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Selective induction of p53 and chemosensitivity in RB-deficient cells by E1A mutants unable to bind the RB-related proteins

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ABSTRACT **The adenovirus E1A oncoprotein renders primary cells sensitive to the induction of apoptosis by diverse stimuli, including many anticancer agents. E1A-expressing cells accumulate p53 protein, and p53 potentiates drug-induced apoptosis. To determine how E1A promotes chemosensitivity, a series of E1A mutants were introduced into primary human and mouse fibroblasts using high-titer recombinant retroviruses, allowing analysis of E1A in genetically normal cells outside the context of adenovirus infection. Mutations that disrupted apoptosis and chemosensitivity separated into two complementation groups, which correlated precisely with the ability of E1A to associate with either the p300/CBP or retinoblastoma protein families. Furthermore, E1A mutants incapable of binding RB, p107, and p130 conferred chemosensitivity to fibroblasts derived from *RB*-deficient mice, but not fibroblasts from mice lacking p107 or p130. Hence, inactivation of RB, but not p107 or p130, is required for chemosensitivity induced by E1A. Finally, the same E1A functions that promote drug-induced apoptosis also induce p53. Together, these data demonstrate that p53 accumulation and chemosensitivity are linked to E1A's oncogenic potential, and identify a strategy to selectively induce apoptosis in RB-deficient tumor cells.**

Despite the widespread use of cytotoxic agents to treat cancer, the molecular mechanisms underlying drug sensitivity and resistance remain poorly understood. Most anticancer agents induce apoptosis, suggesting that tumor-cell chemosensitivity is influenced by the efficiency with which anticancer agents activate apoptotic programs (1, 2). This hypothesis implies that responsive tumors must be more susceptible to apoptosis than normal tissue, and that resistant tumors are unable to efficiently engage apoptotic programs. Tumorigenic mutations can have different effects on apoptosis. For example, activation of the c-*myc* oncogene enhances apoptosis (3) whereas inactivation of the *p53* tumor suppressor gene suppresses cell death (reviewed in ref. 4). These diverse effects suggest that tumor-cell chemosensitivity is determined, in part, by the combined effects of oncogenic mutations on apoptosis (1, 5).

Given the varied impact of oncogenic mutations on apoptosis, it is difficult to study the molecular determinants of chemosensitivity in the unknown genetic background of tumor cells. However, cells expressing the adenovirus early region 1A (*E1A*) oncogene provide a simple model for studying cellular processes that modulate chemosensitivity. E1A promotes apoptosis in nontumorigenic cells (6). As a consequence, E1A-expressing cells become extremely sensitive to toxic agents and readily undergo apoptosis following treatment with anticancer agents (1, 7).

E1A can impinge on a variety of other cellular processes, including transcription, differentiation, and tumor necrosis factor cytolysis (reviewed in ref. 8). During adenovirus infection, E1A makes quiescent cells permissive for virus replication by promoting S phase entry (8). Consequently, E1A has oncogenic potential: E1A facilitates the immortalization of primary rodent cells and cooperates with viral (e.g., *E1B*) or cellular (e.g., oncogenic *ras*) genes to transform primary cells to a tumorigenic state (9). E1B prevents the apoptosis associated with E1A (6), whereas E1A prevents a senescent-like cell cycle arrest provoked by oncogenic Ras (10). Consequently, these transforming interactions illustrate the compensatory mechanisms normal cells possess to suppress transformation (reviewed in ref. 11).

The *E1A* gene expresses several alternatively spliced transcripts, including the 12S and 13S messages encoding 243 (243R) and 289 (289R) amino acid oncoproteins, respectively (reviewed in ref. 12). The 289R protein contains three regions that are conserved between different adenovirus serotypes, designated conserved regions 1, 2, and 3 (CR1, CR2, CR3). CR3 encodes a domain required for transcriptional activation of other viral genes and is absent in the 243R protein, whereas CR1 and CR2 are present in both E1A proteins and are essential for many E1A activities, including oncogenic transformation (13, 14).

E1A 243R associates with a series of cellular proteins, including the retinoblastoma gene product (RB), the RB-related proteins p107 and p130, the p300 and CREB binding protein (CBP) transcriptional coactivators, cyclin A, and certain cyclin-dependent kinases (cdk) (refs. 13 and 14; reviewed in refs. 15 and 16). Because most of these interactions also require residues in CR1 and CR2, the ability of E1A to disrupt the function of these proteins may be crucial for its transforming activities. For example, E1A associates with RB (14, 17) and mutations in either CR1 or CR2 that disrupt this interaction also abolish oncogenicity (13). By binding RB, E1A disrupts RB-E2F heterodimers, thereby relieving repression and promoting transactivation of S phase genes (reviewed in refs. 15 and 16). Mutational inactivation of *RB* achieves a similar effect; consequently, E1A mimics mutational events that occur in familial retinoblastoma and many sporadic tumors (reviewed in ref. 18).

In adenovirus-infected cells, E1A expression appears sufficient for apoptosis (19–21). However, cells tolerate ectopic E1A expression but become extremely prone to apoptosis (1, 7). E1A-expressing cells accumulate p53 protein, and both p53 and Bax—a pro-apoptotic member of the Bcl-2 family—contribute to apoptosis in this setting (1, 7, 22–25). p53 and Bax are inefficient at inducing apoptosis in normal cells lacking E1A (7); indeed, p53 functions to promote cell-cycle arrest (26). Furthermore, E1A-expressing cells possess a discrete factor, absent in normal cells, that is capable of activating the

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Abbreviations: CBP, CREB-binding protein; RB, retinoblastoma; CR1, -2, -3, conserved regions 1, 2, and 3; MEF, mouse embryonic fibroblast; puro, puromycin; hygro, hygromycin.
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apoptotic machinery in a cell-free system (27). Hence, E1A enables cells to more efficiently engage the apoptotic machinery.

In this study, we examined how E1A promotes p53 accumulation and chemosensitivity. To this end, we stably expressed E1A or a series of E1A mutants in primary human and mouse fibroblasts using high-titer recombinant retroviral vectors. Using this approach, we genetically defined multiple E1A activities that act in concert to promote p53 accumulation and chemosensitivity and demonstrate that one of these functions involves inactivation of the *RB* gene product.

MATERIALS AND METHODS

Cells and Cell Culture. Mouse embryonic fibroblasts (MEFs) were isolated as described (10). Cells were maintained in DMEM (GIBCO) supplemented with 10% fetal bovine serum and 1% penicillin-G/streptomycin sulfate (Sigma). $RB^{-/-}$ MEFs were obtained from T. Jacks (28), $p107^{-/-}$ and $p130^{-/-}$ MEFs were from N. Dyson (29, 30). IMR90 cells overexpressed the murine ecotropic receptor, allowing infection with ecotropic retroviruses (10). MEFs were used between passages three and six, IMR90 cells between 20–30 population doublings.

E1A Mutants, Retroviral Vectors, and Infections. The 12S E1A cDNA and 12S E1A deletion or point mutants (31, 32) were subcloned into pLPC (10) or pWZLHygro (unpublished data; J. P. Morgenstern, M. J. Zoller, and J. S. Brugge, Ariad Pharmaceuticals, Cambridge, MA). pLPC-12S coexpresses an E1A 12S cDNA with puromycin phosphotransferase (*puro*) and pWZL-12S coexpresses E1A with hygromycin phosphotransferase (*hygro*). The E1A mutant constructs used in this study were as follows: pLPC 12S.ΔN, pLPC 12S.ΔCR1, pLPC 12S.ΔCR2, pLPC 12S.pm47/124, pWZL 12S.ΔN, pWZL 12S.ΔCR1, and pWZL 12S.ΔCR2.

Ecotropic retroviruses were produced using the Phoenix packaging line (provided by G. Nolan, Stanford University) according to a previously described procedure (10). Cells were placed into medium containing 2.5 μg/ml puromycin (Sigma) or 100 μg/ml hygromycin B (Boehringer Mannheim) to eliminate uninfected cells. When two separate E1A mutants were coexpressed, they were introduced sequentially, the first using LPC and the second using WZLHygro, with drug selection for 2–3 days after each infection.

Cell Viability. Cells (1×10^5) were plated into 12-well plates 24 h before treatment. Twenty-four hours following treatment with adriamycin, or 48 h after serum withdrawal, adherent and nonadherent cells were pooled and analyzed for viability by trypan blue exclusion. At least 200 cells were counted for each point. Null mutant fibroblasts were compared with cells derived from wild-type littermate controls.

Protein Expression. Proteins were extracted in Nonidet P-40 lysis buffer [150 mM NaCl/1% Nonidet P-40/50 mM Tris·HCl, pH 7.5/1 mM phenylmethylsulfonyl fluoride/1 mM EDTA/2 μg/ml CLAP (chymostatin, leupeptin, antipain, and pepstatin)] for 1 h on ice with frequent vortex mixing. Lysates were normalized by Bradford method (Bio-Rad), and 20 μg (for p53) or 10 μg (for E1A) of total protein was loaded in each lane. After electrophoresis, proteins were transferred to Immobilon-P membranes (Millipore) using standard "wet" transfer procedures. E1A was detected using either the M58 or M73 (1:100 dilution) mAbs (33), the latter recognizes an epitope retained in all E1A mutants studied (34). The CM1 and CM5 polyclonal antibodies were used (1:1,000) to detect human and mouse p53, respectively (Novocastra, Newcastle, U.K.). Proteins were visualized by ECL (Amersham), and equal sample loading was confirmed by India Ink.

RESULTS

To determine how E1A promotes chemosensitivity, we began a structure–function analysis to identify the regions of E1A required for this effect. A series of recombinant retrovirus vectors coexpressing various E1A mutants (Fig. 1*A*) with either *puro* or *hygro* were constructed. Earlier studies demonstrated that the 243-amino acid protein encoded by the E1A 12S cDNA was sufficient for apoptosis and chemosensitivity (7, 22); hence, all mutants were derived from an E1A 12S cDNA (31, 32). These mutants were chosen because they are compromised in their ability to physically associate with either the p300/CBP (ΔN and ΔCR1) or RB/p107/p130 (pm47/124 and ΔCR2) family of cellular proteins (Fig. 1*A*) (31).

High-titer ecotropic retroviruses were generated using a transient retrovirus packaging system (35). Virus supernatants were used to infect either normal diploid IMR90 human lung fibroblasts or primary MEFs, and pure populations of E1A-expressing cells were isolated by brief selection in the presence of puromycin or hygromycin B. All E1A mutant proteins were efficiently expressed (Fig. 1*B*). Using this approach, we were able to stably express E1A in primary cell populations in the absence of additional adenoviral proteins—i.e., in a genetically normal background.

Multiple E1A Regions Are Required for Apoptosis and Chemosensitivity. Full-length E1A rendered both human and mouse fibroblasts sensitive to the induction of apoptosis by a variety of agents (Fig. 2; data not shown). As expected, mouse cells expressing E1A lost viability in a dose-dependent manner

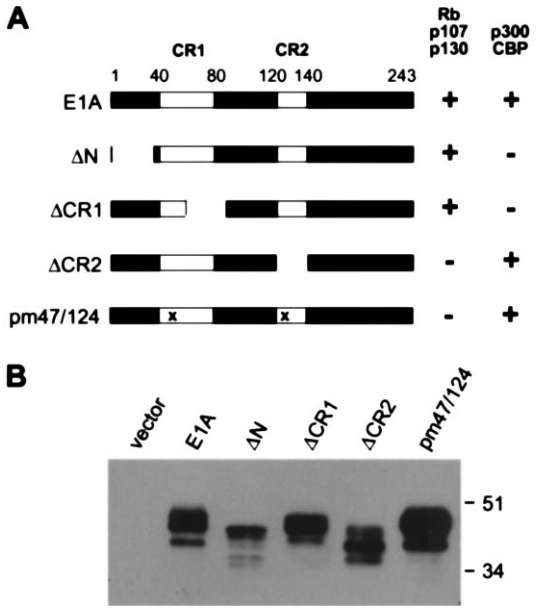

FIG. 1. Structure and expression of E1A mutants. (*A*) The E1A 243R contains two conserved regions (CR1 and CR2, white boxes). In the E1A mutants, deletions are indicated by gaps, and point mutations by an "x." ΔN, ΔCR1, and ΔCR2 are deletions of amino acids 2–36, 68–85, and 120–140, respectively. pm47/124 mutant has a tyrosine to histidine and cysteine to glycine changes at amino acids 47 and 124, respectively. Cellular proteins able to interact with each E1A mutant in coimmunoprecipitations are indicated (31). (*B*) E1A was introduced into IMR90 cells using LPC-based retroviral vectors. After selection in puromycin, E1A levels were determined by Western blot analysis. E1A is highly phosphorylated and undergoes posttranslational modification, which accounts for variable migration in SDS gels (33). Note that the ΔN mutant was able to coimmunoprecipitate similar levels of RB as wild-type E1A (data not shown). Infection efficiencies were >50% before selection and >95% of the cells in the selected cells expressed E1A as determined by immunofluorescence (data not shown). Each E1A mutant localized to the nucleus (data not shown).

following adriamycin treatment or serum withdrawal (Fig. 2 *B* and *C*). Under these conditions cell death is largely p53-dependent, because p53-deficient MEFs expressing E1A remained viable (data not shown; see also refs. 1 and 7). Human cells also lost viability following adriamycin treatment (Fig. 2*A*), but not after serum withdrawal (data not shown). In both cell types, the dying cells displayed features of apoptosis (1, 7). Fibroblasts infected with an empty vector did not undergo apoptosis after either treatment (Fig. 2).

All of the E1A mutants were defective in promoting chemosensitivity in both human and mouse fibroblasts (Fig. 2). IMR90 cells expressing ΔN, pm24/147, or ΔCR2 were completely insensitive to adriamycin treatment (Fig. 2*A*). Although IMR90 cells expressing the ΔCR1 mutant lost viability in a dose-dependent manner, cell death was substantially reduced compared with full-length E1A (35% vs. 11% viable at 0.5 μg/ml, respectively) (Fig. 2*A*). Like IMR90 cells, MEFs expressing ΔN or ΔCR1 remained completely or partially insensitive to adriamycin treatment, respectively (Fig. 2*B*). By contrast, MEFs expressing either the pm47/124 or ΔCR2 mutants displayed modest levels of cell death, but only at the higher doses (Fig. 2*B*). MEFs expressing each E1A mutant were also defective in apoptosis following serum withdrawal, a treatment not known to produce cellular damage (Fig. 2*C*). The behavior of each E1A mutant was independent of the apoptotic stimulus, because similar results were obtained following treatment of human and mouse cells with etoposide, cisplatin, 5-fluorouracil, or γ-radiation (data not shown). Therefore, multiple regions of E1A are required for apoptosis following treatment with diverse agents.

Functionally Distinct Regions of E1A Cooperate to Confer Chemosensitivity. Each E1A mutant defective in apoptosis is also impaired for binding either the p300/CBP or RB-related proteins (see Fig. 1) (31), raising the possibility that these processes are related. However, the observations are correlative, and it is also possible that these mutations affect one or more unknown E1A activities. To establish whether multiple E1A functions contribute to apoptosis, combinations of E1A mutants were expressed in a trans complementation assay. If two E1A mutants were defective because they lacked the same function(s), they would be unable to function in trans to confer chemosensitivity. Conversely, if two mutants were defective owing to loss of *separate* functions, then coexpressing these mutants might restore chemosensitivity. Therefore, E1A mutants were introduced sequentially into IMR90s and MEFs using retroviruses coexpressing different selectable markers (*puro* and *hygro*).

In both human and mouse fibroblasts, E1A mutants that bound different classes of cellular proteins acted in trans to restore chemosensitivity, whereas those that bound the same class did not (Fig. 3). For example, although cells expressing either the ΔN or ΔCR2 mutant alone were insensitive to adriamycin-induced apoptosis, the levels of apoptosis in cells coexpressing these mutants approached those observed in cells expressing full-length E1A (Fig. 3 *A* and *B*). Similar results were observed when cells were treated with other anticancer agents or following serum withdrawal (data not shown). Likewise, cells coexpressing the ΔCR1 and ΔCR2 mutants were as sensitive to adriamycin-induced apoptosis as cells expressing full-length E1A (Fig. 3 *C* and *D*). No increase in chemosensitivity was observed when cells were infected sequentially with the same E1A mutant (e.g., ΔN or ΔCR1) compared with cells infected only once (data not shown). This finding indicates that the cooperativity between ΔN or ΔCR1 with ΔCR2 did not result from increased gene dosage, but rather was due to synergy between separate E1A functions. Thus, multiple E1A activities contribute to chemosensitivity.

In contrast, the ΔN and ΔCR1 mutants failed to restore chemosensitivity when expressed in trans: cells coexpressing ΔN and ΔCR1 behaved identically to cells expressing the partially defective ΔCR1 mutant alone (Fig. 3 *E* and *F*). As discussed above, both ΔN and ΔCR1 restored chemosensitivity when coexpressed with ΔCR2, implying that the ΔN and ΔCR1 mutations did not produce global aberrations in E1A structure, but rather, disrupted the same function(s). The fact that two E1A mutants that fail to bind p300/CBP (see Fig. 1) (31) are defective for apoptosis because they affect overlapping functions suggests that binding of one or more of these proteins is required for chemosensitivity.

Role of CR2 in Chemosensitivity. CR2 is required for the physical association between E1A and the RB-related proteins (14). In principle, CR2 could contribute to chemosensitivity by inactivating one or more of these proteins or by affecting some other cellular activity. If CR2 promotes chemosensitivity by inactivating a single RB-related protein, then the ΔCR2 mutant should behave like full-length E1A in cells lacking this crucial target. Because all of the *RB*-related genes have been

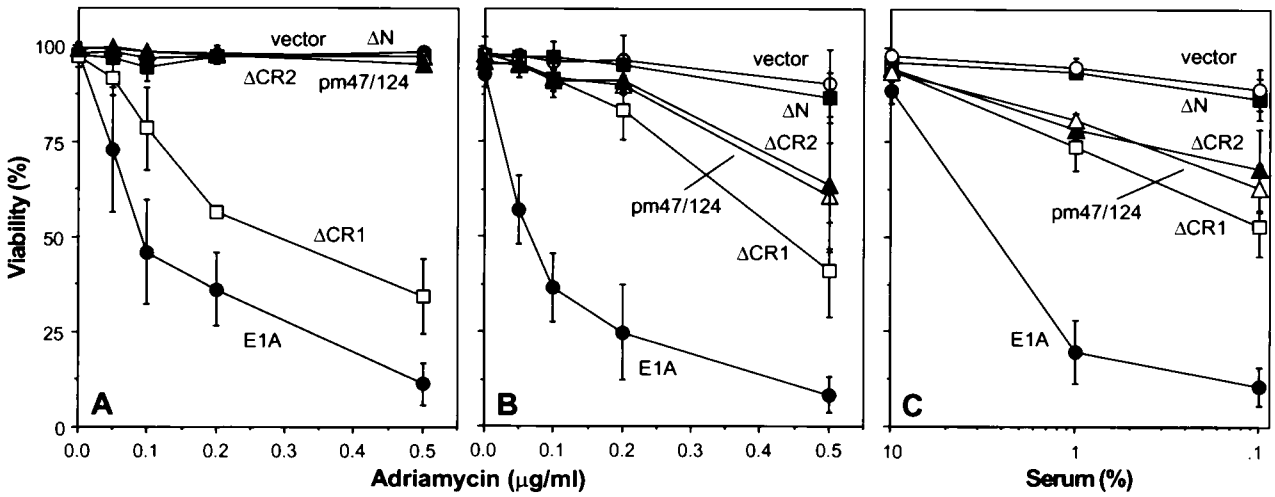

FIG. 2. Multiple regions of E1A are required for chemosensitivity. Primary mouse (MEF) (*A* and *C*) or human (IMR90) (*B*) fibroblasts were infected with an empty vector (vector, ○), vectors expressing full-length E1A (●), or the following mutants: ΔN (■), ΔCR1 (□), ΔCR2 (▲), and pm47/pm124 (△). Infected populations were plated in multiwell dishes and treated with the indicated concentrations of adriamycin (*A* and *B*) or serum (*C*). Cell viability was determined 24 h following adriamycin treatment or 48 h after serum withdrawal. Previous studies demonstrated that cell death under these conditions results from apoptosis (1, 7), and this was confirmed by visualizing chromatin condensation using 4′,6-diamidino-2-phenylindole (data not shown). Each value represents the mean ± SD from at least three separate experiments.

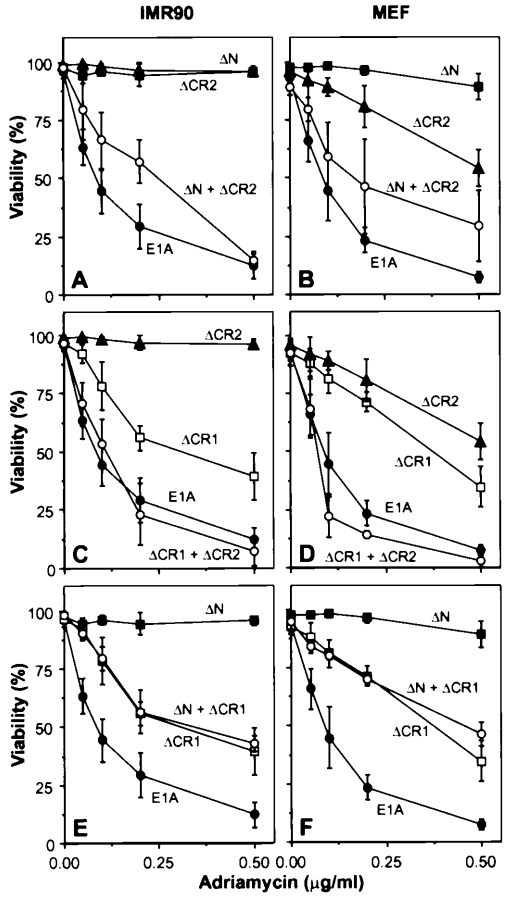

FIG. 3. Separate E1A functions cooperate to confer chemosensitivity. IMR90 or MEF cell populations expressing E1A (●), ΔN (■), ΔCR1 (□), ΔCR2 (▲), ΔN and ΔCR2 (○ in *A* and *B*), ΔCR1 and ΔCR2 (○ in *C* and *D*), or ΔN and ΔCR1 (○ in *E* and *F*) were generated by retroviral infection. Multiple E1A mutants were introduced sequentially as described in *Materials and Methods*. Cell populations were treated with adriamycin and viability was determined 24 h later by trypan blue exclusion. Each value represents the mean ± SD of the data from at least three separate experiments.

disrupted in mice and all are expressed in MEFs (28–30, 36), this hypothesis could be tested definitively.

E1A and the ΔCR2 mutant were introduced into wild-type, $RB^{-/-}$, $p107^{-/-}$, or $p130^{-/-}$ MEFs, and the resulting populations were treated with apoptosis-inducing stimuli (Fig. 4). Adriamycin treatment induced similar levels of apoptosis in cells expressing full-length E1A, irrespective of their genotype. Thus, as expected, loss of the RB-related proteins does not impair apoptosis. Furthermore, MEFs infected with the empty vector were insensitive to adriamycin treatment, demonstrating that loss of either RB, p107, or p130 was not sufficient to produce chemosensitivity (data not shown).

Concordant with previous results, wild-type MEFs expressing ΔCR2 are relatively insensitive to adriamycin treatment (Fig. 4 *Upper Left*). Likewise, $p107^{-/-}$ and $p130^{-/-}$ cells expressing ΔCR2 remained insensitive to adriamycin treatment. By contrast, $RB^{-/-}$ cells expressing ΔCR2 (Fig. 4) or pm47/124 (data not shown) were as sensitive to adriamycin-induced apoptosis as cells expressing full-length E1A. This synergy was specific for ΔCR2 and pm47/124, because the ΔN mutant remained defective in all cell types (Fig. 4 *Lower*). Thus, inactivation of RB—but not p107 or p130—is the critical function of CR2 important for apoptosis. Furthermore, E1A mutants unable to bind RB are defective in normal cells but promote apoptosis in cells with mutant *RB* genes.

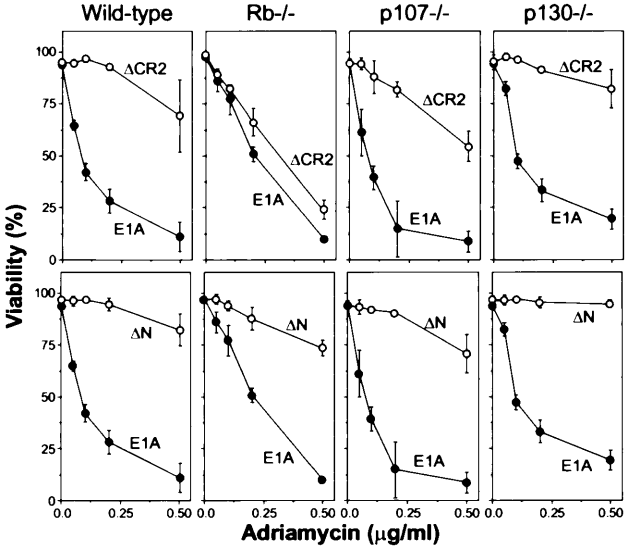

FIG. 4. Inactivation of RB by CR2 is required for chemosensitivity. Wild-type, $RB^{-/-}$, $p107^{-/-}$, and $p130^{-/-}$ MEFs expressing either E1A (●), ΔCR2 (○, *Upper*), or ΔN (○, *Lower*) were generated by retroviral infection. E1A and E1A mutants were expressed at similar levels (data not shown). Cell viability was determined 24 h after adriamycin treatment. Each point represents the mean ± SD from at least three separate experiments.

p53 Accumulation and Chemosensitivity Involve the Same E1A Functions. Cells expressing E1A accumulate p53 protein due, in part, to increased p53 stability (22). To determine whether p53 accumulation and chemosensitivity involve the same E1A functions, we examined the ability of each E1A mutant to induce p53. Cells expressing full-length E1A displayed a 20- to 30-fold increase in steady-state p53 protein levels (Fig. 5*A*). The ΔN and ΔCR2 mutants produced only a slight increase in p53 levels in IMR90 cells, and no increase in MEFs. However, coexpression of both mutants induced p53 to levels observed in cells expressing full-length E1A (Fig. 5*A*). Remarkably, ΔCR2 induced p53 when expressed in $RB^{-/-}$ MEFs (30-fold increase), but not in $p107^{-/-}$ or $p130^{-/-}$ MEFs (Fig. 5*B*). $RB^{-/-}$ cells infected with the empty vector displayed no increase in p53 levels (data not shown). Thus, the same E1A functions that promote apoptosis and chemosensitivity also induce p53.

DISCUSSION

Despite the widespread use of cytotoxic agents to treat cancer, molecular factors that influence tumor-cell chemosensitivity remain largely unknown. The E1A oncoprotein displays a remarkable ability to enhance chemosensitivity, and acts to promote drug-induced apoptosis (1, 7, 37). In this study, we demonstrate that at least two independent E1A functions act in concert to promote apoptosis and chemosensitivity, and that one function involves inactivation of the retinoblastoma gene product. Of note, the regions of E1A that promote apoptosis are similar, if not identical, to those previously shown to facilitate oncogenic transformation (13, 14). Consequently, our results underscore the association between factors that influence tumorigenesis and tumor-cell chemosensitivity.

In the context of adenovirus infection, the E1A regions involved in binding the p300/CBP and RB-related proteins have been associated with apoptosis. However, depending on the setting, only the p300/CBP binding region, either the p300/CBP or RB-related protein binding region, or both regions were required (38–40); hence, the results are contradictory. Moreover, in one study, p53 accumulation did not correlate with apoptosis (39). Another study examined the

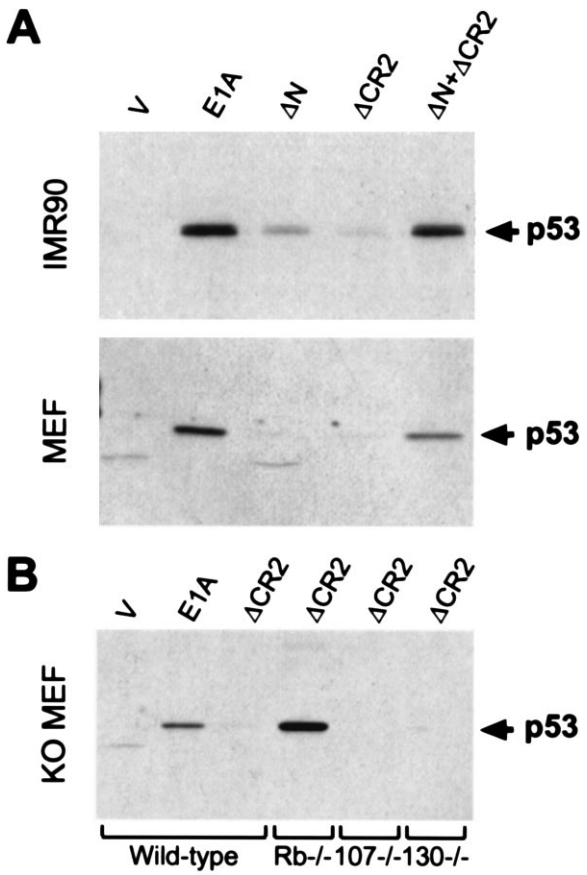

FIG. 5. E1A and p53 accumulation. (*A*) p53 expression in populations of IMR90s or MEFs expressing the empty vector (V), full-length E1A, ΔN, ΔCR2, or coexpressing ΔN and ΔCR2 was examined by immunoblotting using polyclonal antibodies specific for human or mouse p53, respectively. (*B*) p53 expression in wild-type, $RB^{-/-}$, $p107^{-/-}$, or $p130^{-/-}$ MEF populations expressing the indicated E1A proteins. No increase in p53 was observed in $RB^{-/-}$, $p107^{-/-}$, or $p130^{-/-}$ MEFs without E1A, and E1A induced p53 in MEFs of all three genotypes (data not shown.)

relationship between E1A and chemosensitivity in a tumorigenic cell line. Here, the regions of E1A capable of conferring chemosensitivity varied with the agent tested (41). However, even full-length E1A did not substantially enhance apoptosis in these cells, and no correlation between p53 accumulation and chemosensitivity was observed. Furthermore, this study did not support an absolute requirement for RB inactivation for chemosensitivity. All previous studies have been correlative: none provides direct evidence that the cellular proteins targeted by E1A participate in apoptosis.

The studies described above examined E1A in immortal or tumor-derived lines, often in the context of adenovirus infection. However, E1A is a transforming oncogene that itself facilitates immortalization (9); hence, immortal or tumor-derived cells may already have alterations in processes affected by E1A. This may explain why the RB-binding domain of E1A was dispensable for apoptosis in HeLa cells (38, 39)—these tumor cells express papillomavirus E7, an oncoprotein that inactivates RB. Likewise, adenovirus contains several genes that affect apoptosis in addition to EIA (6, 42) and can induce apoptosis in the absence of E1A (39). In this study, retrovirus-mediated gene transfer was used to introduce E1A into whole populations of primary cells, allowing E1A to be studied in the absence of other adenoviral genes and unknown host-cell mutations.

All E1A mutants tested showed marked reduction in apoptotic potential in both primary human and mouse fibroblasts, and the requirement for each E1A region was independent of the apoptotic stimulus. These regions correlated precisely with the ability of E1A to associate with the p300/CBP or RB-related proteins. Coexpression of E1A mutants binding separate classes of cellular proteins functioned in trans to confer chemosensitivity, whereas coexpression of mutants binding the same cellular proteins did not. Thus, this study genetically defines at least two E1A functions that act in concert to promote apoptosis and chemosensitivity.

As has been observed during adenovirus-induced apoptosis (38–40), our results provide genetic evidence that E1A's interaction with the p300/CBP proteins is critical for chemosensitivity. Here, we used a genetic complementation test to demonstrate that two spatially separate E1A mutations, both known to disrupt p300/CBP binding (ΔN and ΔCR1), affect the same E1A function(s) involved in chemosensitivity. Whereas ΔCR1 is unable to associate with p300/CBP in immunoprecipitations, it retains some capacity to affect p300/CBP functions in cells (32, 43). By contrast, the ΔN mutant is completely defective in p300/CBP interaction using both immunoprecipitations and functional assays. Perhaps this explains why the ΔN and ΔCR1 mutants displayed a complete and partial defect in apoptosis, respectively (see Fig. 3). p300 and CBP are both transcriptional coactivators and histone acetyltransferases (ref. 44; reviewed in ref. 45), and E1A binding to p300 produces global changes in transcription (reviewed in ref. 16). Recent studies suggest that p300 and CBP physically associate with p53 and contribute to p53's transcriptional activity, raising the possibility that E1A binding to p300 modulates p53 function to promote apoptosis (46–48). Alternatively, the critical target may not be p300/CBP itself, but another molecule displaced or altered by the E1A–p300/CBP interaction.

In addition to the apparent p300/CBP binding activity, a second E1A function is required for apoptosis and chemosensitivity. Using primary fibroblasts derived from $RB^{-/-}$, $p107^{-/-}$, or $p130^{-/-}$ mice, we conclusively demonstrate that this function involves inactivation of RB, but not p107 or p130. Interestingly, inactivating mutations in the *RB* gene occur in many human cancers; by contrast, mutations in p107 or p130 have not been observed (18). The fact that E1A promotes chemosensitivity by inactivating a tumor suppressor underscores the utility of viral oncogenes to identify processes relevant to human cancer. Furthermore, the critical role of RB inactivation for apoptosis reiterates the fundamental relationship between tumorigenesis and chemosensitivity.

How RB inactivation contributes to apoptosis and chemosensitivity remains to be determined. *RB*-deficient mice display elevated apoptosis in the embryonic lens, fetal liver, and the developing nervous system, implying RB inactivation alone can promote apoptosis in some settings (49, 50). Furthermore, overexpression of RB in HeLa cells can suppress cell death (51). The interaction between E1A and RB releases E2F transcription factors; similarly, overexpression of E2F-1 overcomes RB binding and induces apoptosis in a p53-dependent manner (52). This finding suggests that one or more E2Fs might mediate this aspect of E1A function. However, E2F-$1^{-/-}$ MEFs expressing E1A display no defects in apoptosis (L. Yamasaki, A.V.S., and S.W.L., unpublished data), indicating that E2F-1 is dispensable for this effect.

We have previously shown that p53 protein accumulates in cells expressing E1A, which correlates with the involvement of p53 in apoptosis (22, 23). Here we demonstrate that the same E1A functions that promote apoptosis and chemosensitivity also induce p53 (see also ref. 38). These regions are also required for E1A's transforming activities (13, 14), implying that p53 accumulation, chemosensitivity, and oncogenic potential arise from the same E1A functions. This suggests that p53 accumulation is a cellular response to oncogenic "stress" rather than a direct effect of E1A on p53. Interestingly, extracts

from E1A-expressing cells possess a discrete factor that reproduces some of the pro-apoptotic activities of E1A in cell-free systems (27). The nature of this factor may shed light on the links between p53, chemosensitivity, and cell-cycle control.

The *RB* gene is mutated in many human cancers, and the RB pathway is disrupted in the vast majority of cancer cells (reviewed in ref. 18). Our results suggest a strategy to specifically kill cancer cells with defective RB function. In normal cells, at least two processes affected by E1A are necessary to promote chemosensitivity—RB inactivation and apparently disruption of some p300/CBP function. The RB-inactivating function of E1A is dispensable for chemosensitivity in RB-deficient cells, consequently such E1A mutants, or small molecules that mimic their action, might synergize with standard chemotherapeutic agents to specifically induce apoptosis in *RB* mutant tumor cells. Although p53 potentiates apoptosis under the conditions used in this study, E1A can promote chemosensitivity in p53-deficient cells (refs. 1 and 7, and unpublished results). Consequently, this therapeutic approach may not strictly depend on the presence of wild-type p53. Experiments to test this strategy are underway.

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