AS cells co-transfected with 10 µg of either p73α, p73γ, p73δ, p73ε (upper) or ΔNp73 (lower) expression constructs. β-Actin was used as a control for protein loading. D, same as A, except SK-N-AS cells were co-transfected with 1.0 µg of pMDR1-HT and 0.5 µg of either pRC/CMV, p63γ, ΔNp63γ, pCDNA.3, p73α, or ΔNp73α. E, same as D, except using H1299 NSCLC. F, SW620 colorectal adenocarcinoma cells were co-transfected with 1.0 µg of pMDR1-HT and 0.5 µg of either pRC/CMV, p63γ, pCDNA.3, p73α. G, same as F, except using HepG2 hepatocellular carcinoma cells. H, RT-PCR of total RNA isolated from SK-N-AS cells transfected with 12 µg of pCDNA.3 (lane 1), p63γ (lane 2), ΔNp63γ (lane 3), ΔNp73α (lane 4), or p73α (lane 5). Arrows indicate MDR1 (upper) and G3PDH (lower) mRNA.

RESULTS

p63 and p73 Activate Rather than Repress MDR1 Transcription—We have shown previously that wild-type p53 represses transcription of MDR1 through direct binding to a novel head-to-tail (HT) element within the proximal MDR1 promoter (39). To assess a role for the p53 family members p63 and p73 on MDR1 transcription, we evaluated the effect of different isoforms of each protein on MDR1 promoter activity in a transient transfection assay. The human neuroblastoma cell line SK-N-AS (which does not express either p63 or p73) was co-transfected with the wild-type MDR1 luciferase expression construct pMDR1-HT and either empty vector or one of the predominant p63 or p73 isoforms (Fig. 2A). Western blot analysis verified equivalent concentrations of both p63 isoforms (Fig. 2B, upper panel) and p73 isoforms (Fig. 2C, upper panel) in the transfectants. Most surprisingly, all three p73 isoforms (α, β, and γ) activated rather than repressed the MDR1 promoter; the relative strength of activation by the various p73 isoforms was consistent with what has been observed in the analyses of the effects of these isoforms on other promoters (7, 8, 44). p63γ was also shown to activate MDR1 transcription, whereas p63α was a very weak activator; again, this difference in activation potential for p63 isoforms is consistent with what was observed for regulation of the promoter of p21 and other p53 target genes (45).

The failure of p63α to efficiently activate the MDR1 promoter suggested that MDR1 activation is dependent upon a transcriptionally competent form of the protein. To address this more...
fully, the ΔN forms of p63 and p73 that lack the amino-terminal TAD and are therefore transcriptionally compromised (Fig. 1) were co-transfected into SK-N-AS cells along with pMDR1-HT and protein expression quantitated by Western blot (Fig. 2, B and C, lower panels). As shown in Fig. 2D, ΔNp63γ was impaired in its ability to activate the MDR1 construct, resulting in approximately half the level of activation as was observed with p63γ; a similar reduction in activity was observed in a co-transfection analysis of the p21 promoter (data not shown and see Ref. 28). That all activity is not lost upon deletion of the TAD is not completely unexpected, because it has been suggested that ΔNp63 may contain a second TAD and can in fact activate the transcription of a subset of genes (6, 46, 47). The effect of deletion of the TAD was even more striking with respect to p73α, because ΔNp73α was unable to activate either the MDR1 promoter (Fig. 2D) or the p21 promoter (data not shown and see Ref. 21). Taken together, these data indicate that activation of the MDR1 promoter by the p63 family members is dependent on the transcriptional activation competence of the protein. Most interestingly, when a similar experiment was performed in p53-deficient H1299 cells (Fig. 2E), neither p63 nor p73 isoforms were able to activate MDR1, indicating that activation may be cell line/tissue-specific. Although one difference between the SK-N-AS and H1299 cells is the status of p53 (wild-type and null, respectively), this does not appear to be the primary basis for the difference in activation by p63 and p73 because the SW620 cell line expressing mutant p53 (R273H) was able to support p63 and p73 activation (Fig. 2F), whereas the wild-type p53-expressing HepG2 cell line could not (Fig. 2G).

To evaluate activation of the endogenous MDR1 gene by the p63 family members, either p63γ or p73α expression vectors were transiently transfected into SK-N-AS cells. RT-PCR analysis of MDR1 mRNA from transfected cells lines showed a substantial increase in MDR1 RNA in cells that expressed either p63γ (Fig. 2H, lane 2) or p73α (lane 5) as compared with the empty vector control (lane 1). However, no increase in MDR1 levels was observed in ΔNp63γ (lane 3) or ΔNp73α (lane 4) transfectants. G3PDH expression was unaffected by these proteins (Fig. 2H, lower panel). Thus, both the endogenous MDR1 gene as well as a transfected MDR1 promoter construct can be activated by the p53 homologues, and this activation is dependent on the amino-terminal activation domain.

p63 and p73 Activate MDR1 Transcription through a Novel Upstream Element (the APE)—To address whether the HT site (Fig. 3A) mediates activation of MDR1 transcription by the other p53 family members, SK-N-AS cells were co-transfected with either p63γ or p73α expression vectors and either pMDR1-HT or an MDR1 luciferase reporter construct mutated in the HT site (pMDR1-HTmut). We have shown previously (39) that pMDR1-HTmut neither interacts with nor is repressed by wild-type p53. As shown in Fig. 3B, both p63γ and p73α induced transcription from the pMDR1-HTmut at least as well as they induced the wild-type promoter, indicating that the HT site is dispensable for this activation. To delineate the site through which p63 and p73 mediate their effect, MDR1 promoter deletion mutants were constructed and analyzed in co-transfection experiments (Fig. 3C). SK-N-AS cells were transfected with either p63γ or p73α and pMDR1-HT, pMDR1−221, pMDR1−190, or pMDR1−136. Both p63γ and p73α activated pMDR1-HT and pMDR1−221 constructs with equal efficiency; however, deletion of sequences between −221 and −190 markedly reduced activation, indicating involvement of this region in the regulation of MDR1 by these proteins. We have designated this region as the APE (alternate p63/p73 element).

Activation through the APE Requires the DBD but Is Independent of DNA Binding—Like p53, both p63 and p73 have been shown to activate transcription by interacting with the consensus p53 element through their DBDs. Several critical amino acids have been identified that are essential for DNA binding. By using proteins containing mutations of these amino acids in their DBDs, we sought to determine the importance of this region in MDR1 activation. A co-transfection experiment was performed in SK-N-AS cells with pMDR1-HT and constructs expressing the established DBD mutant proteins p63γ (R304H) and p73α (R293H) (43). As shown in Fig. 4A, mutations within the DBD of either protein rendered it incompetent for MDR1 activation.

Examination of the 32 bp encompassing the APE did not reveal the presence of a consensus p53-binding site; however, a region was identified that exhibited only 60% similarity to the consensus p53 site but maintained the critical bases and spacing required for DNA binding (Fig. 4B). Because the DBD mutant data implicated DNA binding in activation, we sought
to determine whether direct DNA-protein interaction could occur in this region. EMSA was performed by using whole cell extracts from SK-N-AS cells transfected with either pCDNA, p63, or p73 and the APE as a probe. As shown in Fig. 4C, the addition of p63 and p73 to cells resulted in the formation of several DNA-protein complexes (compare lanes 1–4, p63 and lanes 5–9, or p73). Supershift assays were performed using 100 ng of the following antibodies: mouse IgG (lanes 2, 6, and 11), p63 (4A4) (lane 7), or p73 (Ab-2) (lane 12). Competition assays utilized 50 ng of cold APE (lanes 3, 8, and 13) or nonspecific HT oligonucleotide (lanes 4, 9, and 14). Arrows indicate formation of new DNA-protein complexes.

fig. 4. Activation by p63 and p73 requires the DBD but does not involve direct binding to the MDR1 APE. A, SK-N-AS cells were co-transfected with 1.0 μg of pMDR1-HT and 0.5 μg of either pRC/CMV, p63, or p73 (mutated in the DBD). B, sequence of the MDR1 APE (−154 to −225). C, EMSA competition assay using 32P-labeled APE oligonucleotide as a probe and 10 μg of nuclear extract from SK-N-AS cells transfected with 10 μg of either pcDNA (lanes 1–4), p63 (lanes 5–9), or p73 (lanes 10–14). Supershift assays were performed using 200 ng of the following antibodies: mouse IgG (lanes 2, 6, and 11), p63 (4A4) (lane 7), or p73 (Ab-2) (lane 12). Competition assays utilized 50 ng of cold APE (lanes 3, 8, and 13) or nonspecific HT oligonucleotide (lanes 4, 9, and 14). Arrows indicate formation of new DNA-protein complexes.

fig. 5. Replacement of the p53DBD with the p63DBD converts p53 from a repressor to an activator. A, SaOS-2 osteosarcoma cells were co-transfected with 1.5 μg of pMDR1-HT or pMDR1–190 and 0.5 μg of either pCMV.5 (empty vector), wt-p53 (wild-type p53 expression construct), p53/63DBD (the p53DBD is replaced with the p63DBD), or p53/73DBD (the p53DBD is replaced with the p73DBD). B, SaOS-2 cells were co-transfected with 1.5 μg of pMDR1-HT and 0.5 μg of either pCMV.5, p53/63DBD, or p53/63DBD-mut (the p53/63DBD containing the R304H mutation within the DBD).

shown in Fig. 5A, replacement of the p53DBD with either the p63DBD or the p73DBD converted p53 from an MDR1 transcriptional repressor to an MDR1 transcriptional activator; removal of the upstream APE (pMDR1–190) greatly reduced this activation. Most importantly, these proteins retained their ability to activate p21 either as well as (p53/63DBD) or better than (p53/73DBD)-wild-type p53 (data not shown). Moreover, mutation of the p63DBD in the context of the p53 protein abrogated this effect (Fig. 5B). Most interestingly, when the reciprocal chimeras were analyzed (the p63DBD or p73DBD replaced by the p53DBD), global repression of transcription was observed (data not shown); the basis for this repression is under further investigation. Taken together, these data indicate that the ability of p53 to selectively repress MDR1 transcription, as well as the ability of p63 and p73 to activate MDR1 transcription, is at least in part determined by the nature of their respective DBDs.

**DISCUSSION**

Over the past few years it has become increasingly apparent that, despite considerable structural and functional similarities, there are critical differences among the p53 family members with respect to their roles in tumorigenesis and development (11). The mechanistic basis for these differences remains largely unexplored, yet it is likely that subtle distinctions in their ability to regulate the transcription of select target genes will be found to play a major role (7, 8, 44). In the present report, we have examined the effect of p63 and p73 on transcription of MDR1 (P-glycoprotein), a gene involved in transport, drug response, and cellular growth and death decisions (48). The ability of wild-type p53 to repress MDR1 transcription was demonstrated over a decade ago (38), and we have recently shown that this repression is mediated by direct bind-
indicated that both the activation domain and the DBD of each protein were necessary for activation to occur. Analysis of mutant proteins upstream of the p63/p73 element designated the alternative p63/p73 Element (APE), located between –221 and –184, is necessary for activation to occur. Analysis of mutant proteins indicated that both the activation domain and the DBD of each protein were required for MDR1 activation. Moreover, EMSAs indicated that transfection of either p63 or p73 resulted in the formation of new APE-specific complexes. Paradoxically, examination of the APE reveals only a very weak homology to a p53-binding site; mutation of bases within this sequence had no effect on the ability of p63 or p73 to activate MDR1 (data not shown), and neither p63 nor p73 was able to form a complex with an oligonucleotide that included the APE. Taken together, these data suggest that activation of MDR1 by p63/p73 is indirect and suggest two possible models. In the first model (Fig. 6A), p63 and p73 are part of the complex that binds the APE, but they do not interact directly with promoter DNA; this model predicts that the complex would be destabilized during electrophoresis, explaining the lack of a p63/p73 supershift in EMSAs. In this scenario, the p63/p73DBD would be required for protein-protein interactions rather than direct interactions with DNA, which has been shown previously (51) for the DBD of other transcription factors, including p53. In the second model (Fig. 6B), p63/p73 activate gene X, which encodes protein X. Protein X may be a transcription factor that binds directly to the APE (pathway 1) or may induce the formation of a complex at the APE either by direct protein–protein interaction as shown (pathway 2) or by modifying an APE complex member. Further experiments are underway to test these models and to identify the proteins of the APE complex.

Perhaps the most interesting observation made during the course of these studies was that, despite the homology among the DBDs of the different p53 family members, it is this region of the proteins that dictates the disparate transcriptional effects on the MDR1 promoter. Thus, when the p53DBD was replaced with the DBD from either p63 or p73, p53 was converted from a protein that repressed MDR1 transcription by direct DNA binding to the HT element to an activator that mediated its effect through the APE. Yet the p53/p63 chimera retained its ability to activate the p21 promoter through the consensus p53-binding site, consistent with the homology shared between the DBDs of the two proteins. Notably, the corollary was not true; replacement of the p63DBD or p73DBD with the p53DBD did not convert the homologues into MDR1 transcriptional repressors, although it did prevent activation through the APE. Therefore, it appears that although similar in their ability to bind to and activate through the consensus p53 element, there are unique features shared by the p63/p73DBDs that allow activation through the APE, and unique features of the p53DBD that permit interaction with and repression through the HT site.

It is interesting to speculate on a possible link between the expression of the p53 family members during development and tumorigenicity and the expression of MDR1/Pgp in normal and tumor cells. Various isoforms of both p63 and p73 are expressed in epithelial cells, with different isoforms predominating in different cell types (52). MDR1/Pgp is also expressed in a variety of epithelial cell types, where it is involved in the protection of critical tissues from xenotoxic compounds. Although a role for the p63 and p73 isoforms in cancer remains controversial (44, 53, 54), it is possible that their overexpression in certain tumor types may also impact on MDR1 expression, which could in turn impact on tumor progression and drug resistance (55–60). For example, elevated levels of p63 (particularly ΔNp63) have been implicated in squamous cell carcinoma (1, 28, 61, 62), a cancer in which increased MDR1 levels are associated with a more aggressive phenotype. Overexpression of p73 has also been observed in a number of tumor types, including those in which overexpression of Pgp has been implicated (53, 63–65). Given both the distinct and interdependent functions for the p53 family members, predicting the effect that altered expression of one homologue would have on MDR1 expression in the context of a normal or tumor cell will not be trivial. Indeed our studies show a cell/tissue-specific activation of MDR1 by p63 and p73 (Fig. 2). Initial attempts to determine whether repression by p53 or activation by p63/p73 is dominant were complicated by our observation in immunoprecipitation/Western analyses that p53 interacts with both p63 and p73 when overexpressed in cultured cells, effectively sequestering both proteins from their transcriptional targets (data not shown). Further studies are underway to address this important question.

In summary, we have shown that the p53 family members differentially regulate the MDR1 promoter. Although differen-
functional differences with respect to development and tumor-roles of the different family members explain some of their and/or interactions with a subset of promoters not regulated by permitting recognition of the p53 consensus sequence, the yet to be identified, our results suggest that, in addition to Hijikata for providing p73

observation of regulation by different mechanisms (direct and indirect) through independent promoter regions. To our 11. Yang, A., and McKeon, F. (2000)

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