Apoptosis, Reproductive Failure, and Oxidative Stress in Chinese Hamster Ovary Cells with Compromised Genomic Integrity

Charles L. Limoli, Andreas Hartmann, Lee Shephard, Chin-rang Yang, David A. Boothman, Jim Bartholomew, and William F. Morgan

ABSTRACT

Chromosomal instability and persistent reproductive cell death show a significant correlation after cells are exposed to ionizing radiation. To examine the possible role of apoptosis in persistent reproductive cell death, we analyzed subsets of chromosomally stable and unstable clones for relationships between chromosome stability, reproductive integrity, and apoptosis. All clones were generated from the GM10115 cell line and derived from single progenitor cells surviving 10 Gy of X-rays, and all measurements were made ~60–80 generations after irradiation. The incidence of apoptosis, as measured by both annexin V binding of phosphatidylserine residues and terminal deoxynucleotidyl transferase labeling of DNA strand breaks, was significantly higher in chromosomally unstable clones than it was in chromosomally stable clones (P < 0.05; ANOVA and Student’s t test). Furthermore, statistical analyses of the biological end points of persistent reproductive cell death and apoptosis were consistent, showing R² values of 0.78 and 0.76, respectively. These results suggest that persistent reproductive cell death can, in part, be explained by the predisposition of a fraction of the clonal population to undergo apoptosis or necrosis. Immunological blot analyses of protein levels and DNA bandshift assays confirmed the mutant status of p53 in the host cell line, implying an apoptotic pathway that is independent of p53. Induction of apoptosis by agents such as actinomycin D, etoposide, and staurosporine and induction of necrosis by sodium azide were accompanied by an increase in the level of intracellular peroxy radicals and lipid peroxidation products, two independent end points that are typically associated with oxidative stress. Similar findings were observed in several subclones showing persistent apoptosis. These results suggest that the elevated levels of free radical damage that we detected were derived from the fraction of cells dying by apoptotic or necrotic processes. Possible mechanisms whereby oxidative stress may contribute indirectly to the perpetuation of chromosomal instability are discussed.

INTRODUCTION

The progression of cancer is believed to result, in part, from the accumulation of subtle genetic alterations that predispose an individual to develop the abnormalities commonly associated with cancer. Ionizing radiation is a tool with which to study carcino genesis by inducing the onset of many of these abnormalities within a reasonable time frame. A long history links radiation exposure and the elevated incidence of cancer (1), and the potential molecular mechanisms of radiation oncogenesis have recently been described (2). Not surprisingly, substantial interest has focused on the role of genomic instability in radiation carcinogenesis (3).

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One pathway that is believed to prevent cells from acquiring these so-called chaotic changes is apoptosis. Apoptosis involves a complex series of biochemical reactions that result in the eventual elimination of a cell from the proliferating population. This mode of cell death can be induced by various cellular, chemical, and physical agents and is regulated by intracellular enzymes acting on membrane, protein, and nucleic acid substrates (9). The role of the tumor suppressor gene p53 is important in promoting apoptosis in compromised cells (10). Many cell types that contain mutant versions of p53 are less likely to undergo apoptosis; thus, they are more likely to survive a given insult and acquire further deleterious change (11, 12). Although the precise molecular mechanisms by which p53 mediates apoptosis are not known, there are suggested links between p53, oxidative stress, and the production of reactive oxygen species (13, 14). However, the dependence of apoptosis on p53 is not absolute because apoptosis has been reported in p53 null and mutant cells (15). In the study reported here, we investigated apoptosis as a factor that not only contributes to the persistence of reproductive cell death but also influences the state of oxidative stress and the amount of free radical damage in irradiated cells mutant in p53.

MATERIALS AND METHODS

Cell Culture. All experiments used the human/hamster hybrid cell line GM10115, which contains one copy of human chromosome 4 in a background of 20–24 hamster chromosomes. The growth of these cells has been described (16). For X-irradiation, cells were exposed at a dose rate of 2.5 Gy/min at ambient temperature using a Philips RT250 X-ray machine (250 kVp, 15 mA; half-value layer 1.0 mm Cu).
Cell Populations. Three groups of GM10115 cells were studied: unirradiated control cell clones, clones of cells surviving 10 Gy of X-rays that showed no chromosomal instability, and clones surviving 10 Gy of X-rays that exhibited significant and persistent chromosomal instability. All of the chromosomally unstable clones used in this study had at least five distinct metaphase subpopulations of cells with rearrangements of human chromosome 4. Clones with fewer than three such subpopulations were considered chromosomally stable. Details concerning the isolation, expansion, determination of plating efficiency, and cytogenetic characterization of all subclones have been described in detail (17).

Measurement of Apoptosis. To determine whether GM10115 cells could be induced to undergo apoptosis in response to ionizing radiation, we exposed exponentially growing cells to 5 or 10 Gy of X-rays and then cultured the cells for 24, 48, or 72 h. To determine whether GM10115 cells could be chemically induced to undergo apoptosis, we exposed exponentially growing cells to staurosporine (8 μM), actinomycin D (50 μM), or etoposide (100 μM) for 24 h. Sodium azide (15 mM) was used over the same time period as a control for inducing necrosis, a nonapoptotic mode of cell death (18). Populations of cells treated under each condition were then examined for apoptosis by means of two assays: (a) annexin V-FITC binding of phosphatidylserine residues externalized to the outer leaflet of the plasma membrane and (b) addition of dNTP-FITC moieties to the 3’-hydroxyl termini of DNA strand breaks by TdT.

Annexin V Binding and TdT Labeling Assays. Logarithmic-phase cultures of all clones used in the annexin V binding and TdT labeling assays were harvested by incubating cells for 10 min at ambient temperature in a solution of versene [1 mM EDTA, 40 mM Tris (pH 7.5), and 150 mM NaCl]. This procedure constitutes a gentle harvest treatment that does not compromise cell membrane integrity. Annexin V binding and TdT labeling assays were performed using the ApoAlert Annexin V Apoptosis Kit (Clontech Laboratories, Palo Alto, CA) and the ApoTag in situ Apoptosis Detection Kit (Oncor, Gaithersburg, MD), following the instructions provided by the manufacturers. After each assay, cells were fixed for 15 min in 50 μl of 4% neutral buffered formalin, dropped onto glass slides, allowed to dry, covered with 20 μl of Antifade (Oncor), and fitted with glass coverslips. Slides were stored at 4°C until analysis by fluorescence microscopy using a Zeiss Axioskop microscope equipped with a dual-band pass FITC/Texas Red filter set.

Assessment of p53 Status. Nuclear extracts were prepared from cells exposed to various doses of ionizing radiation at selected times, after which immunoblotting and DNA bandshift assays were performed as described previously (19, 20). Briefly, immunoblot analysis of nuclear extracts was performed for alterations in p53 protein levels by using an antibody to p53 (DO-1, horseradish peroxidase-conjugated) purchased from Santa Cruz Biotechnologies (Santa Cruz, CA). Nuclear protein (10 μg) from unirradiated or X-irradiated cells was separated on denaturing 8% SDS-polyacrylamide gels, transferred to Immobilon-P membranes (Millipore, Bedford, MA), probed with the antibody against p53, and detected by enhanced chemiluminescence (Amersham, Arlington Heights, IL).

Electromobility supershift assays were performed by using an oligonucleotide constructed to contain a consensus binding site for wild-type p53: 5′-GATCCGGACATGCCCGGGCATGTCCG-3′. The 20 bases underlined is the consensus binding site for wild-type p53. These DNA oligomers were then end-labeled with 32P and incubated with nuclear extracts prepared from unirradiated and irradiated cells. p53 antibody was used to detect the supershifted p53-DNA complexes. Where applicable, DNA bandshift and immunoblot analysis of DNA bandshifts were quantified by PhosphorImager (Molecular Dynamics) or Bio-Rad Gel Doc analysis, respectively. All experiments were performed three times, and human melanoma cells known to contain wild-type p53 (U1-Mel) were used as positive controls in these assays (19, 20). Equal amounts of protein were loaded on the gels based on protein concentrations determined by Bradford assays of each nuclear extract sample.

Determination of Peroxy Radicals. Levels of intracellular peroxy radicals were assessed in clones by FACS analysis with the use of the dye H2DCFDA (Molecular Probes, Eugene, OR). Exponentially growing cultures of clones were trypsinized, counted, and resuspended in complete medium at 2 × 106 cells/ml. Cells were incubated with 10 μM H2DCFDA for 30 min at ambient temperature; after dye loading, cells were quenched on ice to minimize efflux of the dye until FACS analysis.

As positive controls for peroxy radical production, cells were preloaded with H2DCFDA and placed on ice, and then 100-1000 μM hydrogen peroxide was added 10 min before FACS analysis. As positive controls for apoptosis and necrosis, exponentially growing cells were treated with actinomycin D (50 μM, 16 or 24 h) or etoposide (100 μM, 24 h) to induce apoptosis or sodium azide (15 mM, 16 or 24 h) to induce necrosis. After these chemical treatments, cells were loaded with H2DCFDA dye and analyzed by FACS.

FACS analysis was performed with an instrument constructed basically according to the design of Steinkamp et al. (21). Signals from the photomultiplier were collected in an Oxford multichannel analyzer after analog-to-digital conversion and transferred into Microsoft Excel format for display and calculations.

Lipid Peroxidation Assays. Products of lipid peroxidation were measured colorimetrically by using a Lipid Peroxidation Assay Kit (Calbiochem, San Diego, CA), after which the extent of lipid peroxidation was determined by MDA and 4-HNE assays according to the manufacturer’s instructions. Total cellular content of MDA and 4-HNE was derived from standard curves generated on the day of assay, and all determinations were done in duplicate and corrected for the amount of protein in each sample.

Treatment of Cells with Xanthine/Xanthine Oxidase. Logarithmic-phase cells were resuspended in PBS containing 1 mM xanthine and then treated for 15 min with 0–5 × 10−2 units of xanthine oxidase (Sigma Chemical Co., St. Louis, MO) at 37°C. After treatment, cell survival assays, colony isolation, and potential chromosomal instability analysis were performed in selected clones as described previously (16).

RESULTS

Cytogenetic and Clonogenic Characterization. After exposure of cells to 10 Gy of X-rays, the status of chromosomal stability in the GM10115 subclones was assessed by fluorescence in situ hybridization of a labeled probe to the human chromosome. Details concerning the analysis of these clones has been published (17). As a group, unstable clones had significantly lower plating efficiencies than did both the irradiated but chromosomally stable clones and the unirradiated control cells (P < 0.05; ANOVA and Student’s t test).

Chemical and X-Ray Induction of Apoptosis. To investigate whether some fraction of the persistent reproductive cell death phenotype could be a consequence of the persistent manifestation of apoptosis, we conducted an extensive series of control experiments. GM10115 cells were indeed capable of undergoing apoptosis after exposure to X-rays or to a variety of chemicals (Table 1). Apoptosis was determined by assays for annexin V binding of phosphatidylserine residues and TdT labeling of DNA strand breaks. Treatment with sodium azide was accompanied by much lower levels of apoptosis, consistent with the observation that this agent induces necrotic cell death (18).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose/concentration</th>
<th>Assay time after treatment (h)</th>
<th>Annexin V-positive cells*</th>
<th>TdT-positive cells*</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Actinomycin D</td>
<td>50 μM</td>
<td>24</td>
<td>4.9</td>
<td>0.3</td>
</tr>
<tr>
<td>Etoposide</td>
<td>100 μM</td>
<td>24</td>
<td>41</td>
<td>26</td>
</tr>
<tr>
<td>Staurosporine</td>
<td>8 μM</td>
<td>24</td>
<td>56</td>
<td>6.5</td>
</tr>
<tr>
<td>Sodium azide</td>
<td>15 mM</td>
<td>24</td>
<td>56</td>
<td>50</td>
</tr>
<tr>
<td>X-rays</td>
<td>5 Gy</td>
<td>24</td>
<td>17</td>
<td>1.8</td>
</tr>
<tr>
<td>X-rays</td>
<td>3 Gy</td>
<td>24</td>
<td>14</td>
<td>2.8</td>
</tr>
<tr>
<td>X-rays</td>
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<td>X-rays</td>
<td>10 Gy</td>
<td>23</td>
<td>23</td>
<td>35</td>
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</table>

* A total of 1000 cells were scored.

The abbreviations used are: TdT, terminal deoxynucleotidyl transferase; FACS, fluorescence-activated cell sorting; H2DCFDA, 2′,7′-dichlorodihydrofluorescein diacetate; MDA, malonaldehyde; 4-HNE, 4-hydroxy-2(E)-nonenal; DCF, 2′,7′-dichlorofluorescein.
Analysis of Apoptosis in Chromosomally Stable and Unstable Subclones. Having established that apoptosis could be induced in GM10115 cells, we sought to determine whether apoptosis was persisting over multiple generations in clones surviving radiation exposure. Subclones of GM10115 cells were maintained in exponential growth and examined for apoptosis measured by the annexin V and TdT assays ~60 and ~80 generations after irradiation (Table 2). For individual clones, the amounts of apoptotic cells found at each of these time points were not significantly different. However, unirradiated controls and 14 chromosomally stable subclones had significantly fewer apoptotic cells than did the 12 chromosomally unstable subclones (P < 0.05; ANOVA and Student’s t test). The morphological appearance of apoptotic cells is shown in Fig. 1.

**Apoptosis and Persistent Reproductive Cell Death.** The correlation between plating efficiency and the two assays used to measure apoptosis is shown in Fig. 2. Correlation coefficients obtained between plating efficiency and annexin V binding (0.78) or TdT labeling (0.76) indicate an interdependence of these assays and, thus, an association between apoptosis and persistent reproductive cell death.

**p53 Protein Levels.** To investigate whether the observed apoptosis involved the p53 gene product, we used immunological blot analysis with an antibody probe directed against the p53 protein. GM10115 cells showed little change in p53 protein levels 2 and 12 h after cellular exposure to 0, 3, 6, or 12 Gy of X-rays (Fig. 3). This same antibody, however, when used to probe nuclear extracts prepared from human melanoma cells (U1-Mel) known to contain wild-type p53 (19, 20), detected higher basal levels of p53 protein and an increase in p53 protein levels 2 h after exposure to 9 Gy of X-rays (Fig. 3). Sample-to-sample variations in nuclear protein levels were accounted for by comparison with α-tubulin loading controls (data not shown).

**p53 Functionality.** To determine whether the absence of elevated p53 protein levels after irradiation was due to mutant p53, the functionality of p53 in GM10115 cells was investigated by means of a DNA bandshift assay. Nuclear extracts isolated from cells 2 and 12 h after exposure to 0, 3, 6, or 12 Gy of X-rays contained little, if any, p53 protein capable of binding the DNA and retarding its mobility through the gel (Fig. 4). In contrast, nuclear extracts from U1-Mel cells contained p53 protein proficient at binding DNA, as evidenced by the increase in apparent molecular weight of the DNA-p53 protein complex (Fig. 4).

**Intracellular Peroxy Radicals.** To examine the production of intracellular peroxy radicals, a measure of oxidative stress, we monitored DCF fluorescence (derived from the intracellular oxidation of H$_2$DCF, which, in turn, was derived from the enzymatic removal of acetate moieties from H$_2$DCFDA by cellular esterases). Using an agent known to lead to intracellular free radical damage, we observed a dose-dependent increase in the level of DCF fluorescence (as evidenced by a right shift in peak fluorescence) after GM10115 cells

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**Table 2.** Delayed apoptosis in chromosomally stable and unstable clones

<table>
<thead>
<tr>
<th>Clone no.</th>
<th>PE* (%)</th>
<th>Annexin V-positive cellsb (%)</th>
<th>TdT-positive cellsb (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>102</td>
<td>72</td>
<td>14</td>
<td>2.90</td>
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<tr>
<td>103</td>
<td>65</td>
<td>6.5</td>
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<td>110</td>
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<td>13</td>
<td>2.50</td>
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<tr>
<td>114</td>
<td>74</td>
<td>14</td>
<td>1.10</td>
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</tr>
<tr>
<td>130</td>
<td>57</td>
<td>12</td>
<td>4.30</td>
</tr>
<tr>
<td>132</td>
<td>69</td>
<td>18</td>
<td>1.50</td>
</tr>
<tr>
<td>133</td>
<td>84</td>
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</tr>
<tr>
<td>141</td>
<td>51</td>
<td>10</td>
<td>12.8</td>
</tr>
<tr>
<td>145</td>
<td>47</td>
<td>12</td>
<td>14.0</td>
</tr>
<tr>
<td>146</td>
<td>59</td>
<td>16</td>
<td>8.10</td>
</tr>
<tr>
<td>152</td>
<td>51</td>
<td>22</td>
<td>8.00</td>
</tr>
</tbody>
</table>

* PE, plating efficiency.

* A total of 1,000 cells were scored.

* A total of 6,000 cells from unirradiated GM10115 subclones were scored.

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Fig. 1. Morphological appearance of apoptotic cells detected by annexin V binding and TdT labeling, a and b, annexin V binding assay; c and d, TdT labeling assay; a and c, untreated control cells; b and d, cells assayed 72 h after exposure to 5 Gy of X-rays.
Lipid Peroxidation. To determine whether the observed apoptosis was associated with lipid peroxidation (another end point commonly linked to oxidative stress), we assayed cellular levels of MDA and 4-HNE. To ascertain the cellular response and sensitivity of the assay, we first treated control cells with various concentrations of H$_2$O$_2$ for 30 min at 34°C. Increasing the H$_2$O$_2$ concentration from 0.25 to 10 mM resulted in a concomitant increase in the levels of MDA and 4-HNE, indicating lipid peroxidation (Fig. 6a). Having established the response of the assay to a known oxidizing agent, we sought to determine whether those chemicals that induced apoptosis or necrosis would also elicit an increase in lipid peroxidation. As observed with intracellular peroxy radicals, cells treated with actinomycin D, etoposide, staurosporine, or sodium azide all showed a marked increase in levels of MDA and 4-HNE (Fig. 6a). Lipid peroxidation was also analyzed in several chromosomally stable and unstable subclones. As with intracellular peroxy radicals, several subclones exhibited elevated levels of MDA and 4-HNE (Fig. 6b).

Persistent Chromosomal Instability after Xanthine/Xanthine Oxidase Treatment. As a further investigation of whether perturbations to the oxidative state of cells would result in chromosomal instability, we treated cells with xanthine and xanthine oxidase, a system known to generate superoxide anions (22). Thirty clones isolated from treatments resulting in three logarithmic orders of cell kill and 20 clones isolated from treatments resulting in four logarithmic orders of cell kill failed to show persistent chromosomal instability (data not shown).

DISCUSSION

Chromosomally stable and unstable subclones of GM10115 cells with significant differences in plating efficiency (17) were used to address the hypothesis that a reduction in plating efficiency in the unstable clones was due to the persistence of apoptosis. Apoptosis was measured by the binding of annexin V to externalized phosphatidylserine residues and the labeling of DNA strand breaks by TdT. The reduced signal emanating from cells induced to undergo necrosis by sodium azide, compared to the signal observed after treatment with radiation or chemicals inducing apoptosis, suggests that these assays detect specific end points associated with apoptosis. We did not use the appearance of annexin V- and TdT-positive cells to distinguish qualitatively between treatments or between chromosomally stable and unstable cells; rather, we used these assays as quantitative measures of variations in the frequency of apoptosis induced by various agents or between distinct classes of subclones.

The induction of apoptosis through the use of X-rays and chemical treatments confirmed that GM10115 cells can apoptosis at early times after insult (24–72 h). This mode of cell death is, thus, a normal pathway for eliminating stressed or damaged cells. Furthermore, the cell clones analyzed were found to be undergoing apoptosis ~60 and ~80 generations after irradiation. Because apoptosis can occur days to
COMPROMISED GENOMIC INTEGRITY OF CHO CELLS

Fig. 4. Assessment of p53 functionality by DNA bandshift assay. 32P-labeled DNA oligomers containing a consensus binding site for p53 were incubated with nuclear extracts isolated from irradiated or unirradiated GM10115 or U1-mel cells at various times after irradiation.

Fig. 5. Detection of intracellular peroxy radicals. a, chemical-mediated induction of DCF fluorescence. Cells were treated with actinomycin D (50 μM), etoposide (100 μM), or sodium azide (15 mM) for 16 or 24 h, loaded with H2DCFDA dye (10 μM; 30 min), and analyzed by FACS. b, DCF fluorescence in chromosomally unstable subclones. Exponentially growing clones were harvested, loaded with dye (10 μM; 30 min), and quenched on ice until FACS analysis. All results shown for FACS analysis represent the average of at least two independent experiments.

months after insult suggests a basis for the persistence of reproductive cell death after exposure to X-rays. Given the significant correlation obtained between chromosomal instability and the persistence of reduced plating efficiency in cells surviving irradiation (17, 23), our results suggest that persistent reproductive cell death can be explained in part by the predisposition of some fraction of the population to undergo apoptosis. It is unlikely that apoptosis can account entirely for the persistence of reproductive cell death, however. The de novo formation of lethal mutations (24, 25), chromosome aberrations incompatible with cell survival (17, 26, 27), and epigenetic factors such as the bystander effect (28, 29) are also likely to contribute to this persistent failure in reproductive integrity.

Analysis of cells induced to undergo apoptosis by treatment with chemicals and X-rays indicated that the externalization of phosphatidylinerine residues is a step that tends to precede the formation of DNA strand breaks. Although most clones showing relatively high levels of annexin V binding also showed relatively high levels of TdT labeling, some did not (i.e., LS12). The implications of this are uncertain but suggest that the cascade of events transpiring during the process of apoptosis may be disrupted in chromosomally unstable cells. These disruptions might further enhance interclonal variability in the expression of a given apoptotic end point and/or lead to differences in the manifestation of such end points over time.

The ability to induce apoptosis in GM10115 cells by treatment with several agents and the capacity of these cells to apoptose several generations after the initial insult prompted investigation of possible molecular mechanisms underlying this phenomenon. We were unable to detect increased levels of p53 protein after X-irradiation, suggesting that GM10115 cells contain a defect in p53 or a dysfunctional pathway regulating the p53 response to radiation. If GM10115 cells contain a mutant p53, the mutation is likely to be within the DNA-binding region of the protein, a region that is known to be hypermutable (10, 30). The relationship between oxidative stress and apoptosis was determined in a p53 mutant cell line suggesting, in parallel with recent reports (13, 14), that there are p53-independent pathways for modulating the redox state of a cell. It should be stressed that chromosomal instability induced by exposure of cells to ionizing radiation appears to be independent of the cells’ p53 status (31).
To understand other ramifications of apoptosis in our stable and unstable clones, we investigated whether oxidative stress could be construed as a process capable of driving persistent chromosomal instability and reproductive cell death. Oxidative stress has been proposed to be a determinant for apoptosis in a number of cellular systems (13, 14, 32, 33). When we treated cells with a series of nonoxidizing chemical agents known to induce either apoptosis or necrosis, we observed a time-dependent increase in the levels of intracellular peroxy radicals and lipid peroxidation products. Because the onset of induced cell death coincided with elevations in oxidative stress, we propose that the increased intracellular free radical damage we detected was derived from the fraction of cells destined to die. Further support for this idea comes from studies showing that the accumulation of peroxidation by-products is implicated in the loss of membrane function and integrity (13, 18, 32, 33), that increased levels of peroxides were found in cell populations containing apoptotic cells (13, 32, 34), and that increased peroxide production did not precede the onset of apoptosis but rather coincided with it (13). It is important to emphasize that, in these studies, the persistent increase in oxidative stress was measured 60–80 generations after radiation exposure. Furthermore, oxidative stress in chromosomally unstable clones was also related to lower plating efficiencies and higher fractions of annexin V- and TdT-positive cells.

We have demonstrated that H₂O₂ (16) or superoxide anions do not induce chromosomal instability, suggesting these reactive oxygen species may not be a major factor in initiating this phenotype. However, Clutton and coworkers (35) have implicated oxidative stress as a mechanism involved in maintaining genomic instability after cellular exposure to radiation. The results presented here indicate that, within some clones of cells surviving radiation exposure, subpopulations of cells are continuously being eliminated by apoptosis or necrosis. This can contribute to the well-characterized phenotype of lethal mutations or persistent reproductive cell death (24, 27). These dying cells can also contribute to the increased levels of intracellular peroxy radicals and lipid peroxidation end products associated with oxidative stress. We point out that oxidative stress is not always lethal.
nor is it only detected in chromosomally unstable clones. We suggest that any population of cells containing a subpopulation undergoing apoptotic or necrotic elimination has the potential to show increased levels of damage indicative of oxidative stress. Thus, dying cells represent a potential source of oxidative stress, in which one ramification of dying cells within a viable population may include further compromise to the fraction of cells destined to proliferate.

Reconciling a mechanism whereby oxidative stress serves to directly perpetuate chromosomal instability is difficult, especially in light of the limited diffusion distances imposed on the reactive oxygen species mediating the biological effects of oxidative stress by their inherently short half-lives (<1 s; Ref. 4). However, oxidative stress may serve to indirectly perpetuate chromosomal instability by the production of long-lived clastogenic factors (36, 37). Moreover, chronic exposure to reactive oxygen species and their byproducts may trigger changes in signal transduction, gene expression, or gap junction communications within a cell or alter the extracellular environment in ways that could influence the phenotype of chromosomal instability (3, 37). Because of the variable nature of genomic instability, it is possible that many of the biological endpoints associated with this phenomenon are governed by a state of chaos; this chaotic state, which a cell may enter after incurring genotoxic or cytotoxic stress, could perturb the basal regulatory and signaling pathways within a cell and disrupt cellular homeostasis.

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REFERENCES


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