Cell Cycle Response to DNA Damage Differs in Bronchial Epithelial Cells and Lung Fibroblasts

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ABSTRACT

Epithelial cells along the conducting airways can be more or less continuously exposed to DNA-damaging agents, which should limit their proliferation by inducing cell cycle checkpoints. Yet, paradoxically, airway epithelial cells frequently show a hyperplastic response when exposed to such agents. In this in vitro study, we assessed the hypothesis that normal human bronchial epithelial cells (BECs) are more resistant to the cell cycle-arresting effects of DNA damage than are human lung fibroblasts (HLFs), a cell type often investigated in the context of cell cycle checkpoints. Using ionizing radiation as a DNA-damaging insult, we have found that BECs indeed show less pronounced G1 and G2 delays than do fibroblasts. Unlike the HLFs, which ultimately enter a condition of apparently terminal arrest in the G1 phase of the cell cycle, BECs continue proliferating following their initial, transient G1 and G2 delays. Radiation-induced p53 and p21(WAF1/CIP1) increases were greater in HLFs than in BECs, whereas preexposure, basal levels of p53 were higher in BECs than in HLFs. The results of this investigation indicate that BECs may be less susceptible to the cell cycle-arresting effects of DNA-damaging agents, perhaps because of their higher basal levels of p53. Extension of these findings to the in vivo condition provides a possible explanation for airway epithelial cell hyperplastic responses that occur in a background of DNA-damaging stresses. Moreover, the attenuated DNA damage-induced, cell cycle checkpoint responses in BECs potentially may favor the transmission of DNA lesions to cell progeny.

INTRODUCTION

Epithelial cells lining the conducting airways of the lower respiratory tract are exposed to particulate and gaseous contaminants contained in inhaled air, with the variety of exposure materials and the extent of exposure being dependent on the environmental setting. Many inhaled pollutants, e.g., numerous tobacco smoke constituents, ozone, nitrogen dioxide, and α particle-emitting radon/ radon progeny, have been shown to cause DNA damage, including DNA strand breaks, by direct and/or indirect mechanisms (e.g., see Refs. 1–10). Additionally, airway epithelial cells can be exposed to other DNA-damaging agents that arise from “normal” lung defense mechanisms and biochemical pathways associated with injurious cellular responses. As examples, superoxide anions and hydrogen peroxide, which cause DNA strand breaks and other DNA lesions (e.g., see Refs. 11–13), are produced by intraluminal and infiltrating macrophages, polymorphonuclear leukocytes, and eosinophils in response to a range of stimuli (14, 15), and several frankly clastogenic materials are produced commonly during inflammatory and injurious responses induced by environmental stresses, e.g., the cytokine tumor necrosis factor-α (16) and 4-hydroxynonenol, an aldehydeic end product of lipid peroxidation (17). Moreover, airway epithelial cells themselves evidently can produce reactive oxygen metabolites in response to various substances, e.g., cigarette smoke and ozone (18–21). If not repaired with complete fidelity, DNA damage arising from sources such as those cited above presumably may contribute to mutations in cell cycle-regulating genes and their products and/or the onset of a genomically unstable condition in a manner ultimately favoring uncontrolled cell growth and the emergence of neoplastic phenotypes.

Responses of normal cells to DNA damage can take on many forms. These include: (a) apoptosis or programmed cell death (e.g., see Ref. 22); (b) the induction of a nonproliferating, “senescence-like” condition in which cells ultimately appear to be maintained in G0-G1 (23–25); (c) differentiation (e.g., see Ref. 26); and (d) delays or arrests in the G1 and G2 phases of the cell cycle (27–30). With regard to (d), DNA damage-induced delays in the cell cycle, or what are commonly referred to as cell cycle checkpoints, are thought to provide cells with additional time to repair their damaged DNA before further cell cycle exposure, so that the probability of transmittal of DNA abnormalities to daughter cells is diminished (31–34). Delay or arrest in G1 phase after exposure to DNA-damaging insults is characteristic of cells that contain a wild-type p53 pathway (31–34).

Given the cell cycle-disturbing effects of DNA-damaging agents observed in vitro, one might expect that exposure to such stresses would curtail cell proliferation in vivo as well. Yet, this does not appear to always be the case, at least in terms of the proliferation of bronchial epithelial cells. As examples, it has long been known that acute or chronic exposure to DNA-damaging cigarette smoke results in a proliferation of the bronchial epithelium (35, 36) and that an enhanced state of BEC proliferation occurs as well with a range of other inhaled pollutants that would be expected to directly or indirectly damage DNA (e.g., see Refs. 36–38). Along this same line, Taya et al. (39) have reported that exposure of rats to α particle-emitting radon and radon progeny, which would be expected to cause both single- and double-strand DNA breaks (40) in cells along the respiratory tract, actually increased the proliferation of BECs. However, we have obtained the opposite result with human fibroblasts, in that we have shown that α particles quite capably cause delays and arrests in both G1 and G2 phases of the cell cycle in vitro, with the G1 cell cycle perturbations appearing to be mediated by the p53 pathway (30). Such observations suggest that BECs may be less sensitive to the cell cycle delaying effects of DNA damage compared to other cell types that have been much more well studied in this context, e.g., fibroblasts and lymphocytes. In the present study, we examined this possibility by comparing the cell cycle perturbations that occur in normal human BECs in response to a DNA-damaging stress, ionizing radiation, with those that occur in normal HLFs. Evidence presented herein suggests that BECs have less pronounced and shorter-lived G1 and G2 cell cycle-delaying responses to a DNA-damaging insult than do lung fibroblasts. Although elucidation of the mechanistic bases for these differences requires additional study, the attenuated G1-arresting response in the BECs, at least, was found to correlate with a higher basal level of cellular p53 and comparatively lesser p53 accumulations following exposure to ionizing radiation than what was observed with the lung fibroblasts. Our findings, accordingly, suggest that the epithelial proliferative response

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observed in the conducting airways that can occur in a background of exposure to DNA-damaging insults may be related to the cell cycle-delaying characteristics of the epithelial cells. Our results also suggest the possibility that airway epithelial cells are more prone to transforming events by DNA-damaging agents because their limited checkpoint responses may not provide sufficient time for DNA repair.

MATERIALS AND METHODS

Cell Cultures and Irradiations. Diploid fibroblasts from human fetal lung were obtained from American Type Culture Collection (clone CCL 153; Rockville, MD). Mycoplasma-free HLFs from passages 15–18 were cultured at 37°C under 5% CO2-95% air in α-MEM (Life Technologies, Inc., Gaithersburg, MD) supplemented with 10% fetal bovine serum (HyClone Laboratories, Inc., Logan, UT), 100 μg/ml streptomycin sulfate (Life Technologies, Inc.), and 100 units/ml penicillin (Life Technologies, Inc.). Normal human BECs, lineage NHBE 2169 from an 8-year-old male donor and lineage NHBE 2129 from a 15-year-old male donor, were obtained from Clonetics (San Diego, CA). Passage 3–5, Mycoplasma-free BECs were cultured at 37°C under 5% CO2-95% air in BEC growth medium (BEGM; Clonetics).

Subconfluent, proliferating cultures were irradiated with a Mark I high-dose rate 17Cs Source Chamber Irradiator (J.L. Shepherd and Associates) for the γ-ray exposures. The instrument delivers a beam of 0.66 MeV γ-rays. Cells were irradiated with 0 or 2 Gy of γ-rays at a dose rate of 99 cGy/min at room temperature; the 2-Gy exposure dose induces about 68 double-strand breaks and approximately 20 times that number of single-strand breaks per G1 genome (41, 42). For the cell cycle analyses, medium was replaced with culture medium containing 5 μM BrdUrd immediately after irradiation.

The data shown in this report are representative of results obtained from experiments that were performed three or more times with the NHBE 2169 lineage of BECs. We did not attempt to average data obtained from all experiments because of the variability that occurs as a result of even slight variations in initial cell cycle starting conditions that are encountered with subculturing primary cells. Instead, the numerical data presented portray directional results that were obtained consistently in all experiments. It should be noted that the cell cycle patterns for the NHBE 2169 cells shown here were observed similarly in preliminary studies undertaken with the NHBE 2129 lineage.

Flow Cytometric Analyses of the Cell Cycle. Cells were harvested at 8, 25, and 48 h after the irradiations using trypsin. The protocol used to stain the cells for the flow cytometric analyses of DNA content and incorporation of BrdUrd has been described elsewhere (30, 43). Briefly, cells were fixed in 70% ethanol and stained at room temperature for a minimum of 30 min in PBS containing 0.5 μg/ml HO (Calbiochem, San Diego, CA), 5 μg/ml MI (Pfizer Corp., Groton, CT), and 5 mM MgCl₂ at a cell concentration of 7.5 × 10⁶ cells/ml.

A two-laser flow cytometer (44) was used for the cell cycle analyses, with one laser operating in the UV (333.6–363.8 nm) and one tuned to 457.9 nm. The lasers were separated by 250 μm to provide sequential excitation and analysis of each fluorochrome (i.e., HO and MI, respectively). HO emissions were measured over a 400–495-nm range, and MI fluorescence was measured above 495 nm. The electronic gains were adjusted so that the G1 peaks of the HO-DNA and MI-DNA content histograms were initially in the same channel number. The fluorescence signals were then compared electronically on a cell-by-cell basis (45). Such comparisons reflect the quenching of the HO fluorescence by BrdUrd, which is proportional to cellular BrdUrd content in cells that had synthesized DNA during the labeling period (43).

For the p53 and p21Waf1 analyses, postirradiated and unirradiated cells were harvested by trypsinization and washed two times with PBS, and cell pellets were stored at −70°C until processed further. Frozen cell pellets were suspended in lysis buffer (50 mM Tris(hydroxymethyl)aminomethane (pH 7.9), 150 mM NaCl, 20 mM EDTA, 0.5% NP40, 20 μg/ml phenylmethylsulfonyl fluoride, 1 μg/ml aprotinin, 1 μg/ml leupeptin, 1 μg/ml pepstatin A, 10 μg/ml soybean trypsin inhibitor, 20 mM sodium fluoride, 20 mM β-glycerophosphate, 0.1 mM sodium orthovanadate, and 1 mM DTT) and incubated for 30 min at 4°C. The supernatants were collected after centrifugation at 14,000 × g for 30 min at 4°C. For direct immunodetection, proteins in supernatants from equal numbers of cells were resolved on SDS-polyacrylamide gels and transferred to nitrocellulose. Porcine S staining of the membranes ensured that equal amounts of protein were loaded and transferred in all lanes. Proteins were detected using mouse monoclonal antibodies to the DO-1 epitope of p53 (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) or to p21Waf1 (Oncogene Science, San Diego, CA) and horseradish peroxidase-conjugated secondary antibodies (Amersham Corp., Arlington Heights, IL) in conjunction with chemiluminescence reagents (Amersham; Ref. 30).

RESULTS

Early Cell Cycle Perturbations after Exposure to γ-Rays. Flow cytometric analysis of BrdUrd-labeled cells was used to characterize the cell cycle disturbances that occur in normal HLFs and normal BECs after exposure to γ-irradiation. With the DNA labeling and flow cytometric techniques used in this study, cycling cells can be distinguished from noncycling cells, because the HO fluorescence is quenched stoichiometrically by the incorporation of BrdUrd into DNA synthesized during cell culture (43), as indicated previously. MI fluorescence, on the other hand, is only slightly altered by BrdUrd incorporation into DNA. Accordingly, BrdUrd− cells can be resolved from BrdUrd+ cells in bivariate histograms of HO fluorescence versus MI fluorescence (e.g., see Fig. 1A, BEC sample, 0 Gy, 8 h postirradiation), and the percentage of labeled and unlabeled cells in each phase of the cell cycle can be quantitated by appropriate computer analysis (46).

Using this method, generally similar cell cycle perturbations were observed in BECs and HLFs as of 8 h after irradiation (Fig. 1A). By this earliest time point, the percentages of cells in G2 of the cell cycle increased substantially in both the HLF and BEC populations (Fig. 1A, G2, boxes). In this regard, computer analyses indicated that the percentage of cells in G2-M in the irradiated HLFs and BECs had increased ~1.6-fold and ~1.8-fold above control (0 Gy) levels, respectively. However, virtually all of the HLFs present in G1 at 8 h after irradiation were unlabeled with BrdUrd, whereas ~11% of the BECs in the G1 compartment were BrdUrd− (Fig. 1A, BEC LG, box). Hence, some BECs were able to complete S phase and G2-M traversal as of 8 h after exposure to the γ-rays; such findings are consistent with a shorter radiation-induced G2 delay in BECs than in HLFs. Both the BEC and HLF populations showed substantial depletions in the percentages of cells in S phase at 8 h after irradiation, particularly in the numbers of cells in early S phase (Fig. 1A, arrows). These decreases in early S-phase cells, along with the presence of predominantly BrdUrd− cells in the G1 phase of the cell cycle, indicate that both normal BECs and HLFs delayed in G1 phase after γ-irradiation. However, there were more BECs in early S phase compared to HLFs, suggesting that radiation did not cause BECs to block as tightly in G1 as did HLFs.

Later Cell Cycle Perturbations. Although the early cell cycle responses of BECs and HLFs to γ-irradiation shared some similarities, especially their G1−delaying responses, notable distinctions in the cell cycle responses of the γ-rays occurred at later times in the two different cell types. By 25 h after irradiation, a near-normal percentage of BrdUrd− BECs was present in the G1 compartment (Fig. 1B, LG1, boxes; Table 1), because cells that were delayed previously in G1 or G2 reentered the cell cycle. That the BECs capably continued to proliferate after the initial radiation-induced G1 and G2 delays was well revealed by the occurrence of BECs in the subsequent S phase (Fig. 1B, arrows) and in the latest G1 phase (Fig. 1B, LG2, boxes). The pattern of BrdUrd incorporation in HLFs at 25 h after irradiation, on the other hand, deviated markedly from that of the BECs. First, ~63% of the irradiated HLFs in G1 remained BrdUrd−, whereas only ~25% of the irradiated BECs in G1 were BrdUrd− at this postirradiation time (Table 1). Thus, a substantial percentage of HLFs that were originally in G1 at the time of exposure to the γ-rays failed to leave G1 phase after irradiation. Secondly, although many of the HLFs that were delayed initially in G2 subsequently divided and entered the G1 phase as indexed by the occurrence of BrdUrd+ cells in G1, they then
rearrrested in the G1 phase of the cell cycle. This conclusion is supported by a lack of S-phase cells in the irradiated HLF culture compared to the unirradiated HLF control (Fig. 1B, arrows) and by the low number of labeled G2 cells in the latest G2 phase of the irradiated HLF cultures (Fig. 1B, LG2 boxes). Although BrdUrd* G2 cells were present in the irradiated HLF culture, the flow cytometrically resolved patterns of such labeling indicated that these cells originated predominately from the previous cell cycle (Fig. 1B).

As described above, the BECs showed initial G1 and G2 delays after irradiation, but the majority of the cells commenced proliferation by 25 h after irradiation. Like the BECs, the HLFs were found to delay in both the G1 and G2 phases at early times postirradiation, but then most of the HLFs that were originally in G1 remained in G1, and irradiated HLFs that delayed in G2 divided and then rearrested in the following G1 phase. These response patterns continued up to the 48-h postirradiation time point (Fig. 1C; Table 2). By this time, most of the G1 cells in the unirradiated BECs, the irradiated BECs and the unirradiated HLF cultures were BrdUrd* (Table 2), and the pattern of G1 BrdUrd labeling was resolvable into two regions that denote the passage of cells through two cycles of DNA synthesis (Fig. 1C, double arrows). However, only 44% of the irradiated HLFs present in G1 were BrdUrd* (Table 2), with the pattern of BrdUrd incorporation occurring in only one area of the lower BrdUrd-labeling region, i.e., the region of higher HO fluorescence (Fig. 1C), because only one round of DNA replication was completed after exposure of the HLFs to the γ-rays. This, along with a lack of cells in S phase in the irradiated HLF samples, shows that the HLFs remained arrested in G1 phase even at 48 h after irradiation, whereas the BECs continued to proliferate until approaching confluence. It should be noted that the prolonged and apparently terminal arrest of the HLFs in G1 following exposure to ionizing radiation has been observed with other human fibroblast types as well (24, 25).

**p53 and p21Cip1 Expression in BECs and HLFs after Exposure to γ-Rays.** It is well recognized that p53 can be induced by γ-irradiation and several other DNA-damaging stresses and that p53 is involved in mediating delays in the G1 phase of the cell cycle in response to DNA damage (29, 31, 32), especially DNA strand breaks (47). As a mediator of G1 delays, p53 is functionally a transcription factor for p21Cip1, which forms inhibitory complexes with many different cyclins and their associated cdks (33, 48–50) and binds with proliferating cell nuclear antigen, a replication factor (51). As described earlier, both BECs and HLFs delayed initially in G1 after γ-irradiation, but a comparatively much higher percentage of HLFs ultimately and evidently permanently arrested in G1, whereas BECs were capable of reentering the cell cycle. Conceivably, these dissimilar cell cycle responses to low linear energy transfer ionizing radiation might be due to differences in the extents of p53 accumulations and further downstream p21Cip1 expression in the two cell types. Accordingly, we assessed whether p53 and p21Cip1 are commonly up-regulated in BECs and HLFs to the same extent in response to γ-ray exposure. Because p53 is induced within hours after γ-irradiation and subsides thereafter (52), we irradiated cells and collected whole-cell extracts at 1, 2, and 4 h after the exposures for assessment of their p53 and p21Cip1 phase of the cell cycle are illustrated in the bivariate histogram of the unirradiated BEC sample as follows: UG1, BrdUrd* (unlabeled) G1-phase cells; LG1, BrdUrd* (labeled) G1-phase cells; LS, BrdUrd* (labeled) S-phase cells; UG2, BrdUrd* (unlabeled) G2 cells; and LG2, BrdUrd* (labeled) G2 cells. Arrows, decreasing numbers of early S-phase cells following exposure to the γ-rays. A, 8 h postirradiation. Arrows, lack of S-phase cells in the irradiated HLF samples compared to the control BECs, control HLFs, and the irradiated BECs. LG1 and LG2 are as in A, 48 h postirradiation. Open arrows refer to the large number of HLFs that never left G1 phase after irradiation compared to the control HLFs. Double arrows, BrdUrd* G1 cells. The top arrow in each pair points to BrdUrd* G1 cells derived from the first round of DNA replication. The bottom arrows in each pair points to BrdUrd* G1 cells from the second round of DNA replication (irradiated HLFs do not contain cells in this region).
Table 2. BrdUrd Labeling of G1-phase HLFs or BECs at 48 h after exposure to 2 Gy γ-rays

<table>
<thead>
<tr>
<th></th>
<th>Total % G1</th>
<th>% BrdUrd+</th>
<th>% BrdUrd−</th>
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<tbody>
<tr>
<td>HLF, 0 Gy</td>
<td>88</td>
<td>93</td>
<td>7</td>
</tr>
<tr>
<td>HLF, 2 Gy</td>
<td>81</td>
<td>44</td>
<td>56</td>
</tr>
<tr>
<td>BEC, 0 Gy</td>
<td>91</td>
<td>93</td>
<td>7</td>
</tr>
<tr>
<td>BEC, 2 Gy</td>
<td>85</td>
<td>86</td>
<td>14</td>
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p21Cip1 contents by Western analyses. As shown in Fig. 2, p53 increased substantially in HLFs as of 1 h after exposure to the γ-rays. The increase in p53 peaked by 2 h and then decreased to an intermediate level by 4 h postirradiation. In contrast, only minor increases in p53 were observed in BECs at 1 and 2 h after γ-irradiation, and such increases subsided to control levels as of 4 h after exposure (Fig. 2).

γ-Rays also caused an increase in p21Cip1 protein in HLFs, but the maximal expression of p21Cip1 occurred 4 h after irradiation in a manner consistent with a p53 transcriptional up-regulation of p21Cip1 (Fig. 3). Much of the p21Cip1 appeared as a faster migrating protein band in the irradiated HLF samples; we have also observed this faster migrating band of p21Cip1 in γ-irradiated human skin fibroblasts (30). Additional experiments are under way to determine whether the faster migrating band is a phosphorylated form of p21Cip1 or, perhaps, another protein that shares a common epitopic feature with p21Cip1. Regardless, unlike with the HLFs, p21Cip1 was not induced appreciably in BECs in response to the γ-rays (Fig. 3), which is consistent with the relatively low amount of p53 induction in this cell type.

Basal Levels of p53 in BECs and HLFs. Recently, Blattner et al. (53) have reported evidence suggesting that the amount of induction of p53 following exposure to DNA-damaging insults may be inversely proportional to the basal level of p53 in the cells. Therefore, we analyzed the basal level of p53 in HLFs and BECs to assess whether or not the basal levels of p53 in the two cell types might be directionally associated with their varying cell cycle responses to the γ-rays. In these experiments, protein extracts from equal numbers of either exponentially growing HLFs or BECs were resolved on a single polyacrylamide gel and transferred to the same nitrocellulose membrane to ensure identical photographic exposure conditions. Ponceau S staining of the membrane was used to ascertain that equal amounts of protein were being analyzed from each cell type. We found that the basal amount of p53 in proliferating HLFs was substantially lower than the basal level of p53 in cycling BECs (Fig. 4). These results collectively lend additional support to the possibility that the amount of induction of p53 in response to DNA damage is in some manner related to the preexisting or basal level of p53 in cells. Whatever the mechanism, our previously described findings suggest that the extent of p53 accumulations following DNA damage may play an important role in regulating the durations of cell cycle delays or arrests in G1, in that BECs with a lesser p53 response only transiently showed delay in G1, whereas the prolonged G1 arrest observed with HLFs was associated with a much more pronounced p53 response.

**DISCUSSION**

Epithelial cells in the conducting airways are more or less continuously exposed to inhaled materials and endogenous chemical species, many of which either directly or indirectly cause DNA damage, including cell cycle-perturbing DNA strand breaks. Rather unexpectedly, exposure of the airways to such agents results frequently in a proliferation of epithelial cells, as opposed to frank cell cycle arrests observed with other cell types in response to DNA damage in vitro (23, 24). This seemingly paradoxical response to DNA-damaging insults prompted us to hypothesize that BECs may be relatively less sensitive to the cell cycle delaying/arresting effects of DNA damage compared to other cell types, such as fibroblasts, which have received far more experimental attention in investigations of cell cycle checkpoints. In the present study, we used a relatively well characterized DNA-damaging stress, ionizing radiation, to compare how cell cycle perturbations induced by the stress may differ between BECs and lung fibroblasts. We have found that BECs indeed have less pronounced and shorter-lived G1 and G2 cell cycle-delaying responses to DNA damage than do lung fibroblasts, which arrest ultimately in G1 in an apparently terminal manner.

Presently, we can only speculate about the mechanism(s) that may underlie the differing cell cycle responses we have observed with lung fibroblasts and airway epithelial cells. Our results suggest that the extents of early p53 increases in response to DNA damage may play, in an as yet to be elucidated manner, decisive roles in the regulation of the durations and persistencies of cell cycle arrests, especially G1-S phase transitional events, that follow DNA injury. It is well recognized that p53 protein accumulates in the nucleus in response to damaged DNA and/or during the repair of damaged DNA (29), where it functionally serves as a transcription factor for p21Cip1 (50). p21Cip1, in turn, mediates the G1 arrest by forming inhibitory complexes with numerous cdks (33, 48), e.g., cdk2/cyclin E, thereby precluding the hyperphosphorylation of pRb and the release of the E2F and E2F-like proteins that are required as transcription factors for further traverse into S phase (54). Another DNA...
replication inhibiting function of p53 may include interactions with replication protein A (RPA) in a manner that inhibits the binding of RPA to DNA (55). DNA damage-induced delays in G2, on the other hand, have been associated with an inhibition of p34^{cdk2} activity, which correlates with an accumulation of the tyrosine-phosphorylated form of p34^{cdk2} protein and, in some cell types, an aberrant expression of cyclin B (56, 57). Unlike the G1 checkpoint, a relationship between p53 and delays in G2 is presently somewhat less clear. Although cells that do not contain wild-type p53 retain the ability to arrest in G2 phase in response to DNA damage (27–29, 31), some evidence indicates an association between increased levels of p53 or an alternative splice form of p53 and G2-M arrests under some circumstances (e.g., see Refs. 58 and 59).

Conceivably, the cell cycle response differences seen in our study with BECs and HLFs may reflect metabolic and corresponding functional differences in the two cell types aside from cell type-specific variations in their cell cycle-regulating machinery. Within limits, p53 elevations in normal cells scale generally with the extent of DNA damage (e.g., see Ref. 60). In our investigation, we used a standard DNA-damaging stress that presumably would cause, with all other variables being equal, the same extent of DNA strand breaks in the target cells. However, the magnitude of p53 increases in the HLFs, which showed the more prolonged and persistent cell cycle arrests, was higher than in irradiated, more transiently cell cycle-delaying BECs. We did not measure the extents of DNA damage in the BECs and HLFs after exposure to the γ-rays in our study. Nevertheless, the observed differences in the p53 responses suggest that the level of initial DNA damage in the two cell types may have differed or, perhaps, the repair of the damaged DNA in the two cell types varied kinetically. On the other hand, the differences in the p53 responses by the two cell types may be due to relative differences in the abilities of BECs and HLFs to mount a p53 response to a common level of DNA damage. Regardless, consistent with our in vitro findings of limited p53 accumulations in BECs in response to DNA-damaging ionizing radiation, no evidence for an up-regulated accumulation of p53 was found in airway epithelial cells of chronic cigarette smokers (61), in whom DNA damage would be expected to be essentially continuously ongoing.

Little information is currently available about how DNA damage by a common insult may vary in severity or how the repair of such damage may contrast in BECs and lung fibroblasts. In an earlier study, Fornace et al. (62) examined the repair of X-irradiation-induced single-strand DNA breaks in BECs and fibroblasts and found that both cell types generally repaired this form of injury equivalently. However, the exposure dose in that study was markedly higher, i.e., 50 Gy, than in our study (2 Gy). Hence, the exposure dose used by Fornace et al. may have precluded the detection of kinetic differences in DNA repair that might be observable at lesser radiation doses. Moreover, their investigation did not assay for the repair of DNA double-strand breaks, which are most prominently involved in inducing p53 accumulations (47). In addition to causing p53 increases, unrejoined breaks are thought to be the signal for G2-phase delays (63–66). Consistent with lesser initial DNA damage and/or more rapid repair, the BECs in our study showed delays in G2-M that were of lesser duration than those occurring with irradiated HLFs. Even so, additional study is required to determine whether differences in DNA damage repair or the initial level of DNA damage in the epithelial cells and fibroblasts in fact contribute to their differing cell cycle responses observed in this investigation. With regard to the latter, it remains possible that the differences may be related to the relative abilities of the two cell types to cope with an oxidative stress such as ionizing radiation. Unlike fibroblasts, for example, pulmonary epithelial cells are able to take up GSH from blood or tissue culture medium, and they also have high levels of γ-glutamyl transpeptidase, which allows them to salvage amino acids from extracellular GSH for intracellular GSH synthesis (67–69). Relatively higher levels of GSH in the BECs presumably could afford them greater protection against the DNA-damaging free radicals produced by ionizing radiation.

Finally, the possibility exists that the levels of initial DNA damage in the BECs and HLFs may not have differed significantly, but instead, the differing cell cycle responses were due to another variable, such as the basal levels of p53 that existed in the cells at the time of exposure to the DNA-damaging insult. Postexposure p53 and downstream p21^{Cip1} elevations in HLFs were much more pronounced than the corresponding responses seen in the more transiently arresting BECs. Consistent with the earlier findings by Blattner et al. (53), the magnitudes of the p53 responses in the two cell phenotypes, at least, appeared to be inversely related to the basal levels of p53 in each. If basal p53 amounts observed in vitro with the BEC lineage we examined reflect the in vivo condition, our results would suggest that airway epithelial cells may be less sensitive to the cell cycle-arresting effects of DNA-damaging agents than other cell types that may have lesser basal p53 levels. This possibility may have important implications in terms of why BECs are involved frequently in the development of bronchogenic carcinomas. It is widely believed that the G1 and G2 cell cycle checkpoints provide cells with extended periods of time to repair their damaged DNA before further traversing the cell cycle, so that DNA lesions are not fixed and passed on to cell progeny (29, 34). Conceivably, the relatively short G1 and G2 delays observed in BECs may not provide sufficient time for the complete repair of environmentally induced DNA damage before subsequent DNA and cell replication.

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REFERENCES


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