Regulation of the p53 Homolog p73 by Adenoviral Oncogene E1A*

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p73 is a p53 homolog, as they are similar structurally and functionally. Unlike p53, p73 is not inactivated by the products of viral oncoproteins such as SV40 T antigen and human papilloma virus E6. Here we show that the product of adenoviral oncoprotein E1A inhibits the transcriptional activity of both p73a and p73b. Electro- phoretic mobility shift assays revealed that E1A does not inhibit the sequence-specific DNA binding by p73. Transcriptional activation by a fusion protein containing the Gal4 DNA-binding domain and either of the activation domains of p73 was inhibited by wild-type (WT) E1A, but not by the N-terminal deletion mutant E1A(Δ2–36). E1A(Δ2–36), which does not bind to the p300/CBP family of coactivators, failed to inhibit p73-mediated transcription, whereas E1A(ΔCR2), a deletion mutant that does not bind to the pRB family of proteins, inhibited p73-mediated transcription as efficiently as WT E1A. Consistent with these observations, growth arrest induced by p73 expressed from a recombinant adenovirus was abrogated by WT E1A, which correlated with inhibition of p73-mediated induction of p21WAF1/CIP1 by E1A. However, p73 was able to induce p21WAF1/CIP1 and to mediate growth arrest in the presence of E1A(Δ2–36). Furthermore, the expression of either wild-type E1A or E1A(Δ2–36) resulted in the stabilization of endogenous p73. However, p73 stabilized in response to the expression of E1A(Δ2–36), but not WT E1A, was able to activate the expression of p21WAF1/CIP1. These results suggest that the transcriptional activation function of p73 is specifically targeted by E1A through a mechanism involving p300/CBP proteins during the process of transformation and that p73 may have a role to play as a tumor suppressor.

The product of the tumor suppressor gene p53, which is often described as the “guardian of the genome,” is a transcription factor involved in maintaining genomic integrity by controlling cell cycle progression and cell survival (1). Consistent with this view, mutations in p53 are the most frequently seen genetic alterations in human cancers (2, 3). Similarly, p53 knockout mice as well as transgenic mice carrying mutant p53 alleles are highly prone to developing spontaneous and carcinogen-induced tumors (4, 5).

Several p53 family members have been identified recently.

EXPERIMENTAL PROCEDURES

Plasmids—pG13-Luc, WWP-Luc, pCEP4-p53, pcDNA3-p73a, pcDNA3-p73b, WT E1A, E1A(Δ2–36), E1A(ΔCR2), GST-E1A, G5E1BCAT, and pCMV-LacZ were described previously (16, 22, 25–27). WT E1A refers to 12 S form of E1A, and all of the mutant forms of E1A were derived from it. The fusion protein constructs consisting of the Gal4 DNA-binding domain by cloning the fragment into the Bacillus subtilis expression vector pQE70 (Qiagen) (22, 28). These fusions were successfully expressed in E. coli and purified on amylose affinity resin (Novagen).

1 The abbreviations used are: WT, wild-type; GST, glutathione S-transferase; Ad, adenovirus; BrdUrd, bromodeoxyuridine; m.o.i., multiplicity of infection; PBS, phosphate-buffered saline; CBP, CAMP-responsive element-binding protein-binding protein; FACS, fluorescence-activated cell sorting; ARP, alternate reading frame; Rb, retinoblastoma protein.

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drives the expression of Gal4 fusions in pM, is inhibited by E1A, we transferred the entire fragment carrying Gal4-activation domain fusions as BglII/XbaI fragments into the BamHI and XbaI sites of pCDNA3 (Invitrogen).

**Cell Lines, Transfections, and Reporter Assays**—The mutant p53-expressing human colon cancer cell line SW480 was maintained in culture as described previously (16). The human lung carcinoma cell line H1299 carries a homozygous deletion of p53 and was kindly provided by Dr. Tapas Kumar Kundu (Jawaharlal Nehru Centre for Advanced Scientific Research, Bangalore, India). Transfections were carried out using Lipofectin (Invitrogen) or Escort (Sigma) following the manufacturers’ recommendations or by the calcium phosphate method as described (26). In all transfections, 1 μg of pCMV-LacZ DNA was added to normalize the transfection efficiency variation between samples. Luciferase, β-galactosidase, and chloramphenicol acetyltransferase assays were performed as described previously (16, 26).

**Electrophoretic Mobility Shift Assay**—Electrophoretic mobility shift assay was carried out essentially as described previously (16). A p53-binding site (5′-AGGTAGGCATGTCATGGCATGTTA-3′) from the human p21 promoter was used as probe (28). The following reaction conditions were used: 25 mM Tris, pH 7.5, 20% glycerol, 50 mM KCl, 50 mM dithiothreitol, 1 mM/ml bovine serum albumin, and 0.1% Triton X-100. The sequence of the nonspecific competitor was as follows: 5′-GATCCGAATTCGGTACC-3′. The p73 proteins were made by in vitro coupled transcription/translation (Promega), and E1A was bacterially synthesized and purified as a GST fusion protein (27).

**Adenoviral Reagents and Infections**—The adenoviruses used in this study were as described previously (16, 29, 30). Ad-E1A/WT refers to Ad5Δ520E1B−, which lacks E1B, but expresses wild-type 12 S E1A only. Ad-E1A/1101 refers to Ad5Δ520E1B−/1101, which lacks E1B, but expresses 12 S E1A lacking amino acids 4–25. Ad-LacZ lacks both E1A and E1B, but carries β-galactosidase (25). Ad-p73 lacks both E1A and E1B, but carries simian p73β. (The details of the construction and characterization of Ad-p73 will be published elsewhere.) Adenoviral infections were carried out as described (16).

**Western and Immunohistochemical Analyses**—Western analysis and immunohistochemical staining were performed as described previously (16) with mouse anti-human p53 monoclonal antibody (Ab-1, Oncogene), mouse anti-Ad2 E1A monoclonal antibody (M73, Ab-1, Oncogene), mouse anti-human p73 monoclonal antibody (Ab-2, Oncogene), mouse anti-human Rb monoclonal antibody (clone LM95.1, Ab-5, Oncogene), and goat anti-human actin polyclonal antibody (I-19, Santa Cruz Biotechnology). Cells were harvested or fixed 24 h after viral infection and subjected to analysis. Under conditions in which two viral infections were carried out using Lipofectin (Invitrogen) or Escort (Sigma) following the manufacturers’ recommendations or by the calcium phosphate method (a–d) or using Escort transfection reagent (e). Lysates were prepared and analyzed for luciferase reporter activity 48 h post-transfection as described under “Experimental Procedures.” pUC18 plasmid DNA was added to keep the total amount of DNA per transfection at 7 μg. In d, increasing amounts of wild-type 12 S E1A expression plasmid DNA (0.25, 0.5, 0.75, and 1 μg) were used (bars 3–6).

**RESULTS**

**E1A Inhibits p73-mediated Transcriptional Activation**—E1A has been shown to inhibit p53-mediated transcriptional activation (15, 16). To determine the effect of E1A on p73-mediated transcriptional activation, we studied the ability of p73 to activate transcription in the presence of E1A. We used the p53-specific reporter pG13-Luc to assay p73-mediated transcription. pG13-Luc contains a synthetic promoter with 13 copies of p53-binding sequence cloned into a p53 null or mutant cell line. Neither p73α nor p73β activated transcription in the presence of WT E1A (Fig. 1, a and c). Moreover, the inhibition by WT E1A was dose-dependent (Fig. 1d). When activated, p53 has been shown to induce p21WAF1/CIP1, which is the major mediator of p53-mediated growth arrest (1). Because p73 also induces p21WAF1/CIP1 upon overexpression, p21WAF1/CIP1 may play an important role in p73-mediated growth arrest. WT E1A inhibited the transcriptional activation of the p21WAF1/CIP1 promoter by p73β (Fig. 1e). These results suggest that adenovirus E1A inhibits p73-mediated transcription very effectively.

**E1A Requires Its p300/CBP-binding Region to Inhibit p73-mediated Transcription**—E1A is believed to transform cells by binding and abrogating the activities of two sets of cellular proteins: the p300/CBP family of coactivators and the pRb family of proteins (33). Inhibition of p53-mediated transcription by E1A requires its p300/CBP-binding region (16, 34–36). To determine the domain of E1A required for the inhibition of p73 function, we tested the ability of two deletion mutants of E1A to inhibit p73 function. The N-terminal deletion mutant E1A(ΔΔ–36), which does not bind to the p300/CBP family of coactivators, failed to inhibit p73-mediated transcription (Fig. 2, a, bar 4; and b, bar 4). However, the deletion mutant E1A(ΔCR2), which does not bind to the pRb family of proteins,
inhibited p73 function very efficiently (Fig. 2, a, bar 5; and b, bar 5). Both WT E1A and its deletion mutants were detected in transfected cells (Fig. 2c, lanes 2–4). These results suggest that an intact p300/CBP-binding region is required for E1A to inhibit p73 function.

**E1A Does Not Inhibit Sequence-specific DNA Binding by p73**—p73 binds to a sequence similar to p53-binding sequence and activates transcription (21). We investigated the effect of E1A on the sequence-specific DNA binding by p73 proteins, as it is essential for transactivation (10). p73α and p73β proteins synthesized in vitro using coupled transcription/translation system were used in this assay. p73 proteins bound to the p53-binding site (Fig. 3, a, lane 3; and c, lane 4). The binding was specific, as it was competed efficiently by an unlabeled p53 oligonucleotide (Fig. 3, a, lanes 4–6; and c, lanes 5–7), but not by a nonspecific oligonucleotide (a, lanes 7–9; and c, lanes 8–10). E1A did not inhibit the sequence-specific DNA binding by p73β (Fig. 3b, lane 5) and p73α (c, lane 12). These results suggest that the sequence-specific DNA binding by p73 is not targeted by E1A during its inhibition of p73 function.

**E1A Targets Activation Domains of p73**—p73 has two activation domains: one in the N-terminal region, which has ∼25% similarity to the N terminus of p53, and the other in the C-terminal region (37). Whereas the N-terminal activation domain is identical in p73α and p73β, the C-terminal activation domain, which extends from amino acids 380 to 513 in p73α, is terminated at amino acid 495 in p73β due to differential splicing (37). Because the sequence-specific DNA binding by p73 was not affected by E1A, we thought that E1A could inhibit p73-dependent transcription by targeting the activation domains. To examine this possibility, we studied the effect of E1A on the transcriptional activation by a fusion protein comprising the Gal4 DNA-binding domain and either of the activation domains of p73. E1A inhibited transcription from the N-terminal activation domain (Fig. 4, a, compare lanes 2 and 3; and c, compare bars 1 and 2) as well as from the C-terminal activation domain (b, compare lane 1 and 2; and d, compare bars 1 and 2) of p73α. The inhibition of Gal4 fusion proteins by WT E1A was specific because of the following reasons. Transcription by the Gal4-VP16 fusion protein was not inhibited by WT E1A (16).

The N-terminal deletion mutant E1A(Δ2–36) failed to inhibit transcription from either the N-terminal activation domain (Fig. 4, a, compare lanes 2 and 4; and c, compare bars 1 and 3) or the C-terminal activation domain (b, compare lanes 1 and 3; and d, compare bars 1 and 3) of p73. These experiments suggest that E1A specifically inhibits the activation domains of p73 and that the p300/CBP-binding region of E1A is required for this inhibition.

**E1A Inhibits p73-mediated Induction of p21<sup>WAF1/CIP1</sup>**—Overexpression of p73 by transient transfection leads to induction of p21<sup>WAF1/CIP1</sup> and growth arrest (10). To study the importance of inhibition of p73 function by E1A, we studied the ability of p73 to transactivate p21<sup>WAF1/CIP1</sup> in the presence of E1A. We used a replication-deficient recombinant adenovirus carrying p73β (Ad-p73) for this purpose. We first checked the ability of p73 expressed from Ad-p73 to induce p21<sup>WAF1/CIP1</sup> in the presence of E1A. We used the human lung carcinoma cell line H1299 for this experiment because it carries a homozygous deletion of p53. H1299 cells infected with a control virus (Ad-LacZ) showed very little or no expression of p73, E1A, and p21 proteins (Fig. 5a, panels A, E, and I, respectively). Infection of H1299 cells with Ad-p73 led to accumulation of high levels of p73 and p21 (Fig. 5a, panels B and J, respectively). However, when Ad-p73 infection was preceded by Ad-E1A/WT infection, cells expressed high levels of p73 and E1A, but not p21<sup>WAF1/CIP1</sup> protein (Fig. 5a, panels C, G, and K, respectively). Results from reporter assay experiments suggested that an intact p300/CBP-binding region is required for E1A to inhibit p73 function (Fig. 2, a and b). To confirm this observation, we checked the effect of an N-terminal deletion mutant of E1A on p73-mediated induction of p21<sup>WAF1/CIP1</sup>. We used Ad-E1A/1101 for this purpose because it expresses an E1A protein with a
deletion of amino acids 24 and 25, which makes it deficient for binding to p300/CBP proteins (29, 30). Unlike WT E1A, the N-terminal deletion mutant failed to inhibit p73-mediated induction of p21\textsuperscript{WAF1/CIP1} (Fig. 5a, panel L). We also analyzed the p73 and p21\textsuperscript{WAF1/CIP1} levels by Western blotting in a similar experiment (Fig. 5b), the results of which were comparable to those of the immunohistochemical staining experiment. The presence of WT E1A, but not its N-terminal deletion mutant, inhibited the induction of p21\textsuperscript{WAF1/CIP1} by p73 (Fig. 5b, compare lanes 5 and 6). These results suggest that E1A inhibits p73-mediated induction of p21\textsuperscript{WAF1/CIP1} efficiently through its p300/CBP-binding region.

**E1A Inhibits p73-mediated Cell Cycle Arrest**—To correlate the immunohistochemical and Western blot data with changes in cell cycle phases, we studied the cell cycle profile under the same conditions. We first monitored cellular DNA synthesis by measuring BrdUrd and \[^{3}H\]thymidine incorporation. The ability of p73 to inhibit DNA synthesis in the absence or presence of either WT E1A or its N-terminal deletion mutant was analyzed. Infection of H1299 cells with Ad-p73 resulted in a drastic reduction in the percentage of cells incorporating BrdUrd in comparison with Ad-LacZ-infected cells (Fig. 6a, compare panels B and D), suggesting inhibition of cellular DNA synthesis upon overexpression of p73. However, prior expression of E1A by infecting cells with Ad-E1A/WT before Ad-p73 infection abrogated the p73-mediated DNA synthesis inhibition (Fig. 6a, compare panels D and F). The results obtained in Fig. 6a were quantified, and percent BrdUrd incorporation under different conditions is shown in Fig. 6b. Cellular DNA synthesis was also measured by \[^{3}H\]thymidine incorporation under different conditions. Infection of cells with Ad-p73 resulted in a drastic reduction in \[^{3}H\]thymidine incorporation, which is indicative of DNA synthesis inhibition (Fig. 6c, compare bars 1 and 2). However, expression of E1A before Ad-p73 infection resulted in the abrogation of DNA synthesis inhibition as seen by increased \[^{3}H\]thymidine incorporation (Fig. 6c, compare bars 2 and 3). In both of the above experiments, the N-terminal deletion mutant of E1A failed to abrogate the DNA synthesis inhibition by p73 (Fig. 6, a, compare panels D and H; b, compare bars 2 and 4; and c, compare bars 2 and 4). The phosphorylation status of Rb correlated with the extent of cellular DNA synthesis (Fig. 5c) under the conditions used in the above experiments. The hypophosphorylated form of Rb was seen in Ad-p73-infected cells.
Phase cells. Thus, in concurrence with our earlier data (Fig. 6), WT, but not with Ad-E1A/1101, abrogated the decrease in S phase cells at different time points after infection (Fig. 7a). Ad-p53-infected cells were used as a positive control. Upon Ad-p73 infection, cells with less than 2N DNA, representing apoptotic cells, appeared only after 48 h (34.9% versus 4.2%) in comparison with Ad-LacZ-infected cells. However, the proportion of cells actively replicating DNA, representing S phase cells, decreased by ~50% as early as 24 h (8.8% versus 16.6%) and to negligible levels by 48 h (0.9% versus 19.3%) in the Ad-p73-infected sample in comparison with the Ad-LacZ-infected sample. Thus, it is apparent that growth arrest as seen by a decrease in cells undergoing DNA synthesis occurs much earlier than apoptosis. Next, we checked the ability of WT E1A to abrogate p73-mediated growth arrest by FACS (Fig. 7b). Ad-p73 infection led to a significant decrease in S phase cells in comparison with Ad-LacZ infection (26.2 to 5.2%) with a concomitant increase in cells containing 2N DNA (58.8 to 81.3%), suggesting a potent G1 phase growth arrest mediated by p73. However, prior infection of Ad-p73-infected cells with Ad-E1A/WT, but not with Ad-E1A/1101, abrogated the decrease in S phase cells. Thus, in concurrence with our earlier data (Fig. 6), WT E1A inhibits p73-mediated growth arrest through its p300/CBP-binding region.

Stabilization of Endogenous p73 by E1A—Expression of wild-type E1A leads to stabilization of the p53 protein in rat embryo fibroblast cells (38). p53 stabilization by wild-type E1A does not require either the p300/CBP- or Rb-binding region of E1A (16). To study the relation between inhibition of p73 function and transformation by E1A, we studied the ability of E1A to stabilize endogenous p73. Infection of H1299 cells with Ad-E1A/WT resulted in the stabilization of p73β in a time-dependent fashion (Fig. 8, lanes 2 and 5). However, endogenous p73 stabilized in response to WT E1A expression was unable to activate p21WAF1/CIP1 very efficiently (Fig. 8, lanes 2 and 5). Expression of an N-terminal deletion mutant of E1A (Ad-E1A/1101) also resulted in the stabilization of endogenous p73β, but the difference was that p73 stabilized by the N-terminal deletion mutant was able to activate p21WAF1/CIP1 very efficiently (Fig. 8, lanes 3 and 6). p73β expressed from an exogenous source by infection of H1299 cells with Ad-p73 resulted in the activation of p21WAF1/CIP1 (Fig. 8, lane 7). These results suggest at least two important points. E1A also inhibits activation of p21WAF1/CIP1 by endogenously stabilized p73β in a p300/CBP-dependent manner, and the p300/CBP-binding region of E1A is not required for stabilization of endogenous p73, whereas it is required for inhibition of p73-mediated transcription. This observation is very important with respect to transformation by E1A because p73 stabilized by E1A would otherwise suppress DNA synthesis and induce growth arrest by activating p21WAF1/CIP1.

**DISCUSSION**

In this study, we found that E1A inhibits p73-mediated transcription, confirming earlier observations by others. In addition, we have shown that the sequence-specific DNA binding by p73 is not affected by E1A, whereas the transactivation...
domains of p73 are targeted by E1A. A mutant of E1A carrying a deletion in the N terminus failed to inhibit p73-mediated transcription. Furthermore, we have shown that E1A abrogated p73-mediated growth arrest, which correlated with its ability to inhibit p73-mediated activation of p21WAF1/CIP1. E1A also stabilized endogenous p73 in a time-dependent fashion. The N-terminal region of E1A, which is needed for binding to the p300/CBP family of coactivators, was required for inhibition of p73 function, but not for stabilization of endogenous p73 by E1A.

E1A has been shown to inhibit p53-mediated transcription through its p300/CBP-binding region (15, 16). Subsequently, it was shown that p300/CBP molecules act as coactivators for p53 and that E1A binds to p300/CBP coactivators, thereby inhibiting p53-mediated transcription (34–36). In our study, we found that wild-type E1A, but not an N-terminal deletion mutant of E1A, inhibited p73-mediated transcription. Because the N-terminal deletion mutant does not bind to the p300/CBP family of proteins (33), it is evident that E1A inhibits p73-mediated transcription by binding to the p300/CBP family of proteins. In fact, p300/CBP has been shown to bind to p73 and to act as a coactivator (39).

Our results also show that E1A targets the transactivation domains of p73 and not the sequence-specific DNA binding by p73. p300/CBP has been shown to interact with the N terminus of p73 and to coactivate p73-mediated transcription (39). In good correlation with this, wild-type E1A, but not the N-terminal deletion mutant, inhibited transcription mediated by the N-terminal activation domain of p73. E1A also inhibited transcription mediated by the C-terminal activation domain of p73.

Fig. 7. Flow cytometry analysis of Ad-p73-infected cells. a, SW480 cells were infected with either Ad-LacZ or Ad-p73 at m.o.i. = 10. The cells were harvested 12, 24, 36, and 48 h after infection and subjected to flow cytometry analysis as described under “Experimental Procedures.” The cells were allowed to incorporate BrdUrd for the last 4 h of the time points at which they were collected. S indicates cells undergoing DNA synthesis (S phase), and A indicates cells containing less than 2N DNA (apoptotic cells). The quantified values are indicated. b, SW480 cells were infected with Ad-LacZ, Ad-E1A/WT, or Ad-E1A/1101 at m.o.i. = 10. After 6 h of infection, the cells were again infected with either Ad-LacZ or Ad-p73 as indicated. 20 h after the second viral infection, BrdUrd incorporation was carried out for 4 h, and the cells were harvested and subjected to flow cytometry analysis as described under “Experimental Procedures.” G1 indicates cells containing 2N DNA, and G2/M indicates cells containing 4N DNA. The quantified values are indicated.
Although we do not know the actual mechanism of this inhibition, one possibility is that there may be an additional site for p300/CBP binding on p73, as the N-terminal deletion mutant of E1A failed to inhibit transcription from the C-terminal activation domain of p73. Another possibility is that another protein, p400, which is known to bind to the N terminus of E1A, could also be involved in the inhibition of the C-terminal activation domain of p73 by E1A (40). p400 is related to SWI/SNF factors, and binding of E1A to p400 could recruit SWI/SNF complexes to chromatin, leading to either transcriptional activation or repression (40).

The level of p73 protein is increased in response to expression of certain cellular and viral oncogenes. Cellular oncogene E2F is a direct transcriptional activator of p73 by binding to several E2F1-responsive elements within the P1 promoter of p73 (41, 42). The level of p73 protein, similar to p53, is increased upon adenoviral infection (23, 43). Adenoviral E1A and E1B proteins are responsible for this p73 stabilization. Upon activation, p73 induces p21\textsuperscript{WAF1/CIP1} and mounts a strong G\textsubscript{1} arrest by inhibiting DNA synthesis, which would prevent transformation. Our study has also shown that endogenous p73 was stabilized by the expression of WT E1A. In addition, we have shown that the N-terminal region of E1A, which is essential for binding to the p300/CBP family of proteins, was not needed for p73 stabilization (Fig. 8). However, p73 stabilized by WT E1A was unable to activate p21\textsuperscript{WAF1/CIP1}, whereas p73 stabilized by the N-terminal deletion mutant of E1A was able to activate p21\textsuperscript{WAF1/CIP1}. These results suggest a mechanism whereby E1A could bring about a successful transformation by inhibiting the transcriptional activation of p21\textsuperscript{WAF1/CIP1} by endogenous p73 induced in response to expression of WT E1A. The N-terminal deletion mutant, which did not inhibit p73-mediated transcription, was also able to stabilize endogenous p73, but with a concomitant activation of p21\textsuperscript{WAF1/CIP1}, thus explaining the fact that this N-terminal deletion mutant is unable to transform cells successfully.

Our study suggests that E1A bypasses important cell cycle checkpoints by inhibiting transcriptional activation by p73. The benefits from inhibition of p73-mediated p21\textsuperscript{WAF1/CIP1} induction are 2-fold. First, inhibition of p73-mediated induction of p21\textsuperscript{WAF1/CIP1} would relieve a block at the G\textsubscript{1}/S boundary because complexes between cyclin and cyclin-dependent kinase associated with G\textsubscript{1}/S progression would remain activated. Second, it would also relieve a block during DNA synthesis, as it has been shown that p21\textsuperscript{WAF1/CIP1} interacts with proliferating cell nuclear antigen and affects processive DNA synthesis (44).

Increases in p53 levels have been observed frequently in primary tumors. However, p53 is inactivated by mutational inactivation in the majority of these cases. p53 is also inactivated by the ARF-MDM2-p53 pathway (45). This pathway includes amplification of MDM2, homozygous deletion of ARF, or methylation silencing of ARF expression. The levels of p73 in normal human tissues are generally very low (46, 47). In contrast, overexpression of p73 has been shown in variety of primary tumors and tumor cell lines (48–54). Unlike p53, p73 is very rarely mutated in primary tumors, suggesting the existence of other mechanisms that functionally inactivate stabilized p73. Binding of MDM2 and MDMX proteins to p73 inhibits the transcriptional activation by p73 and affects the subcellular localization of p73 (55–57). It remains to be seen whether ARF regulates the function of p73. Thus, it is evident that there are cellular mechanisms of inhibition of p73 function during transformation similar to transcriptional inactivation of p73 by E1A.

p73 and p63 have been shown to regulate p53 function, in particular p53-dependent apoptosis in response to DNA damage (58). p53 also utilizes the p300/CBP family of proteins as coactivators (34–36), similar to p73. This raises the question of whether the inhibitory effect of E1A on p73 function is mediated through a mechanism involving p53. Because our experiments were carried out in cells in which p53 is either mutated (SW480) or deleted (H1299), the inhibition of p73-mediated transformation by E1A is p53-independent.

In summary, we conclude that adenoviral oncogene E1A inhibits p73-mediated transformation in a manner similar to inhibition of p53 activity by E1A. However, inhibition of p73 function by E1A is independent of p53. Our findings suggest that inhibition of p73-mediated growth arrest by E1A could be an additional mechanism for a successful transformation. In the p53 family, few other members, such as p63 and p51, in addition to p73, have been identified. It will be interesting to determine whether these members are up-regulated in response to oncogene expression and whether E1A inhibits the function of these p53 homologs as well.

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