Upon DNA damage, p53 can induce either cell-cycle arrest or apoptosis. Here we show that monocytic leukemia zinc finger (MOZ) forms a complex with p53 to induce p21 expression and cell-cycle arrest. The levels of the p53-MOZ complex increased in response to DNA damage to levels that induce cell-cycle arrest. MOZ−/− mouse embryonic fibroblasts failed to arrest in G1 in response to DNA damage, and DNA damage-induced expression of p21 was impaired in MOZ−/− cells. These results suggest that MOZ is involved in regulating cell-cycle arrest in the G1 phase. Screening of tumor-associated p53 mutants demonstrated that the G279E mutation in p53 disrupts interactions between p53 and MOZ, but does not affect the DNA binding activity of p53. The leukemia-associated MOZ-CBP fusion protein inhibits p53-mediated transcription. These results suggest that inhibition of p53/MOZ-mediated transcription is involved in tumor pathogenesis and leukemogenesis.

p53 is an important component of pathways mediating cellular responses to different forms of stresses, and induces expression of numerous target genes that regulate diverse cellular processes including cell-cycle arrest, apoptosis, and genome stabilization (1, 2). Many p53-targeted genes involved in cell-cycle arrest or apoptosis have been identified, including p21 (3), Bax (4), and Puma (5). Regulation of p53 transcriptional activity is essential for cellular responses to genotoxic stress, because p53 responds to DNA damage or checkpoint failure either by arresting the cell cycle in the G1 phase for damage repair or by initiating an apoptotic pathway to eliminate the damaged cell (6–9). Several factors, such as TIP60, Hzf, and hCAS/CSE1L are involved in the selection of p53 target genes (10–13). However, the molecular mechanisms by which p53 chooses cell-cycle arrest versus apoptosis are not fully understood.

The principal post-translational modifications of p53 in response to DNA damage are phosphorylation and acetylation (14–17). Phosphorylation of Ser15 and Ser37 stabilizes p53 (16, 18, 19). Modification of p53 may also control the interactions of p53 with basal transcription factors (20), mediators (21), or coactivators, including histone acetyltransferases (HATs) (2). p53 interacts with components of several different HAT complexes, including p300/CREB (18), P/CAF (22), GCN5 (23), TRRAP (24), and TIP60 (11, 13). Additionally, p53-mediated transactivation of p21 correlates with increased histone acetylation (25).

These results suggest that selective interactions between p53 and HATs may regulate target gene expression in response to diverse signals.

The monocytic leukemia zinc finger protein (MOZ) is a MYST-type HAT and functions as a co-activator of the AML1 transcription factor (8, 27). MOZ is involved in leukemia-associated chromosome rearrangements such as t(8;16)(p11;p13) (26), t(8;22) (27, 28), and inv(8) (29, 30), which result in fusion of MOZ to the transcriptional co-activators CBP, p300, and TIF2, respectively. Although MOZ and p300/CBP act as co-activators for AML1, MOZ-CBP inhibits AML1-mediated transcription (28, 31, 32). MOZ is essential for self-renewal of hematopoietic stem cells (33, 34). The MOZ-TIF2 fusion induces acute myeloid leukemia in irradiated recipient mice after transplantation (35, 36). MOZ-TIF2 also inhibits transcription by nuclear receptors and p53 by impairment of CBP function (37).

In this report, we demonstrate that MOZ directly interacts with p53. The level of the p53-MOZ complex increases after DNA damage, contributing to cell-cycle arrest in the G1 phase.

**EXPERIMENTAL PROCEDURES**

**Plasmids—cDNAs encoding FLAG-tagged or HA-tagged human MOZ, CBP, MOZ-CBP, and AML1 were cloned into the pLNCX retroviral mammalian expression vector, as previously described** (32). The sequences of the above constructs were checked by DNA sequencing. N- and C-terminal deletion mutants of MOZ cDNAs were generated by PCR with human wild-type MOZ as the template. The PCR-amplified deletion MOZ fragments were cloned into the HA-tagged pcDNA3.1(+) vector (Invitrogen). S15A, S15D, S20A, S20D, S46A, S46D, K382R, and K382Q mutants of p53 were derived from FLAG-tagged p53-pLNCX by site-directed mutagenesis using a QuikChange Site-directed Mutagenesis Kit (Stratagene).
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Cells—Wild-type and MOZ−/− mouse embryonic fibroblasts (MEFs) were prepared from E13.5 embryos and cultured in Dulbecco’s modified Eagle’s medium with 10% fetal bovine serum in the presence of β-mercaptoethanol. Bosc 23 and Saos-2 cells were cultured as previously described (32).

Purification of the MOZ Complex and Mass Spectrometry—Bosc 23 cells were transiently transfected with FLAG-MOZ-pLNCX by using the calcium phosphate precipitation method. Transfected cells (1 × 10^6) were solubilized by incubation at 4 °C for 30 min in 50 ml of lysis buffer (20 mM sodium phosphate, pH 7.0, 250 mM NaCl, 30 mM sodium pyrophosphate, 0.1% Nonidet P-40, 5 mM EDTA, 10 mM sodium fluoride, 0.1 mM Na3VO4, and 1 mM phenylmethylsulfonyl fluoride) supplemented with protease inhibitors (Complete, Roche). The lysates were cleared by centrifugation at 30,000 g for 30 min at 4 °C and incubated with 2.5 ml of anti-FLAG monoclonal antibody (M2)-conjugated affinity beads with rotation at 4 °C for 4 h. The beads were washed eight times, each with 50 ml of lysis buffer. The MOZ complexes were selectively eluted by incubating with 0.2 mg/ml FLAG peptide in lysis buffer for 1 h. The eluates were concentrated and separated using 10% SDS-PAGE. Proteins were stained with Coomassie Brilliant Blue, excised, destained with 25 mM ammonium bicarbonate and 50% acetonitrile, dried, digested with sequence grade modified trypsin (Promega) in 50 mM Tris (pH 7.6), extracted with 5% trifluoroacetic acid and 50% acetonitrile, and subjected to LC/MS/MS analysis. LC/MS/MS analysis was performed as previously described (32).

Immunoprecipitation and Immunoblot Analysis—Immunoprecipitation (IP) and immunoblot analyses were performed as previously described (38). In short, cells were solubilized with lysis buffer as described above, and sonicated 6 times for 10 s using a BioRuptor (Cosmo Bio) at the high power setting. Cell lysates were incubated with anti-FLAG monoclonal antibody (M2)-conjugated affinity beads (Sigma) for 4 h at 4 °C. The beads were washed five times with lysis buffer, and immunoprecipitates were selectively eluted by incubating with 0.2 mg/ml FLAG peptide in lysis buffer for 1 h. The eluates were concentrated and separated by SDS-PAGE. The rabbit polyclonal antibody to MOZ has been described previously (28). Commercially purchased antibodies to human p53 (DO-1), p21 (Santa Cruz Biotechnology), phospho-p53 (Ser15, 16G8), phospho-p53 (Ser20), phospho-p53 (Ser46), FLAG M2, β-actin (Sigma), HA (3F10) (Roche), and mouse p53 (Ab-1) (Calbiochem) were also used in this study.

GST Pull-down Assays—For GST pull-down assays, in vitro translated products were generated using the TNT Quick Coupled System (Promega). In vitro translated [35S]methionine-labeled N- and C-terminal deletion mutants of MOZ were incubated in the presence or absence of GST-tagged p53 and in the presence of glutathione-Sepharose 4B (Amersham Biosciences) in IP buffer overnight at 4 °C. The affinity beads were washed five times with IP buffer. The precipitates were subsequently resolved by SDS-PAGE and the [35S]methionine-labeled proteins visualized by autoradiography (BAS2000, FUJIX).

Flow Cytometry—For flow cytometry analysis, cells were first fixed with 70% ethanol and then incubated with RNase A at 37 °C for 30 min. The cells were stained with propidium iodide (50 µg/ml propidium iodide in 0.1% sodium citrate and 0.1% Nonidet P-40) for 30 min at 4 °C. The cells were then analyzed using a FACS Calibur instrument (BD Biosciences) and Cell Quest software.

Luciferase Assays—Saos-2 cells were transfected in 24-well plates using the calcium phosphate precipitations method, and luciferase activity was assayed after 24 h using a luminometer (Veritas Microplate Luminometer, Turner Biosystems), according to the manufacturer’s protocol (Promega). The results presented are the mean ± S.D. of relative luciferase activity generated from three independent experiments normalized against the activity of the internal control enzyme from pRL-CMV.

Semiquantitative RT-PCR Analysis—Total RNA was extracted using an RNasy Mini Kit (Qiagen) according to the manufacturer’s instructions. RT-PCR experiments were carried out with cDNAs generated from 2 µg of total RNA using a GeneAmp RNA PCR Kit (Applied Biosystems). The RT-PCR exponential phase was determined from 30 cycles of amplification to allow semiquantitative comparisons of cDNAs developed from identical reactions. All reactions involved initial denaturation at 95 °C for 5 min followed by 30 cycles of 95 °C for 30 s, 60 °C for 30 s, and 72 °C for 1 min using a GeneAmp PCR System 9700 (Applied Biosystems). Primers used in the RT-PCR study were as follows: mouse p21, 5′-CCGACGAGAAAAGTTGTG-3′ and 5′-GAATCTTCAGCGGCGCTACGAC-3′, mouse Bax, 5′-GCTGATGGCAACTTCAACTG-3′ and 5′-GCACGATGGTCTACGTC-3′, mouse Mdm2, 5′-CTCTCCCAGAGAGTGTGCGAC-3′ and 5′-GATTGCTGTTTCACACTG-3′; mouse Puma, 5′-AGGCAAACCTGACCTGCACTAG-3′ and 5′-GTGGTCACAGTGTCCTCTC-3′; control glyceraldehyde-3-phosphate dehydrogenase, 5′-CTTCACCACATTGGAGAACGC-3′ and 5′-GGCATCGGTGAGTGGGAG-3′.

DNA Binding Assay—Nuclear extracts were prepared from Bosc 23 cells 24 h after transfection with wild-type or mutant p53. Procedures for DNA binding reactions were similar to those previously described (39). Briefly, reaction mixtures contained 4 µg of extract in 18 µl of binding buffer (50 mM KCl, 20 mM HEPES, pH 7.5, 10 mM MgCl2, 10% glycerol, 0.5 mM dithiothreitol, 0.1% Nonidet P-40), plus 1 µg of sonicated DNA from salmon testes. The double-stranded p21 oligonucleotide probe 5′-TCAGTTATACGTCCATCGTCTGCT-3′ was derived from the p21waf1 promoter. Reaction mixtures were incubated for 15–20 min at 23 °C with the addition of 1 ng of radiolabeled probe. Each reaction mixture was then loaded onto a native 4% polyacrylamide gel (acrylamide: Bis, 50:1; 0.5 × TBE) and electrophoresed in 0.25 × TBE at 180–220 V for 3 h at 4 °C.
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**RESULTS**

**Interaction between p53 and MOZ**—To identify MOZ-interacting proteins, we purified MOZ complexes. The complexes of MOZ were partially purified using affinity beads conjugated with anti-FLAG M2 antibody (Fig. 1A). The MOZ-purified fraction contained multiple proteins that were absent from the mock-purified fraction, suggesting that they interact specifically with MOZ. To identify these proteins in the MOZ fraction, we analyzed these specific proteins using mass spectrometry and found that the spectrum obtained from peptides was identified as p53, as well as BRPF1 (40), C23, HPST70, and B23 (Fig. 1A). Co-immunoprecipitation analysis confirmed that these putative associated proteins, including p53, were co-precipitated with MOZ (Fig. 1B). Reciprocal co-immunoprecipitation showed that MOZ was co-precipitated with p53 (Fig. 1C).

**Interacting Domains on p53 and MOZ**—To determine the p53-interacting domains of MOZ, the interaction between MOZ and p53 was examined by IP immunoblot analysis using deletion mutants of MOZ. Bosc 23 cells, which exhibit very high transfection efficiency, were transfected with FLAG-tagged p53 and HA-tagged wild-type or mutant MOZ. The expression of p53 deletion mutants of MOZ. Bosc 23 cells, which exhibit very high transfection efficiency, were transfected with FLAG-tagged p53 and HA-tagged wild-type or mutant MOZ. The expression of p53 deletion mutants and results of the IP immunoblot analysis are shown in supplemental Fig. S1, and summarized in Fig. 1D. These results suggest that there are at least two p53-interacting domains in MOZ, one lies in the basic domain between amino acids 312 and 664, and another lies in the serine-rich domain between amino acids 1517 and 1741. Similarly, to determine the region of p53 required for binding to MOZ, IP immunoblot analysis was conducted using a series of p53 deletion mutants. Each of the p53 mutants was immunoprecipitated by using anti-FLAG antibody M2 (supplemental Fig. S2), and the results are summarized in Fig. 1E. These results suggest that the p53-core DNA binding domain (amino acids 98–180) is required for its interaction with MOZ. To further examine whether these interactions are direct or indirect, we performed in vitro GST pull-down assays, and showed that either the N or C terminus of MOZ could interact directly with p53 in vitro (Fig. 1F).

**Impaired G1 Arrest in MOZ−/− Cells**—To investigate the role of MOZ in DNA damage responses, we tested the effects of ADR on cell cycle progression in wild-type and MOZ−/− MEFs. Analysis by flow cytometry showed that wild-type cells were...
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arrested in the G1 phase of the cell cycle, and that the number of cells in G1 peaked 12 h after ADR treatment (Fig. 2, A and B). However, in MOZ-deficient cells, the number of cells in the G1 phase decreased after ADR treatment, and the number of apoptotic cells in the sub-G1 phase increased (Fig. 2, A and C). These differences in sensitivity to ADR-induced DNA damage were also observed in TUNEL assays (Fig. 2D) and in cytotoxicity assays (Fig. 2E). These results suggest that in response to DNA damage, MOZ-deficient cells failed to arrest in G1 phase and tended to undergo apoptosis.

Impaired Expression of the p21 Gene in MOZ−/− Cells—The altered response of MOZ−/− MEFs to DNA damage suggests that the p53 pathway might be altered in MOZ−/− MEFs. To test this hypothesis, we examined the expression of the p53 target gene p21 after ADR treatment. In wild-type MEFs, p21 expression increased after ADR treatment in a dose-dependent manner and reached a maximum at 6–12 h (Fig. 3, A and B, supplemental Fig. S3, A–C). However, expression and induction of p21 were profoundly impaired in MOZ−/− MEFs as assessed by immunoblot analysis, Northern blotting, and real-time RT-PCR analyses. Furthermore, expression of the other p53 target genes, such as Mdm2, Bax, and Puma, were induced by ADR treatment both in wild-type and MOZ−/− cells (supplemental Fig. S3, D and E). Thus, these results suggested that MOZ is required for p53-induced expression of p21, but not for those of Bax, Mdm2, and Puma.

MOZ Induces the Expression of the p21 Gene—To test whether MOZ directly affects p53-mediated transcription, we used reporter plasmids under the control of p21 promoters. MOZ strongly stimulated p53-mediated activation of p21-luc rather than p300 (Fig. 3C). However, BAX-luc and AIP1-luc were activated by p300 but not by MOZ (supplemental Fig. S3, F and G). ChIP assays showed that after UV radiation, MOZ...
associated with the RE1 and RE2 p53-response elements of the p21 promoter, but not with an unrelated region of p21 (Fig. 3D).

Knockdown of MOZ expression by pretreatment of MCF-7 cells with a MOZ-specific siRNA partially inhibited the recruitment of p53 to the p21 promoter by DNA damage, but not MDM2, Bak, and Puma (Fig. 3E, and supplemental Fig. S3, H–J). Furthermore, binding of MOZ to the p21 promoter was detected in p53+/−/ HCT116 cells but not in p53−/− HCT116 cells (Fig. 3F). Thus, these results suggest that the p53-MOZ interaction is involved in recruitment to and activation of the p21 gene promoter, and that the recruitment of the p53-MOZ complex to the p21 promoter is p53 dependent.

Increase in the Level of the p53-MOZ Complex in Response to DNA Damage—To test interactions between endogenous p53 and MOZ proteins, levels of MOZ, as well as p300, in p53 immunoprecipitates were monitored in γ irradiation-exposed MCF-7 cells. A p53-MOZ complex was nearly undetected in non-irradiated cells, but appeared following irradiation of MCF-7 cells and reached a maximum at 2 h (Fig. 4A). An increase in p53-p300 complex was also observed after irradiation. Immunoblot analysis indicated that these increases were associated with increases in overall p53 protein level, as well as in p53 phosphorylation on Ser15, and were followed by an increase in p21 expression. These results indicate that p53 interacts with MOZ in response to DNA damage.

Post-translational Modification of p53 Correlates with p53-MOZ Interaction—p53 is stabilized and activated upon DNA damage primarily through post-transcriptional modifications including phosphorylation and acetylation (14–19, 22, 41–43). Differential modifications of p53 may be important for differential activation of target genes (44–46). To test the effects of such modifications on p53 interactions, we performed co-immunoprecipitation analysis using p53 mutant proteins, in which potential modification sites were altered. Interaction of p53 with MOZ was impaired by mutation of Ser15 to Ala (S15A) and Ser20 to Ala (S20A) (Fig. 4B). In contrast, the p53-MOZ interaction was enhanced by mutation of Ser15 to Asp (S15D) and Ser20 to Asp (S20D), which mimic serine phosphorylation. However, mutation of Ser46 to Asp (S46D) showed defects in enhancement of the interaction. These results indicate that phosphorylation of p53 on Ser15 and Ser20 enhances its interaction with MOZ. Furthermore, substitution of the major acetylation site (Lys382) with Arg (K382R) strongly enhanced the p53-MOZ interaction, but not K382Q (Fig. 4B), suggesting that Lys382 acetylation also plays an important role in the p53-MOZ interaction. These results suggest that the interaction between p53 and MOZ correlates with specific post-translational modifications, such as phosphorylation and acetylation.

p53-MOZ Complex Is Associated with p21 Expression—To further investigate this hypothesis, we compared interactions of p53-MOZ and p53-p300 following various doses of UV irradiation in MCF-7 cells. Levels of the p53-p300 complex increased in a dose-dependent manner (Fig. 4C). In contrast, levels of the p53-MOZ complex increased after MCF-7 cells irradiation of 30 J/m², but not 50 J/m². The lower irradiation dose was correlated with induction of p21 expression and G1 arrest (Fig. 4, C and D). Thus these results suggest that interactions of p53-MOZ and p53-p300 are differentially regulated, and that the p53-MOZ complex is associated with p21 expression and induction of G1 arrest.

Oncogenic G279E Mutation Inhibits the p53-MOZ Interaction—Mutations in p53 are frequently found in human cancers. Although ~1400 different p53 mutations have been reported, the large majority of mutations are located in the core domain that is essential for binding to specific DNA sequences (47, 48), suggesting that impaired interaction of p53 with DNA
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is associated with cancer. In fact, most of these mutations in the core domain abolish p53 DNA binding and activation of transcription (Fig. 5, A and B). The G279E mutant, however, lacks the ability to activate transcription, but still has the ability to bind DNA. The MOZ-interacting domains also map to the p53 core domain (Fig. 1D and supplemental S2); therefore, we tested the interaction of this G279E mutant with MOZ, and found that the G279E mutant is unable to bind MOZ (Fig. 5C).

A MOZ-CBP fusion gene is produced by the t(8;16) translocation associated with acute monocytic leukemia. We tested the effects of MOZ-CBP on p53-dependent transcription, and found that the MOZ-CBP fusion protein, as well as adenovirus E1A, strongly inhibited p53-dependent transcription of p21 in a dose-dependent manner (Fig. 5D). Deletion analysis showed that the bromo and HAT domains in the CBP region of the fusion protein are important for MOZ-CBP inhibition of p53-mediated transcription (Fig. 5, E and F).

DISCUSSION

The data presented here demonstrate that MOZ, a MYST-type histone acetyltransferase, directly interacts with p53, and that the interaction is regulated by phosphorylation and acetylation. Like other co-factors, such as p300/CBP and PCAF, MOZ functions as a co-activator of p53. However, MOZ stimulates p53-mediated transcription of p21 to induce cell-cycle arrest, but not other p53 target genes, such as Bax or AIP1. We partially purified MOZ complexes from transfected Bosc 23 cells that transiently expressed FLAG-tagged MOZ. Mass spectrometry and IP immunoblot analyses revealed that MOZ could interact with p53. In contrast with previous reports in which levels of the p53-p300 or p53-CBP interaction increased in a dose-dependent manner with increasing DNA damage (16, 18, 19), levels of the p53-MOZ complex increased with levels of DNA damage to induce cell-cycle arrest (Fig. 4C).

Among the most distinctive features of p53 is the ability to induce either cell-cycle arrest or apoptotic death. Although this differential activity may be partially explained by the molecular mechanisms of promoter discrimination, the precise mechanism of the selective activation of p53 target genes remains
unknown. In response to severe DNA damage, MYST acetyltransferases, such as TIP60 and MOF, acetylate Lys\(^{120}\) of p53. This acetylation results in preferential accumulation of p53 on proapoptotic promoters, such as Bax and PUMA (50, 52). CAS/CSE1L, which suppresses histone H3K27 methylation, selectively associates with the PIG3 and AIP1 promoters, but not the p21 promoter (12). In addition, Hzf, which is a p53 target gene, interacts directly with the p53 DNA binding domain, and induces preferential expression of p53 target genes that block the cell cycle, such as p21 and 14-3-3 (10).

In this report, we demonstrated that MOZ could interact with p53 and that the p53-MOZ complex increases p21 expression in cells exposed to doses of DNA damage that induce cell-cycle arrest. In response to DNA damage, p53 is phosphorylated on Ser\(^{15}\) and Ser\(^{39}\) to recruit MOZ, and subsequently the p53-MOZ complex preferentially induces transcription of the cell cycle-related p21 gene. The interaction between p53 and MOZ is regulated by post-translational modification, such as phosphorylation and acetylation, of N- and C-terminal residues of p53, respectively (Fig. 4B). In addition, deletion of either the N- or C-terminal of p53 enhances its interaction with MOZ (supplemental Fig. S2). These results suggest that the MOZ-binding domain of p53 is masked by its N- and C-terminal domains and that post-translational modifications, such as phosphorylation and acetylation, of N- and C-terminal regions of p53 have an effect on its conformation to regulate interactions with MOZ. Furthermore, p53-MOZ interaction was also slightly affected by Nutlin-3, the antagonist of MDM2, suggesting that the stabilization of p53 is also important for regulation of p53 and MOZ interaction (Fig. 4E) (49, 50).

ChIP analysis and reporter analysis showed that DNA damage could induce recruitment of the p53-MOZ complex to the p21 promoter. Experiments using MOZ\(^{-/-}\)MEFs revealed that MOZ-deficient cells failed to arrest in the G\(_1\) phase in response to DNA damage and were more sensitive to DNA damage. As shown by immunoblot and RT-PCR analyses, the expression of p21 was profoundly impaired in MOZ\(^{-/-}\)MEFs after ADR treatment (Fig. 3, A and B, supplemental Fig. S3, A–C). These results suggest that MOZ stimulates p53-dependent transcription of the p21\(^{^{14\text{a}}}\) gene, thereby regulating cell-cycle arrest.

MOZ\(^{-/-}\) mice die at around embryonic day 15 (E15) (33). The response of MOZ\(^{-/-}\)MEFs to DNA damage suggests that the p53 pathway may be altered in MOZ\(^{-/-}\)MEFs. In fact, induced expression of p21 was profoundly impaired in MOZ\(^{-/-}\)MEFs as assessed by immunoblot and RT-PCR analyses.

A previous report demonstrated that p21 expression was not induced in p53\(^{-/-}\) cells after ADR treatment, and Bax expression was only slightly affected (51). DNA damage-induced cell-cycle arrest was impaired in MOZ\(^{-/-}\) MEFs (Fig. 2A), as observed in p53-null and p21-null cells. These results suggest that the enhanced apoptosis found in MOZ\(^{-/-}\) MEFs may be due to reduced induction of p21 and impaired cell-cycle arrest. In fact, reporter analysis indicated that MOZ did not increase the activity of the Bax and AIP1 promoters (supplemental Fig. S3, F and G). Although the specific expression mechanisms are currently unknown, there are two possibilities. Because the p53-MOZ complex prefers the p21 promoter to other promoters (Fig. 3D), formation of the p53-MOZ complex may inhibit transcription of apoptotic genes by reducing levels of available p53. Alternatively, MOZ may enhance p53-mediated cell-cycle arrest, thereby inhibiting transcription of apoptotic genes indirectly. p300\(^{-/-}\)MEFs retain the ability to respond to UV irradiation by stabilization of p53 and induction of p21 (52). Because CBP functions as a co-activator to p53 as well as p300, CBP may compensate for p300 function in p300\(^{-/-}\)MEFs.

Somatic mutations in p53 are found in \(\approx 50\%\) of all human cancers. Approximately 1400 different p53 mutations have been reported, with the large majority of such mutations located in the DNA binding core domain (47, 48). Domain analysis using p53 deletion mutants indicated that the DNA-binding core domain of p53 is required for interactions with MOZ, which suggests that the p53-MOZ complex controls access of p53 to binding sites in target gene promoters and that MOZ may play a role in the selectivity of p53-mediated activation of transcription. In the process of screening somatic tumor-associated p53 mutants, we found that the G279E mutation of p53, which did not affect DNA binding to p53 responsive elements but impaired transcription of p21, disrupted the interaction between p53 and MOZ. These results suggest that MOZ functions as a co-activator of p53, and that activation of p53-dependent transcription of p21 may depend on the interaction of p53 with MOZ to recruit p53 to the p21 promoter.

The MOZ-CBP leukemic fusion gene is produced by the t(8;16) translocation, which is associated with acute myeloid leukemia, suggesting that MOZ-CBP may affect the growth and differentiation of hematopoietic cells. We found that the MOZ-CBP fusion protein inhibits p53-mediated transactivation of p21 (Fig. 5, D and F). Because MOZ-CBP fusion protein showed reduced activity for acetylation of p53 as compared with either CBP or MOZ alone, MOZ-CBP might suppress p53 acetylation by competing with other HAT protein such as CBP and MOZ (supplemental Fig. S4, A and B).

Another leukemia-associated fusion, MOZ-TIF2, also inhibits p53-dependent transcription (37). ChIP analysis indicated that MOZ-CBP directly inhibits binding of p53 to the p21 promoter (supplemental Fig. S4C). These results suggest that p53 dysfunction associated with MOZ gene translocations and inhibition of p53/MOZ-mediated transcription are involved in the pathogenesis of leukemia and other cancers.

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