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p53 Down-Regulates Matrix Metalloproteinase-1 by Targeting the Communications Between AP-1 and the Basal Transcription Complex

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Abstract We have previously reported that human matrix metalloproteinase-1 (MMP1) is a *p53* target gene subject to down-regulation (Sun et al. [1999]: J Biol Chem 274:11535–11540]. In the present study, we demonstrate that the down-regulation of the human −83MMP1 promoter fragment by p53 was abolished when the −72AP-1 site was eliminated and that a GAL4-cJun-mediated but not a GAL4-Elk1-mediated induction of pFR-luci was effectively inhibited by p53 suggesting an AP-1 dependent but AP-1 binding independent mechanism. Results from gel mobility shift assays were consistent with an AP-1 binding independent mechanism. We also demonstrate that both p300 and TATA box binding proteins cooperated with the transcription factor AP-1 to induce the promoter of MMP1; however, p53 only inhibited the p300-mediated induction of the MMP1 promoter and the inhibition was −72AP-1 dependent. Furthermore, the down-regulation of the MMP1 promoter and mRNA by p53 could be reversed by p300 and by a p53 binding p300 fragment that had no coactivator activity. Taken together, these results indicate that p53 down-regulates MMP1 mainly by disrupting the communications between the transactivator AP-1 and the basal transcriptional complex, which are partially mediated by p300. Finally, by using p53 truncated mutant constructs, we demonstrate that both the N-terminal activation domain and the C-terminal oligomerization domains of p53 were required for the down-regulation of MMP1 transcription. J. Cell. Biochem. 92: 258–269, 2004.

Key words: matrix metalloproteinase-1; tumor suppressor protein p53; coactivator protein p300; transcription

Tumor suppressor protein p53 plays an important role in cell growth and cell-cycle control, DNA repair and apoptosis, and is a key element in the maintenance of genome integrity. Thus, it is no surprise that p53 mutation is a common event in human tumors [Hollstein et al., 1991; Vogelstein and Kinzler, 1992]. In normal cells, upon exposure to DNA damaging agents, the cellular level of p53 increases and affects the decision to either enter a cell-cycle arrest to allow cells to repair the damaged DNA or enter apoptosis. This decision is in part dependent upon the activation and repression of cellular genes involved in growth control, DNA repair, survival, and apoptosis [Levine, 1997]. The best-characterized target for p53-dependent transactivation is p21, an inhibitor of cyclin dependent kinase. The induction of p21 prevents cell-cycle progression arresting cells in G_1 phase [El-Deiry et al., 1993]. p53 also negatively regulates transcription [Deb et al., 1992; Shiio et al., 1992; Miyashita et al., 1994; Sun et al., 1999]. It has been reported that p53 can interact with many transcription factors to negatively affect transcription including TATA box binding protein (TBP), general transcription factor TFIIB, transcription factors Sp1, CCAAT-bind-

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ing factor, NF-κB, and coactivator p300 [Seto et al., 1992; Borellini and Glazer, 1993; Liu et al., 1993; Avantaggiati et al., 1997; Ravi et al., 1998; Yun et al., 1999].

Transcriptional coactivators are a group of transcriptional regulators that facilitate the communications between transactivators and the basal transcriptional complex. p300 is one of well studied coactivators and it has been shown to interact with several transactivators including MyoD, AP-1, NF-κB, and Ets and to potentiate their transactivation [Albanese et al., 1996; Yuan et al., 1996; Avantaggiati et al., 1997; Ravi et al., 1998; Yang et al., 1998]. p300 is also required for p53-dependent transactivation, and the C-terminal domain of p300 (amino acids 1,514–1,737) contains a p53 a binding site [Avantaggiati et al., 1997; Lill et al., 1997].

Degradation of the extracellular matrix around tumor cells is an essential step in the process of tumor invasion and metastasis. There is growing evidence indicating that a high level of expression of matrix metalloproteinases (MMPs) is correlated to the invasiveness of tumor cells [Nuovo et al., 1995; Basset et al., 1997; Johnsen et al., 1998]. Increased expression of MMP1 has been observed in many tumor cells including lung carcinomas [Bolon et al., 1995], squamous cell carcinomas of the head and neck [Johansson et al., 1997], and colorectal tumors [Murray et al., 1996]. Recently, we have demonstrated that p53 down-regulates the expression and the promoters of human MMP1 and MMP13 and that certain human tumor-derived p53 mutants lost such wt-p53 repressive activity [Sun et al., 1999, 2000]. In the present study, we report that p53 down-regulates the promoter of human MMP1 mainly by disrupting the communications between the transcription factor AP-1 and the basal transcription complex.

MATERIALS AND METHODS

Plasmid Constructs

The pCMV-p53 plasmid and the deletion and mutation constructs of the MMP1 promoter have been previously described [Sun et al., 1999, 2002]. Additional deletion or mutation constructs of the MMP1 promoter were prepared by a similar method. N-terminal or C-terminal truncated p53 mutant expression plasmids were prepared by PCR-directed deletion mutagenesis. All PCR generated p53 mutant c-DNAs were inserted into the EcoRI/Xba I sites of the pCI vector (Promega, Madison, WI). The pFR-luci and fusion transactivator plasmids pFA2-c-jun, pFA2-Elk1, and pFA2-CHOP were obtained from Stratagene. The coactivator p300, mutant p300 (1,514–1,922), human TATA box binding protein (TBP) and transcriptional factor II B (TFIIB) expression plasmids were kindly provided by Dr. Antonio Giordano (Thomas Jefferson University). The human c-Jun and JunB expression plasmids pRSV-c-jun and pRSV-junB were kindly provided by Dr. Yang Shi (Harvard Medical School).

Cell Culture and Transient Transfection

Cervical carcinoma HeLa cell line was obtained from the ATCC and cultured in McCoy's 5A medium supplemented with 15% fetal bovine serum and L-glutamine. Transfections were performed using LipofectAMINE 2000 reagent following the manufacturer's instructions (Invitrogen, Carlsbad, CA). A typical transient transfection procedure is as follows. Briefly, exponentially growing HeLa cells were plated at a density of 4.5×10^5 cells/well in six-well cluster plates and grown until 60–70% confluent. The cells were cotransfected with 1.0 μg of MMP1luci plasmid and 0.1 μg of pCMV-p53 plasmid using 3 μl of LipofectAMINE 2000 reagent per well or 0.1 μg of the empty vector pCMV as control (triplicate). Eighteen hours later, fresh medium containing 10% serum was added and cells were incubated for another 24 h. The cells were then harvested and lysed using reporter lysis buffer (Promega) and luciferase activity was determined using an EG&G Berthold Autolumat LB953 Rack Luminometer. The reporter activities were normalized to the amount of protein in the lysate as determined by using a total protein assay kit from Pierce (Rockford, IL). Three independent transfections were performed. Data were expressed as means ± SEM and the relative promoter activities in cells transfected with pCMV-p53 were calculated based on the promoter activities in cells transfected with empty vector pCMV.

Gel Mobility Shift Assay

An oligonucleotide probe spanning from the −80 to the −57 base pair of the human MMP1 promoter containing the −72AP-1 site was used in gel mobility shift assays (top: 5′-GATCA-TAAAGCATGAGTCAGAC-ACCTCT-3′, bottom:

5′-GATCAGAGGTGTCTGACTCATGCTTTAT-3′). Oligonucleotides were annealed by boiling 5 min and cooling down slowly to room temperature. The probe was labeled by a filling-in reaction containing α-^{32}P ATP and Klenow DNA polymerase and purified using a G-25 Sepharose (Sigma, St. Louis, MO) spin column. HeLa cells in 100 mm plates were transfected with either 12 μg of pCMV (four plates) or 12 μg of pCMV-p53 plasmids (four plates) using the LipofectAMINE 2000 transfection reagent (Invitrogen) and incubated in McCoy's 5A medium/10% serum for an additional 24 h. Cells were harvested and nuclear cell extracts were prepared using NE-PER™ Nuclear Extraction reagents following the manufacturer's instructions (Pierce). Nuclear proteins (5 μg per assay) were incubated with 5 ng of the labeled probe in a binding buffer (20 mM HEPES [pH 7.9], 20% glycerol, 100 mM KCl, 0.5 mM EDTA, 0.5 mM DTT, 1 μg poly dI:dC) for 20 min at room temperature. Samples were applied on native 5% polyacrylamide gels in TBE buffer. Gels were dried on Whatman 3MM paper and exposed to X-ray film.

RT-PCR

Total RNA was isolated using the Trizol Reagent according to the manufacturer's instructions (Invitrogen). Reverse transcription (RT) was carried out with 1 μg of total cellular RNA at 50°C for 60 min, followed by enzyme inactivation at 85°C for 5 min using the ThermoScript™ RT-PCR System (Invitrogen). The resulting cDNA samples were then amplified by PCR method. PCR primers for MMP1 were synthesized based on published sequence for human MMP-1 (sense, 5′-GATCATCGGGACAACTCTCCT-3′ and antisense, 5′-TCCGGGTAGAAGGGATTTGTG-3′). Amplifications were carried out for 30 cycles by denaturing at 95°C for 30 s, annealing at 55°C for 30 s, and extending at 72°C for 45 s, with a final extension at 72°C for 10 min. As an internal control, a 353-bp fragment of the constitutively expressed housekeeping gene, β-actin, was also amplified. The PCR products were analyzed by electrophoresis on 2% agarose gel containing ethidium bromide.

Western Blot Analysis

HeLa cells were transfected either with 1.5 μg pCMV or pCMV-p53 plasmid (triplicate) in 6-well cluster plates. Eighteen hours post-transfection fresh McCoy's medium containing 10% serum was added and incubated for another 24 h. The cells were then harvested, pooled, lysed, and protein extracts subjected to Western blot analysis using antibody specific for phosphorylated c-Jun or p53. Briefly, the proteins derived from pCMV and pCMV-p53 transfected cells were separated on 7.5% SDS–PAGE minigels and transferred to nitrocellulose membrane. The membranes were blocked with 5% skim milk for 1 h, and then probed with human phosphorylated c-Jun specific antibody (Santa Cruz Biotechnology, Santa Cruz, CA) overnight. The membrane was washed three times and incubated with horseradish-peroxidase-conjugated anti-mouse immunoglobulin G for 2 h. After the membrane was washed, immunolabeled bands were visualized with a color development solution containing 3,3′-diaminobenzidine and hydrogen peroxide. The same membrane was then probed with human p53 specific antibody DO-1 (Santa Cruz Biotechnology).

RESULTS

The −72AP-1 Site Is Critical for the Down-Regulation of MMP1 Promoter by p53

To identify the *cis*-elements that were involved in the down-regulation of the human MMP1 promoter, we first analyzed a series of 5′ deletion constructs of the human MMP1 promoter in HeLa cells. We chose HeLa cells because they were easy to transfecte and they have been used to study MMP1 transcription previously in many laboratories. As we can see from Figure 1, the promoter activities of all deletion promoter fragments, from −357MMP1 to −83MMP1, were down regulated by p53, resulting in approximately a threefold to fourfold repression. In contrast, the minimal promoter −61/+68MMP1luci that only contained a lone TATA box was not down regulated by p53. Further deletions (down to −12/+38MMP1) yielded similar results. These results suggested that at least one of the *cis*-elements involved in the p53-mediated down-regulation of the MMP1 promoter resided between the −83 and the −61 base pair region of the human MMP1 promoter.

It was likely that the −72AP-1 site, which was critical to both the basal and induced promoter activity of MMP1 [Gutman and Wasylyk, 1990; Vincenti et al., 1996], was involved in the p53-mediated downregulation of the MMP1 promoter. We examined this possibility further by site directed mutagenesis to eliminate the −72AP1 site. As shown in Figure 1B, elimination of the

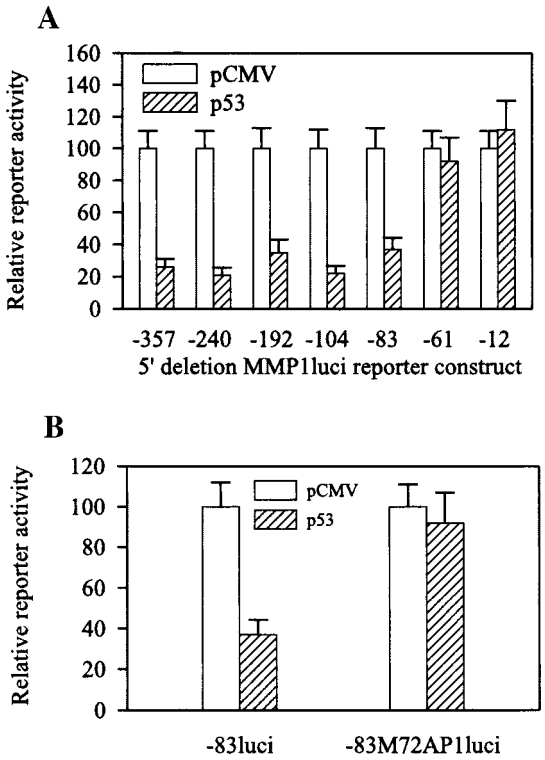

Fig. 1. The −72AP-1 site is critical for the down-regulation of matrix metalloproteinase-1 (MMP1) promoter by p53. **A**: HeLa cells were transiently cotransfected with a series of 5′ deletion human MMP1 promoter/luciferase reporter plasmids (1 μg/per well) together with 0.1 μg of pCMV-p53 plasmid or pCMV as control as described in Materials and Methods. Twenty-four hours post-transfection cells were harvested and protein extracts were assayed for luciferase activity. **B**: HeLa cells were cotransfected with 1 μg of −83MMP1luci or −83M72AP1luci together with 0.1 μg of pCMV-p53 plasmid or 0.1 μg of pCMV as control. Twenty-four hours post-transfection, cells were lysed and luciferase activities were assayed. Three to six independent transfections were performed and the results expressed as the means ± SEM. Promoter activities are presented as relative reporter activities calculated by arbitrarily setting the activity of the control as 100.

−72AP-1 site by mutation abolished the p53-mediated down-regulation of the MMP1 promoter, confirming that the −72AP-1 site was indeed involved in the p53-mediated down-regulation of the MMP1 promoter. Similar results were obtained using several other cell lines including a breast cancer cell line, MCF-7, and a chondrosarcoma cell line HTB 94 (data not shown).

The results above indicated that the transcriptional down-regulation of MMP1 by p53 was −72AP-1 dependent; however, the possibility that some other sites upstream the −83 base pair were also involved in the transcriptional inhibition of MMP1 by p53 could not be completely excluded. The most relevant part of the human MMP1 promoter is shown in Figure 2A. The −192/+68 base pair promoter region contains several sites including a −181AP-1 and −88PEA3 and the −72AP-1 sites which are known to be important in the transcriptional control of the human MMP1 promoter [Gutman and Wasylyk, 1990; Chapman et al., 1999; Sun et al., 2002]. To examine their possible involvement, we prepared a series of mutant −192MMP1 promoter/luciferase reporter constructs. As shown in Figure 2B, only mutation at the −72AP-1 site relieved the down-regulation

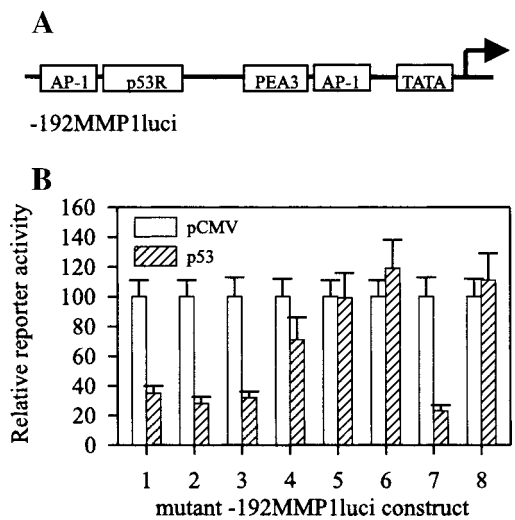

Fig. 2. The −181AP1, −88PEA3, and −161p53 sites are not involved in the p53-mediated down-regulation of the MMP1 promoter. **A**: The −192/+68 base pair promoter region contains several sites that are known to be important in the transcriptional control of the MMP1 transcription: −181AP-1, −88PEA3, and −72AP-1 sites. It also contains a sequence (p53R) that closely matches the sequence G(G/C)AA(G/C)TGA which has been reported to mediate the down-regulation of the Rb promoter by p53 [Shiio et al., 1992]. **B**: HeLa cells were transiently cotransfected with a series of mutant −192MMP1luci (1 μg/per well) together with 0.1 μg of pCMV-p53 plasmid or pCMV as control (triplicate). Twenty-four hours post-transfection cells were harvested and protein extracts were assayed for luciferase activity. **Bar group 1**: −192MMP1luci; **bar group 2**, −192M181AP1luci (mutation at the −181AP-1 site); **bar group 3**, −192M88PEA3luci (mutation at the −88PEA3 site); **bar group 4**, −192M72AP1luci (mutation at the −72AP-1 site); **bar group 5**, −192M181-72AP1luci (mutation at both the −181AP-1 and −72AP-1 sites); **bar group 6**, −192M88PEA3-72AP1luci (mutation at both the −88PEA3 and −72AP-1 sites); **bar group 7**, −192M181AP1-88PEA3luci (mutation at both the −181AP-1 and −88PEA3 sites); **bar group 8**, −192M3luci (mutation at all three sites). Three independent transfections were performed and the results expressed as the means ± SEM. Promoter activities are presented as relative reporter activities calculated by arbitrarily setting the activity of the control as 100.

of the MMP1 promoter by p53 substantially (compare the bar groups 1 with 4), which was consistent with the results shown in Figure 1B. Although the −88PEA3 site was also critical to the basal and the induced activity of the promoter [Chapman et al., 1999; Sun et al., 2002], mutation at this site alone had no detectable effect on the down-regulation of the −192MMP1 promoter by p53 (compare bar group 1 with 3). Similarly, mutation at the −181AP1 site alone had no detectable effect on the down-regulation of the −192MMP1 promoter by p53 (compare the bar group 1 with 2).

It has been reported that the down-regulation of the Rb promoter by p53 is mediated by a putative *cis*-acting element, G(G/C)AA(G/C)TGA [Shiio et al., 1992]. Examination of the human MMP1 promoter indicates that there are several sites that closely match the sequence G(G/C)AA(G/C)TGA. One of such sites is located at the −161 base pair region and has the sequence CCAAGTGA. We prepared a mutant construct of −192M161luci where the −162 site was mutated. Results from using the mutant construct indicated that the site was not involved (data not shown).

p53 has Little Effect on the Binding of AP-1 to the −72AP-1 Site

Since the results shown above suggested that the down-regulation of the MMP1 promoter by p53 was dependent on the −72AP-1 site, we decided to perform gel mobility shift assays using an oligoprobe spanning from the −80 to the −57 base pair of the human MMP1 promoter containing the −72AP-1 site. HeLa cell nuclear extracts prepared from HeLa cells transfected with either a p53 expression plasmid, pCMV-p53, or the empty vector pCMV plasmids. The efficiency of transfections was in the range of 75–85% judged by immunostaining with an anti-p53 antibody (data not shown) or by β-gal staining after transfecting with a pCMV-β-gal plasmid (Fig. 3A). Figure 3B shows a representative gel mobility shift assay. No detectable difference was observed between the binding of the −72AP-1 site to the AP-1 transcription factor in the nuclear extract prepared from pCMV-p53 transfected cells and to the AP-1 transcription factor in the nuclear extract prepared from pCMV transfected cells (lanes 1 and 2). Both extracts formed a specific complex with the labeled probe and the binding was efficiently competed for by the cold −72AP-1 probe (lane 4) but not by a sp-1 probe (lane 3). Gel mobility assays were also performed using nuclear extracts prepared from a p53 inducible Saos-2 cell line established using the Ecdysone-Inducible System (Invitrogen) and similar results were obtained (data not shown). In parallel experiments, we extracted total RNA from HeLa cells transfected with a p53 expression plasmid and examined the levels of MMP1 mRNA using RT-PCR. Results indicated that the endogenous MMP1 was down-regulated by p53 and that p53 deletion mutant

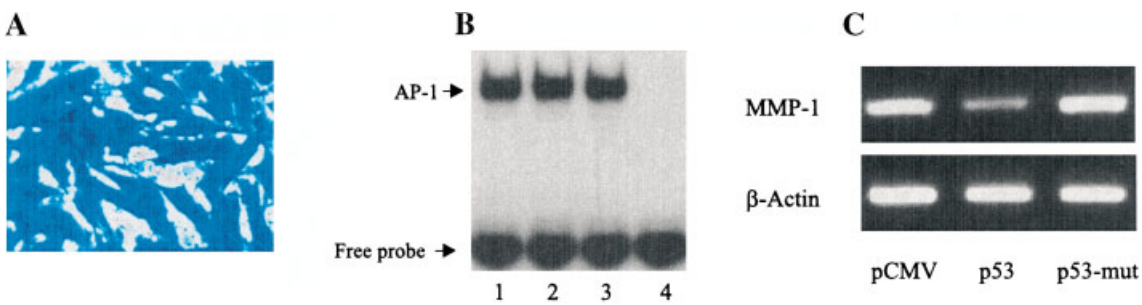

Fig. 3. p53 has little effect on the binding of AP-1 to the −72AP1 site. **A**: HeLa cells in 60 mm plates were transfected with 6 μg of pSV40-β-galactosidase plasmid using 18 μl of LipofectAMINE 2000 transfection reagent. Twenty-four hours later, cells were stained with 1 mg/ml 5-bromo-4-chloro-3-indoyl-β-D-galactoside. **B**: Gel mobility shifts were performed using a labeled oligoprobe containing the −72AP-1 site. Extract prepared from pCMV plasmid transfected cells was incubated with the labeled −72AP-1 probe (**lane 1**). Extract prepared from pCMV-p53 transfected cells was incubated with the labeled −72AP-1 probe alone (**lane 2**), with the labeled −72AP-1 probe plus a tenfold excess of unlabeled sp-1 probe (**lane 3**) or plus a tenfold excess of unlabeled −72AP-1 probe (**lane 4**). **C**: Total RNA was isolated from HeLa cells transfected with p53 expression plasmids and subjected to RT-PCR analysis for MMP1 mRNA. Level of MMP1 mRNA in HeLa cells transfected with pCMV plasmid (**1st lane**), with pCMV-p53 plasmid (**2nd lane**), and with pCMV-p53-del (1–159) plasmid (**3rd lane**). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

p53-del (1–159) lost the wild-type p53 repressive activity (Fig. 3C).

p53 Inhibits the c-Jun-Mediated Induction of pFR-Luci and −83MMP1luci

It is conceivable that p53 could inhibit the transcription of MMP1 in an AP-1 dependent manner either by affecting the AP-1 binding to the −72AP-1 site or by interfering with the communications between the AP-1 transcription factor and the basal transcriptional complex formed on the MMP-1 promoter. Since the gel mobility shift assay indicated that p53 had little effect on the AP-1 binding, it was likely that p53 inhibited the transcription of MMP-1 by interfering with the communications between the AP-1 transcription factor and the basal transcriptional complex. To examine this theory, we cotransfected HeLa cells with the following three plasmids: a reporter plasmid pFR-luci that contains a GAL4 binding site and a TATA box upstream of the luciferase c-DNA, a pFA2-c-Jun vector that encodes a GAL4-c-Jun fusion protein, and pCMV-p53. When expressed in mammalian cells, the GAL4 binding domain of the fusion protein GAL4-c-Jun binds to the GAL4 site on the pFR-luci plasmid, puts the activation domain of c-Jun in close contact with the basal transcription complex formed on the TATA, and activates the expression of luciferase. Since it is unlikely that p53 affects the binding of the fusion protein GAL4-cJun to the GAL4 site on the pFR-luci reporter plasmid, the effect of p53, if any, can be attributed to its action on the communications between the transactivator c-Jun and the basal transcription complex. As shown in Figure 4A, the fusion protein GAL4-cJun activated the

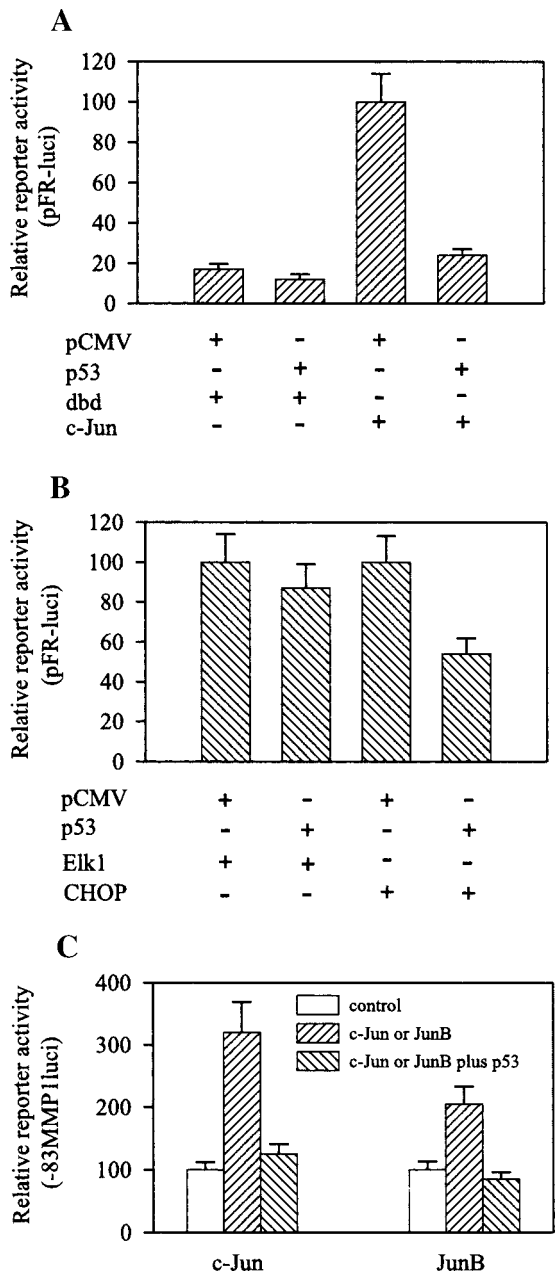

Fig. 4. p53 inhibits the c-Jun-mediated induction of the pFR-luci reporter and the −83MMP1 promoter. **A**: HeLa cells were cotransfected with 1 μg of pFR-luci, 0.1 μg of pCMV and 20 ng of pFA2-dbd plasmid, which is the parent plasmid for pFA2-c-Jun (the **first bar**), 1 μg of pFR-luci, 0.1 μg of pCMV-p53 and 20 ng of pFA2-dbd plasmid (the **second bar**), 1 μg of pFR-luci, 0.1 μg of pCMV and 20 ng of pFA2-c-Jun plasmid (the **third bar**), or 1 μg of pFR-luci, 0.1 μg of pCMV-p53 and 20 ng of pFA2-cJun plasmid (the **fourth bar**). Twenty-four hours post-transfection, cells were harvested and protein extracts were assayed for luciferase activity. **B**: Similar experiments were performed using pFA2-Elk1 or pFA2-CHOP to replace the pFA2-cJun plasmid. HeLa cells were cotransfected with 1 μg of pFR-luci, 0.1 μg of pCMV and 20 ng of pFA2-c-Elk 1 plasmid (the **first bar**), 1 μg of pFR-luci, 0.1 μg of pCMV-p53, and 20 ng of pFA2-Elk 1 plasmid (the **second bar**), 1 μg of pFR-luci, 0.1 μg of pCMV, and 20 ng of pFA2-c-CHOP plasmid (the **third bar**), or 1 μg of pFR-luci, 0.1 μg of pCMV-p53, and 20 ng of pFA2-CHOP plasmid (the **fourth bar**). **C**: HeLa cells were cotransfected with 1 μg of −83MMP1luci together with 0.4 μg of pcDNA3 plasmid and 0.1 μg of pCMV (the **blank bars**), or together with 0.4 μg of pSRV-cJun (or pSRV-JunB) and 0.1 μg of pCMV (the **middle shaded bars**), or together with 0.4 μg of pSRV-cJun (or pSRV-JunB) and 0.1 μg of pCMV-p53 (the **right-hand side shaded bars**) in six-well plates (duplicate). Twenty-four hours later, cells were harvested and protein extracts were assayed for luciferase activity. Three independent transfections were performed and the results expressed as the means ± SEM. Promoter activities are presented as relative reporter activities calculated by arbitrarily setting the activity of the control as 100.

expression of luciferase up to sevenfold (compare bar 1 with 3) and the activation was inhibited almost completely by p53 (compare bar 3 with 4). It is worth noting that p53 only had a minor effect on the basal activity of the minimal promoter in the pFR-luci reporter plasmid (compare bar 1 with 2). These results suggested that p53 inhibited the GAL4-cJun mediated induction of pFR-luci by blocking the communications between c-Jun and the basal transcription complex. Significantly, the inhibition of transactivator-mediated induction of pFR-luci by p53 was specific to the transactivator c-Jun; p53 either had no effect or a much weaker effect on the GAL4-Elk1-mediated and the GAL4-CHOP-mediated induction of pFR-luci (Fig. 4B, compare bar 1 with 2, and bar 3 with 4). We also cotransfected HeLa cells with the −83MMP1luci and a c-Jun expression plasmid, pRSV-c-Jun, or a JunB expression plasmid, pRSV-junB, with or without the pCMV-p53 plasmid. As expected, the induction of −83MMP1luci by the ectopically expressed c-Jun or JunB was inhibited effectively by p53, again suggesting that similar to the GAL4-cJun mediated induction of pFR-luci, p53 inhibited the c-Jun mediated induction of −83MMP1luci by blocking the communications between c-Jun and the basal transcription complex (Fig. 4C).

To exclude the possibility that p53 inhibited the c-Jun-mediated induction of pFR-luci by modulating the phosphorylation of c-Jun, Western blot analysis was performed using antibody specific to phosphorylated c-Jun. Results indicated that p53 had no detectable effect on the level of phosphorylated c-Jun (Fig. 5).

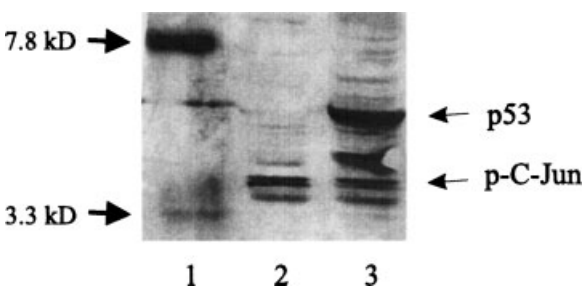

Fig. 5. p53 has no detectable effect on the level of phosphorylated c-Jun. HeLa cells were transfected with pCMV or pCMV-p53 plasmid and cell lysates subjected to Western blot analysis as described in Materials and Methods. **Lane 1**: Molecular marker; **lane 2**, lysates prepared from cells transfected with 1.5 μg of pCMV plasmid; **lane 3**, lysates prepared from cells transfected with 1.5 μg of pCMV-p53 plasmid.

p53, but not its Mutants, Inhibits the p300-Mediated Induction of −83MMP1luci

The general transcription factors TBP, TFIIB, and the coactivator protein p300 have been implicated in p53-mediated transcriptional inhibition [Seto et al., 1992; Liu et al., 1993; Liu and Berk, 1995; Avantaggiati et al., 1997]. To examine their potential involvement in the p53-mediated transcriptional inhibition of MMP1, we cotransfected HeLa cells with the −83MMP1luci plasmid and pcDNA3-TBP, pcDNA3-TFIIB, pCMV-p300, or pCMV-p300 (1,514−1,922), respectively. Results indicated that both TBP and p300, but not the TFIIB or the p300 (1,514−1,922) mutant, induced the promoter of −83MMP1luci (Fig. 6A). To further examine the involvement of p300 in the transcriptional control of MMP-1, we analyzed the levels of MMP1 mRNA in cells transfected with p300 expression plasmids using RT-PCR. As shown in Figure 6B, p300 induced the transcription of MMP1 and the induction was abolished when both of the N- and C-terminal activation domains of p300 were deleted (Fig. 6B). After confirming that p300 was involved in the transcriptional control of MMP1, we then examined how p53 and its mutants could affect the p300-mediated induction of −83MMP1luci. As we expected, p53 inhibited the p300-mediated induction of −83MMP1luci while p53 mutants lost the wild-type p53 repressive activity (Fig. 6C, bar group 1), which was consistent with our previous observation that p53 down-regulated the expression of endogenous MMP1 while p53 mutant either lost the repressive activity or up-regulated the expression of MMP1 [Sun et al., 1999]. It is worth noting that the inhibition of the induction of MMP1 was effector specific in that p53 failed to inhibit the TBP mediated induction of −83MMP1luci (Fig. 6C, bar group 2) indicating that p53 down-regulated MMP1 by targeting p300.

Since the p53-mediated down-regulation of MMP1 was −72AP-1 dependent, we decided to examine whether the induction of the MMP1 promoter by p300 and the inhibition of the p300-mediated induction of MMP1 promoter by p53 were −72AP-1 dependent. As shown in Figure 7, although still able to induce transcription, the induction of −83MMP1luci by p300 was decreased to threefold (Fig. 7, compare bar 1 with 2 within the first bar group) from about sixfold

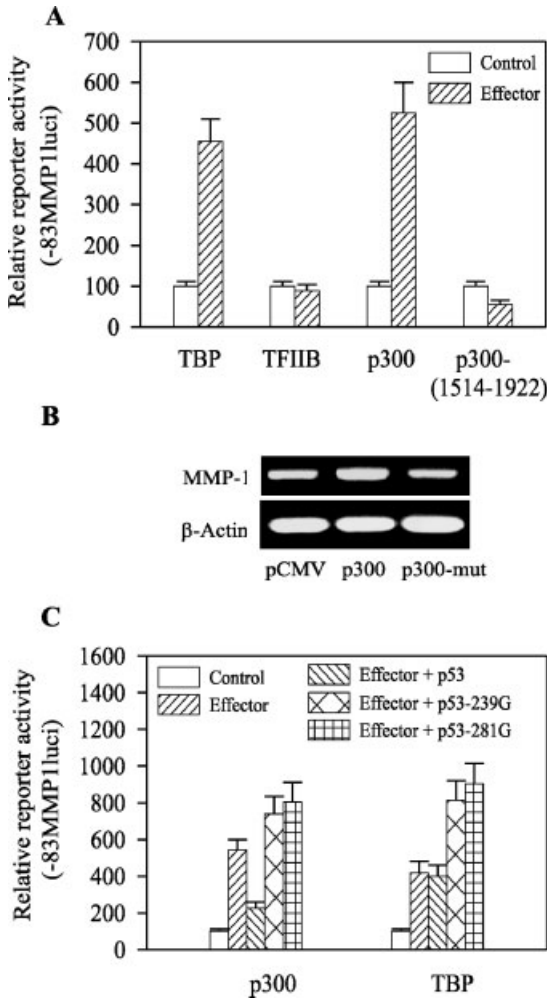

Fig. 6. p53 but not p53 mutants inhibits p300-mediated induction of −83MMP1luci. **A**: HeLa cells were cotransfected with 1 μg of −83MMP1luci together with 0.4 μg of pcDNA3 (the **blank bars**), or together with 0.4 μg of pcDNA3-TBP, pcDNA3-TFIIB, pcDNA3-p300, or pcDNA3-p300 (1,514–1,922) (the **shaded bars**). **B**: Total RNA was isolated from HeLa cells transfected with p300 expression plasmids and subjected to RT-PCR analysis for MMP1 mRNA. Level of MMP1 mRNA in HeLa cells transfected with pCMV plasmid (**1st lane**), with pCMV-p300 plasmid (**2nd lane**), and with pCMV-p300 (1,514–1,922) plasmid (**3rd lane**). **C**: HeLa cells were cotransfected with 1 μg of −83MMP1luci together with 0.4 μg of pcDNA3 and 0.1 μg of pCMV plasmids, or together with 0.4 μg of pcDNA3-p300 or pcDNA3-TBP and 0.1 μg of pCMV plasmids, or together with 0.4 μg of pcDNA3-p300 or pcDNA3-TBP and 0.1 μg of pCMV-p53 or pCMV-p53 mutants expression plasmids as indicated. Twenty-four hours post-transfection, cells were harvested and protein extracts were assayed for luciferase activity. Three independent transfections were performed and the results expressed as the means ± SEM. Promoter activities are presented as relative reporter activities calculated by arbitrarily setting the activity of the control as 100.

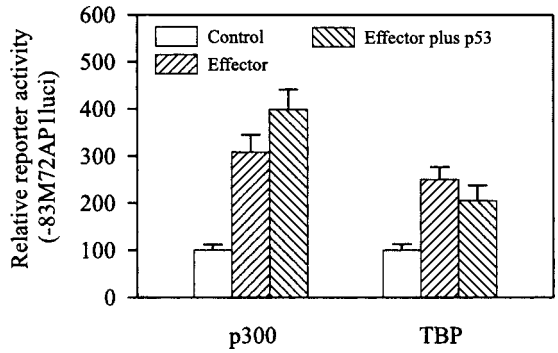

Fig. 7. Mutation at the −72AP-1 site abolishes the inhibition of the p300-mediated induction of −83MMP1luci by p53. HeLa cells were cotransfected with 1 μg of −83M72AP1luci (the −72AP-1 site was mutated) together with 0.4 μg of pcDNA3 and 0.1 μg of pCMV plasmids (**blank bars**), or together with 0.4 μg of pcDNA3-p300 and 0.1 μg of pCMV, or 0.4 μg of pcDNA3-TBP and 0.1 μg of pCMV (**middle shaded bars**), or together with 0.4 μg of pcDNA3-p300 and 0.1 μg of pCMV-p53, or 0.4 μg of pcDNA3-TBP and 0.1 μg of pCMV-p53 (**right-hand side shaded bars**). Three to six independent transfections, each run in triplicate, were performed, and the results expressed as the means ± SEM. Relative promoter activities were calculated by arbitrarily setting the activity of −83M72AP1luci without transactivator protein as 100.

(Fig. 6A, the third bar group) when the −72AP-1 site was mutated, indicating that p300 was indeed a coactivator of AP-1 in the transcriptional regulation of MMP1. Interestingly, the TBP-mediated induction was also associated with the −72AP-1 site. The induction of −83MMP1luci by TBP was decreased to two-fold (Fig. 7, compare bar 1 with 2 within the second bar group) from about fourfold to five-fold (Fig. 6A, the second bar group) when the −72AP1 site was mutated. Most importantly, the inhibition of p300-mediated induction of −83MMP1luci by p53 was completely abolished after the −72AP-1 site was mutated (Fig. 7, the first bar group), confirming that the inhibition of the p300-mediated induction of MMP1 promoter by p53 was −72AP-1 dependent.

As expected, increasing amounts of p300 rescued the transcriptional inhibition of −83MMP1luci by p53 (Fig. 8, bar group 1). However, we could not exclude the possibility that the inhibition of the −83MMP1luci by p53 and the p300-mediated reversion were two separate unrelated events because p300 itself induced the promoter of MMP1. To clarify the issue, we took advantage of the findings that the p300 (1,514–1,922) mutant has no coactivator activity (Fig. 6A, the last bar group) but

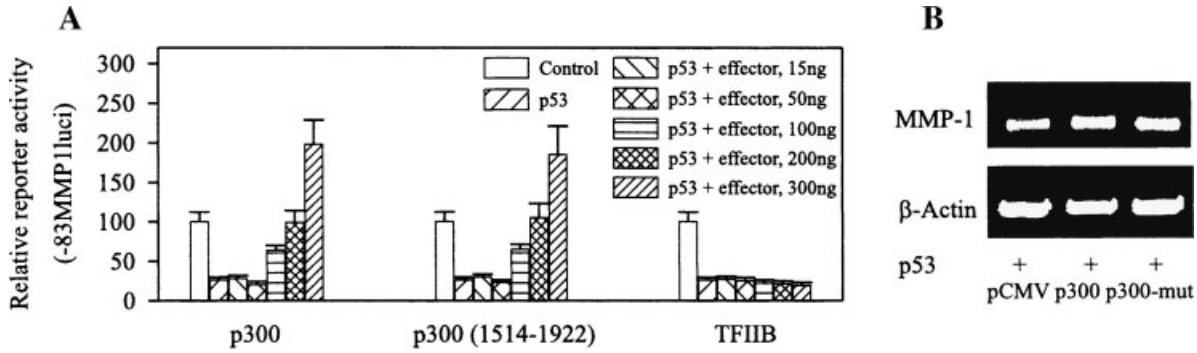

Fig. 8. p300 and the inactive coactivator p300 (1,514–1,922) reverse the down-regulation of −83MMP1luci and MMP1 mRNA. **A**: HeLa cells were cotransfected with 0.8 μg of −83MMPluci together with 0.3 μg of pcDNA3 and 0.1 μg of pCMV plasmids (**blank bars**), or together with 0.3 μg of pcDNA3 and 0.1 μg of pCMV-p53 (the **second bars** in all three bar groups), or together with increased amounts of pcDNA3-p300, pcDNA3-300 (1,514–1,922) or pcDNA3-TFIIB as indicated (**bars 3–7** in the three bar groups). The total amount of plasmid transfected was kept constant by pcDNA3. Transfected cells were subsequently incubated with fresh medium containing 10% serum for 24 h. Cells were then harvested and protein extracts were assayed for luciferase activity. Three independent transfections, each run in triplicate, were performed, and the results expressed as the means ± SEM. Relative promoter activities were calculated by arbitrarily setting the control as 100 (the **blank bars**). **B**: Total RNA was isolated from HeLa cells cotransfected with p53 and p300 expression plasmids and subjected to RT-PCR analysis for MMP1 mRNA. Level of MMP1 mRNA in HeLa cells (60 mm plates) cotransfected with 3 μg of pCMV-p53 and 3 μg of pCMV plasmids (**1st lane**), with 3 μg of pCMV-p53 and 3 μg of pCMV-p300 plasmids (**2nd lane**), and with 3 μg of pCMV-p53 and 3 μg of pCMV-p300 (1,514–1,922) plasmids (**3rd lane**).

contains a p53 binding site [Avantaggiati et al., 1997]. We cotransfected HeLa cells with the −83MMP1luci, pCMV-p53 and increasing amounts of pcDNA3-p300 (1,514–1,922). We figured that if the inhibition of the −83MMP1luci by p53 and the p300-mediated reversion were two separate unrelated events, p300 (1,514–1,922) should fail to rescue the transcriptional inhibition of −83MMP1luci by p53. However, results revealed that excess amounts of p300 (1,514–1,922) rescued the transcription inhibition of −83MMP1luci by p53 just as effectively as wild-type p300 (Fig. 8, bar group 2) indicating that p300 reversed the p53-mediated inhibition of MMP1 by directly targeting p53. A parallel experiment was performed using pcDNA3-TFIIB to serve as a negative control (Fig. 8, bar group 3). In addition, we extracted total RNA from HeLa cells transfected with p53 and p300 expression plasmids, and examined the levels of MMP1 mRNA using RT-PCR. Results confirmed that both p300 and p300 (1,514–1,922) could reverse the transcriptional inhibition of MMP1 by p53 (Fig. 8B).

The N-Terminal Activation and the C-Terminal Oligomerization Domains of p53 Are Required for the Inhibition of MMP1 Transcription

Lastly, we decided to determine the p53 domains required for p53-mediated down-regulation of the MMP1 promoter. We cotransfected HeLa cells with the −83MMP1luci and wild-type p53 expression plasmids or a series of truncated p53 mutants expression plasmids respectively. As shown in Figure 9, p53 down-regulated the −83MMP1luci expression but all the N-terminal truncated mutants lost the inhibitory activity. However, both the C-terminal truncated mutants p53-C376 and p53-C354 retained the wild-type p53 inhibitory activity and only further deletion to 324 amino acids abolished the inhibitory activity of p53. These results indicated that the N-terminal 1–25 (25 amino acids) and the C-terminal 324–354 (30 amino acids) regions were required for the transcription inhibition of MMP1.

DISCUSSION

We have previously shown that MMP1 is a *p53* target gene subject to down-regulation [Sun et al., 1999]. However, the detailed mechanisms are incompletely studied. In the present study, we demonstrated that the down-regulation of the human MMP1 promoter by p53 was mediated by a −72AP-1 dependent mechanism. The down-regulation of human MMP1 promoter by p53 was abolished after the proximal −72AP1 site was deleted or mutated. The question then is how does p53 modulate the promoter activity

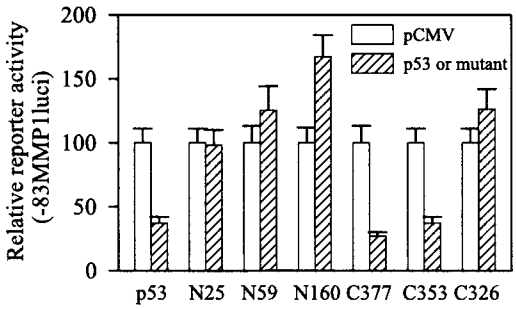

Fig. 9. Effect of wild-type p53 and deletion mutants on MMP-1 promoter activity. HeLa cells were transfected with 1.0 μg of −83MMP1luci together with 0.1 μg of wt-p53 and a series of truncated p53 mutant expressing plasmids. Transfected cells were subsequently incubated with fresh medium containing 10% serum for 24 h. Cells were then harvested and protein extracts were assayed for luciferase activity. Three independent transfections, each run in triplicate, were performed, and the results expressed as the means ± SEM. Relative promoter activities were calculated by arbitrarily setting the control as 100 (the **blank bars**).

of MMP1 through an AP-1 dependent mechanism. It is conceivable that this can be accomplished through multiple pathways: (1) p53 can act by down-regulating the expression of genes that compose the AP-1 transcription factor; (2) p53 can modulate the binding of AP-1 transcription factor to its binding site; and (3) p53 can interfere with the communications between the transcription factor AP-1 and the basal transcription complex. Since p53 equally inhibited the endogenous AP-1-mediated (Fig. 1B) and the ectopically expressed c-Jun-mediated (Fig. 4C) induction of −83MMP1luci, the first pathway was unlikely. p53 effectively inhibited the GAL4-cJun mediated-induction of pFR-luci; it suggested that pathway 2 did not play a major role either. Gel mobility shift assays supported the above conclusions. It is noteworthy that p53 had only a minor effect on the basal activity of the pFR-luci reporter (Fig. 4A, compare the first and the second bars) and on the GAL4-Elk1 mediated induction of pFR-luci (Fig. 4B) demonstrating that p53 acted specifically by interfering with the communications between the transactivator c-Jun and the basal transcription complex.

The general transcription factors TBP, TFIIB, and the coactivator p300 have been implicated in p53-mediated inhibition of target genes [Seto et al., 1992; Liu et al., 1993; Liu and Berk, 1995; Avantaggiati et al., 1997]. In the present study, we found that both p300 and TBP but not the TFIIB stimulated the promoter of −83MMP1luci. These results indicate that similar to the coactivator p300, human TBP also can function as a coactivator of AP-1. It has been proposed that p53 binds to TBP to inhibit transcription [Seto et al., 1992; Liu et al., 1993]. In our study, we found that p53 only marginally inhibited the minimal promoter −62MMP1 and failed to inhibit the TBP-mediated induction of −83MMP1luci, indicating that this type of mechanism did not play a major role, at least, in HeLa cells. Our finding was consistent with a previous study reporting that p53 was unable to repress a basal TATA promoter stimulated by overexpression of TBP [Farmer et al., 1996]. We found that p53 effectively inhibited the GAL4-cJun mediated-induction of pFR-luci and the p300-mediated induction of −83MMP1luci suggesting that p53 down-regulated the MMP1 promoter mainly through the third pathway. Several additional lines of experimental evidence supported this mechanism. First, the inhibition of the p300-mediated induction of −83MMP1luci by p53 was completely abolished after the −72AP-1 site was eliminated. Second, a p53 binding p300 fragment, p300 (1,514–1,922) that was inactive as a coactivator, could reverse the down-regulation of the MMP1 promoter by p53. Third, wild-type p53 but not p53 mutants could inhibit the p300-mediated induction of −83MMP1.

It is conceivable that a passive depletion model can be used to explain the reversion of the MMP1 promoter by p300. In this model, p300 first depletes the excess amount of free p53 (Fig. 8, bars 3–4 in the first bar group) and then inactivates the p53 that is involved in the actual repressive process (Fig. 8, bars 5–6 in the first bar group); after all the p53 is inactivated by p300 through physical interaction, extra p300 protein induces the promoter of MMP1luci by functioning as a coactivator of the transcription factor AP-1 (Fig. 8, bar 7 in the first bar group). Although the passive depletion model fits well with the p300-mediated reversion of the down-regulation of the MMP1 promoter by p53, it cannot explain how the p300 (1,514–1,922) mutant, an inactive coactivator, could reverse the down-regulation of MMP1 promoter by p53 beyond its basal level. An alternative model is that p300 and its mutants bind to p53 and form a transcriptionally active complex $p300_i - p53_j$. The transcriptional activity of this complex varies depending on the ratio of i/j in the complex and the nature of the p300 mutant. Under certain circumstances, p300 and its mutants

may convert wild-type p53 from a transrepressor to a transactivator for promoters containing no p53 binding site. It has been speculated that p300 functions as a tumor suppressor [Giles et al., 1998]. This speculation is based on the observations that p300 is a target for viral oncoproteins adenovirus E1A and SV40 Large T antigen [Eckner et al., 1994; Avantaggiati et al., 1996] and that p300 mutations are found in several types of human malignancies [Muraoka et al., 1996; Gayther et al., 2000]. It is noteworthy that p300−/− cells have severe growth defects [Yao et al., 1998; Yuan et al., 1999] while a similar effect has not been observed in cancer cells containing truncated p300 mutants [Gayther et al., 2000], indicating that those truncated p300 mutants found in human cancer cells are not "loss of function" mutants. Our findings that p300 and its mutants under certain circumstances may convert wild-type p53 from a transrepressor to a transactivator for promoters containing no p53 binding site raise the possibility that aberrant p300 may promote or contribute to tumor development by impairing the tumor suppressive function of p53.

Finally, by using p53 truncated mutant constructs, we demonstrated that both the N-terminal region of p53 (1–25 amino acids) and the C-terminal region of p53 (324–354 amino acids) were required for the transcription inhibition of MMP1. Since the region of p53 (1–25 amino acids) overlaps with the activation domain of p53 [Ko and Prives, 1996] and the region of p53 (324–354 amino acids) is the same as the minimum region for p53 oligomerization (325–355) [Clore et al., 1995], it suggests that both the activation and the oligomerization domains of p53 are required for the transcription inhibition of MMP1.

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