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Bcl-2 Blocks p53-Dependent Apoptosis

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Adenovirus E1A expression recruits primary rodent cells into proliferation but fails to transform them because of the induction of programmed cell death (apoptosis). The adenovirus E1B 19,000-molecular-weight protein (19K protein), the E1B 55K protein, and the human Bcl-2 protein each cause high-frequency transformation when coexpressed with E1A by inhibiting apoptosis. Thus, transformation of primary rodent cells by E1A requires deregulation of cell growth to be coupled to suppression of apoptosis. The product of the p53 tumor suppressor gene induces apoptosis in transformed cells and is required for induction of apoptosis by E1A. The ability of Bcl-2 to suppress apoptosis induced by E1A suggested that Bcl-2 may function by inhibition of p53. Rodent cells transformed with E1A plus the p53(Val-135) temperature-sensitive mutant are transformed at the restrictive temperature and undergo rapid and complete apoptosis at the permissive temperature when p53 adopts the wild-type conformation. Human Bcl-2 expression completely prevented p53-mediated apoptosis at the permissive temperature and caused cells to remain in a predominantly growth-arrested state. Growth arrest was leaky, occurred at multiple points in the cell cycle, and was reversible. Bcl-2 did not affect the ability of p53 to localize to the nucleus, nor were the levels of the p53 protein altered. Thus, Bcl-2 diverts the activity of p53 from induction of apoptosis to induction of growth arrest, and it is thereby identified as a modifier of p53 function. The ability of Bcl-2 to bypass induction of apoptosis by p53 may contribute to its oncogenic and antiapoptotic activity.

Apoptosis is a fundamentally important part of normal development (24); it is a defense against cancer (39), and it modulates viral pathogenesis and latency (10, 16, 35). The regulation of apoptosis is poorly understood, although the expression of agents which deregulate cell growth such as E1A (25, 37) and c-*myc* (7, 41) initiates the apoptotic response. Negative growth signals such as growth factor withdrawal (24) and accumulation of p53 protein levels (17) are similarly associated with apoptosis, which may arise from incompatible or conflicting growth signals (4, 43). These conflicting signals are likely to arise during the transformation process; this has been confirmed with the identification of two oncogenes, *bcl-2* (12) and the adenovirus E1B 19,000-molecular-weight-protein (19K-protein) (4, 25, 37), which function as inhibitors of apoptosis. In transformation assays E1B cooperates with E1A (33) and *bcl-2* cooperates with both E1A (25) and c-*myc* (2, 8, 31). Although the mechanism by which c-*myc* activates, or *bcl-2* inhibits, apoptosis is not known, induction of apoptosis by E1A is associated with deregulation of cell growth control (34) and is mediated by wild-type p53 (4). Expression of either dominant-interfering mutant alleles of p53 or the E1B gene, which encodes two redundant, overlapping functions which inhibit p53, blocks apoptosis by E1A (4). The E1B 55K protein binds p53 and directly inhibits its function (27, 42), whereas the E1B 19K protein affects p53 by an indirect mechanism (4).

The ability of *bcl-2* to suppress apoptosis and cooperate with E1A in a transformation assay, and the dependence of E1A-mediated apoptosis on p53, suggested that *bcl-2* could block p53-dependent apoptosis. The human Bcl-2 protein was expressed in cells transformed with E1A plus temperature-sensitive p53(Val-135), which undergo apoptosis when p53 assumes the wild-type conformation. Bcl-2 completely prevented the induction of apoptosis by wild-type p53 and diverted cells into a predominantly growth-arrested state without affecting either the levels or localization of the p53 protein. Thus, Bcl-2 has been identified as a modifier of p53 function, and this may be an important aspect of its role in oncogenesis.

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MATERIALS AND METHODS

Plasmids and tissue culture. The p53A cell line (4) was derived by transfection of primary baby Fisher rat kidney cells with a cytomegalovirus promoter construct to express E1A (pCMVE1A) (34) and with plasmid pLTRcGval135 (22) to express murine mutant p53. The p53(Val-135) protein is temperature sensitive, and it exists predominantly in the mutant conformation at 37.5 to 38.5°C and predominantly in the wild-type conformation at 32°C (9, 20, 22). Continuous propagation of p53A and derivatives containing the p53(Val-135) protein was carried out at 38.5°C. Cell lines were maintained in Dulbecco modified Eagle medium with 10% fetal bovine serum.

Transfections, antibodies, and Western blotting (immunoblotting). The E1A-plus-p53(Val-135)-transformed BRK line p53A was transfected by electroporation with a neomycin resistance marker only (pSV2neo) or with the human Bcl-2 expression vector pSFFV*bcl-2* containing a neomycin resistance marker (12). Transformants were screened for Bcl-2 expression by Western blotting (by enhanced chemiluminescence) with a monoclonal antibody directed against human Bcl-2 (12). Three independent p53A clones that expressed Bcl-2 (3B, 4B, and 1A) were identified in parallel with two independent control p53A derivatives that were only neomycin resistant (neo4 and neo5) and were maintained at the restrictive temperature.

Monoclonal antibodies directed against murine p53 (pAb248 and pAb2C2) were generously provided by Arnold J. Levine (Princeton University, Princeton, N.J.). The E1A-specific monoclonal antibody M73 was generously provided by

Ed Harlow (Massachusetts General Hospital, Charlestown). Cell extracts for Western analysis were prepared from subconfluent cultures, and 20 μg of protein from each cell line was analyzed by polyacrylamide gel electrophoresis and semidry blotting onto nitrocellulose membranes by standard procedures. Following antibody incubations, immune complexes were detected by enhanced chemiluminescence according to the manufacturer's specifications (Amersham). The E1A-plus-E1B-transformed BRK cell line 4P (33) was utilized as a reference for E1A expression levels.

Viability and DNA fragmentation analysis. Transformed BRK cell lines were plated at a density of 5×10^5 cells per 6-cm plate at 38.5°C. At 40 h postplating, when the cells were completely attached to the substrate, the cells were trypsinized and the viable cell number per plate was determined by trypan blue exclusion. The remaining plates were shifted to 32°C, and the viable cell number was determined following incubation for increasing lengths of time. For DNA fragmentation assays, apoptotic cells were harvested from the culture medium of transformed BRK cell lines by low-speed centrifugation, and low-molecular-weight DNA was extracted by a modified Hirt procedure which permits the selective isolation of low-molecular-weight, degraded DNA from high-molecular-weight intact chromosomal DNA (11, 36). Low-molecular-weight Hirt supernatant fractions from BRK lines were equalized with respect to the original viable cell number at the time of the shift to 32°C. Hirt DNA was analyzed by electrophoresis in a 1% agarose gel and visualized by ethidium bromide staining.

Flow cytometry analysis. Cells (10^6) in monolayer culture were harvested, washed, fixed, and stained with propidium iodide as previously described (38). Fluorescence intensities were determined by quantitative flow cytometry, and profiles were generated on a Coulter EPICS-PROFILE II analyzer.

Indirect immunofluorescence. Bcl-2-expressing and control cell lines were plated on glass coverslips at 38.5°C and shifted to 32°C, after which they were fixed and processed for indirect immunofluorescence as previously described (33). The murine p53-specific pAb248 monoclonal antibody was used to visualize p53. Slides were examined and photographed with a Nikon FXA microscope equipped with epifluorescence optics.

RESULTS

Human Bcl-2 expression blocks induction of apoptosis by wild-type p53. Primary BRK cells are transformed at a high frequency when transfected with the cooperating oncogenes E1A and murine mutant p53(Val-135) (4). The p53(Val-135) protein is temperature sensitive, and it exists predominantly in the mutant conformation at the restrictive temperature (38.5°C) and predominantly in the wild-type conformation at the permissive temperature (32°C) (22). Stable, transformed cell lines which rapidly and completely undergo apoptosis upon being shifted to the permissive temperature (4) were generated at the restrictive temperature. Expression of the E1B 19K protein, which functions as an inhibitor of apoptosis caused by E1A, or tumor necrosis factor alpha, or Fas antigen prevents this p53-dependent apoptosis, demonstrating that the induction of apoptosis in this setting can be specifically repressed (4). The human *bcl-2* gene was introduced into one E1A-plus-p53(Val-135)-transformed line, p53A (4), at the restrictive temperature by selection for neomycin resistance, and transformants were screened for human Bcl-2 expression. Eighteen independent Bcl-2-expressing clones were isolated. Preliminary characterization of these clones indicated that they behaved similarly. Detailed characterization of three independent clones was pursued.

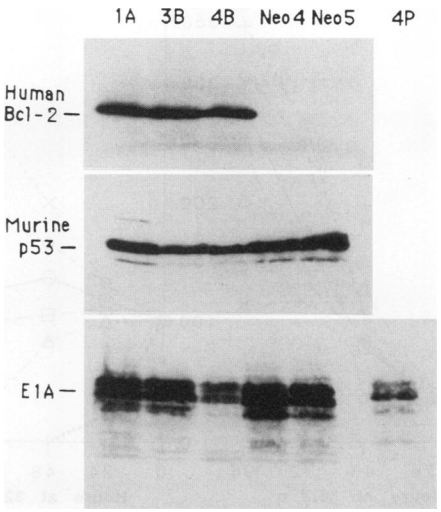

FIG. 1. Expression of Bcl-2 in E1A-plus-p53(Val-135)-transformed rat cells. The E1A-plus-p53(Val-135)-transformed line p53A was derived from transformed foci of primary BRK cells transfected with E1A and p53(Val-135) expression vectors as previously described (4). A quantity of 20 μg of cell extract from each line was analyzed for E1A, murine p53, and human Bcl-2 levels, with monoclonal antibodies M73 (E1A), pAb248 (murine p53), and 6C8 (human Bcl-2), by Western blotting. Cell line 4P is an E1A-plus-E1B-transformed BRK line used as a reference for E1A levels (33), and Saos2 cells, which do not express p53, were used as a negative control for p53 levels.

Three independent cell lines, 1A, 3B, and 4B, that expressed high levels of the human Bcl-2 protein (Fig. 1) comparable to those found in E1A-plus-*bcl-2*-transformed BRK lines (25) were identified. Two control lines were selected in parallel by transfection of p53A with the drug resistance marker alone. These control lines (neo4 and neo5) did not express human Bcl-2 but were drug resistant (Fig. 1). All lines expressed p53 and E1A at levels comparable to those of the parental p53A cell line (Fig. 1). The level of E1A expression in the 4B line was similar to that found in the E1A-plus-E1B transformant 4P, whereas E1A levels were slightly higher in the other lines (Fig. 1). Murine p53 was not expressed and, therefore, was not detectable in the 4P cell line (Fig. 1).

The viabilities of the Bcl-2 transformants and controls were monitored at the permissive and restrictive temperatures. All transformants were viable and possessed similar rapid growth rates at 38.5°C (Fig. 2). Viability was rapidly lost by the Bcl-2-negative control lines at 32°C (Fig. 2) and was comparable to those of the parental p53A line and other independently selected neomycin-resistant transformants (4). The kinetics of viability loss among the control lines were slightly different, with one control line (neo4) losing viability more rapidly (Fig. 2). This is consistent with previous observations that the growth rate is inversely proportional to the death rate among a group of E1A-plus-p53(Val-135) transformants (4). After prolonged incubation at 32°C (7 days) the survival rate for both control lines was less than 1 in 10^6 cells, as previously reported for other independently isolated lines (4). In contrast, all three Bcl-2-expressing lines remained viable, and in one case a line (1A) was able to sustain limited cell division at 32°C during the course of the experiment (Fig. 2). Subtle differences in the levels of E1A and Bcl-2 expression in the cell lines may account for these observations.

Pronounced morphological changes and DNA fragmentation are indicative of cell death by apoptosis (40) and occur

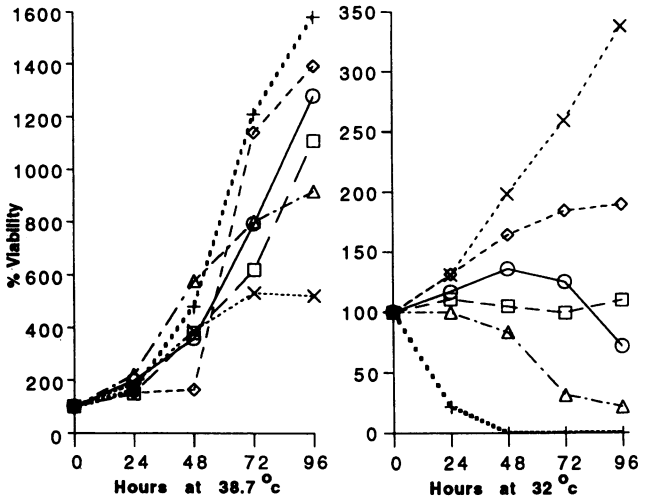

FIG. 2. Bcl-2 prevents viability loss induced by wild-type p53. The viabilities of E1A-plus-p53(Val-135) transformants (neo4 and neo5) and E1A-plus-p53(Val-135)-plus-Bcl-2 transformants (3B, 4B, and 1A) at permissive and restrictive temperatures are shown. Transformed BRK cell lines were plated at a density of 5×10^5 cells per 6-cm plate at 38.5°C. At 40 h postplating the cells were trypsinized, and the viable cell numbers per plate were determined by trypan blue exclusion. The remaining plates were shifted to 32°C, and the viable cell numbers were determined following incubation for 24, 48, 72, and 96 h. Viability is expressed as the percentage of the original viable cell number at the time of the shift to 32°C in a single experiment. Symbols: +, neo4; △, neo5; □, 3B; ○, 4B; ◇, 1A; ×, 4P.

upon conformational shift of p53 from the mutant to the wild-type form in E1A-plus-p53(Val-135)-transformed BRK lines (4). At the restrictive temperature, control and Bcl-2-expressing transformants were healthy and morphologically indistinguishable from each other (Fig. 3). After 24 h at 32°C, the control cell lines began rounding up and detaching from the surface, and this process was nearly complete by 72 h (Fig. 3); these results were analogous to those for previously isolated lines (4). Staining the nuclei for DNA revealed chromatin condensation, which is often associated with cells undergoing apoptosis (data not shown). The Bcl-2-expressing transformants 1A, 3B, and 4B did not undergo any gross morphological change when p53 assumed the wild-type conformation that was seen in the control cell lines (Fig. 3). The absence of any morphological indication of cell death in the Bcl-2-expressing cell lines at the permissive temperature indicated that the sustained viability was a result of the inhibition of apoptosis and not an increased rate of proliferation relative to the rate of cell death (Fig. 3).

DNA fragmentation in the characteristic nucleosome ladder pattern of cells undergoing apoptosis was coincident with viability loss and morphological changes in the control cell lines (Fig. 4). Thus, the Bcl-2-expressing lines maintained their morphology (Fig. 3) and DNA integrity (Fig. 4) as well as their viability (Fig. 2) at the permissive temperature. Bcl-2 expression can apparently suppress cell death by wild-type p53-dependent apoptosis in a manner indistinguishable from that of the expression of the E1B 19K protein (4).

Cell cycle analysis of growth-arrested cells. The variable abilities of the Bcl-2-expressing cell lines to become growth arrested at the permissive temperature were examined further in long-term incubations at the permissive temperature. The Bcl-2-expressing cell line 4B, which displayed complete growth

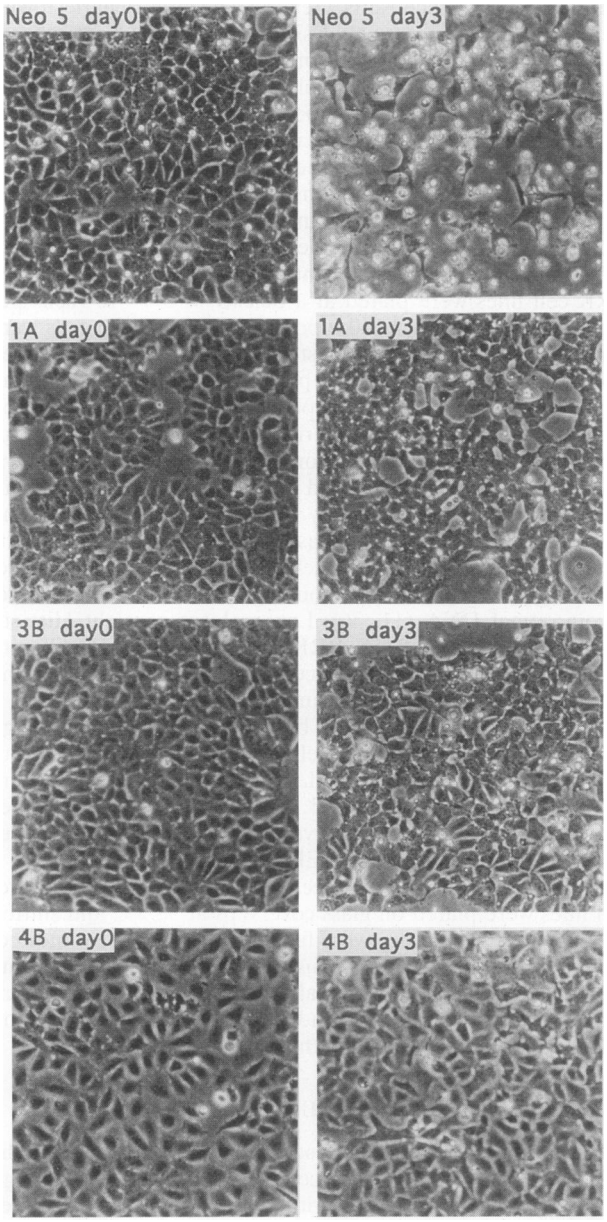

FIG. 3. Bcl-2 expression blocks the induction of the cytopathic effect that accompanies p53-dependent apoptosis. The neomycin-resistant control cell line neo5 and the Bcl-2-expressing lines 1A, 3B, and 4B derived from E1A-plus-p53-transformed line p53A are described in the legend to Fig. 2. Cells were photographed at the restrictive temperature (day 0) or after incubation for 96 h at the permissive temperature (day 3). Magnification, ×25.

arrest over 96 h, did so even after extended incubation (10 days) at 32°C (Fig. 5). The Bcl-2-expressing cell line 1A, which was capable of producing a slight increase in cell numbers at 32°C, was unable to sustain cell division and assumed a growth arrest phenotype after 6 days (Fig. 5). No apoptotic cells were apparent in the Bcl-2-expressing cell lines even after extended incubation at the permissive temperature. The lack of cell death and the absence of a significant increase in viable cell numbers suggest that Bcl-2 expression confers a growth-arrested state to cells expressing high levels of wild-type p53. The growth arrest phenotype at the permissive temperature

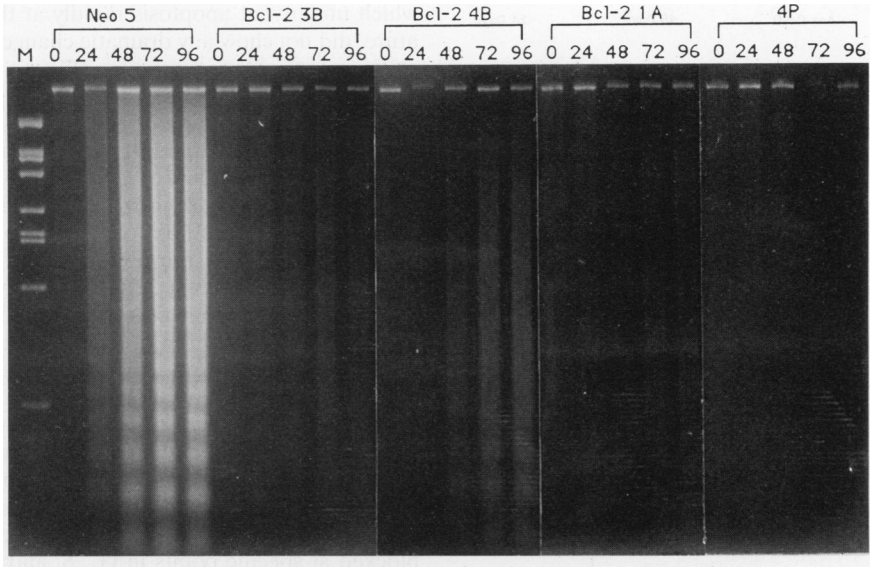

FIG. 4. Bcl-2 expression blocks DNA fragmentation during wild-type p53-dependent apoptosis. DNA fragmentation was monitored with a modified Hirt assay (4) in parallel with the viability assay used for Fig. 2. DNA was isolated from cells at 38.5°C at the time of the shift to 32°C (lanes 0) or after incubation for 24, 48, 72, or 96 h at 32°C, as indicated. Lane M, Ad5*dl*309 DNA digested with *Hin*dIII as molecular weight markers.

was reversible even after 10 days, with viable cell numbers increasing upon the return of the Bcl-2-expressing cell lines to the restrictive temperature (Fig. 5). At day 5 following their return to the restrictive temperature, the 4B and 1A cell lines reached confluence and could be passaged at least two more times at the restrictive temperature (data not shown). Thus,

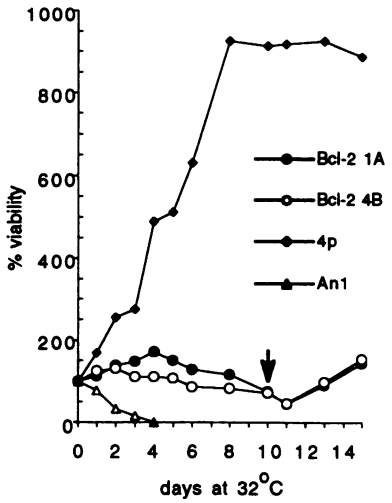

FIG. 5. Induction of long-term growth arrest in apoptosis-resistant cell lines. Viability of Bcl-2-expressing E1A-plus-p53(Val-135) transformants, as described in the legend to Fig. 2, was determined in a single experiment over an extended period of 14 days. The arrow indicates the point at which the Bcl-2-producing lines 4B and 1A were returned to the restrictive temperature of 38°C. Cell line 4P, an E1A-plus-E1B transformant which does not contain a temperature-sensitive mutation, was used as a positive control for viability at the permissive temperature. The neomycin-resistant E1A-plus-p53(Val-135)-transformed line p53AN1, which undergoes apoptosis at the permissive temperature (4), served as a control for the induction of apoptosis.

bcl-2 expression, although sufficient to block apoptosis caused by p53, produced only a limited capacity to overcome reversible growth arrest mediated by p53.

Expression of wild-type p53, when not associated with the induction of apoptosis, is most often associated with the induction of growth arrest at the G_1/S boundary of the cell cycle (5, 20, 43). The Bcl-2-expressing cell lines were therefore examined for evidence of a particular cell cycle arrest point, i.e., a point at which growth was arrested at the permissive temperature, in parallel with the viability analysis for Fig. 5. This experiment was repeated three times, and the same results were obtained in each case. The results from one of the experiments are shown.

By fluorescence-activated cell sorter (FACS) analysis the

TABLE 1. Cell cycle analysis of Bcl-2-expressing cells[a]

Cell line	Day	% of cells at cell cycle phase[b]:			
		G_1	S	G_2/M	>G_2/M
4P	0	58.3	18.3	21.4	2.0
	1	66.4	18.6	13.6	1.4
	2	62.4	17.2	18.1	2.3
	4	59.6	16.0	20.6	3.8
	6	62.9	16.4	17.5	3.2
4B	0	51.9	19.5	22.0	6.6
	1	61.5	17.3	16.4	4.8
	2	52.0	17.4	21.5	9.1
	4	55.0	12.0	24.4	8.6
	6	42.5	12.9	30.1	14.4
1A	0	50.6	16.5	26.5	6.4
	1	55.0	14.9	22.5	7.6
	2	50.8	13.5	25.4	10.3
	4	51.1	14.8	19.5	14.6
	6	49.1	21.7	16.1	13.1

[a] Quantitation of the cell cycle analysis data in Fig. 6.
[b] Determined by DNA content at each time point.

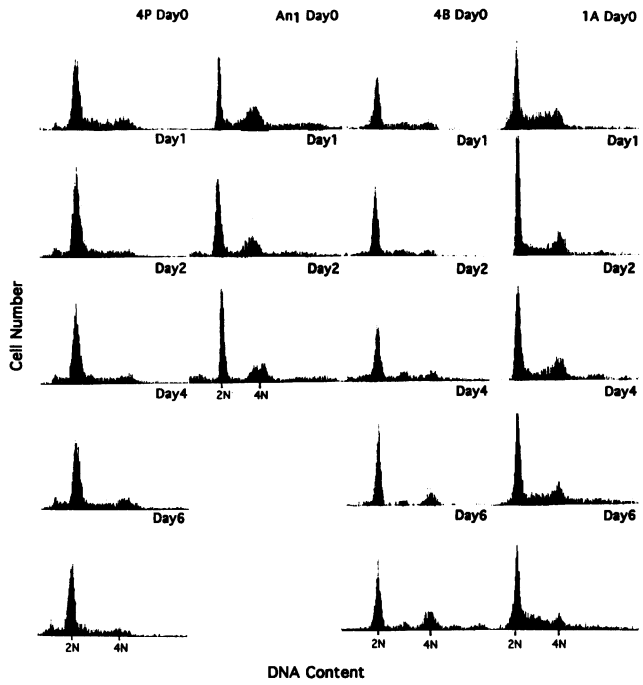

FIG. 6. Cell cycle analysis of apoptosis-resistant cell lines. Shown are FACS profiles of E1A-plus-p53(Val-135)-transformed line p53AN1 (4), E1A-plus-p53(Val-135)-plus-Bcl-2-transformed lines 4B and 1A, and E1A-plus-E1B transformant 4P. Transformed BRK lines were plated at a density of 10^6 cells per 6-cm plate at 38.5°C. At 40 h postplating the cells were trypsinized, fixed with ethanol, and stained with propidium iodide. Remaining plates were shifted to 32°C and processed for FACS analysis following incubation for 1 to 6 days as indicated. The *x* axis represents relative fluorescence intensity, which is proportional to DNA content. The positions of 2N (G_1) and 4N (G_2/M) DNA contents are indicated. Data for one of three experiments which yielded the same results are shown.

E1A-plus-E1B-transformed control cell line 4P, which is not temperature sensitive for growth at 32°C, displayed a typical distribution of cells with G_1 (2N), S, and G_2/M (4N) DNA contents at the restrictive and permissive temperatures for the duration of the experiment (Table 1; Fig. 6). FACS analysis of the E1A-plus-p53(Val-135)-transformed control line neo5, which underwent apoptosis rapidly at the permissive temperature, did not show any dramatic changes following incubation at the permissive temperature (Fig. 6). If growth arrest at a particular point in the cell cycle was a prerequisite for apoptosis, then accumulation of cells with a specific DNA content should have been apparent, but this was not the case. There was a slight diminution of cells with S-phase DNA contents, which is consistent with previously published observations that cell cycle arrest does not occur prior to apoptosis but cells in G_1 prior to S phase are preferentially sensitive to apoptosis (26, 43). Beyond 48 h at 32°C there were insufficient numbers of intact cells of the neo5 cell line to obtain a FACS profile because of the induction of apoptosis (data not shown).

The Bcl-2-expressing cell line 4B, which underwent complete growth arrest at the permissive temperature, did not show specificity to arrest at any single point in the cell cycle (Fig. 6). Rather, there was a gradual accumulation of discrete peaks at S and G_2 and a diminution of the continuous distribution of cells with DNA contents variable between G_1 and G_2/M (Fig. 6). This suggests that cell cycle progression is blocked at specific points in G_1, S, and G_2/M. From day 0 to day 6 at 32°C there was a reduction in the percentage of cells with G_1 DNA contents (from 51.9 to 42.5%) and an increase in the percentage of those with G_2/M DNA contents (from 22.0 to 30.1%) (Table 1; Fig. 6). A fraction of the cells must be capable of escaping the cell cycle block and accumulating in G_2, suggesting that cell cycle arrest not only occurs at multiple points but also is leaky. Finally, there was a modest accumulation of cells with DNA contents greater than 4N, from 6.6% at day 0 to 14.4% at day 6 (Table 1; Fig. 6). The presence of cells with DNA contents greater than 4N suggests that some of the cells must undergo another round of DNA synthesis without cell division. The accumulation of cells with quantities of DNA greater than 4N took 6 days to become apparent, indicating that cell cycle progression was predominantly arrested but that with time a small percentage of the cells could escape the cell cycle block and undergo another round of DNA synthesis.

The Bcl-2-expressing line 1A, which grew slowly at the permissive temperature before entering growth arrest, displayed no dramatic changes in the distribution of cells across the cell cycle (Fig. 6). Between days 0 and 4 there was a slight increase in viable cell numbers (Fig. 5), indicating that these cells are capable of gradually progressing through the cell cycle. There was also a small increase in numbers of cells with

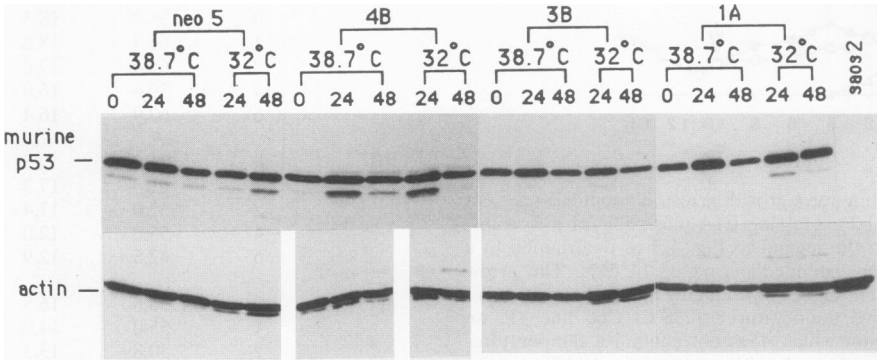

FIG. 7. p53 protein levels in Bcl-2-expressing cell lines. The Bcl-2-expressing E1A-plus-p53(Val-135) transformants 1A, 3B, and 4B and the neo5 control were examined for p53 protein levels at both the permissive and restrictive temperatures by Western blot analysis. Monoclonal antibody pAb248 against murine p53 was used to visualize p53. Actin levels in parallel Western blots were monitored as controls for relative protein levels in each sample. The numbers above each lane represent hours at the indicated temperatures.

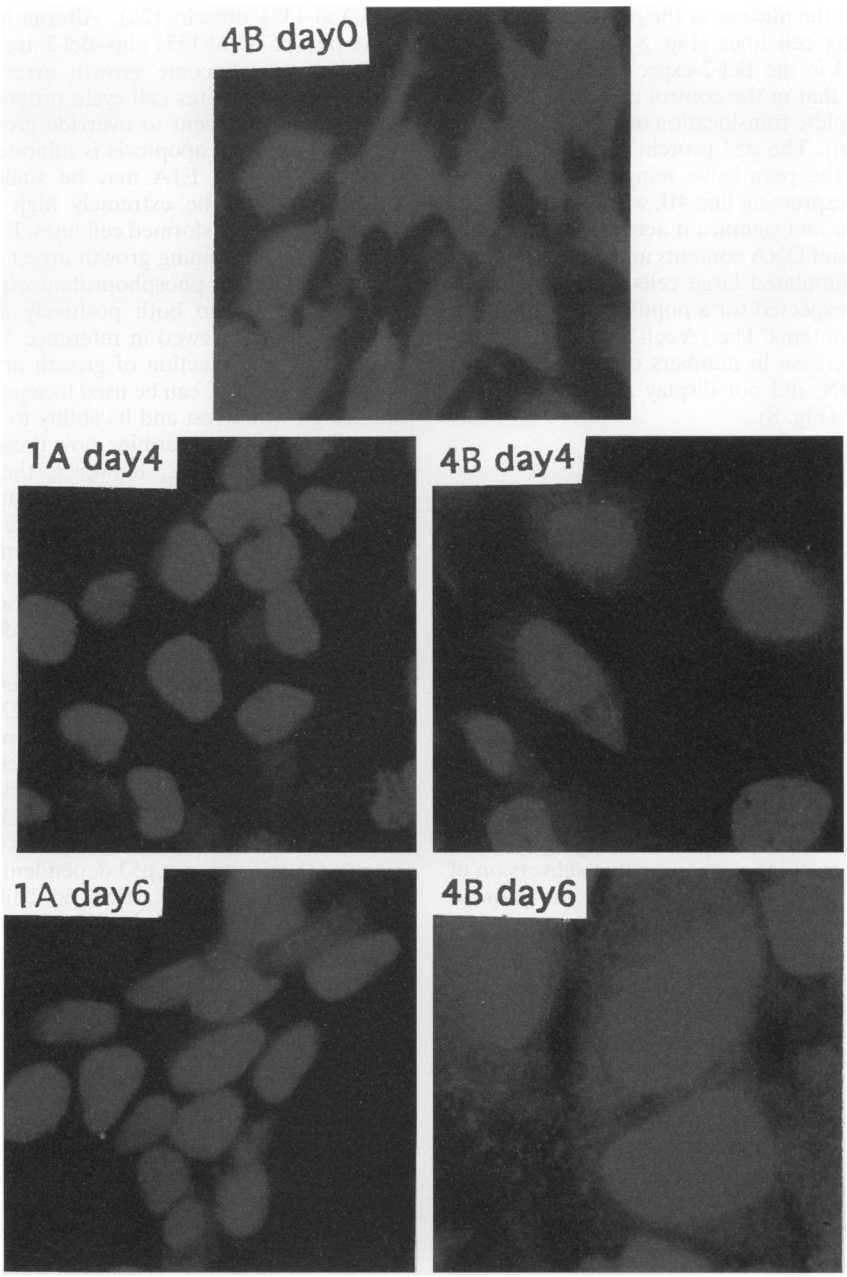

FIG. 8. Localization of p53 in Bcl-2-expressing cell lines. p53 localization in Bcl-2-expressing E1A-plus-p53(Val-135) transformants was examined by indirect immunofluorescence. The murine p53-specific pAb248 monoclonal antibody was used to visualize p53 at the restrictive temperature (day 0) and subsequently at the permissive temperature (day 4 and day 6). pAB248 did not detect the endogenous rat p53 by indirect immunofluorescence (data not shown). Magnification, ×600.

DNA contents greater than 4N, similar to the increase observed with the Bcl-2-expressing line 4B (Table 1; Fig. 6). These findings are consistent with the existence of multiple cell cycle arrest points and a leaky growth arrest phenotype.

p53 protein levels and nuclear localization are not affected by Bcl-2. p53 function can be modulated in vivo at the level of protein half-life and localization (reviewed in reference 45). p53 levels and localization in the Bcl-2-expressing cell lines at the permissive temperature were examined. Western blotting of cell extracts from controls neo4 (data not shown) and neo5 and from Bcl-2-expressing cell lines 4B, 3B, and 1A showed similar levels of murine p53 at both the restrictive and permissive temperatures (Fig. 7). A slight reduction in p53 levels occurred at 32°C, which coincides with a decrease in the half-life of wild-type versus mutant p53 protein, but this was independent of Bcl-2 expression (Fig. 7).

The p53(Val-135) protein is predominantly cytoplasmic when in the mutant conformation at the restrictive temperature and is rapidly transported to the nucleus upon being returned to the wild-type conformation at the permissive temperature (9, 20). In control cell lines (neo4 and neo5) complete nuclear translocation of the p53 protein took 4 h of incubation at the permissive temperature (data not shown). p53 was still cytoplasmic at the restrictive temperature and was

efficiently transported to the nucleus at the permissive temperature in Bcl-2-expressing cell lines (Fig. 8). The kinetics of nuclear transport of p53 in the Bcl-2-expressing cell lines 1A and 4B was identical to that in the control cell lines neo4 and neo5, with virtually complete translocation occurring within 4 h at 32°C (data not shown). The p53 protein remained nuclear for at least 6 days at the permissive temperature (Fig. 8). Interestingly, the Bcl-2-expressing line 4B, which displayed the growth arrest phenotype and significant accumulation of cells with G_2 DNA contents and DNA contents and greater than G_2 (44.6%) (Table 1), accumulated large cells with large nuclei (Fig. 8). This would be expected for a population of cells with abnormally high DNA contents. The 1A cell line, which did not show as dramatic an increase in numbers of cells with DNA contents greater than 4N, did not display as many enlarged cells and nuclei at 32°C (Fig. 8).

DISCUSSION

p53 mutations are the most prevalent genetic alterations found in human tumors (13, 32). Loss of p53 function greatly accelerates the frequency of tumor formation in humans (19) and animal models (6), demonstrating the importance of p53 as a tumor suppressor. There is evidence that p53 regulates both cell cycle control (5, 20) and apoptosis (44). In response to DNA damage p53 levels are increased when p53 either acts as a cell cycle checkpoint, inducing G_1 arrest to permit repair (15), or triggers apoptosis (3, 18). E1A expression also induces p53 accumulation (17), but it induces apoptosis rather than growth arrest (25). The ability of p53 to induce growth arrest or apoptosis may depend on the cellular context in which it is expressed. Induction of apoptosis by E1A cosegregates with induction of DNA synthesis (34), suggesting that subversion of cell cycle control by E1A is incompatible with the growth-suppressive activity of wild-type p53 (4). There was no evidence of cell cycle arrest prior to apoptosis, indicating that growth arrest is not a prerequisite for apoptosis but is a distinct process. Bcl-2 can modify the activity of p53 from induction of apoptosis to induction of growth arrest, suggesting that these two functions of p53 are discrete and separable. The E1B 19K protein has the identical ability to inhibit p53-dependent apoptosis in E1A-plus-p53(Val-135)-transformed rodent cells (4), suggesting that the E1B 19K and Bcl-2 proteins are functionally similar.

Induction of growth arrest by ectopic expression of wild-type p53 is most often associated with arrest at G_1/S (5, 20, 43). In cells expressing Bcl-2, p53 did not produce an accumulation of cells at any one specific point in the cell cycle, suggesting participation of p53 in several cell cycle control points in these transformed cells. The growth arrest phenotype of p53 in the Bcl-2-expressing cells was somewhat leaky, with a small percentage of the cells escaping through to another round of DNA synthesis before entering growth arrest again. It may be possible for Bcl-2 to overcome cell cycle arrest caused by p53 if expressed at sufficient levels, which may explain the paradox that E1A and *bcl-2* cooperate to transform primary BRK cells (25). As E1A-induced apoptosis in this situation is exclusively p53 dependent, Bcl-2 should be able to overcome the growth-suppressive activity as well as the apoptotic activity of p53. One explanation is that in the presence of large amounts of wild-type p53, which the E1A-plus-p53(Val-135)-transformed cells have, Bcl-2 may not be sufficient to completely overcome the growth arrest activity of p53. During the course of transformation of BRK cells with E1A and *bcl-2*, the levels of p53, although p53 protein accumulation is induced by E1A, do not nearly approach the levels found in cells expressing the p53(Val-135) protein (2a). Alternatively, E1A levels in the E1A-plus-p53(Val-135)-plus-Bcl-2 transformants may not be sufficient to overcome growth arrest caused by p53. E1A expression stimulates cell cycle progression (14, 29) and may normally be sufficient to override growth suppression caused by p53 as long as apoptosis is inhibited. Thus, if apoptosis is averted by Bcl-2, E1A may be sufficient to overcome p53 cytostasis. With the extremely high levels of p53 in these p53(Val-135)-transformed cell lines, E1A may be only partially effective at overcoming growth arrest caused by p53.

p53 is a nuclear phosphoprotein with specific DNA-binding capability that can both positively and negatively regulate transcription (reviewed in reference 45). How these activities are related to induction of growth arrest or apoptosis is not known. Since Bcl-2 can be used to separate the ability of p53 to induce growth arrest and its ability to induce apoptosis, it will be informative to determine how these other activities of p53 are affected by Bcl-2. However, the functional interaction between p53 and Bcl-2 could be quite indirect. The proteins reside in different cellular compartments, so the potential for direct interaction between them is transient at best. Bcl-2 does not act by diminishing p53 protein levels, nor does it prevent nuclear translocation of p53. These observations are consistent with Bcl-2 modifying the function of p53 but not eliminating its activity completely.

In animal models Bcl-2 overexpression in lymphoid cells produces extended B-cell memory (23) and lymphomas (21). Bcl-2 has a variable ability to block negative selection in the thymus, indicating the existence of Bcl-2-dependent and independent pathways (28, 30). Bcl-2 blocks apoptosis in response to DNA damage (1, 28), for which p53 function is required (3, 18). However, Bcl-2 inhibits apoptosis in response to glucocorticoids (1), which is not p53 dependent (18). As Bcl-2 does not alter p53 levels or localization, Bcl-2 likely acts downstream of p53 by suppressing both p53-dependent and independent pathways.

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