Proc. Natl. Acad. Sci. USA
Vol. 91, pp. 2026-2030, March 1994
Cell Biology

Abrogation of oncogene-associated apoptosis allows transformation of p53-deficient cells

(tumor suppressor gene/adenovirus E1A/adenovirus E1B)

SCOTT W. LOWE*, TYLER JACKS*, DAVID E. HOUSMAN*, AND H. EARL RULEY†‡

*Center for Cancer Research and Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139; and ‡Department of Microbiology and Immunology, Room AA5206 MCN, Vanderbilt University School of Medicine, 1161 21st Avenue South, Nashville, TN 37232-2363

Communicated by Peter M. Howley, November 12, 1993

ABSTRACT **p53-deficient mouse embryonic fibroblasts were used to establish a direct mechanism of tumor suppression by p53 involving the destruction of oncogene-expressing cells by apoptosis. The absence of p53 enhanced cell growth, appeared sufficient for immortalization, and allowed a single oncogene [adenovirus early region 1A (E1A)] to transform cells to a tumorigenic state. p53 suppressed transformation of E1A-expressing cells by apoptosis. Apoptosis was associated with p53 stabilization and was triggered by environmental signals that normally suppress cell growth. Absence of even a single p53 allele significantly enhanced cell growth and survival. Although abrogation of apoptosis allowed transformation by E1A alone, escape from apoptosis susceptibility was not a prerequisite for tumor growth. Consequently, p53 mutation could enhance the survival of malignant cells expressing oncogenes activated early in tumor progression.**

The p53 tumor suppressor is the most frequently mutated gene in human tumors (1). Presently, the only model to account for the action of p53 as a tumor suppressor views p53 as a "guardian of the genome" (2). According to this model, p53 is an essential component of a DNA damage control system that, when operating normally, reduces the likelihood that cells will sustain oncogenic mutations. This view stems from the observations that p53 expression and stability are induced in cells exposed to DNA-damaging agents (3), leading to either cell cycle arrest [which may facilitate DNA repair (4)] or cell death by apoptosis (5, 6). Failure to activate p53 expression after DNA damage may account for the high cancer incidence in individuals with ataxia–telangiectasia (4) and in mice lacking p53 (5).

Nevertheless, there are reasons to doubt that this indirect mechanism is the only means by which p53 mutation contributes to cancer. First, loss of p53 typically occurs late in tumor progression, after oncogenic mutations have already occurred (7). Second, mutant p53 alleles, which can inhibit normal p53 function (8), enable *ras* oncogenes to transform both primary and established cells (9, 10). Transformed foci appear within days, and stable transformation requires continuous expression of mutant p53 (11). Thus, p53 may directly influence both the initiation and maintenance of transformed phenotypes. This view is supported by the transforming interactions between the adenovirus early region 1A (E1A) gene and other oncogenes. E1A, while unable to transform alone, collaborates with either adenovirus E1B or activated *ras* oncogenes to oncogenically transform primary cells (12). We recently demonstrated that p53 levels and stability increase in response to E1A and suggested that stabilized p53 suppressed transformation by enhancing apoptosis (13). Consequently, proteins that either block p53 transactivation (adenovirus $p55^{E1B}$ and mutant p53) or protect against E1A-associated apoptosis (adenovirus $p19^{E1B}$ and mutant p53) can collaborate with E1A in oncogenic transformation (8, 14–16).

The evidence that p53 directly suppresses oncogenic transformation is circumstantial, since it has not been possible to assess the physiological activities of endogenous p53. While forced overexpression of wild-type or mutant p53 can reveal potential p53 activities, the relevance of this approach to circumstances in which endogenous p53 suppresses transformation is unknown. Certainly, proteins not normally involved in proliferation might suppress growth or viability when sufficiently overexpressed. Furthermore, mutant p53 alleles can transform p53-deficient cells, indicating that they are not simply dominant-negative suppressors of wild-type p53 (17).

In this study, transforming interactions between endogenous p53 and transfected oncogenes were analyzed by using embryonic fibroblasts derived from mice carrying disrupted p53 genes. Since the recipient cells differed only in their p53 status, differences between cellular responses could be unambiguously attributed to p53 function. Embryonic fibroblasts also provide a well-characterized model of multistep carcinogenesis in which oncogenic transformation typically requires two or more oncogenes acting in concert (12). These studies indicate that p53 can directly suppress oncogenic transformation by its involvement in apoptosis. Consequently, p53 loss allows transformation of primary cells by a single oncogene.

MATERIALS AND METHODS

Cells, Plasmids, and Gene Transfer. $p53^{+/+}$, $p53^{+/-}$, and $p53^{-/-}$ mouse embryonic fibroblasts (MEFs) were cultured in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum (FBS) and used between passages 3 and 5. p1AHygro contains the adenovirus type 5 E1A gene (nucleotides 1–1834) inserted into pY3 [expressing hygromycin phosphotransferase (18)]. pT24neo encodes a *ras* oncogene (T24 H-*ras*; designated *ras*) (19) and p5XX encodes the E1B gene. pLTRKH215 and pLTRcGala expressed a dominant-transforming p53 allele ($p53^{KH215}$) and mouse wild-type p53, respectively (9). Stable lines expressing E1A were generated by calcium phosphate precipitation (19) using 1 μg of p1AHygro and a 10-fold molar excess of pT24neo, p5XX, pLTRKH215, or pLTRcGala. Alternatively, pY3 was used at a molar amount equivalent to 1 μg of p1AHygro. For each precipitation, the total mass of DNA was adjusted to 20 μg by using pBluescript. Transfected cells were subcultured into medium containing either 100 μg ($p53^{-/-}$ MEFs) or 15 μg ($p53^{+/+}$ and $p53^{+/-}$ MEFs) of hygromycin B per ml (Sigma), concentrations that were determined empirically. After 2–3

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: E1A, adenovirus early region 1A; E1B, adenovirus early region 1B; MEF, mouse embryonic fibroblast; FBS, fetal bovine serum; BrdU, 5-bromo-2′-deoxyuridine.

‡To whom reprint requests should be addressed.

weeks, several clones from each transfection were expanded and colony numbers were estimated.

Viability Assays. E1A-expressing colonies (which are morphologically distinct) were marked and transferred to 0.1% FBS, photographed at various times, and scored for regression. Alternatively, cell lines were seeded at 1–2 × 10^6 cells per 100-mm plate and subsequently transferred to 0.1% FBS. Later, adherent and nonadherent cells were pooled for viability measurements using fluorescein isothiocyanate staining and flow cytometry (5). For analyzing DNA fragmentation, low molecular weight DNA was purified from pools of adherent and nonadherent cells 24 hr after transfer to 0.1% FBS and analyzed by agarose gel electrophoresis (20).

p53 Expression and Cell Cycle Analysis. p53 half-life was estimated by p53 immunoprecipitation of ^{35}S-labeled cell lysates as described (13). Western blot analysis was performed with lysates derived from 10^6 cells (5). For cell cycle analysis, cells were incubated with 5-bromo-2'-deoxyuridine (BrdU) for 4 hr, beginning 14 hr after transfer to 0.5% FBS. Subsequently, cultures were washed to remove dead cells, and the adherent cells were prepared for flow cytometry (20). Proliferation was assessed by DNA content (propidium iodide staining) and DNA synthesis (BrdU immunostaining).

Tumorigenicity Experiments. Male nude mice (Swiss *nu/nu*; Taconic) (4–6 weeks old) were injected with 2 × 10^6 cells and monitored for tumors at the injection sites for ≈10 months. Tumors were scored positive when they became clearly visible (diameter, ≈2 mm). To avoid selection for a transformed phenotype, hygromycin-resistant clones were expanded minimally before inoculation.

RESULTS

Introduction of E1A into $p53^{+/+}$, $p53^{+/-}$, and $p53^{-/-}$ MEFs. Both E1A and mutant p53 alleles facilitate immortalization of primary cells in culture (21, 22). As an initial step in analyzing interactions between E1A and endogenous p53, we compared the ability of E1A (alone or with other oncogenes) to promote clonal outgrowth. E1A was introduced into $p53^{+/+}$, $p53^{+/-}$, and $p53^{-/-}$ MEFs by using p1AHygro, a plasmid that coexpresses adenovirus type 5 E1A and hygromycin phosphotransferase. Thus, colonies arising in hygromycin B have a high probability of expressing E1A. A plasmid expressing only hygromycin phosphotransferase (pY3) was used to assess the effects of endogenous p53 genes on clonal outgrowth. While $p53^{+/+}$ and $p53^{+/-}$ MEFs transfected with pY3 produced very few colonies (3 and 22 colonies per 10^6 cells, respectively), $p53^{-/-}$ MEFs generated many colonies (466 colonies per 10^6 cells). Thus, the absence of endogenous p53 resulted in efficient clonal outgrowth.

The ability of E1A to produce colonies correlated with p53 dosage. E1A was inefficient at promoting clonal outgrowth in $p53^{+/+}$ MEFs; transfection of p1AHygro averaged only 8 colonies per 10^6 cells (Fig. 1*A*). A 5- to 10-fold increase in colonies was obtained when p1AHygro was cotransfected with plasmids expressing either adenovirus type 5 E1B, *ras*, or a mutant p53 allele. $p53^{+/-}$ MEFs express less p53 than wild-type MEFs (23), and transfection of p1AHygro into $p53^{+/-}$ cells produced many more colonies than in $p53^{+/+}$ MEFs (254 colonies per 10^6 cells). Still, colony numbers increased ≈2-fold when E1A was cointroduced with either E1B, *ras*, or mutant p53 (Fig. 1*B*). Transfection of p1AHygro into $p53^{-/-}$ MEFs generated as many colonies as any oncogene combination (526 colonies per 10^6 cells). Wild-type p53 significantly reduced colony numbers in $p53^{-/-}$ MEFs (Fig. 1*C*), and of those that did emerge and were analyzed none (0/3) expressed detectable p53 immunofluorescence (data not shown). Since endogenous p53 levels are increased in response to E1A (13) and p53 overexpression causes either growth arrest or apoptosis (1, 24), the increase in p53 levels

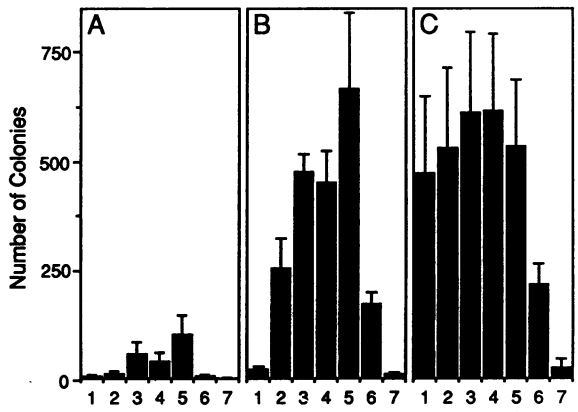

FIG. 1. Introduction of genes into MEFs. Transfection of various plasmids into $p53^{+/+}$ (*A*), $p53^{+/-}$ (*B*), and $p53^{-/-}$ (*C*) embryonic fibroblasts (10^6 cells per transfection). The transfected plasmid(s) expressed the following: 1, hygromycin phosphotransferase; 2, E1A; 3, E1A and E1B; 4, E1A and *ras* (T24 H-*ras*); 5, E1A and a mutant p53; 6, E1A and wild-type p53; 7, no DNA. The E1A expression vector (p1AHygro) coexpressed the adenovirus type 5 E1A gene and hygromycin phosphotransferase, allowing isolation of E1A-expressing colonies in medium containing hygromycin B. Colony numbers were estimated ≈2 weeks after transfection and represent the average ± SD determined from at least three transfections.

that accompanies E1A expression may suppress clonal outgrowth.

Attempts to expand E1A-expressing colonies into stable cell lines revealed striking differences among fibroblast types. Most $p53^{-/-}$ colonies expressing E1A (12/15) or hygromycin phosphotransferase (3/4) were established into permanent lines. By contrast, E1A-expressing colonies derived from $p53^{+/+}$ MEFs rarely reached a size suitable for transfer (500–1000 cells), and none (0/3) could be established. $p53^{+/+}$ colonies were established when E1A was coexpressed with either E1B (5/6), *ras* (6/10), or mutant p53 (9/16). Although many E1A-expressing $p53^{+/-}$ colonies were obtained (see Fig. 1*B*), only one clone (1/10) produced a permanent line. Immunoprecipitation analysis indicated that this clone did not express p53 (data not shown). Therefore, in the absence of other oncogenes, the combination of E1A and endogenous p53 was incompatible with long-term growth.

p53-Dependent Death of Cells Expressing E1A. E1A can induce apoptosis, particularly after serum depletion, and E1B inhibits apoptosis (13, 15). Since E1A increases p53 levels (13) and p53 is necessary for some forms of apoptosis (5, 6), we tested whether p53 was required for E1A-associated cell death. Although cell lines coexpressing E1A and endogenous p53 were not obtained, the availability of unexpanded colonies allowed analysis of E1A effects on cell viability. E1A-expressing colonies (with or without E1B) were marked and transferred to medium containing 0.1% FBS. As illustrated in Fig. 2, the majority of $p53^{+/+}$ and $p53^{+/-}$ colonies expressing E1A alone completely regressed by 72 hr after serum withdrawal (8/8 and 23/25 colonies regressed, respectively). In contrast, $p53^{-/-}$ colonies remained viable in 0.1% FBS (2/25 colonies regressed) and many continued to grow. Although E1B enhanced the viability of $p53^{+/+}$ colonies (6/12 colonies regressed), the effect was less than that of p53 absence. Therefore, p53 deficiency substituted for E1B in suppressing E1A-associated cell death. Because colonies were analyzed prior to significant growth in culture, resistance to death was not due to genetic alterations occurring upon clonal expansion.

***ras* Oncogenes Do Not Inhibit p53-Dependent Apoptosis.** Like E1B, coexpression of *ras* allowed establishment of E1A-expressing colonies containing endogenous p53. To test

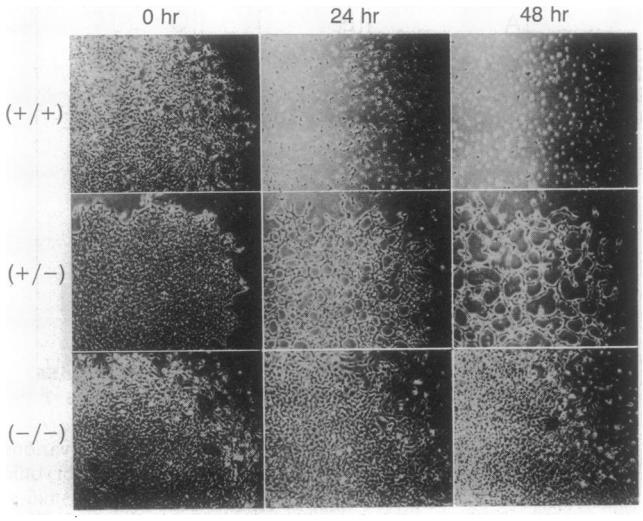

FIG. 2. Regression of E1A-expressing colonies upon serum depletion. p1AHygro was introduced into $p53^{+/+}$ (+/+), $p53^{+/-}$ (+/−), and $p53^{-/-}$ (−/−) MEFs and colonies were isolated by selection in hygromycin B. After 3 weeks, E1A-expressing colonies were marked and photographed at the indicated times after transfer to medium containing 0.1% FBS (without hygromycin B). Both $p53^{+/+}$ and $p53^{-/-}$ untransfected MEFs remained viable (≈90% viability) for at least 6 days after transfer to 0.1% FBS (data not shown).

whether *ras* also prevented p53-dependent cell death, cell lines expressing E1A were transferred to medium containing 0.1% FBS and viability was measured at various times thereafter (Fig. 3*A*). While all cells remained viable in 10% FBS, $p53^{+/+}$ cells coexpressing E1A and *ras* died rapidly in 0.1% FBS. $p53^{+/-}$ cells coexpressing E1A and *ras* also died in 0.1% FBS but less rapidly than wild-type cells. In contrast, all $p53^{-/-}$ lines remained viable in low serum, as did $p53^{+/+}$ cells coexpressing E1A and E1B. $p53^{+/+}$ cells coexpressing E1A and *ras* contained large amounts of degraded DNA after transfer to 0.1% FBS, whereas cells lacking p53 or expressing E1B did not (Fig. 3*B*). The degraded DNA was present in multiples of 180–200 bp, consistent with internucleosomal cleavage and cell death by apoptosis (25).

p53 Expression and Cell Proliferation During Apoptosis. Wild-type cells coexpressing E1A and *ras* contained elevated p53 levels, resulting from a 5- to 10-fold increase in protein stability (Fig. 4*A*). E1A (but not *ras*) increased p53 levels in transient assays, indicating that p53 stabilization was due to E1A (data not shown). For comparison, p53 levels in cells coexpressing E1A and *ras* were higher than in untransfected MEFs after γ-radiation [which also stabilizes p53 (3)] (Fig. 4*B*). However, p53 levels did not increase further upon transfer to 0.1% FBS, indicating that high p53 levels were not sufficient for apoptosis.

Since E1A sequences required for apoptosis are identical to those required for induction of DNA synthesis (26), we tested whether p53-dependent apoptosis might be triggered by unscheduled proliferation. $p53^{+/+}$ and $p53^{-/-}$ MEFs and their E1A/*ras*-expressing derivatives were incubated with BrdU for 4 hr beginning 14 hr after transfer to medium containing 0.5% FBS. Cells were analyzed for DNA content (by propidium iodide staining) and DNA synthesis (by BrdU incorporation) by using multiparameter flow cytometry. $p53^{+/+}$ MEFs arrested rapidly upon serum withdrawal (Fig. 5 *A* and *B*), although $p53^{-/-}$ fibroblasts exited the cell cycle more slowly (Fig. 5 *C* and *D*). In 0.1% FBS, $p53^{+/+}$ cells coexpressing E1A and *ras* continued to proliferate with no reduction in BrdU-positive cells, even though many cells had initiated apoptosis (compare Fig. 5 *B* and *F*). Moreover, cells that tolerate E1A expression alone also proliferate after

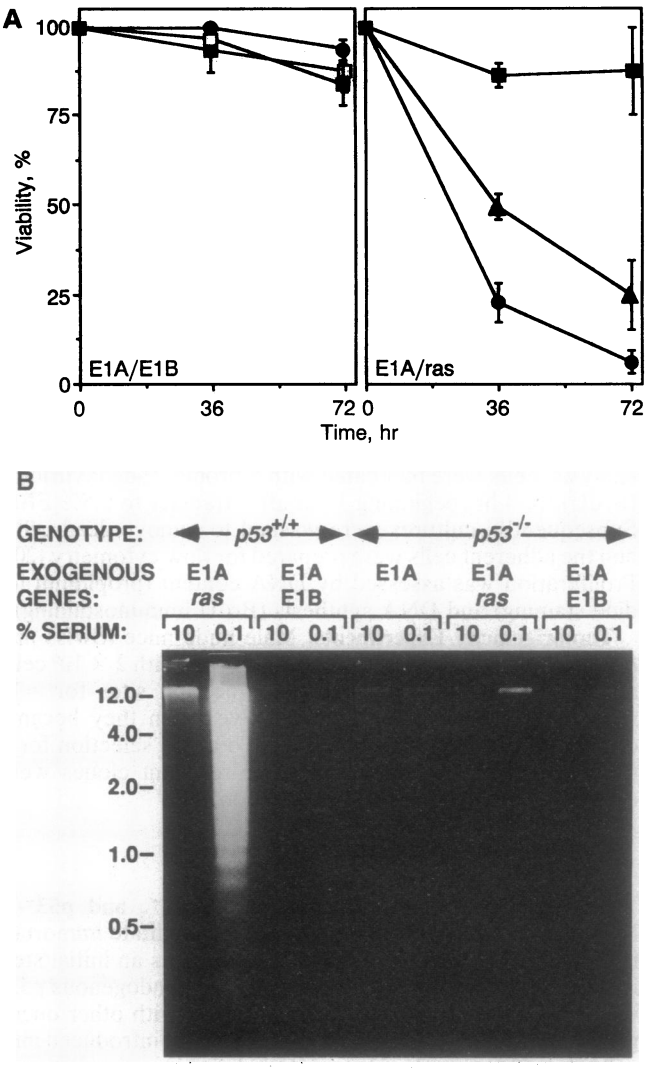

FIG. 3. Viability of cell lines upon serum withdrawal. (*A*) Viability of cells expressing E1A, E1A and E1B, and E1A and *ras* in 0.1% FBS was measured by uptake of fluorescein isothiocyanate and fluorescence-activated cell sorting analysis. Cell lines were derived from $p53^{-/-}$ (■), $p53^{+/-}$ (▲), and $p53^{+/+}$ (●) MEFs. The viability of $p53^{-/-}$ lines expressing E1A alone is indicated (□). Values represent average ± SD obtained from at least three independent clones and were normalized to the percentage of viable cells at the start of the experiment (generally >90%). The parental MEFs retained viability in 0.1% FBS (data not shown). (*B*) Low molecular weight DNA was isolated from 2×10^6 cells 24 hr after transfer to 0.1% FBS. DNA was resolved on 1% agarose gels and visualized by ethidium bromide staining. Since samples were normalized by cell number, viable cells contained almost no low molecular weight DNA. Numbers on left indicate mobilities of size standards (in kb).

serum withdrawal, indicating that E1A is sufficient to circumvent growth arrest (M. Raggozino and H.E.R., unpublished observations). Therefore, p53-dependent apoptosis coincided with continued cell cycle progression under conditions that normally suppress growth.

p53-Deficient Cells Expressing E1A Are Tumorigenic. Since apoptosis provides a mechanism whereby p53 can act as a tumor suppressor (5, 6, 24), we investigated whether reduced susceptibility to apoptosis would increase tumorigenic potential. Nude mice were injected with $p53^{+/+}$ and $p53^{-/-}$ cells and monitored for tumors at the sites of injection (Table 1). $p53^{-/-}$ cells expressing E1A were tumorigenic, with latency periods similar to $p53^{+/+}$ cells coexpressing E1A and E1B. Untransfected MEFs and $p53^{-/-}$ MEFs expressing only hygromycin phosphotransferase were not tumorigenic,

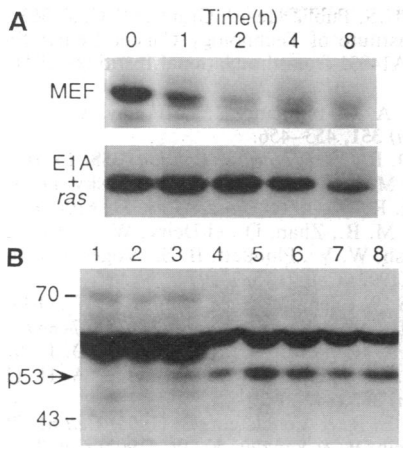

FIG. 4. p53 expression in cells coexpressing E1A and *ras* during apoptosis. (*A*) Cells were pulse-labeled for 1 hr with 100 μCi of ^{35}S-labeled amino acids per ml (1 Ci = 37 GBq) and chased with excess unlabeled methionine. At various times, cells were lysed and p53 was immunoprecipitated with PAb421 (13). p53 half-life was estimated by a PhosphorImager (0.5 hr in untransfected MEFs; 4–5 hr in cells coexpressing E1A and *ras*). (*B*) p53 levels were estimated by Western blot analysis (5). Lanes: 1, $p53^{-/-}$ MEFs; 2, $p53^{+/+}$ MEFs; 3, $p53^{+/+}$ MEFs 8 hr after exposure to 5 Gy of ionizing radiation; 4 and 5, untreated $p53^{+/+}$ cells coexpressing E1A and *ras*; 6–8, $p53^{+/+}$ cells coexpressing E1A and *ras* 1, 4, and 8 hr, respectively, after transfer to 0.1% FBS.

indicating that tumorigenicity required E1A. Because all clones were derived from drug-resistant colonies, no prior selection for a transformed phenotype was imposed. Therefore, abrogation of p53-dependent apoptosis permits transformation of MEFs by E1A alone, and the absence of p53 substituted for E1B in transformation.

The latency of tumors derived from $p53^{-/-}$ cells coexpressing E1A and *ras* was significantly less than tumors derived from wild-type cells (Table 1). Nevertheless, cells coexpressing E1A and *ras* were highly tumorigenic. Since these cells were sensitive to apoptosis *in vitro*, it was possible that tumors arose from resistant variants. To test this, cells derived from a $p53^{+/+}$ tumor coexpressing E1A and *ras* were reestablished in culture and transferred to 0.1% FBS. The tumor-derived cells lost viability as rapidly as the original clone, indicating that tumorigenicity did not result from mutations that suppressed apoptosis (data not shown). Thus,

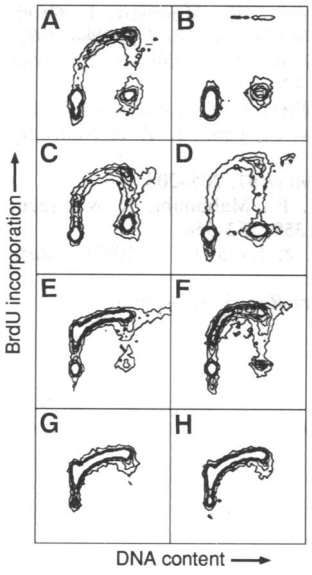

FIG. 5. Cell cycle analysis by flow cytometry. $p53^{+/+}$ (*A* and *B*) and $p53^{-/-}$ (*C* and *D*) MEFs, or $p53^{+/+}$ (*E* and *F*) and $p53^{-/-}$ (*G* and *H*) lines coexpressing E1A and *ras* were placed in medium containing either 10% FBS (*A*, *C*, *E*, and *G*) or 0.5% FBS (*B*, *D*, *F*, and *H*). Fourteen hours later, cells were incubated in BrdU for 4 hr. Cell proliferation was assessed by DNA content (propidium iodide fluorescence intensity) and BrdU incorporation (anti-BrdU fluorescence intensity; i.e., DNA synthesis) was assessed by flow cytometry. Contour plots from representative samples are shown.

Table 1. Tumorigenicity of E1A-expressing cells

	$p53^{-/-}$			$p53^{+/+}$		
Genes*	Clone	Tumors†	Onset‡	Clone	Tumors†	Onset‡
None	MEF	0/4	NA	MEF	0/4	NA
Hygro	3	0/4	NA			
	6	0/4	NA			
1A	1	2/4	194			
	3	2/4	136			
	4	1/4	133			
	6	3/4	144			
1A/1B	2	3/4	117	4	0/4	NA
	4	2/4	117	5	2/4	125
	6	3/4	140	6	0/2	NA
1A/*ras*	6	4/4	8	1	4/4	14
	8	4/4	6	2	3/4	16
	9	3/4	8	3	4/6	21

Mice injected with cells derived from $p53^{+/+}$ or $p53^{-/-}$ MEFs were monitored for tumor growth as described. Hygro, hygromycin phosphotransferase; 1A, adenovirus E1A; 1B, adenovirus E1B; *ras*, T24 H-*ras*; NA, not applicable.
*Exogenous genes.
†Number of tumors/number of injected sties.
‡Average number of days from inoculation to tumor detection.

escape from apoptosis is not a prerequisite for transformation or tumor growth.

DISCUSSION

The present study establishes a direct mechanism of tumor suppression in which p53 participates in the destruction of aberrantly growing cells by apoptosis. We show that p53 levels and stability increase in response to E1A, p53 is required for E1A-associated apoptosis, and p53 suppresses transformation by E1A. This suggests that p53 mutations, which typically occur late in tumor progression (7), could enhance the survival of cells expressing oncogenes activated early in tumor progression. p53 loss may also increase the likelihood that cells acquire oncogenic mutations by allowing inappropriate cell proliferation after DNA damage (2, 4). In either case, p53 action is required to protect the organism from the deleterious consequences of genetic damage.

The effects of p53 deficiency on cell growth and survival were surprisingly dose dependent. Thus, $p53^{+/-}$ cells transfected with either E1A or E1A and *ras* formed almost as many colonies as the $p53^{-/-}$ cells, and $p53^{+/-}$ cells transformed by *ras* and E1A displayed an intermediate level of resistance to apoptosis. These observations imply that mutations leading to partial loss of p53 functions could allow the growth of expanded cell populations from which p53-deficient variants might arise.

Adenovirus E1A and mutant p53 alleles facilitate immortalization of primary cells (21, 22, 27). In this study, p53 deficiency had a greater effect on the growth potential of embryonic fibroblasts than any of the oncogenes tested, suggesting that p53 loss may be sufficient for immortalization. By contrast, while E1A promotes colony outgrowth, the establishment of permanent cell lines appears to require additional genetic changes (28). Our results indicate that p53 loss and escape from E1A-associated apoptosis contribute to immortalization by E1A.

Two region E1B products, $p55^{E1B}$ and $p19^{E1B}$, separately collaborate with E1A to transform cultured cells (29). We show in this study that p53 loss and E1B have equivalent effects on cell growth, survival, and transformation. Thus, the primary role of E1B is to bypass p53 stabilization, which precludes transformation by E1A alone. Two E1B-encoded proteins are involved, since $p55^{E1B}$ binds p53 and $p19^{E1B}$ prevents apoptosis upon p53 overexpression (15, 16).

Although E1A-associated apoptosis occurs during normal propagation of cells, cell death is greatly enhanced upon removal of growth factors. Similarly, *myc*-expressing fibroblasts lose viability when exposed to growth-limiting conditions (30). It has been suggested that *myc* "primes" cells for apoptosis; thus, *myc*-expressing cells are able to immediately execute the apoptotic program while normal cells are not (31). However, the ultimate fate of the cell—proliferation or apoptosis—is determined by environmental signals. We suggest that p53 stabilization is part of the mechanism whereby oncogenes prime cells for apoptosis.

Elevated p53 levels appear necessary for suppressor activity, since p53 is normally expressed at low levels without adversely affecting cell growth or survival (1). However, p53 stabilization is not sufficient for apoptosis, since exposure of normal cells to ionizing radiation also stabilizes p53 but induces growth arrest without apoptosis (4). E1A promotes proliferation despite high p53 levels, suggesting that E1A prevents p53-dependent growth arrest. Similarly, the failure of E1A-expressing cells to undergo p53-dependent growth arrest after γ-irradiation accompanies apoptosis (20). Thus, stimuli that normally limit proliferation instead induce apoptosis in cells unable to respond appropriately due to the expression of an oncogene. In this manner, p53 could function as part of a general mechanism to selectively destroy aberrantly growing cells.

The present study demonstrates that tumorigenicity is enhanced by genetic changes that promote cell survival. A similar mechanism accounts for cotransformation by *myc* and *bcl-2* (32, 33). While the involvement of p53 in *myc*-associated apoptosis has not been examined, both activation and escape from apoptosis appear to be of fundamental importance to multistep carcinogenesis and tumor progression. In contrast, cells transformed by E1A and *ras* are highly tumorigenic yet remain sensitive to apoptosis, even when passaged as tumors and placed back in culture. Therefore, escape from apoptosis is neither a prerequisite for, nor a consequence of, oncogenic transformation. This is perhaps not surprising, since apoptosis is a common feature of malignant tumors (34).

We suggest that cells can acquire tumorigenic phenotypes by various routes that alter the balance of growth, differentiation, and survival in different ways. Oncogenes such as $p19^{E1B}$ and *bcl-2* block apoptosis directly, whereas the enhanced growth rate of *ras*-transformed cells may simply compensate for cell losses due to apoptosis. *ras* cotransformation may also protect cells from environmental conditions that trigger apoptosis—for example, through the production of autocrine growth factors. In either case, tumor growth can occur while the cells remain genotypically susceptible to apoptosis. This may be a factor in limiting tumor progression and metastatic spread and, as described elsewhere, appears to modulate the cytotoxicity of anticancer agents (20).

We thank G. Paradis for expert assistance; R. Weinberg and P. Hinds for helpful discussions; M. Wigel and S. Dell for assistance with the manuscript; and M. McCurrach for expert technical assistance and helpful advice. T.J. is a Lucille P. Markey Scholar and was supported in part by a grant from the General Cinemas Charitable Foundation. This work was supported by Grants R01CA40602 (H.E.R.) and 5R27CA17575 (D.E.H.) from the National Cancer Institute, by U.S. Public Health Grant PO1-CA42063, and by Massachusetts Institute of Technology Cancer Center Support (core) Grant P30-CA14051 from the National Institutes of Health.

1. Levine, A. J., Momand, J. & Finlay, C. A. (1991) *Nature (London)* **351**, 453–456.
2. Lane, D. P. (1992) *Nature (London)* **358**, 15–16.
3. Kastan, M. B., Onyekwere, O., Sidransky, D., Vogelstein, B. & Craig, R. W. (1991) *Cancer Res.* **51**, 6304–6311.
4. Kastan, M. B., Zhan, Q., el-Deiry, W. S., Carrier, F., Jacks, T., Walsh, W. V., Plunkett, B. S., Vogelstein, B. & Fornace, A., Jr. (1992) *Cell* **71**, 587–597.
5. Lowe, S. W., Schmitt, E. M., Smith, S. W., Osborne, B. A. & Jacks, T. (1993) *Nature (London)* **362**, 847–849.
6. Clarke, A. R., Purdie, C. A., Harrison, D. J., Morris, R. G., Bird, C. C., Hooper, M. L. & Wyllie, A. H. (1993) *Nature (London)* **362**, 849–852.
7. Fearon, E. R. & Vogelstein, B. (1990) *Cell* **61**, 759–767.
8. Vogelstein, B. & Kinzler, K. W. (1992) *Cell* **70**, 523–526.
9. Hinds, P., Finlay, C. & Levine, A. J. (1989) *J. Virol.* **63**, 739–746.
10. Hicks, G. G., Egan, S. E., Greenberg, A. H. & Mowat, M. (1991) *Mol. Cell. Biol.* **11**, 1344–1352.
11. Zambetti, G. P., Olson, D., Labow, M. & Levine, A. J. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 3952–3956.
12. Ruley, H. E. (1990) *Cancer Cells* **2**, 258–268.
13. Lowe, S. W. & Ruley, H. E. (1993) *Genes Dev.* **7**, 535–545.
14. Yew, P. R. & Berk, A. J. (1992) *Nature (London)* **357**, 82–85.
15. Rao, L., Debbas, M., Sabbatini, P., Hockenbery, D., Korsmeyer, S. & White, E. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 7742–7746.
16. Debbas, M. & White, E. (1993) *Genes Dev.* **7**, 546–554.
17. Dittmer, D., Pati, S., Zambetti, G., Chu, S., Teresky, A. K., Moore, M., Finlay, C. & Levine, A. J. (1993) *Nature Genet.* **4**, 42–46.
18. Blochinger, K. & Diggelmann, H. (1984) *Mol. Cell. Biol.* **4**, 2929–2931.
19. Franza, B. R., Jr., Maruyama, K., Garrels, J. I. & Ruley, H. E. (1986) *Cell* **44**, 409–418.
20. Lowe, S. W., Ruley, H. E., Jacks, T. & Housman, D. E. (1993) *Cell* **74**, 957–967.
21. Houweling, A., van den Elsen, P. J. & van der Eb, A. J. (1980) *Virology* **105**, 537–550.
22. Jenkins, J. R., Rudge, K. & Currie, G. A. (1984) *Nature (London)* **312**, 651–654.
23. Livingstone, L. R., White, A., Sprouse, J., Livanos, E., Jacks, T. & Tlsty, T. D. (1992) *Cell* **70**, 923–935.
24. Yonish-Rouach, E., Resnitzky, D., Lotem, J., Sachs, L., Kimchi, A. & Oren, M. (1991) *Nature (London)* **352**, 345–347.
25. Wyllie, A. H. (1980) *Nature (London)* **284**, 555–556.
26. White, E., Cipriani, R., Sabbatini, P. & Denton, A. (1991) *J. Virol.* **65**, 2968–2978.
27. Harvey, D. M. & Levine, A. J. (1991) *Genes Dev.* **5**, 2375–2385.
28. Zerler, B., Moran, B., Maruyama, K., Moomaw, J., Grodzicker, T. & Ruley, H. E. (1986) *Mol. Cell. Biol.* **5**, 3084–3091.
29. Bernards, R., de Leeuw, M. G. W., Houweling, A. & van der Eb, A. J. (1986) *Virology* **150**, 126–139.
30. Evan, G. I., Wyllie, A. H., Gilbert, C. S., Littlewood, T. D., Land, H., Brooks, M., Waters, C., Penn, L. Z. & Hancock, D. C. (1992) *Cell* **69**, 119–128.
31. Wyllie, A. H. (1993) *Br. J. Cancer* **67**, 205–208.
32. Bissonnette, R. P., Echeverri, F., Mahboubi, A. & Green, D. R. (1992) *Nature (London)* **359**, 552–554.
33. Fanidi, A., Harrington, E. A. & Evan, G. I. (1992) *Nature (London)* **359**, 554–556.
34. Wyllie, A. H. (1985) *Anticancer Res.* **5**, 131–136.