p53 Physically Interacts with Mitochondrial Transcription Factor A and Differentially Regulates Binding to Damaged DNA[1]

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ABSTRACT

Mitochondrial transcription factor A (mtTFA; also designated Tfam) is necessary for both transcription and maintenance of mitochondrial DNA. mtTFA preferentially recognizes cisplatin-damaged DNA, as well as oxidized DNA. Increased apoptosis has been observed in mtTFA knockout animals, suggesting that mtTFA is involved in apoptosis. A fraction of p53 protein localizes to mitochondria at the onset of p53-dependent apoptosis, but not during p53-independent apoptosis. Using immunochemical coprecipitation, we observed binding of mtTFA and p53. Interaction between mtTFA and p53 required the high mobility group-box1 or high mobility group-box2 of mtTFA and amino acids 363–376 of p53. Binding of mtTFA to cisplatin-modified DNA was significantly enhanced by p53, whereas binding to oxidized DNA was inhibited. Our findings suggest that the interaction of p53 with mtTFA may play an important role in apoptosis.

INTRODUCTION

mtTFA[3] is a member of the HMG-box proteins family (1) and stimulates transcription of mitochondrial genes by binding to the mitochondrial D-loop region. Nuclear HMG-box proteins, such as HMG1/HMG2, are ubiquitous in higher eukaryotic cells and bind preferentially to cisplatin-damaged DNA (2–5). We demonstrated previously that mtTFA preferentially recognizes cisplatin-damaged DNA and oxidized DNA (6). mtTFA is essential not only for mitochondrial gene expression but also for maintenance and repair of mtDNA (7). In addition, increased apoptosis has been observed in cells lacking mtDNA gene expression, suggesting that mtTFA is involved in apoptosis (8).

Although the mechanism of p53-mediated apoptosis after cellular stress remains unclear, p53 has been detected in mitochondria (9–14). Current evidence suggests that the death signal induces transfer of p53 to mitochondria (11, 12). In some cell types, p53-mediated apoptosis occurs in the absence of transcription or protein synthesis (15, 16). The perturbation of mitochondria observed during p53-mediated apoptosis was preceded by transactivation of various oxidoreductases and reactive oxygen species production (17, 18). Oxidative damage attributable to oxygen metabolism and reactive oxygen species production, as well as the associated repair mechanisms, are critical to the mitochondrion and its genome. Another potentially significant role of p53 is its possible involvement in DNA repair. p53 preferentially associates with damaged DNA (19, 20) and interacts with nucleotide excision repair factors such as nucleotide excision repair protein B and nucleotide excision repair protein D (21).

In this study, we investigated physical and functional interactions between mtTFA and p53. We found that binding of mtTFA to cisplatin-modified DNA was enhanced by p53, whereas the binding of mtTFA to oxidized DNA was inhibited. Our findings suggest that interaction of p53 with mtTFA may play an important role in apoptosis.

MATERIALS AND METHODS

Cell Culture and Antibodies. KB human epidermoid cancer cells and HCT116 human colon adenocarcinoma cells were grown in modified Eagle's medium, and McCoy's 5A medium supplemented with 10% fetal bovine serum, respectively.

Antibodies to p53 (Do-1), caspase-9 p35 (H-170), topoIIα (C-15), and Sp1 (PEP2) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Antithio antibody and anticytochrome oxidase subunit I antibody were purchased from Invitrogen (San Diego, CA) and Molecular Probes (Eugene, OR), respectively. Polyclonal antibody was raised against mtTFA by multiple immunizations of a New Zealand White rabbit with a synthetic peptide. The sequence of the synthetic peptide for mtTFA was KRTIKKQRKYGAEEC (K plus amino acids 233–246).

Plasmid Preparation. Full-length cDNAs for human mtTFA, p53, MYH, and HMG1 were amplified from total RNA extracted from the human epidermoid cancer line, KB, by reverse-transcription PCR. The construction of GST-mtTFA, GST-mtTFAΔ1.2, GST-mtTFAΔ2 and GST-mtTFAΔ1, GST-MYH, GST-HMG1, GST-p53, GST-p53 N124, GST-p53 160C, ThioHis-p53, ThioHis-p53 N376, ThioHis-p53 N362, ThioHis-p53 N124, ThioHis-p53 224C has been described previously (6, 22, 23). GST-mtTFAΔ1′ was obtained by PCR using the following primer pairs: 5′-CCAAAAAGACCTCGT-TCAGC-3′ and 5′-TTTTAACACTCCTCAGCACC-3′.

Cell Fractionation for Western Blotting. KB cells were incubated with or without 20 μM cisplatin (Sigma) for 12 h, and HCT116 cells were treated with 375 μM 5-FU (Sigma) or 0.34 μM doxorubicin (Sigma) as described (24). Briefly, cells were Dounce-homogenized in buffer M [0.25 M sucrose, 10 mM Tris-HCl (pH 8.0), 3 mM MgCl$_2$, 1 mM PMSF, 1 mM DTT]. After removing the nuclear fraction by centrifugation at 2,000g for 10 min, the supernatant was centrifuged at 21,000g for 30 min at 4°C and the pellets resuspended in 0.25 M sucrose. The suspension was layered over a 0.25–2.10-M sucrose step gradient and centrifuged at 105,000g for 1 h. The mitochondrial fraction at the 1.25–2.10-M sucrose interface was collected and subjected to SDS-PAGE, either directly or after coimmunoprecipitation. The samples were transferred to polyvinylidene difluoride membranes, immunoblotted with anti-mtTFA, anti-p53 (Do-1), anti-Sp1 (PEP2), anti-caspase 9 (H-170), anti-topoIIα (C-15), and anticytochrome oxidase subunit I and visualized by chemiluminescence according to the enhanced chemiluminescence protocol (Amersham Pharmacia Biotech).

Coimmunoprecipitation. KB cells growing in 100-mm tissue culture dishes were treated with 20 μM cisplatin (Sigma) for 12 h. HCT116 cells were treated with either 5-FU or doxorubicin for 24 h. Whole cells or the mitochondrial fraction were sonicated for 10 s in binding buffer [50 mM Tris-HCl (pH 8.0), 1 mM EDTA, 120 mM NaCl, 10% glycerol, 0.5% NP40, 1 mM DTT and 0.5 mM PMSF], and centrifuged at 21,000g for 10 min. The supernatant (whole-cell fraction) was incubated with or without 75 units/ml DNaseI (Worthington, Lakewood, NJ) or 10 μg/ml RNaseA (Sigma) in binding buffer containing 1 mM CaCl$_2$ at 37°C for 1 h and incubated for 2 h at 4°C with 20

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RESULTS

A Fraction of p53 Is Localized in Mitochondrial Fraction after DNA Damage. Immunoblots of nuclear extract, cytosol, and the mitochondrial fraction from KB cells incubated with or without cisplatin (20 μM for 12 h) are shown in Fig. 1. Under these conditions, KB cells underwent apoptosis. The results show that mtTFA was present only in the mitochondria, and p53 was mainly located in the nucleus. The mitochondrial fractions were stringently monitored for contamination using markers for nuclei (topoIIα and Sp1), cytoplasm (caspase 9), and mitochondria (cytochrome oxidase subunit I). The nuclear and cytoplasmic markers were not detected in the mitochondrial fractions. Cytochrome oxidase subunit I could not be detected in either the nuclear or cytosolic fraction, indicating that these fractions were not contaminated with mitochondrial proteins. Treatment with cisplatin resulted in the up-regulation of p53, mtTFA, and cytochrome oxidase subunit I. Mitochondrial p53 was only detectable after cisplatin treatment. However, the p53 accumulated in the mitochondria comprised only a small fraction of the total p53 induced.

mtTFA Interacts with p53. Mitochondrial localization of p53 has been observed in several systems (9–14), but understanding of its organelle-based physiological activity remains incomplete. We have shown previously that p53 interacts with the HMG-box of HMG1 (22). To determine whether mtTFA, which is a member of the HMG-box family, can interact with p53 *in vivo*, whole-cell extracts of KB cells were incubated with specific antibodies against p53 and mtTFA. The protein complexes were then examined by sequential immuno-
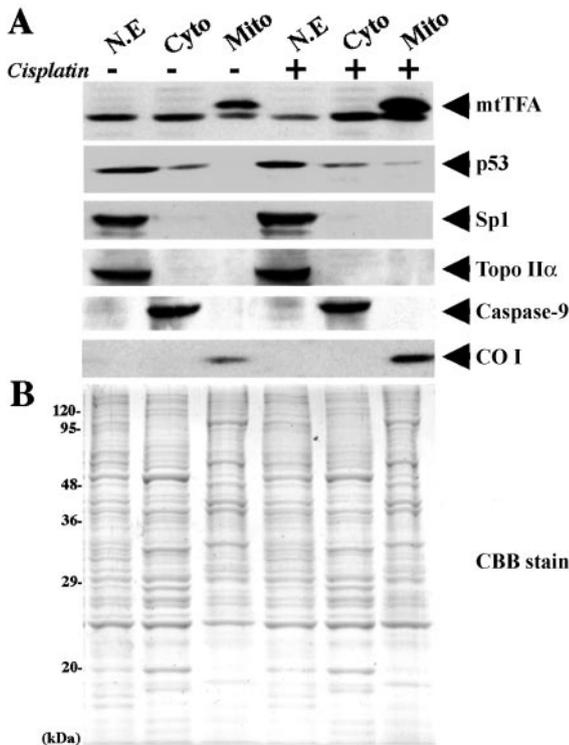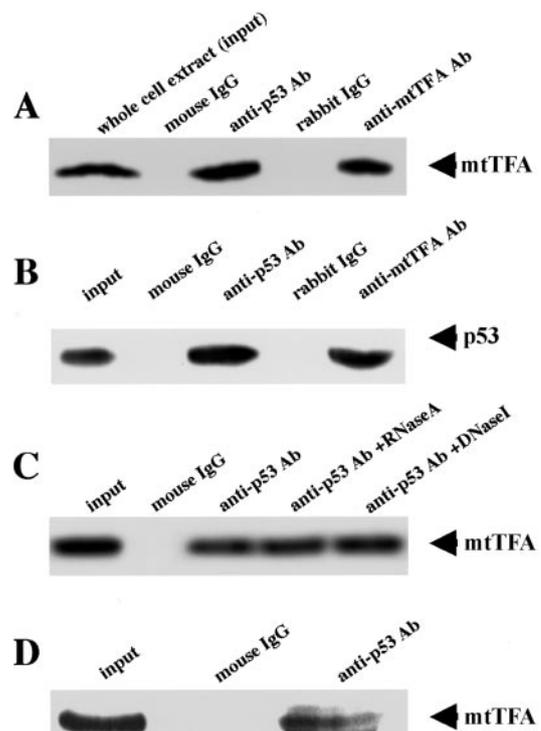

Fig. 1. *A*, Western blots of fractions from KB cells. Nuclear extract (*N.E*; 100 μg), cytosol (*Cyto*) and mitochondrial (*Mito*) fractions from KB cells, incubated with or without 20 μM of cisplatin for 12 h, were analyzed on a 10–15% gel by SDS-PAGE and immunoblotted with antibodies as indicated. The *arrowheads* denote mtTFA (25 kDa), p53 (53 kDa), Sp1 (95 kDa), topoIIα (*Topo IIα*; 170 kDa), Caspase-9 p35 (35 kDa), and cytochrome oxidase subunit I (*CO I*; 57 kDa). *B*, the gel was stained with Coomassie Brilliant Blue (*CBB stain*).

μl of protein A/G-agarose and antibodies as indicated. Immunoprecipitates were washed three times with buffer containing 20 mM HEPES (pH 7.9), 120 mM NaCl, 1 mM EGTA, 1 mM PMSF, and 1 mM DTT followed by centrifugation. They were then subjected to SDS-PAGE and developed as described above.

Pull-down Assay. GST and ThioHis fusion proteins were induced by isopropyl-1-thio-β-D-galactopyranoside and pull-down assays performed as described previously (6, 22). Briefly, *Escherichia coli* cells were sonicated for 10 s in binding buffer and the soluble fraction obtained by centrifugation at 21,000g for 10 min at 4°C. Those GST fusion proteins binding to 15 μl of glutathione-sepharose 4B in a 50% slurry were mixed with ThioHis fusion proteins in binding buffer. The mixtures were incubated for 2 h at 4°C with gentle inversion and washed three times with binding buffer. Pull-down samples representing 10% of the starting material were subjected by SDS-PAGE and analyzed by immunoblotting using anti-Thio antibody.

EMSA. The following annealed 22-mer duplexes were prepared as described previously (6): 5′-GGTGGCCTGACXCATTCCCCAA-3′ and 3′-AC-CGGACTGYGTAAGGGGGTTGG-5′, where X = G or 8-oxo-dG and Y = A, C, G, or T. The 22-mer duplexes were labeled with [α-^{32}P]dCTP, using the Klenow fragment for extension, and gel-purified. Half the volume of each labeled oligonucleotide was treated with 0.3 mM cisplatin at 37°C for 12 h and purified by ethanol precipitation. The mean amount of platinum bound to DNA was 4.3 platinum atoms/oligonucleotide under the reaction conditions used (6). GST fusion proteins binding glutathione-sepharose 4B were washed three times with binding buffer, and eluted with 50 mM Tris-HCl (pH 8.0) and 20 mM reduced glutathione according to the manufacturer's protocol (Pharmacia). Purified GST fusion proteins were used directly for EMSA. Reaction mixtures contained 5% glycerol, 10 mM Tris-HCl (pH 7.5), 50 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 4 ng of ^{32}P-labeled probe DNA, and GST fusion proteins as indicated. Binding reactions were incubated for 5 min at room temperature. The products were analyzed on 4% polyacrylamide gels in 0.5× Tris-borate EDTA buffer using a bioimaging analyzer (BAS 2000; Fuji Photo Film, Tokyo; see Ref. 6).

Fig. 2. *In vivo* interaction between mtTFA and p53. *A*, whole-cell extract (100 μg in the case of rabbit IgG and anti-mtTFA Ab; 600 μg for mouse IgG and anti-p53 Ab) prepared from KB cells treated with 20 μM cisplatin for 12 h was incubated with mouse IgG, rabbit IgG, or antibodies to mtTFA or p53. The immune complexes representing 10% of input were electrophoresed and analyzed by immunoblotting with the antibody to mtTFA. *Ab*, antibody. *B*, whole-cell extract (600 μg for rabbit IgG and anti-mtTFA Ab; 100 μg for mouse IgG and anti-p53 Ab), prepared from KB cells treated with 20 μM cisplatin for 12 h, was incubated with mouse IgG, rabbit IgG, or antibodies to mtTFA or p53. The immune complexes representing 10% of input were electrophoresed and analyzed by immunoblotting with the antibody to p53. *Ab*, antibody. *C*, immunoprecipitation assay using whole-cell extract (600 μg) after treatment with DNaseI and RNaseA. *D*, immunoprecipitation of the mitochondrial fraction (400 μg) was performed with anti-p53 Ab and blotted with anti-mtTFA. *Ab*, antibody.
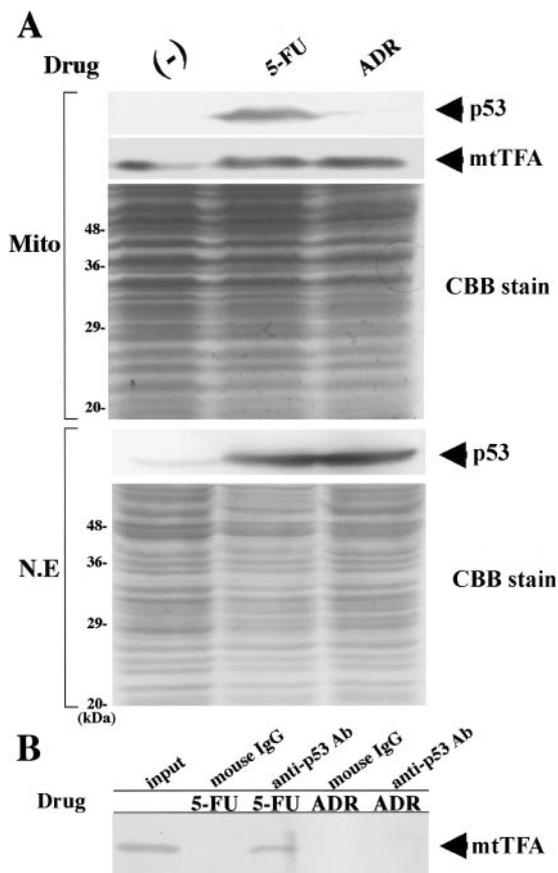
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Fig. 3. *A*, Western blots of fractionated protein from HCT116 cells. One hundred micrograms of nuclear extract (*N.E*) and mitochondrial (*Mito*) fraction from HCT116 cells, treated with 375 μM of 5-FU for 24 h or 0.34 μM of doxorubicin (*ADR*), were analyzed on a 10–15% gel by SDS-PAGE and immunoblotted with antibodies as indicated. The *arrowheads* denote mtTFA (25 kDa) and p53 (53 kDa). The gel was stained with Coomassie Brilliant Blue (*CBB stain*). *B*, a mitochondrial fraction (400 μg), prepared from HCT116 cells treated with 375 μM 5-FU or 0.34 μM doxorubicin (*ADR*) for 24 h, was incubated with mouse IgG or antibodies to p53. The immune complexes and 10% of input were electrophoresed and analyzed by immunoblotting with the antibody to mtTFA.

precipitation and immunoblotting (Fig. 2). To exclude the possibility that p53 and mtTFA might associate to form a ternary complex with nucleic acids, we also tested the immunoprecipitates after treatment with DNaseI and RNaseA, which completely destroy cellular nucleic acids under the conditions used (data not shown). As shown in Fig. 2*C*, a direct association between p53 and mtTFA could be demonstrated when nuclease-treated whole-cell extract was assayed. To determine whether p53 interacts with mtTFA in the mitochondrial fraction, we purified the latter from the KB cells after treatment with cisplatin. As shown in Fig. 2*D*, p53 interacted with mtTFA in the mitochondrial fraction. To test whether this interaction was correlated with apoptosis, we treated HCT116 cells with either 5-FU or doxorubicin and investigated mitochondrial translocation of p53 and the expression level of mtTFA. In a given cell line, such as HCT116 cells, treatment with 0.34 μM doxorubicin induces cell cycle arrest, whereas treatment with 375 μM 5-FU causes apoptosis (25). As shown in Fig. 3, significant amounts of p53 translocated to the mitochondrial fraction when HCT116 cells were treated with 5-FU. On the other hand, only a small amount translocated when the cells were treated with doxorubicin. In both conditions, the amount of nuclear p53 was almost the same. The expression of mtTFA was significantly increased when the cells were treated with these drugs (Fig. 3*A*). Coimmunoprecipitation again showed that mtTFA interacts with p53 in the mitochondrial fraction of HCT116 cells treated with 5-FU (Fig.

3*B*). However, we could not detect this interaction in mitochondria of HCT116 cells treated with doxorubicin because only a small fraction of p53 translocated to the mitochondria when the cells were treated with doxorubicin (Fig. 3*A*). To confirm the association, we performed a pull-down assay using immobilized GST fusion protein. This also showed that mtTFA interacts with p53, but not with GST alone (Fig. 4*B*). Subsequently, we mapped the domain of mtTFA required for interaction with p53 using a pull-down assay with GST fusion proteins containing deletion mutants of mtTFA (Fig. 4*B*) and a ThioHis-p53 fusion protein (Fig. 4*B*). The fusion protein with both HMG-boxes deleted was unable to bind p53, whereas proteins containing either box 1 or box 2 retained full binding activity. We next mapped the domain of p53 responsible for interaction with mtTFA, using GST fusion protein to capture ThioHis-p53 or its deletion derivatives (Fig. 5*A*). This assay demonstrated that the COOH-terminal region of p53 participates in the interaction (Fig. 5*B*). Specifically, a 14-amino-acid region (residues 363–376) of p53 was shown to be critical for the interaction. The COOH-terminal region of p53 was also involved, in precisely the same manner, in the interaction between HMG1 and p53 (22).

Effect of p53 on Binding of mtTFA to Damaged DNA. We have shown previously that interaction with p53 enhances binding of cisplatin-damaged DNA by HMG1 (22). We next examined how p53 affected binding of mtTFA to damaged DNA because mtTFA is known to bind preferentially to damaged DNA (6). We observed that addition of p53 to the mtTFA-DNA binding reaction resulted in significant activation of binding activity for cisplatin-damaged DNA (Fig. 6). The addition of p53 to the mtTFA-DNA binding reaction resulted in a 10–20-fold increase of the DNA binding activity of purified mtTFA. Binding of p53 to mtTFA did not increase the size of the mtTFA-DNA complex, as shown by EMSA. Addition of p53 to a similar reaction significantly inhibited the DNA binding of mtTFA
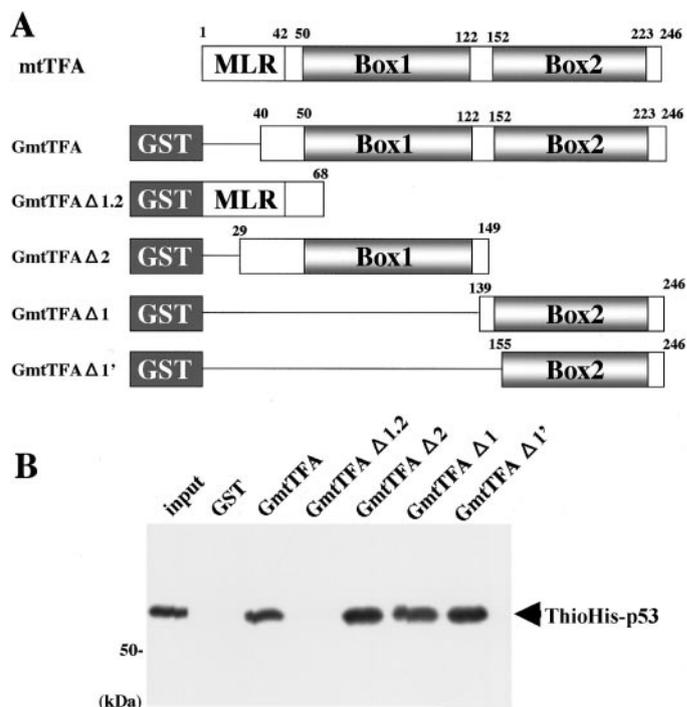

Fig. 4. Mapping of the p53 binding site for mtTFA. *A*, schematic representation of GST-mtTFA fusion protein and the deletion mutants used. *GmtTFA* and *MLR* indicate GST-mtTFA and mitochondrial localizing region, respectively. *Box 1* and *Box 2* are homologous regions of the HMG-box. *B*, ~1 μg of GST fusion proteins immobilized on glutathione-sepharose beads was incubated with ThioHis-p53 expressed in bacteria. Bound protein samples representing 10% of input were analyzed on a 10% gel by SDS-PAGE and immunoblotted with anti-Thio antibody.

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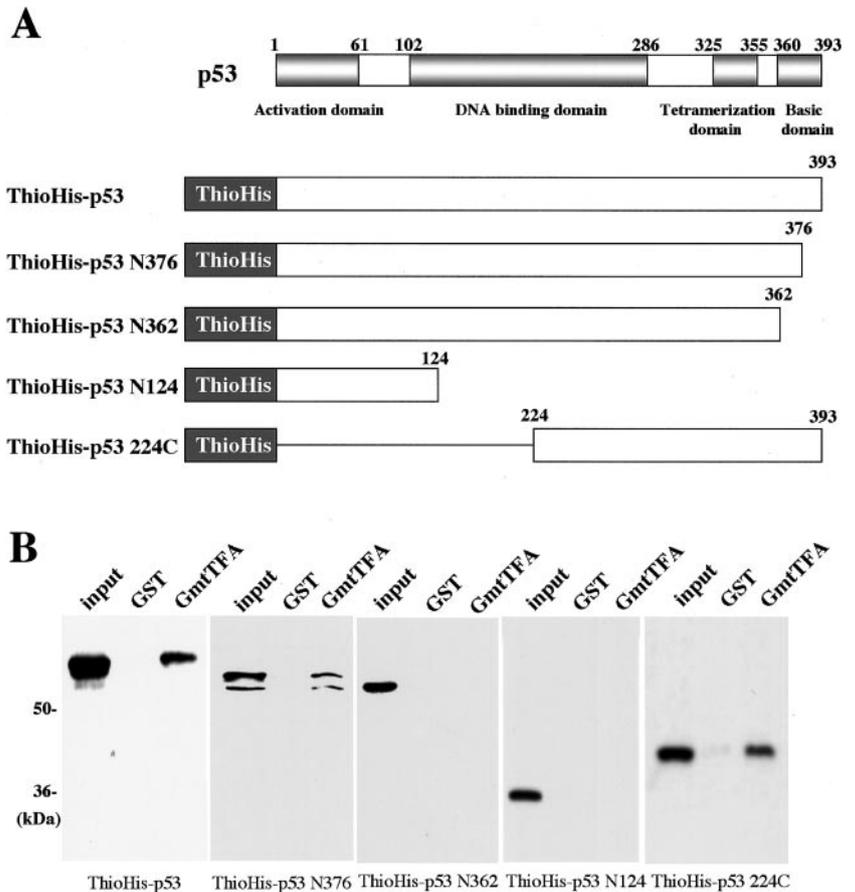
Fig. 5. Mapping of the mtTFA binding site of p53. *A*, schematic representation of the ThioHis-p53 fusion protein and the deletion mutants used. A schematic representation of the functional domains of p53 is also shown (*top panel*). *B*, ~1 μg of GST fusion proteins immobilized on glutathione-sepharose beads was incubated with full-length and truncated forms of ThioHis-p53 expressed in bacteria. Bound protein samples representing 10% of input were analyzed by SDS-PAGE (10% gel) and immunoblotted with anti-Thio antibody. *GmtTFA* indicates GST-mtTFA.

when oxidatively damaged DNA was used as a probe (Fig. 7*A*). Mutant p53, which cannot interact with mtTFA, was unable to inhibit DNA binding by mtTFA; specifically, GST-p53 160C inhibited the DNA binding of mtTFA, whereas GST-p53 N124 did not. HMG1 was unable to recognize oxidatively damaged DNA.

Binding of mtTFA to DNA containing mismatches attributable to the presence of 8-oxo-dG has been observed (6). mtTFA binds efficiently to DNA containing A/8-oxo-dG and C/8-oxo-dG and weakly to T/8-oxo-dG and G/8-oxo-dG. We analyzed the influence of p53 on binding of mtTFA to mismatch-containing DNA. As shown in Fig. 7*B*, p53 inhibited all binding of mtTFA to DNA containing 8-oxo-dG. The GST-MYH bound efficiently to A/8-oxo-dG and G/8-oxo-dG, but weakly to C/8-oxo-dG and T/8-oxo-dG (6). However, p53 did not inhibit the binding of MYH when A/8-oxo-dG was used (Fig. 7*B*), which was probably attributable to lack of interaction of MYH with p53 (data not shown). Two shifted bands were reproducibly observed when GST-MYH was used; this was probably attributable to binding to a degradation product.

In conclusion, binding of mtTFA to cisplatin-modified DNA was enhanced by p53, whereas binding to oxidized DNA was inhibited (Fig. 8).

DISCUSSION

We have demonstrated previously that mtTFA binds preferentially to oxidatively damaged DNA containing 8-oxo-dG (6). mtTFA is a member of the HMG-box protein family, which binds upstream of the light and heavy-strand promoters of mtDNA and activates transcription of mtDNA (1). Members of the HMG protein family bind to bent or distorted structures such as, for example, DNA containing four-way DNA junctions (26), cisplatin adducts, or base bulges (27). mtTFA is

also necessary for mtDNA maintenance and for embryogenesis in mice (7). mtDNA is more susceptible to DNA damage than genomic DNA because mtDNA lacks nucleosome structures. This suggests that
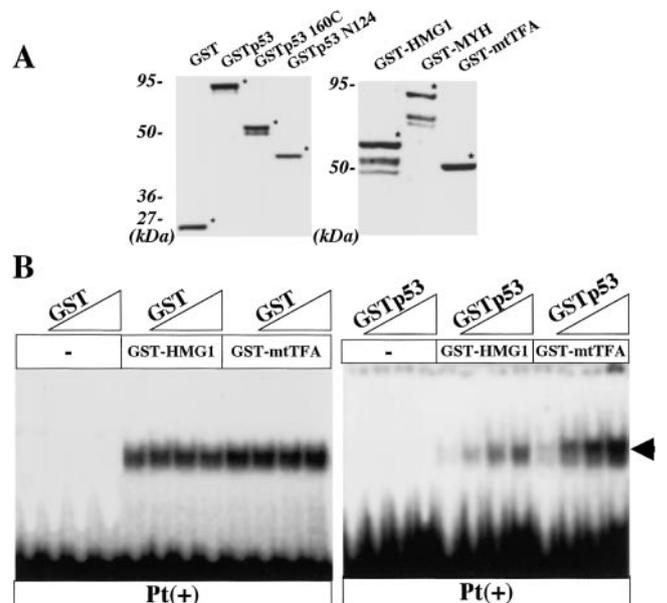

Fig. 6. Stimulation of mtTFA binding to cisplatin-damaged DNA by p53. *A*, Coomassie Brilliant Blue (*CBB*) staining of purified GST-fusion proteins. *Asterisks* indicate full-length GST fusion protein. *B*, binding of purified GST fusion protein to ³²P-labeled double-stranded oligonucleotides (22-mers, 0.4 ng/μl) after cisplatin treatment analyzed by EMSA. Increasing amounts of GST and GST-p53 (10, 30 and 90 ng) were incubated with GST-HMG1 or GST-mtTFA (*right panel*, 30 ng; *left panel*, 100 ng). The *arrowhead* indicates DNA-protein complexes.

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DNA repair activity in mitochondria may be more effective than DNA repair activity in the nucleus.

Mitochondria act as a pivotal decision center in many types of apoptotic response (28–30). Their proteins appear to be involved in apoptosis, and the search for proteins that can specifically bind to damaged DNA is crucial for understanding mtDNA instability and apoptosis. Our finding that mtTFA binds preferentially to oxidatively damaged DNA provides a useful insight into mitochondrial function in this area (6).

Mitochondrial localization of p53 has been correlated recently with the early changes leading to p53-mediated apoptosis in diverse cell types in response to a range of stressors, including DNA damage and hypoxia (11, 12). The present study showed that redirecting the wild-type p53 protein to the mitochondrion provides an enhancer pathway for stress-induced apoptosis involving the direct action of p53 on the organelle. Finally, the expression levels of several genes encoded by mtDNA are affected by the activity of p53 (31–33). In addition, p53 is involved in other mitochondrial functions (34–36). Our data show that a small fraction of cisplatin-induced p53 localizes to mitochondria (Fig. 1). Moreover, mtTFA is up-regulated by treatment with cisplatin. p53 is a multifunctional protein that interacts with a variety of proteins with both positive and negative effects. However, it has not been known previously whether p53 interacts with mtTFA. In this study, pull-down experiments showed that p53 interacts independently with two HMG-boxes. However, these assays also showed that the full-length mtTFA fusion protein did not bind any greater amount of p53, indicating that each HMG-box individually can inter-
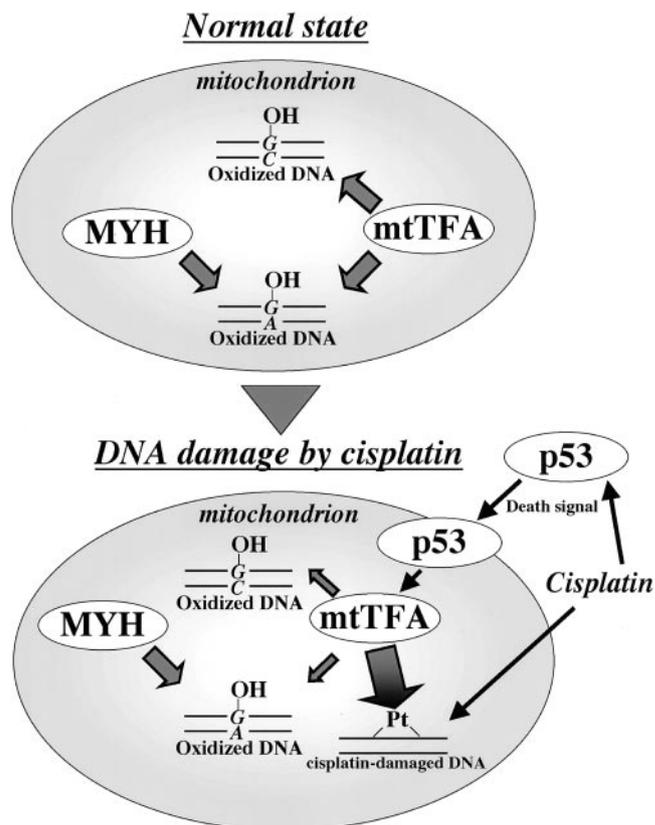

Fig. 8. Schematic summary of interaction between mtTFA and p53 in mitochondria. Binding of mtTFA to cisplatin-modified DNA was significantly enhanced by p53, whereas binding to oxidized DNA was reduced.
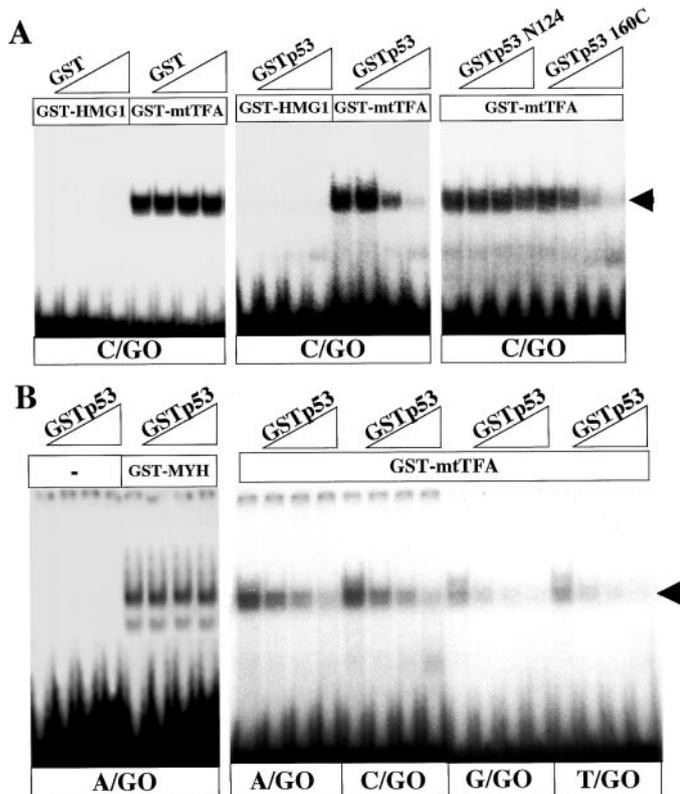

Fig. 7. Effect of p53 on binding of oxidized DNA by mtTFA. *A*, binding of purified GST fusion protein to ^{32}P-labeled double-stranded oligonucleotides (22-mers, 0.4 ng/μl) containing 8-oxo-dG (*C/GO*) analyzed by EMSA. *GO* indicates 8-oxo-dG. Increasing amounts of GST, GST-p53, GST-p53 N124, and GST-p53 160C (10, 30, and 90 ng) were incubated with GST-mtTFA (100 ng). *B*, effect of p53 on binding by MYH of mismatch-containing oxidized oligonucleotides (*left panel*) and mtTFA (*right panel*). Increasing amounts of GST-p53 (10, 30, and 90 ng) were incubated with GST-MYH (90 ng) and GST-mtTFA (90 ng). *A/GO*, *G/GO*, and *T/GO* indicate mismatch-containing oligonucleotides containing 8-oxo-dG. The *arrowhead* indicates DNA-protein complexes.

act with p53. We demonstrated that p53 interacts with mtTFA by both immunoprecipitation and pull-down assays (Figs. 2–5). Interaction between mtTFA and p53 may be involved in transcriptional regulation because, similar to transcription factors, both proteins have DNA-binding activity. Interestingly, we demonstrated that interaction with p53 enhances the binding of cisplatin-damaged DNA, but inhibits the binding of oxidatively damaged DNA (Figs. 6 and 7). The mobility of mtTFA complexed with cisplatin-modified DNA was not altered by p53, indicating that the interaction may be unstable and the p53 may dissociate from the mtTFA-DNA complex during electrophoresis. Taken together, these findings suggest that association with p53 may induce a conformational change of mtTFA. The conformational changes induced by platinum adducts are sharp bends, local denaturation of the duplex, and reduced rigidity (37). The conformational alteration induced by 8-oxo-dG in DNA is different from that induced by cisplatin (37, 38). Therefore, the different effects of p53 on the binding of mtTFA may depend on structural differences in the damaged DNA. Analysis of the mechanism by which mtTFA recognizes damaged DNAs would be desirable for understanding the differential modulation of mtTFA binding by p53.

Two mitochondrial proteins, MYH and mtTFA, bind to oxidatively damaged DNA. MYH has DNA glycosylase activity and can protect mtDNA from the mutagenic effects of oxidized DNA. On the other hand, mtTFA is a transcription factor and binds preferentially to oxidized DNA (6). We did not detect any interaction of mitochondrial repair enzymes such as MYH, 8-oxoguanine-DNA glycosylase 1 (OGG1), or human homologue of *E. coli* endonuclease III (Nth1) with mtTFA *in vitro* (data not shown). mtTFA may recruit repair complexes to damaged regions. Alternatively, it may mask damaged regions to inhibit signaling of the DNA damage until a repair complex

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is recruited. When cells were exposed to cisplatin, interaction with p53 caused an alteration in the way mtTFA recognized DNA (Fig. 6). Up-regulation of expression of mtTFA, and the change in its damage recognition activity may contribute to the avoidance of cisplatin-induced apoptosis. Further study is required to test this idea. Our findings suggest that the inhibition of mtTFA binding to oxidatively damaged DNA, and activation of its binding to cisplatin modified DNA by p53 may play an important role in apoptosis.

p53 has been shown to localize to mitochondria at the onset of p53-dependent apoptosis (11). The COOH-terminus of p53 is necessary for its ability to interfere with growth arrest or apoptosis (39). HCT116 cells undergo apoptosis after treatment with 5-FU but not doxorubicin. Both p53 and mtTFA levels are significantly increased in mitochondria of HCT116 cells treated with 5-FU, indicating that the interaction of p53 with mtTFA might be related to p53-dependent apoptosis (Fig. 3). In addition, apoptosis was increased in cells lacking mtTFA (8). These results suggest a model in which p53 inhibits the ability of mtTFA to execute a cell death signaling. Thus, it is possible that manipulation of mtTFA function may be used as a mitochondria-targeted cancer treatment to enhance apoptosis.

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