Macrophage migration inhibitory factor (MIF) has been shown to functionally inactivate the p53 tumor suppressor and to inhibit p53-responsive gene expression and apoptosis. To better understand the role of MIF in cell growth and tumor biology, we evaluated MIF-null embryonic fibroblasts with respect to their immortalization and transformation properties. Although minor deviations in the growth characteristics of MIF−/− fibroblasts were observed under normal culture conditions, MIF-deficient cells were growth-impaired following the introduction of immortalizing oncogenes. The growth retardation by the immortalized MIF−/− cultures correlated with their reduced susceptibility to Ras-mediated transformation. Our results identify E2F as part of the restraining mechanism that is activated in response to oncogenic signaling and show that the biological consequences of E2F induction in MIF−/− fibroblasts vary depending on the p53 status, inducing predominantly G1 arrest or apoptosis in p53-positive cells. This E2F activity is independent of Rb binding, but contingent on binding DNA. Resistance to oncogenic transformation by MIF−/− cells could be overcome by concomitant interference with p53- and E2F-responsive transcriptional control. Our results demonstrate that MIF plays a role in an E2F/p53 pathway that operates downstream of Rb regulation and implicate MIF as a mediator of normal and malignant cell growth.

Macrophage migration inhibitory factor (MIF) was originally identified for its ability to inhibit the random migration of macrophages in vitro. Subsequent work defined MIF as a potent cytokine with mitogenic and pro-inflammatory functions. However, efforts aimed toward identifying a cellular receptor for MIF have been unsuccessful to date; thus, the process by which extracellular MIF may exert its effect on target cells is not understood.

Early evidence suggesting a role for MIF in cell growth and/or differentiation came from the observations of its expression in developing mouse embryos. The MIF gene is expressed at early embryonic stages prior to implantation (5); and at mid-gestation, its expression pattern parallels tissue specification and organogenesis (6–8). However, MIF appears to be dispensable for normal development because MIF-null mice reproduce and grow normally (9, 10). Among cytokines, MIF is unique in terms of its abundant expression by various cell types (4), as well as storage within the cytoplasm (11). A recent study proposed an intracellular function for MIF in cell cycle regulation via modulation of the transcription factor AP-1. MIF deficiency has also been associated with decreased NF-κB activity (13), whereas Hudson et al. (14) demonstrated that MIF functionally inactivates the p53 tumor suppressor. The p53 gene has become the focus of intense investigation since it became clear that p53 mutations are the most common genetic alteration found in human tumors (15). However, in a proportion of tumors in which p53 is functionally inactivated, the gene remains intact, suggesting altered activity of p53-specific regulators (16, 17). Coincidentally, MIF overexpression has been reported in various tumors (18–20). Immunosuppression of brain tumors revealed predominantly nuclear localization (20), raising the possibility that MIF overexpression may contribute to tumor proliferation via p53 inhibition.

To better understand the role of MIF in cell growth and tumor biology, we analyzed MIF-null embryonic fibroblasts with respect to their immortalization and transformation properties. The data presented here implicate MIF as a mediator of normal and malignant cell growth.

**EXPERIMENTAL PROCEDURES**

**Cells and Tissue Culture**—MIF-knockout mice were kindly provided by Dr. John R. David (Harvard School of Public Health, Boston). Animals were maintained on a mixed 129Sv × C57BL/6 background (F1). Mouse embryonic fibroblasts (MEFs) were prepared from day 14.5 embryos using standard techniques, and passage 4–6 MEFs were used in most experiments. Unless specified, cells were maintained in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and penicillin/streptomycin (Invitrogen).

Fibroblast proliferation was measured by plating equal cell numbers onto six-well dishes in duplicate, followed by direct counting of trypsinized cells using a Coulter counter. Each growth curve represents at least two independent experiments. As an alternative, cell proliferation was assessed by 3H-thymidine incorporation into DNA. Briefly, 3 × 104 cells were plated per well in 96-well plates in DMEM containing 10% FBS and 0.5 μCi/well [3H]thymidine (PerkinElmer Life Sciences). Following 48 h of incubation, the cells were harvested, and radioactive incorporation was determined by β-scintillation counting (Packard-
stment Co.). The data are reported as the average cpm ± S.D. (n = eight wells/condition). Two-tailed Student’s t test was used to determine the statistical significance.

Cell viability was assessed using MEFs that were grown until confluent and either kept in serum-containing DMEM or placed in serum-free DMEM for 48 h. Cells were harvested using enzyme-free dissociation solution (Invitrogen) and analyzed using the TACS™ annexin V/propidium iodide apoptosis kit (R&D Systems) according to the manufacturer’s protocol.

Retroviral Constructs—The replication-defective retroviral expression vector REBNA has been described previously (21). Retroviral vector REBNA/iresGFP contains a poliovirus internal ribosomal entry site element followed by green fluorescent protein cDNA (kindly provided by Dr. Sergei Zolotukhin, University of Florida, Gainesville, FL) placed downstream of the multiple-cloning site in REBNA. Retroviral vector REBNAPuro contains a puromycin resistance cassette and a minimal cytomegalovirus promoter placed upstream of the multiple-cloning site in REBNA. The following CDNAs were used for retroviral expression: mouse c-myc (a gift from Dr. Michael J. Cole, Princeton University); a dominant-negative human p53<sup>H1175</sup> mutant (a gift from Dr. Dan Notterman, Princeton University); SV40 large T-antigen (a gift from Dr. Robert Raleigh, Princeton University); E1A LTag and E1A CR1, which contain deletions of amino acids 2–36 and 68–85, respectively (a gift from Dr. Scott Lowe, Cold Spring Harbor Laboratory); a dominant-negative human E2F1<sup>134T</sup> mutant (a gift from Dr. Stephen F. Dowdy, Washington University School of Medicine, St. Louis, MO); and a human H-ras<sup>Q61K</sup> mutant (a gift from Dr. Dafna Bar-Sagi, State University of New York, Stony Brook). Retroviral stocks were produced as previously described (21). For viral infections, 10<sup>5</sup> virus-containing 10<sup>5</sup> uninfected MEFs and plated on a 6-cm dish were incubated overnight with an appropriate amount of the corresponding retroviral stock. Multiple infections were performed sequentially, with a 12–24-h interval between each infection. When required, infected fibroblasts were selected for 2 days in medium containing 2 µg/ml puromycin. Typically, the efficiency of infection of primary MEFs ranged from 60 to 70%, whereas the efficiency of infection of immortalized cells was >95% as determined using retroviral constructs containing the green fluorescent protein marker. Cells were analyzed for the corresponding protein levels 2–4 days post-infection.

Transformation Assays—Soft agar colony formation was examined in 0.25% Noble agar/DMEM supplemented with 10% FBS and antibiotic/antimycotic (Invitrogen). For focus formation assays, 3 × 10<sup>5</sup> virus-infected fibroblasts were mixed with 10<sup>5</sup> uninfected MEFs and plated onto 6-cm dishes. Cells were maintained in DMEM supplemented with 5% FBS and antibiotic/antimycotic. The growth medium was changed every 3 days. In 12–14 days, transformation efficiency was evaluated by counting individual colonies. All transformation assays were repeated at least three times. Data are shown as the average number ± S.D. of foci/plate using triplicate plates. Representative plates were stained with Giemsa and photographed.

Protein Expression Analysis—Aliquots of whole-cell lysates (50–100 µg of protein) were separated on an 8% SDS-acrylamide gel and blotted onto a Protran BA85 nitrocellulose membrane (Schleicher & Schuell). They were then incubated with antibodies specific for p16 (M-156), p21 (C-19), p33 (FL-393), p107 (C-18), cyclin A (C-19), cyclin D1 (C-20), cyclin E (M-20), CDC6 (130.2), E2F1 (KH-95), E1A (135-5), B-Myb (N-19), and c-Myc (N-262) (Santa Cruz Biotechnology); CDK2 (C18520), CDK2 (C18520), CDK4 (C18720), and Ras (R02120) (Transduction Laboratories); p19ARF (N-19), and c-Myc (N-262) (Santa Cruz Biotechnology); CDK2 (C18520), CDK2 (C18520), CDK4 (C18720), and Ras (R02120) (Transduction Laboratories); p19<sup>ARF</sup> (ab80; Novus Biologicals); MAPK (3A7; Cell Signalling), Rb (554138; Pharmingen); and SV40 T-antigen (Ab-2; Calbiochem) to detect the respective proteins. Secondary antibodies were horseradish peroxidase-conjugated (Amersham Biosciences). The ECL protein detection system (PerkinElmer Life Sciences) was used as recommended by the manufacturer. Nuclear cell extracts were prepared using the NE-PER nucleic extraction reagent (Pierce).

Cell Cycle Analysis—Trypsinized MEFs were washed with phosphate-buffered saline and fixed in 70% ethanol on ice for at least 24 h. The cells were washed with phosphate-buffered saline containing 1% FBS, resuspended in phosphate-buffered saline containing 1% FBS and 25 µg/ml RNase A, and incubated for 30 min at 37 °C. Prior to flow cytometry, 100 µl of propidium iodide solution (50 µg/ml propidium iodide and 0.1% sodium citrate) was added to the cell suspension and incubated for 1 h on ice. The staining was assessed using the FACScan Calibur flow cytometer and CellQuest software following calibration using DNA QC™ beads (BD Biosciences). Data were analyzed using ModFit software (BD Biosciences).

**RESULTS**

Growth Properties of MIF<sup>−/−</sup> Fibroblasts—To examine the role of MIF in cell growth, MEFs were derived from MIF-knockout and wild-type mice and assayed in several experimental settings. Under normal growth conditions, MIF<sup>−/−</sup> fibroblasts proliferated slightly more slowly than wild-type MEFs and became contact-inhibited at cell densities that were 20–25% lower than those of the wild-type controls (Fig. 1A). Immunoblot analysis showed that these differences were not associated with alterations in the expression levels of p53 (Fig. 1B). Moreover, following infection of the cells with retroviruses encoding viral or cellular oncoproteins, such as SV40 large T-antigen (LT), adenoviral E1A, and c-myc, the induction of p53 in both wild-type and MIF<sup>−/−</sup> fibroblasts was similar (Fig. 1B).

To test the possibility that the observed growth differences in cells lacking MIF were related to p53 function, we investigated whether interference with p53 activity can alter these effects. For this purpose, a dominant-negative p53<sup>H1175</sup> allele was introduced into fibroblasts using a replication-defective retrovi-
has functions outside p53 neutralization, these results suggest that MIF affects cell growth, in part, through a p53-independent mechanism.

**MIF**

Fibroblasts Resist Ras-mediated Transformation—

Because the deficiency in tumor suppressor genes, such as p53, facilitates oncogenic transformation, we next determined whether MEFs expressing p53H175 are susceptible to transformation. Introduction of the activated ras (H-rasV12) allele into control wild-type fibroblasts expressing p53H175 led to their clear morphological transformation (Fig. 2B) and the loss of contact inhibition, one of the hallmarks of the tumorigenic state. Subsequently, these cells produced 50 ± 10 transformed colonies/plate in several independent experiments (Fig. 2C). In marked contrast, expression of p53H175 and activated ras in MIF
deficient fibroblasts resulted in enlarged cellular morphology (Fig. 2B) and increased senescence-associated β-galactosidase expression (data not shown). Accordingly, these cells remained contact-inhibited and produced only 1.8 ± 1.5 foci/plate, despite containing equivalent levels of the mutant Ras protein (Fig. 2A) or growth regulatory molecules, such as ARF and p16 (Fig. 2D). Transactivation mediated by p53 was inhibited in cells expressing p53H175 and ras, as evidenced by decreased Apaf-1 and p21 expression (Fig. 2D). However, MIF
deficient fibroblasts accumulated higher levels of the transcription factor E2F1, whereas higher levels of c-Jun and c-Myc were found in the transformed wild-type cells (Fig. 2D). It has been noted that a modest reduction in c-Myc expression dramatically reduces susceptibility to Ras transformation (25).

To exclude the possibility that the observed difference in transformation between wild-type and MIF
deficient cells was independent of MIF loss, we reintroduced the MIF gene into MIF
deficient fibroblasts. Restoration of MIF expression in populations expressing p53H175 and rasV12 led to efficient focus formation (Fig. 2C). However, we were unable to rescue the transformed phenotype of MIF
deficient fibroblasts using various concentrations of recombinant murine MIF purified from bacteria or wild-type MEF-conditioned medium (data not shown). Likewise, MIF
deficient fibroblasts expressing both p53H175 and rasV12 failed to produce transformed colonies when plated on a monolayer of wild-type MEFs. These results suggest that the effects of MIF on cell growth and transformation are independent of its proposed role as a secreted cytokine. Furthermore, our experiments imply that the functional inactivation of p53 is insufficient to render MIF-deficient cells susceptible to ras-mediated transformation. Alternatively, a complete inactivation of p53 requires MIF involvement, even in the presence of excess interfering mutant p53. To address these possibilities, we performed the following experiments.

Viral Oncogenes Cooperate in the Transformation of MIF
deficient Fibroblasts—To rule out the possibility that MIF
deficient fibroblasts are unable to become oncogenically transformed, we examined cells expressing LT, whose activity is associated with the disruption of the p53 and Rb tumor suppressor pathways (24). Following the introduction of oncogenic ras into wild-type and MIF
deficient fibroblasts expressing LT, both cell types were readily transformed and invariably produced numerous foci (Fig. 3A), thus demonstrating that inactivation of the p53 and Rb tumor suppressors renders these cells susceptible to transformation. However, cell cycle analysis revealed that the transformed MIF
deficient fibroblasts accumulated in the G2/M phase (Fig. 3B). Accordingly, when assayed for anchorage-independent growth in soft agar, LT- and ras-transformed MIF
deficient fibroblasts developed colonies with a 2–3-day delay compared with the wild-type cells (data not shown).

MIF
deficient fibroblasts coexpressing the E1A and Ras oncoproteins also showed a 40% lower focus-forming ability compared
with the wild-type cells (Fig. 3A). This correlated with the observed 20–30% slower proliferation by the transformed MIF−/− cultures (data not shown). Because fibroblasts that coexpress E1A and oncogenic ras are prone to p53-mediated apoptosis in response to various insults, such as contact inhibition, we next examined cells expressing E1A deletion mutants E1AΔN and E1AΔCR1 (Fig. 3C). In previous work, these E1A mutants showed a differential capacity to affect the functions of p300/CBP coactivators and displayed a complete and partial defect, respectively, in apoptosis induction (26–28). Remarkably, E1AΔN-expressing wild-type and MIF−/− fibroblasts showed similar growth properties and focus-forming abilities when tested in transformation assays (Fig. 3A). By contrast, E1AΔCR1-expressing MIF−/− fibroblasts consistently produced fewer transformed colonies than the corresponding wild-type cells (Fig. 3A), thus indicating that p53 is more responsive to oncogenic activation in MIF−/− cells.

**MIF Deficiency and Ras-mediated Transformation**

**Fig. 3.** Viral oncogenes cooperate in the transformation of MIF−/− fibroblasts. A, focus formation by wild-type (wt) and MIF−/− (knockout (ko)) fibroblasts coexpressing H-rasV12 and LT, H-rasV12 and E1A, or H-rasV12 and the E1AΔN or E1AΔCR1 deletion mutant. B, flow cytometric analysis of DNA content in wild-type and MIF−/− fibroblasts expressing LT and H-rasV12. C, immunoblot analysis of wild-type and MIF−/− fibroblasts coexpressing H-rasV12 and E1A or H-rasV12 and the E1AΔN or E1AΔCR1 deletion mutant.

**Fig. 4.** e-myc induces a growth inhibitory response in MIF−/− fibroblasts. A, growth curves of myc-expressing wild-type (wt) and MIF−/− (knockout (ko)) fibroblasts before and after infection with retroviruses encoding p53H175. B, representative photographs of myc-expressing wild-type and MIF−/− fibroblasts (magnification ×40).

Myc Induces a Growth Inhibitory Response in MIF−/− Fibroblasts—To explore the association between the p53-dependent and p53-independent growth inhibitory responses resulting from MIF deficiency, we utilized the c-myc proto-oncogene. Unlike E1A, myc promotes cell growth bypassing the normal Rb control (29–31). Under certain conditions, however, overexpression of myc can cause p53-dependent proliferative arrest or augment p53-dependent apoptosis (32, 33). Expression of myc in MIF−/− fibroblasts consistently produced cell populations that proliferated at lower rates compared with the corresponding wild-type controls (Fig. 4A). Morphologically, MIF−/− populations contained many highly condensed cells (Fig. 4B) and were blocked in the G1 phase compared with myc-transduced wild-type cells (data not shown). Although wild-type MEFs generally tolerated higher levels of the exogenous Myc protein than MIF−/− cells, the expression levels of the key functional targets of Myc regulation, such as cyclins D1, cyclin E, CDK2, and p53, were similar in both cell types (data not shown). Of note, equivalent levels of these proteins were also found in wild-type and MIF−/− fibroblasts immortalized by LT and E1A (data not shown). However, combined expression of myc and p53H175 led to a greater increase in proliferation by MIF−/− cells compared with wild-type cells (Fig. 4A). These results are consistent with the reported ability of MIF to bypass p53-mediated growth arrest without altering p53 protein expression (14).

The growth properties of MIF−/− fibroblasts coexpressing myc and H-rasV12 paralleled those of MIF−/− cells transduced with myc alone. Thus, the expression of myc and ras consistently resulted in fewer transformed foci produced by MIF−/− cells compared with the respective wild-type cells (Fig. 5A). This correlated with slower proliferation by myc- and ras-expressing MIF−/− fibroblasts than by the wild-type controls (Fig.
In addition, myc- and ras-transduced MIF−/− cultures contained a larger percentage of apoptotic and necrotic cells (Fig. 5C). Moreover, the proportion of live myc- and ras-expressing MIF−/− fibroblasts rapidly decreased when shifted to low serum conditions (Fig. 5C). Immunoblot analysis of myc- and ras-transformed wild-type and MIF−/− cells showed similar expression levels for several growth regulatory molecules, including p53 and ARF (Fig. 5D). However, the levels of proapoptotic molecules (Apaf-1, caspase-3, and caspase-7) were higher in MIF−/− MEFs coexpressing the exogenous Myc and H-RasV12 proteins (Fig. 5D). Accordingly, sequential introduction of p53H175 and, myc, and ras into MIF−/− MEFs resulted in a 5-fold increase in focus formation, whereas the corresponding wild-type cells produced a 2-fold increase (Fig. 5A). Noteworthy, transformation of wild-type MEFs by coexpressing myc and ras correlated with a significant up-regulation of MIF protein levels (Fig. 5D).

E2F Contributes to the Suppression of the ras-induced Transformed Phenotype in MIF−/− Fibroblasts—Because the proapoptotic activity of c-Myc has been linked to E2F1 expression (32, 34), we examined the possibility that the differences observed in transformation assays were related to E2F1 function. For this purpose, cells transduced with myc and ras were infected with a control green fluorescent protein-expressing retroviral vector or a vector encoding E2F1374, a competitive inhibitor of endogenous E2F activity (Fig. 5E). This E2F1 mutant contains the DNA-binding domain, but lacks the transactivation and Rb-binding regions and has been shown to block E2F-responsive gene activation (35–37). The effect of green fluorescent protein expression on the transforming capacity of the MEFs was insignificant (data not shown). A mild inhibitory effect of E2F1374 expression on focus formation was observed in myc and ras wild-type cells (Fig. 5A), whereas introduction of E2F1374 into myc and ras MIF−/− fibroblasts increased the efficiency of focus formation. These results suggest that myc induces distinct proliferative and growth inhibitory responses in MIF−/− MEFs. Although the intrinsic growth-promoting myc activity cooperates with ras in transformation, the growth inhibitory response observed in MIF−/− cells is likely the result of inappropriate induction of E2F activity and can be partially blocked by interfering with p53 function.

Delayed Induction of E2F Target Genes in MIF−/− Fibroblasts—The Rb/E2F regulatory pathway controls the activity of numerous genes essential for DNA replication and cell cycle progression (37–39). Continuously growing MIF−/− cells showed normal expression levels of several E2F-responsive genes, such as cyclin A, cyclin E, cdc6, B-myb, and p107 (data not shown). To assess the effect of MIF deficiency on cell cycle regulation of E2F target genes, MEFs were synchronized by serum deprivation for 72 h and then serum-stimulated to re-enter the cell cycle. Subsequent examination of protein patterns showed no differences between the wild-type and MIF−/− fibroblasts in serum-induced accumulation of E2F-regulated gene products, such as p107, cyclin A, B-Myb, and proliferating cell nuclear antigen (Fig. 6A) (data not shown). However, the induction of cyclin E and cdc6 was delayed by an average of 4 h as MIF−/− cells progressed toward S phase (Fig. 6A).

The delay in E2F-responsive gene expression observed in MIF−/− MEFs could result from either inadequate E2F-medi-
E2F-responsive genes, including cyclin A, cyclin E, p107 were down-regulated in both E2F1374-expressing cell populations, as was the expression of E2F target genes that play roles in DNA replication, such as cdc6, mcm2, and mcm5. By contrast, c-Myc expression was similar between the corresponding wild-type cells. Thus, the deletion of the E2F3 gene or the combined loss of E2F1-3 results in the suppression of E2F target genes involved in DNA replication and thus affects the ability of cells to progress through mitosis (40, 44). By contrast, the loss of E2F4 or the combined loss of E2F4 and E2F5 has a modest effect on fibroblast cell proliferation (45, 46). Remarkably, sequential introduction of the p53H175 and H-rasV12 mutants into E2F1374-expressing wild-type and MIF−/− MEFs resulted in clear morphological transformation of both cell types (data not shown). In addition, both cell populations coexpressing E2F1374, p53H175, and H-rasV12 produced transformed foci with a similar efficiency (Fig. 7B), thus indicating that interference with the E2F DNA-binding activity contributes to overcoming the effect of MIF deficiency on ras-mediated transformation. Of note, a decrease in focus formation by E2F1374-expressing wild-type fibroblasts is consistent with the role of E2F3 in transformed cell growth (40).

DISCUSSION

We have used a genetic approach to compare MIF-deficient and wild-type fibroblasts with respect to their growth, immortalization, and transformation properties. Several recent studies suggested a role for MIF in cell growth regulation through the modulation of the AP-1, NF-xB, or p53 transcriptional activity (12–14). On the other hand, two groups have used different strategies to generate MIF-knockout mice (9, 10), but no growth-associated deficiencies have been reported in these mutant animals. We found minor deviations in the growth characteristics of MIF−/− MEFs under normal culture conditions. However, major growth-related differences were observed between the wild-type and MIF−/− cell populations following the introduction of immortalizing oncogenes. Moreover, the growth retardation observed with immortalized MIF−/− MEFs correlated with their reduced susceptibility to oncogenic transformation.

Carcinogenesis is a multistep process involving the activation of oncogenes and inactivation of tumor suppressor genes (47–49). The dominant-negative p53H175 allele used in this study has been well characterized with respect to its ability to cooperate with the activated ras mutants in transformation of rodent cells (50, 51). In our experiments, abrogation of p53-dependent transactivation was sufficient to promote ras-induced transformation of wild-type MEFs. However, MIF−/− cultures consistently resisted transformation under similar conditions. It appears that one major difference in the biology of MIF−/− cells is the robustness of the senescence program, which, in the absence of the functional p53 protein, retains the capacity to restrain the inappropriate proliferation induced by constitutively active Ras. The tumorigenic conversion of MIF−/− fibroblasts required an additional cooperating event compared with wild-type cells. Thus, the combined expression of p53H175 and ras or of myc and ras was insufficient to render MIF−/− cells effectively tumorigenic, whereas the concomitant expression of p53H175, myc, and ras resulted in clear oncogenic transformation. Our results identify E2F as part of the restraining mechanism that is activated in MIF−/− cells in response to inappropriate proliferation. The consequences of E2F induction in MIF−/− MEFs vary depending on the p53 status, inducing G1 phase arrest or apoptosis in p53-positive cells or G2/M phase retardation when p53 function is compromised.

In rodent cells, p53 controls both the anti-immortalizing and anti-tumorigenic pathways (15). Several studies suggested a role for the Rb family proteins as the downstream effectors of p53 action (52–54), whereas E2F1, a downstream target of Rb regulation, has been identified as a major cellular regulator of p53 (32, 34, 55). The E2F1374 mutant utilized in this study...
interferes with both E2F-dependent transactivation (35, 36) and recruitment of the Rb-E2F repressor complexes to the corresponding promoters (37). The incorporation of E2F1 into either wild-type or MIF–/– cultures disrupted normal E2F-dependent gene activation and impaired cell growth. However, in two experimental settings involving the expression of either myc or p53(H122), E2F1(H122)-transduced MIF–/– fibroblasts also showed increased susceptibility to ras-mediated transformation. These results suggest that E2F-mediated inhibition of MIF–/– cell growth involves its transcriptional activity. Thus, we found that the cell cycle-regulated induction of cyclin E and cdc6 was delayed by an average of 4 h in MIF–/– fibroblasts compared with the wild-type cells. In addition, the growth inhibition and apoptosis induced by E2F1 in MIF–/– cells likely involves p73 (56), Apaf-1 (57), and caspase proenzymes (58).

E2F1 is the only E2F family member that so far has displayed properties of both a tumor suppressor and an oncogene in different model systems. Thus, E2F1 loss has been found to reduce tumor development in Rb–/– mutant mice and to extend their life span (59, 60). On the other hand, E2F1–/– and E2F1–/–/– mutant mice also show increased tumor susceptibility (61–63). Moreover, tumor formation in E2f1–/– animals occurs without the loss of the wild-type E2F1 allele, indicating that a mere reduction in E2F1 dosage may be sufficient to either induce or sustain tumorigenesis in vivo (61, 62). Although the tumor-suppressive properties of E2F1 are not understood completely, they are believed to result from the ability of E2F1 to induce apoptosis, which can be both p53-dependent (55) and p53-independent (35). Notably, E2F1 plays a role in signaling apoptosis not only as a consequence of the deregulation of the Rb pathway or Myc activation, but also in response to DNA damage through a mechanism involving the ATM pathway (64–70). It has been widely suggested that ARF participates in the p53-dependent apoptosis induced by E2F1. However, recent studies indicated that ARF might be dispensable for E2F1-induced apoptosis (71–74) and that ARF-independent pathways downstream of aberrantly induced E2F are largely responsible for p53 activation and subsequent apoptosis. Moreover, the disruption of E2F-mediated transcription regulation in cells derived from FVB mice causes an increase in the expression of E2F target genes, including ARF (75). In this respect, it will be important to elucidate the pathway that leads to the suppression of tumor cell growth and apoptotic response in MIF–/– cells.

Given the complexity of the E2F-regulated function, we can only speculate as to how MIF deficiency might influence its tumor-suppressive properties of E2F1 are not understood completely, they are believed to result from the ability of E2F1 to induce apoptosis, which can be both p53-dependent (55) and p53-independent (35). In this study, we used the ARF-deficient MEF cells to investigate the role of MIF in E2F-mediated apoptosis in the absence of ARF. The results presented here suggest that MIF plays a role in E2F-mediated apoptosis, independent of ARF.

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