Environmental Chemical-Induced Pro/Pre-B Cell Apoptosis: Analysis of c-Myc, p27 Kip1, and p21 WAF1 Reveals a Death Pathway Distinct from Clonal Deletion

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Environmental Chemical-Induced Pro/Pre-B Cell Apoptosis: Analysis of c-Myc, p27Kip1, and p21WAF1 Reveals a Death Pathway Distinct from Clonal Deletion

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Polycyclic aromatic hydrocarbons (PAH) are common environmental pollutants that suppress the immune system in part by inducing pro/pre-B cell apoptosis. The PAH-induced death signaling pathway resembles the signaling cascade activated during clonal deletion and modeled by B cell receptor cross-linking or by dexamethasone exposure of immature surface IgM B cells in that apoptosis is mediated by NF-κB down-regulation. Because a PAH-induced, clonally nonrestricted deletion of B cells would have important implications for B cell repertoire development, the nature of the PAH-induced intracellular death signal was studied further. Particular emphasis was placed on the roles of growth arrest and c-Myc, p27Kip1, and p21WAF1 expression, because all of these elements contribute to clonal deletion. As in clonal deletion models, and as predicted by the down-regulation of NF-κB, PAH-induced death of pro/pre-B cells was at least partially dependent on c-Myc down-regulation. Furthermore, whereas dexamethasone induced a G0/G1 cell cycle arrest, PAH had no effect on pro/pre-B cell growth, indicating that growth arrest and apoptosis occur by separable signaling pathways in this early phase of B cell development. Finally, in contrast to clonal deletion, PAH-induced pro/pre-B cell death was not dependent on p27Kip1 or p21WAF1 up-regulation but did coincide with p53 induction. These results distinguish the PAH-induced apoptosis pathway from that activated during clonal deletion and indicate that signaling cascades leading to growth arrest and/or apoptosis in pro/pre-B cells differ from those active at later B cell developmental stages. The Journal of Immunology, 2003, 170: 4897–4904.
cytosolic aryl hydrocarbon receptor/transcription factor in neighboring stromal cells, indicating that it is the PAH-exposed stromal cells that deliver the death signal to bone marrow B cells and, potentially, to cells of other hematopoietic lineages (15–20, 30). In the present studies, we used the prototypic PAH, DMBA, as a tool for assessing apoptosis signaling within pro/pre-B cells. In addition to helping us reveal mechanisms of pro/pre-B cell apoptosis, the use of this immuno suppressant provides insights into possible mechanisms through which environmental chemicals suppress immunity.

To the extent that it has been studied, the intracellular pro/pre-B cell death signaling pathway induced by PAH resembles the death pathway induced in immature (WEHI-231) B cells by anti-Ig cross-linking or by dexamethasone in that PAH-induced apoptosis is preceded by and dependent on a decrease in NF-κB activity (19). However, preliminary experiments suggested some distinctions at the two stages of B cell development, including a lack of growth arrest before PAH-induced pro/pre-B cell apoptosis. Therefore, the present studies were designed to evaluate the intracellular, PAH-induced death signaling pathway in nontransformed pro/pre-B cells distal to NF-κB down-regulation. In particular, the contributions of c-Myc, p27Kip1, and p21WAF1 to pro/pre-B cell apoptosis were emphasized. Because apoptosis cannot be induced in the slg- pro/pre-B cells by B cell receptor (BCR) cross-linking, dexamethasone was used as a surrogate inducer of a clonal deletion-like signal.

Materials and Methods

Cell lines

Stromal cell-dependent, CD43+ BU-11 cells expressing rearranged Ig H chains (15, 16, 18, 20) were cocultured on cloned BMS2 bone marrow-derived stromal cells (14) in 50% RPMI 1640 (Life Technologies, Rockville, MD) and 50% DMEM (Mediatech, Washington, DC) containing 5% FBS (Life Technologies), 2 mML -glutamine, 5

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were emphasized. Because apoptosis cannot be induced in the slg- pro/pre-B cells by B cell receptor (BCR) cross-linking, dexamethasone was used as a surrogate inducer of a clonal deletion-like signal.

Immunoblotting

Total cell lysates were prepared using HEDG buffer containing 25 mM HEPES, 1.5 mM EDTA, 1 mM DTT, 10% glycerol, 50 mM NaF, 10 mM sodium phosphate buffer, 20 mM sodium molybdate, and protease inhibitors followed by centrifugation at 14,000 rpm for 10 min at 4°C. Nuclear proteins were prepared using NEBB buffer containing 20 mM HEPES (pH 7.9), 420 mM NaCl, 1.5 mM MgCl2, 1 mM EDTA, 1 mM EGTA, 25% glycerol, 5 mM NaF, 1 mM Na2VO4, 1 mM DTT, 1 mM Na3P2O7, and protease inhibitor mixtures followed by centrifugation at 14,000 rpm for 5 min at 4°C. Total (30 μg) or nuclear (20–50 μg) proteins were electro-phoresed through 10–15% SDS-polyacrylamide gels for 1.5 h at 100 V and transferred to nitrocellulose membranes. Membranes were blocked, incubated with a 1/500 dilution of c-Myc-specific Ab (Upstate Biotechnology, Lake Placid, NY) or 1/1000 dilutions of p27Kip1, p21WAF1, or p53-specific Ab (Santa Cruz Biotechnology, Santa Cruz, CA), washed, and incubated for 1 h at room temperature with HRP-conjugated secondary Ab at a 1/3000 dilution in TBS containing 5% milk and 0.1% Tween 20. Membranes were washed three times for 10 min with TBS. Protein bands were visualized by enhanced ECL (Sigma-Aldrich). Membranes probed for c-Myc, p27Kip1, or p21WAF1 were stripped and reprobed with β-actin-specific Ab (Sigma-Aldrich) for normalization of band densities. Image analyses were performed on digitally scanned (Perfection 1250; Epson) autoradiographs. Relative band intensities were determined with the Kodak Digital Sciences ID program. c-Myc, p27Kip1, or p21WAF1 band intensities were normalized to β-actin band intensities.

Statistical analyses

ANOVA and Dunnett’s or Duncan’s multiple comparison tests were used for statistical analyses of apoptosis and cell cycle data. A paired t test was used to analyze putative changes in c-Myc, p27Kip1, p21WAF1, or p53 expression in Western immunoblots.

Results

Treatment with DMBA or dexamethasone distinguishes pro/pre-B cell growth arrest from apoptosis

Previous studies demonstrated that low doses of the prototypic PAH, DMBA, readily induce apoptosis in pro/pre-B cells cultured on cloned stromal cells (15, 17–20). A role for NF-κB in DMBA-induced apoptosis at this early stage of B cell development (19) suggested the competence of a signaling pathway similar to that activated during BCR-dependent clonal deletion and mimicked by dexamethasone. To test this hypothesis, cocultures of BMS2 stromal cells and BU-11 cells were treated with 0.1–1 μM DMBA or 10 μM dexamethasone for 24–48 h. BU-11 cells were harvested, stained with PI, and analyzed by flow cytometry for apoptosis and cell cycle arrest.

Vehicle-treated cells exhibited a low background of spontaneously apoptotic BU-11 cells, as quantified by calculating the percentage of cells exhibiting a subdiploid DNA peak (7.5 ± 0.4%) (Fig. 1A). Consistent with previous studies, treatment with 0.1 or 1 μM DMBA for 24 h induced a significant (p < 0.01) percentage of BU-11 cells to undergo apoptosis (Fig. 1, A and B). Apoptosis increased when cells were cultured with DMBA for a 48-h period (Fig. 1A, lower panel). Decreases in PI staining were accompanied by morphologic changes, DNA fragmentation, an increase in annexin V staining, and poly(ADP-ribose) polymerase cleavage characteristic of apoptosis (data not shown) (15–17, 20). In contrast, 10 μM dexamethasone did not affect BU-11 cell viability at either the 24 or 48 h time points (Fig. 1, A and B).

Despite the induction of apoptosis, 0.1–1 μM DMBA did not induce growth arrest at 24 or 48 h (Fig. 2, A and B) or at earlier

Stable transfections

BU-11 cells (5 × 104) were washed twice in cold PBS, centrifuged at 1500 rpm for 5 min, and resuspended in 0.8 ml of cold PBS. Plasmid DNA(s) (10 μg pEFl4-zeocin vector alone or 10 μg pM21-c-myc expression vector with 2 μg of pEF4-zeocin vector) were added to the cells. The pM21-c-myc expression vector, which contains the human c-myc gene under the transcriptional control of MoMLV enhancer and promoter, was kindly provided by Dr. G. Sonenshein (Boston University, Boston, MA). After a 20-min incubation on ice, cells were electroporated at 180 V/960 μF and then cultured on BMS2 cell monolayers as above for 48 h. Cells were transferred to pEF4-zeocin-transfected BMS2 monolayers in 48-well culture plates, and 0.6 mg/ml zeocin (Invitrogen) was added. Every 3 days for 2 wk, half of the culture medium was removed and replaced with fresh medium supplemented with 0.6 mg/ml zeocin. Positively selected lines were expanded, and c-Myc expression was assessed by immunoblotting.

Immunoblotting

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time points (4, 8, 12, 18 h; data not shown). Conversely, although not inducing apoptosis, dexamethasone (10 μM) induced a pro-found state of growth arrest (p < 0.01) at the G0/G1 stage as early as 24 h after its addition to the cultures. In contrast to studies with immature sIgB cells, these results indicate a discordance between regulation of cell cycle and induction of apoptosis, which can be dissected with these two immunosuppressants. Furthermore, they suggest that PAH-induced, stromal cell-dependent modulation of B cell development is not likely to occur at the level of cell growth arrest.

c-Myc down-regulation correlates with DMBA-induced apoptosis and dexamethasone-induced growth arrest

Using DMBA and dexamethasone as two immunosuppressants that induce independent outcomes (growth arrest vs apoptosis), we evaluated the extent to which the signaling pathways for growth arrest and apoptosis overlap. Because NF-κB down-regulation is required for DMBA-induced pro/pre-B cell apoptosis (19), and because NF-κB transcriptionally regulates c-Myc as part of the death and growth arrest signaling pathways during clonal deletion (3), it was postulated that c-Myc would decrease in pro/pre-B cells before overt death. Furthermore, because dexamethasone-induced growth arrest and apoptosis in immature slgB+ cells are characterized by c-Myc down-regulation (32), it was important to determine whether changes in c-Myc expression correlated with growth arrest in the absence of apoptosis. To address these issues, c-Myc expression in BU-11 cells was assayed 24 h after treatment of cocultures with 0.1–1.0 μM DMBA or 10 μM dexamethasone, treatments which induce apoptosis or growth arrest, respectively. To ensure that any observed decrease was not due to nonspecific degradation of proteins during apoptosis, c-Myc expression was normalized to β-actin levels. Total cellular c-Myc levels were modestly but significantly reduced to 73 ± 9% (p < 0.05) and 62 ± 6% (p < 0.01) of control levels following treatment with 0.1 or 1.0 μM DMBA, respectively (Fig. 3, A and B). Similarly, c-Myc was significantly reduced in dexamethasone-treated cells to 33 ± 13% of controls (p < 0.05) (Fig. 3, A and B). The DMBA-induced decrease in c-Myc expression was more readily observed when evaluating nuclear c-Myc levels (Fig. 3, C and D) (43 ± 14% of controls; p < 0.03). These results are consistent with a role for
c-Myc down-regulation in both PAH-induced apoptosis and dexamethasone-induced growth arrest.

To determine whether c-Myc down-regulation contributes to DMBA-induced apoptosis, BU-11 cells were stably transfected with pM21-c-myc and pEF4-zeocin or, as a control, pEF4-zeocin alone. Western blotting of three randomly selected pM21-c-myc-transfected and control pEF4-zeocin-transfected clones indicated significant increases in c-Myc expression in all three pM21-c-myc-transfected clones (Fig. 4A). Treatment of pM21-c-myc-transfected BU-11 clones with 0.1–1.0 μM DMBA consistently demonstrated a modest (30 ± 3% overall) but statistically significant reduction in the level of DMBA-induced apoptosis in all three pM21-c-myc clones relative to apoptosis seen in control transfectants (Fig. 4B). c-Myc expression in each of the DMBA-treated pM21-c-myc transfected clones was not significantly affected by treatment with the highest (i.e., 1 μM) DMBA dose (Fig. 4C). Even pooling data from all three pM21-c-myc-transfected, DMBA-treated clones (n = 18) did not reveal significant differences in the levels of c-Myc expression after DMBA treatment in pM21-c-myc-transfected clones (p = 0.41). The data indicate that down-regulation of c-Myc is at least partially responsible for DMBA-induced BU-11 cell apoptosis. They do not rule out contributions of additional c-Myc-independent signaling pathways.

\( p27^{Kip1} \) is not up-regulated during DMBA-induced apoptosis or dexamethasone-induced growth arrest in pro/pre-B cells

In immature sIg\(^+\) B cells, down-regulation of c-Myc following BCR cross-linking or after dexamethasone treatment coincides with de-repression of \( p27^{Kip1} \) (1, 5–7). To determine the role of \( p27^{Kip1} \) in pro/pre-B cell apoptosis and growth arrest, cocultures were treated with DMBA or dexamethasone as above. BU-11 cells were harvested 24 h later, and protein extracts evaluated by Western blotting for \( p27^{Kip1} \) expression. As a positive control, WEHI-231 cells were treated in parallel with dexamethasone. Naive (not shown) or vehicle-treated BU-11 cells expressed significant levels of \( p27^{Kip1} \) (Fig. 5). Surprisingly, and despite the decrease in c-Myc protein (Fig. 3), treatment with 0.1 or 1 μM DMBA significantly (\( p < 0.05 \)) down-regulated \( p27^{Kip1} \) expression to 70 ± 8 or 57 ± 9% of normalized controls, respectively (Fig. 5, A and B). Similarly, dexamethasone not only failed to increase \( p27^{Kip1} \) levels but actually decreased them (\( p < 0.05 \)). This result may be contrasted with the expected increase in \( p27^{Kip1} \) seen in dexamethasone-treated immature WEHI-231 cells (Fig. 5, A and C). The data indicate that \( p27^{Kip1} \) up-regulation is not required for DMBA-induced pro/pre-B cell apoptosis and that the signaling pathway induced in pro/pre-B cells by environmental chemicals is distinct from that active during immature B cell clonal deletion.

\( p21^{WAF1} \) is not up-regulated during DMBA-induced apoptosis or dexamethasone-induced growth arrest in pro/pre-B cells

An alternative but potentially overlapping pathway to apoptosis in several cell types, including anti-lg-treated immature B cells (8), is through induction of p53 and its target, cyclin-dependent kinase inhibitor \( p21^{WAF1/CIP1} \). In immature B cells, \( p21^{WAF1} \) up-regulation also is associated with anti-lg-induced growth arrest (8). To evaluate a potential role for \( p21^{WAF1} \) in DMBA-induced pro/pre-B cell apoptosis or dexamethasone-induced growth arrest, cultures were treated with DMBA or dexamethasone as above, and protein extracts were assayed for \( p21^{WAF1} \) expression by Western blotting. Little or no \( p21^{WAF1} \) protein was detected in untreated or vehicle-treated BU-11 cells (Fig. 6). Furthermore, treatment of BU-11 cultures with DMBA did not induce \( p21^{WAF1} \) expression. Interestingly, the failure to induce \( p21^{WAF1} \) appeared not to be related to a lack of or decrease in p53, because DMBA treatment significantly

**FIGURE 3.** c-Myc is down-regulated in pro/pre-B cells following exposure to DMBA or dexamethasone. A, BU-11 cells were cultured on BMS2 cells and treated for 24 h with vehicle, 0.1 μM DMBA, 1 μM DMBA, or 10 μM dexamethasone. Cells were harvested, total protein was extracted, and 30 μg was loaded into SDS-polyacrylamide gels for c-Myc-specific immunoblotting. Blots were stripped and reprobed with β-actin-specific Ab to control for loading errors. Representative data from one of five experiments are shown. B, c-Myc band densities were normalized to β-actin levels and then compared with normalized c-Myc levels in vehicle-treated cells. Data are presented as the average percentage of normalized c-Myc protein ± SE from five experiments. Statistical significance was determined using the paired t test. * or **. A statistically significant decrease in c-Myc, \( p < 0.05 \) or \( p < 0.01 \), respectively. C, BU-11 cells were cultured on BMS2 cells and treated for 24 h with vehicle or 1 μM DMBA. Cells were harvested, nuclear protein was extracted, and 25 μg was loaded into SDS-polyacrylamide gels for c-Myc-specific immunoblotting. Representative data from one of three experiments are provided. D, Nuclear c-Myc levels from DMBA-treated cells were compared with c-Myc levels in vehicle-treated cells. Data are presented as the average percentage of nuclear c-Myc protein ± SE from three experiments. Statistical significance was determined using the paired t test. * A statistically significant 57.3% decrease in c-Myc, \( p < 0.05 \).
compared with any of the pEF4-zeocin-transfected subclones, pEF4-zeocin-transfected clone 1 or pEF4-zeocin-transfected clone 5, using one-way ANOVA and the Duncan test. The data are presented as the average percentage of DMBA-specific apoptosis.

The average percent reduction in the three pM21-c-myc-transfected clones expressing c-Myc was loaded into SDS-polyacrylamide gels for c-Myc-specific immunoblotting. Blots were stripped and reprobed for β-actin expression to control for loading errors. Normalized c-Myc band densities were compared with c-Myc expression in any of the DMBA-treated clones, and statistical significance was not reached even when data from all three DMBA-treated clones were pooled (n = 18; p = 0.41).

**Discussion**

Environmental pollutants adversely affect the immune system at multiple levels. In particular, the developing immune system is exquisitely sensitive to a wide variety of environmental chemicals, including halogenated and nonhalogenated hydrocarbons (33, 34). In previous studies, we demonstrated that PAH are potent inducers of pre- and pro/pre-B cell apoptosis in vitro and in vivo and that signaling of B cell death is dependent on bone marrow stromal or liver parenchymal cells (15, 16, 18–20). Cell death induced by PAH resembled death induced by cross-linking of the BCR in immature slg+ B cells in that both are mediated by a down-regulation of NF-κB (4, 5, 19). Consequently, it appears that PAH can commandeer at least part of the death pathway activated during clonal deletion of more mature cells but in a non-clonally restricted way, much in the same fashion as glucocorticoids. In the present study, we further characterized the PAH-induced pro/pre-B cell death pathway with the intention of investigating not only the molecular signaling pathway through which environmental chemicals effect early B cell death but also the extent to which this pathway overlaps with that activated in slg+ B cells during clonal deletion and mimicked by glucocorticoid exposure. Particular emphasis was placed on putative modulation of c-Myc. The results reveal two signaling pathways in early B cells, one resulting in apoptosis and the other in growth arrest. Neither pathway appears identical with the classic B cell clonal deletion signaling cascade.

Given that a decrease in NF-κB activity is critical to DMBA-induced pro/pre-B cell apoptosis (19), and that NF-κB influences c-myc transcription, it was predicted that PAH-induced BU-11 cell death would be triggered by a decrease in c-Myc. As predicted, c-Myc levels in BU-11 cells decreased significantly after DMBA treatment and these cells could be partially rescued from death by ectopic expression of c-Myc. The degree of rescue from apoptosis induced with 1 μM DMBA approximated that seen in BU-11 cells transfected with the NF-κB subunit RelA (19), a result consistent with NF-κB transcriptional regulation of c-myc levels during apoptosis. These results indicate a role for c-Myc as a survival factor at an earlier stage in B cell development than previously appreciated. Furthermore, it could be said that c-Myc dysregulation in general contributes to pro/pre-B cell death because overexpression of c-Myc in transgenic mice also induces apoptosis in bone marrow B cells (35). However, the failure of dexamethasone to induce apoptosis, despite an obvious decline in c-Myc expression, indicates that c-Myc down-regulation may not be sufficient for apoptosis induction in pro/pre-B cells and/or that DMBA and dexamethasone effect c-Myc down-regulation by distinct mechanisms.
This result also suggests that there are B cell developmental stage-specific differences in the apoptotic machinery. For example, down-regulation of c-Myc alone by transfection of the inhibitory c-Myc dimerization partner dMax is sufficient to induce apoptosis in immature WEHI-231 cells (36), whereas c-Myc down-regulation in BU-11 pro/pre-B cells by dexamethasone does not induce apoptosis. Despite the decrease in c-Myc following DMBA treatment, BU-11 cells do not undergo growth arrest. Clearly, additional signals, such as those activated following dexamethasone treatment, are required. In WEHI-231 cells, increases in free calcium levels provide that second signal for growth arrest (32). The dissociation between a decrease in c-Myc and growth arrest in BU-11 cells differs from results obtained with melanoma (37), monomyeloid (38), ovarian carcinoma (39), Burkitt’s lymphoma (40), and acute lymphoblastic leukemia (41) cells, in all of which growth arrest is concomitant with a decrease in c-Myc.

p27Kip1 is a cyclin-dependent kinase inhibitor that can block cyclin E and cyclin A activity while positively regulating cyclin D activity (42). In addition to the traditional view of p27Kip1 as a

FIGURE 5. Expression of p27Kip1 is not up-regulated in pro/pre-B cells after DMBA or dexamethasone treatment. A, BU-11 cells were cultured on BMS2 stromal cells and treated for 24 h with vehicle, 0.1 μM DMBA, 1 μM DMBA, or 10 μM dexamethasone for 24 h. WEHI-231 cells were treated with vehicle or 10 μM dexamethasone as a positive control for p27Kip1 induction. Cells were lysed, total protein was extracted, and 30 μg was loaded into SDS-polyacrylamide gels for p27Kip1-specific immunoblotting. Blots were stripped and reprobed with β-actin-specific Ab to control for loading errors. B, p27Kip1 band densities were normalized to β-actin levels and then compared with p27Kip1 levels in vehicle-treated cells. Data are presented as the average percent p27Kip1 levels ± SE from five experiments. *, A significant decrease in p27Kip1 levels relative to vehicle controls, p < 0.05. C, WEHI-231 cells were treated with 10 μM dexamethasone and protein was extracted 24 h later for p27Kip1 and β-actin expression by immunoblotting. p27Kip1 band densities were normalized to β-actin levels and then compared with p27Kip1 levels in vehicle-treated cells. Data are presented as the average percent p27Kip1 levels ± SE from four experiments. *, A significant increase in p27Kip1 levels relative to vehicle controls, p < 0.01.

FIGURE 6. Expression of p21WAF1 is not up-regulated in pro/pre-B cells after DMBA or dexamethasone treatment. A, BU-11 cells were cultured on BMS2 cells and treated with vehicle, 0.1 μM DMBA, 1 μM DMBA, or 10 μM dexamethasone for 24 h. Proteins from vehicle-treated WEHI-231 cells were used as a positive control for p21WAF1 detection. Cells were harvested and lysed, total protein was extracted, and 30 μg was loaded into SDS-polyacrylamide gels for p21WAF1-specific immunoblotting. Blots were stripped and reprobed with β-actin-specific Ab to control for loading errors. Data from a representative experiment (five experiments total) are presented. B, p21WAF1 band densities were normalized to β-actin levels and then compared with p21WAF1 levels in vehicle-treated cells. Data are presented as the average percent p21WAF1 levels ± SE from five experiments.
regulated of cell cycle, it has been shown recently to promote (43) or inhibit (44, 45) apoptosis. Because c-Myc represses p27Kip1 transcription (7) and p27Kip1 induction correlates with WEHI-231 cell apoptosis (6, 32), it was expected that the decrease in c-Myc would be accompanied by an increase in p27Kip1. However, treatment of cocultures with DMBA actually decreased p27Kip1 levels, an observation that could reflect the ability of caspase 8 (46) and/or caspase 3 (47, 48) to cleave p27Kip1. Both caspases 8 and 3 are active in this system (not shown). The functional significance of this decrease is not known, especially because it did not result in an increased rate of proliferation. However, there is significant precedent for a decrease in p27Kip1 expression in B and T cells during apoptosis induced with a variety of stimuli other than dexamethasone or Ag-specific receptor cross-linking (46). The mechanism through which p27Kip1 dysregulation may effect apoptosis is not known. However, its requirement for assembly of elements critical to cell function, such as cyclin D (42), offers insights into potential areas of apoptosis regulation. Experiments are underway to determine whether the observed decrease in p27Kip1 is critical to DMBA-induced pro/pre-B cell apoptosis.

Although de-repression of p27Kip1 following a decrease in c-Myc does not appear to contribute to BU-11 cell apoptosis, other c-Myc-regulated genes may play a role. One such c-Myc target is ornithine decarboxylase (ODC). ODC is important in the production of cellular polyamines, the depletion of which results in apoptosis in several cell types, including WEHI-231 B cells (49–52). In some cases, depletion of constitutive ODC levels coincides with a decrease in c-Myc before cell apoptosis (52). As with BU-11 cell apoptosis induced with DMBA (data not shown), apoptosis induced by polyamine depletion in WEHI-231 cells is characterized by caspase 3 induction and changes in mitochondrial membrane potential (49). Furthermore, there are c-Myc-independent pathways to polyamine synthesis, e.g., through S-adenosylmethionine decarboxylase or spermidine synthase, ectopic expression of c-Myc would be expected to only partially protect cells from apoptosis induced by polyamine depletion.

In a fashion similar to p27Kip1, p21WAF1 is cleaved by caspase 3 during apoptosis. The subsequent reduction in p21WAF1-regulated Cdk2 activity results in endothelial cell apoptosis (53). The opposite effect, e.g., a p34-mediated increase in p21WAF1, induces apoptosis in immature B cells (8). However, neither of these apoptosis pathways appear to be used in BU-11 cells following DMBA exposure, because little or no p21WAF1 was detected in either untreated or DMBA-treated cultures, and because p53 is up-regulated, rather than down-regulated, after DMBA treatment (Fig. 7). This up-regulation may reflect a DNA damage response subsequent to formation of DMBA metabolite-DNA adducts (54). Similarly, p21WAF1 appears not to play a part in the growth arrest observed following dexamethasone treatment of BU-11 cells, despite its contribution to dexamethasone-induced growth arrest in lung alveolar epithelial cells (55).

Finally, experiments with a number of soluble (e.g., TNF-α, TGF-β, and IFN-γ) or cell-associated (e.g., Fas ligand) death factors have not revealed the nature of the PAH-induced, stromal cell-derived death factor(s). However, our ongoing studies indicate that the effects of a stromal cell-derived death signal may be generalizable to other hemopoietic cell types. For example, murine thymocytes readily undergo apoptosis when cocultured with bone marrow stromal cells and PAHs (DMBA or B(a)P) (data not shown). Furthermore, in vivo administration of DMBA or B(a)P results in a rapid depletion of virtually all hemopoietic cell types in murine bone marrow (16). Hence, analysis of the apoptosis pathway induced by PAH in pro/pre-B cells is important for determining how these and other environmental chemicals compromise hemopoiesis in general and how apoptosis contributes to early B cell development under normal physiologic conditions.

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References


