Targeted and Nontargeted Effects of Low-Dose Ionizing Radiation on Delayed Genomic Instability in Human Cells

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Abstract
All humans receive some radiation exposure and the risk for radiation-induced cancer at low doses is based on the assumption that there is a linear non-threshold relationship between dose and subsequent effect. Consequently, risk is extrapolated linearly from high radiation doses to very low doses. However, adaptive responses, bystander effects, and death-inducing effect may influence health effects associated with low-dose radiation exposure. Adaptive response is the phenomenon by which cells irradiated with a sublethal radiation dose can become less susceptible to subsequent high-dose radiation exposure. Bystander effects are nontargeted effects observed in cells that were not irradiated but were either in contact with or received soluble signals from irradiated cells. These non-hit bystander cells can exhibit damage typically associated with direct radiation exposure. Death-inducing effect is a phenomenon whereby medium from human-hamster hybrid cells displaying radiation-induced chromosomal instability is toxic to unirradiated parental cells. In this study, we show that human RKO cells do not exhibit adaptive response, bystander effect, or death-inducing effect, as measured by cell killing, or delayed genomic instability in a stably transfected plasmid-based green fluorescent protein assay measuring homologous recombination and delayed mutation/deletion events. However, growth medium conditioned by some chromosomally unstable RKO derivatives induced genomic instability, indicating that these cells can secrete factor(s) that elicit responses in nonirradiated cells. Furthermore, low radiation doses suppressed the induction of delayed genomic instability by a subsequent high dose, indicative of an adaptive response for radiation-induced genomic instability. These results highlight the inherent variability in cellular responses to low-dose radiation exposure and add to the uncertainties associated with evaluating potential hazards at these low doses. [Cancer Res 2007;67(3):1099–104]

Introduction
Ionizing radiation has been described as a double-edged sword (1). There is considerable concern about the potential detrimental health effects associated with radiation exposure–induced cancers, yet radiation is widely used in cancer therapy. All humans receive some radiation exposure, mostly from natural sources such as cosmic rays and radioactive decay products of radon gas (2). The currently available data on risks associated with radiation exposure come from populations exposed to ionizing radiation, primarily from epidemiologic studies of Japanese A-bomb survivors and also from clinically exposed individuals and survivors of the Chernobyl accident (3, 4). However, those doses, in the range of 0.2 to 2.5 Sv, are much higher than the average human dose from natural sources estimated at 2 to 3.5 mSv/y (5). Risk estimates for radiation-induced cancer at doses <0.2 Sv are based on the assumption that there is a linear non-threshold dose-response relationship, with potentially detrimental health effects extrapolated from comparatively high doses to those doses associated with occupational or environmental exposures (6).

However, significant evidence has accumulated suggesting that risks associated with low doses of radiation might not strictly conform to a linear non-threshold dose-response relationship (7, 8). This evidence includes the influence of cellular responses to low-dose radiation (e.g., DNA repair; ref. 9) and a host of nontargeted effects associated with radiation exposure, including delayed reproductive death, bystander effects, death-inducing effect, and delayed genomic instability (10, 11). Delayed reproductive death is the general failure of the progeny of cells surviving irradiation to thrive and manifests as reduced plating efficiency of cells that survive radiation exposure (12, 13). Bystander effects include a host of effects, including induced micronuclei, chromosomal aberrations, mutations, and cell killing, which occur in unirradiated cells that were either in contact with irradiated cells or incubated with culture medium transferred from irradiated cells (14). Death-inducing effect is similar to bystander effect as it is seen in medium transfer experiments, but death-inducing effect is characterized by dramatically reduced survival of unirradiated cells by conditioned medium from cells showing radiation-induced chromosomal instability (15, 16). Thus, death-inducing effect is seen many generations after the initial radiation exposure; the cells producing death-inducing effect factor(s) are progeny of irradiated cells, and there is no radiation exposure of the transferred growth medium. Radiation-induced genome instability includes the delayed induction of large-scale chromosomal changes (chromosomal instability; ref. 10) and the recently discovered but mechanistically distinct induction of delayed homologous recombination. Cells displaying chromosomal instability usually display delayed reproductive death (17), but this is not true for cells displaying delayed homologous recombination, and cells displaying one type of delayed genomic instability does not necessarily display the other (18).

Nontargeted effects associated with radiation exposure are largely low dose phenomena (19). Another low dose effect is the “adaptive response”—a radioprotective effect that results in cells treated with low dose, with low dose rate radiation becoming
refractory to subsequent challenge with a high dose of radiation (20, 21). There is, however, considerable variability both in vitro and in vivo about whether or not low-dose irradiation will elicit an adaptive response (22). Thus, an adaptive response can protect cells from a subsequent high "challenge" dose of radiation, but other nontargeted effects like bystander effects and death-inducing effect tend to enhance the deleterious effects of radiation.

In this study, we describe several effects of low-dose ionizing radiation exposure. We show that human RKO cells do not display an adaptive response or bystander effects as measured by cell survival. However, an initial low dose of ionizing radiation does reduce the induction of delayed genomic instability by a subsequent high dose (i.e., RKO cells display an adaptive response for induction of delayed homologous recombination). We also show that medium transfer from RKO cells that display radiation-delayed chromosomal instability can induce delayed instability indirectly. However, unlike death-inducing effect, which we observed in human-hamster hybrid GM10115 cells, transfer of medium conditioned by unstable RKO cells does not affect cell survival.

These results highlight the complex and somewhat variable cellular responses to low-dose ionizing radiation and raise important questions about both protective and potentially detrimental effects associated with such exposure.

Materials and Methods

Cell culture. RKO36 cells are derivatives of RKO human colorectal carcinoma cells carrying a green fluorescent protein (GFP) direct repeat homologous recombination substrate (23). One copy of GFP is driven by the cytomegalovirus promoter but is inactivated by an XhoI linker frameshift mutation, and the second copy has wild-type coding capacity but is inactive because it lacks a promoter (23). GM10115 cells are human-hamster hybrid cells, and LS12 and Fe-10-3 cells are chromosomally unstable cell clones of GM10115 cells derived from single cells surviving exposure to ionizing radiation (24, 25). All cells were grown as monolayers in DMEM supplemented with 10% fetal bovine serum (HyClone, Logan, UT) incubated in an atmosphere of 5% CO2 in air.

Radiation-induced genomic instability as measured by mixed GFP colony formation. Genomic instability was evaluated in RKO36 cells by analysis of homologous recombination and mutation/deletion at the GFP direct repeats and as measured by the number of GFP+/− colonies in the total number of colonies scored. RKO36 cells comprise a heterogeneous mixture of both GFP+ cells and GFP− cells. When RKO36 cells are plated, colonies arising from single cells are either homogeneously colorless (GFP−, Fig. 1A) or green (GFP+, Fig. 1B), both of which reflect a stable homologous recombination substrate. A GFP− cell can be converted to GFP+ cell directly by radiation-induced homologous recombination producing a "pure" GFP+ colony. Similarly, a GFP+ cell can be converted to GFP− cell directly by radiation-induced point mutation or deletion. Thus, all pure GFP+ or GFP− colonies reflect either a prior stable phenotype or a new stable phenotype directly induced by radiation. However, if radiation induces delayed instability at the GFP direct repeats, mixed GFP+− colonies will arise (Fig. 1C and D). Such colonies can arise by delayed homologous recombination (GFP− → GFP+−) or delayed mutation/deletion (GFP+ → GFP−). Genomic instability, as measured by this GFP-based assay, is defined by colonies with both GFP+ and GFP− cells, with >10 cells of each type in a colony (23, 26). The frequency of induced instability was calculated as the number of GFP+− colonies per total surviving colonies scored.

X-irradiation. Cells were exposed to various doses of X-rays at ambient temperature using a Pantak HF320 X-ray machine. When cells were exposed to ≥1 Gy, X-rays were delivered at a dose rate of 2.4 Gy/min (250-kV peak, 13 mA; half-value layer, 1.65-mm copper). For doses <1 Gy, the dose rate was 51 cGy/min (100-kV peak, 5 mA).

Analysis of adaptive responses. Four experimental groups were established: (a) nonadapted, nonchallenged, untreated controls; (b) adaptive dose alone; (c) challenge dose alone; and (d) adaptive dose + challenge dose. Twelve hours before treatment, RKO36 cells were seeded into 10-cm-diameter dishes at a density of 200 per dish for groups A and B and 103 cells per dish for groups C and D. For groups B and D, dishes were irradiated with 1, 2, 5, or 10 cGy of X-rays; 4 h later, group C and D cells were exposed to challenge doses of 5 Gy. Cells were incubated for 10 to 14 days and colonies (>50 cells) were scored by fluorescence microscopy for the number of GFP+, GFP−, and GFP+− colonies. These same dishes were then stained with 2% crystal violet in 40% methanol to determine the surviving fraction, calculated as the ratio of the number of colonies obtained to the number of cells plated divided by the plating efficiency of nonirradiated cells.

Analysis of bystander effects by growth medium transfer. Potential bystander effects in RKO36 cells were investigated by medium transfer experiments as described by Mothersill and colleagues (27, 28). A range of doses (10 cGy–10 Gy) and different time intervals (30 min–24 h) between irradiation and medium transfer were used. RKO36 cells were grown to confluence in T75 flasks and irradiated. Thirty minutes to 24 h later, the medium was removed, filtered through a 0.2-μm cellulose acetate filter (Corning, Corning, NY), and transferred to 10-cm dishes containing 200 RKO36 cells seeded 12 h before medium transfer. Fresh medium, irradiated medium without cells, and medium from unirradiated cells (conditioned medium) served as controls. Colonies (>50 cells) were examined 10 to 15 days later for induced instability, as measured by GFP expression, and then stained with 2% crystal violet in 40% methanol to determine plating efficiency.

Analysis of death-inducing effect. RKO36 and seven chromosomally unstable derivatives (23) were grown to ~90% confluence. Fresh medium was then added and the cells were incubated for 48 h; this period of incubation was previously shown to be sufficient to produce death-inducing effect in GM10115 cells (16, 29). After the 48-h incubation, the medium was removed, filtered, and transferred to 10-cm dishes containing 200 RKO36 cells plated 12 h previously. RKO36 cells in fresh medium served as control. Cells were grown for 10 to 14 days and the resulting colonies (>50 cells) were scored as described above for genomic instability (mixed GFP colony formation) and plating efficiency.

Results

RKO36 cells show an adaptive response for radiation-induced delayed genomic instability but not cell survival. Low, nontoxic doses of radiation can increase cell survival after a subsequent high (challenge) dose, although such cell survival adaptive responses are not seen in all mammalian cell types (30). To determine whether RKO36 cells exhibit an adaptive response for cell survival, we treated cells with a low adapting radiation dose, 1 to 10 cGy followed by a 5-Gy challenge dose. The results of seven independent experiments are pooled and shown in Table 1. As expected, the 1 to 10 cGy doses had little or no cytotoxic effect. A single 5-Gy dose resulted in ~95% cell killing, and cell survival was not significantly changed when cells were pretreated with 1 to 10 cGy doses followed by a 5-Gy challenge dose given 4 h later. We varied the time interval between the initial low "adapting" dose and the challenge dose from 4 to 24 h, but this had no effect on the outcome (data not shown).

These results indicate that RKO36 cells do not elicit an adaptive response as measured by cell killing.

Delayed instability, as measured by GFP+− colonies, was induced in up to 4% of RKO36 cells at X-ray doses as low as 1 to 10 cGy. The frequency of this induced genomic instability increases with dose although, typical of nontargeted effects associated with ionizing radiation, the dose response was not linear (31). We were
therefore interested in whether RKO36 would display an adaptive response as measured by induction of GFP+/− colonies. To test this, we pretreated RKO36 cells with 1 to 10 cGy doses of X-rays, followed with a 5-Gy challenge dose 4 h later as described above. The results of these experiments are shown in Table 1. GFP+/− colonies were observed at frequencies of <1% in unirradiated cells, 3% to 4% in cells irradiated with 1 to 10 cGy, and <10% in cells irradiated with 5 Gy. Interestingly, cells pretreated with 1 to 10 cGy

<table>
<thead>
<tr>
<th>X-ray dose (cGy)</th>
<th>Plating efficiency ± SD</th>
<th>GFP+/− colonies ± SD (%)</th>
<th>Increase in GFP+/− colonies compared with control (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.00 ± 0.13</td>
<td>0.99 ± 0.11</td>
<td>—</td>
</tr>
<tr>
<td>1</td>
<td>0.87 ± 0.14</td>
<td>2.42 ± 0.32</td>
<td>2.49</td>
</tr>
<tr>
<td>2</td>
<td>0.93 ± 0.17</td>
<td>3.94 ± 0.46</td>
<td>4.07</td>
</tr>
<tr>
<td>5</td>
<td>0.93 ± 0.17</td>
<td>4.14 ± 0.52</td>
<td>4.28</td>
</tr>
<tr>
<td>10</td>
<td>0.98 ± 0.19</td>
<td>2.86 ± 0.46</td>
<td>2.96</td>
</tr>
<tr>
<td>1 + 500 (4 h)</td>
<td>0.023 ± 0.004</td>
<td>2.59 ± 0.24*</td>
<td>2.67*</td>
</tr>
<tr>
<td>2 + 500 (4 h)</td>
<td>0.028 ± 0.005</td>
<td>2.87 ± 0.14*</td>
<td>2.96*</td>
</tr>
<tr>
<td>5 + 500 (4 h)</td>
<td>0.028 ± 0.004</td>
<td>3.95 ± 0.42*</td>
<td>4.08*</td>
</tr>
<tr>
<td>10 + 500 (4 h)</td>
<td>0.025 ± 0.004</td>
<td>2.91 ± 0.08*</td>
<td>3.01*</td>
</tr>
<tr>
<td>500</td>
<td>0.038 ± 0.003</td>
<td>9.76 ± 1.43</td>
<td>10.08</td>
</tr>
</tbody>
</table>

NOTE: Values represent the mean of seven independent replicate experiments.

*p < 0.05 compared to 500 cGy, ANOVA one way analysis of variance.
and later challenged with 5 Gy showed significantly lower frequencies of GFP\textsuperscript{+/−} colonies than with a single 5-Gy dose ($P < 0.05$, one-way ANOVA). These results indicate that low doses of X-rays can protect RKO36 cells from genomic instability induced by a subsequent high dose of radiation. Our results further indicate that this novel adaptive response is independent of cell survival adaptive response.

In RKO36 cells, most GFP\textsuperscript{+/−} colonies are primarily GFP\textsuperscript{−} with different numbers of GFP\textsuperscript{+} cells reflecting delayed homologous recombination. However, delayed mutation or deletion will also produce GFP\textsuperscript{+/−} colonies in clonally expanded GFP\textsuperscript{+} cells. In contrast to the predominantly GFP\textsuperscript{+} mixed colonies, these events were comparatively rare. Thus, in the present study, the induction of GFP\textsuperscript{+/−} colonies results predominantly from delayed homologous recombination.

RKO36 cells do not display bystander effects for cell survival or delayed genomic instability. An interesting nontargeted effect of radiation is seen when growth medium transferred from irradiated cells causes killing of unirradiated cells as measured by reduced plating efficiency; this cytotoxic bystander effect is seen with some cell types but not with others (28). We wanted to investigate whether RKO36 cells would display a bystander effect after exposure to low LET X-rays. In light of the observed adaptive response for genome instability described above, we were also interested in whether medium transferred from irradiated RKO36 cells would enhance genome instability as measured by the induction of GFP\textsuperscript{+/−} colonies. We used doses ranging from 10 Gy to 10 Gy and postincubation intervals ranging from 0 to 24 h, but there were no observable bystander effects on transfer of filtered medium from irradiated RKO36 cells. Representative data with a 5-Gy dose and incubation times of 30 min and 2 h are shown in Table 2. The data indicate no differences in plating efficiencies with fresh medium, conditioned medium (unirradiated control), or irradiated medium in the presence or absence of cells. Furthermore, medium transfer had no cell-dependent effects on the frequency of genome instability as measured by either an increase or a decrease in the number of GFP\textsuperscript{+/−} colonies (Table 2). Thus, RKO36 cells do not display bystander effects related to cell survival or induced genomic instability.

Growth medium transferred from chromosomally unstable derivatives of RKO36 is not cytotoxic, but medium from some derivatives can enhance delayed genomic instability as measured by mixed GFP\textsuperscript{+/−} colonies. The chromosomally unstable clones LS12 and Fe-10-3 were isolated from GM10115 human hamster hybrid cells following irradiation (24, 25). When growth medium is transferred from these unstable cells to the parent GM10115 cells, it is cytotoxic (16). We previously isolated seven chromosomally unstable derivatives of RKO36 that survived 10-Gy X-irradiation (23) and these are distinct from the unstable RKO36 derivatives that display delayed genomic instability as measured by GFP\textsuperscript{+/−} colonies. We were interested in whether the chromosomally unstable RKO36 cells show a similar death-inducing effect as with GM10115 cells. RKO36 cells cultured in either fresh or conditioned medium have plating efficiencies of 28% and 27%, respectively. Growth medium transferred from the seven chromosomally unstable RKO36 derivatives did not induce death-inducing effect (Table 3). We also tested whether medium from chromosomally unstable GM10115 derivatives LS12 and Fe-10-3 could induce death-inducing effect in RKO36 cells. Conditioned medium from parental GM10115 cells and LS12 had no effect, but medium from Fe-10-3 caused a small but not statistically significant reduction in plating efficiency (Table 3). We next investigated induced instability after medium transfer. Growth medium from one of the unstable clones, C6, significantly increased genomic instability of otherwise untreated RKO36 cells ($P < 0.05$, one-way ANOVA), whereas medium from the stable GM10115 and RKO36 cell lines had no effect on induced GFP\textsuperscript{+/−} colonies. Growth

### Table 2. Bystander effects in RKO36 cells as measured by cell killing and induced genomic instability as measured by GFP\textsuperscript{+/−} colonies

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Surviving fraction normalized to control (%)</th>
<th>% GFP\textsuperscript{+/−} colonies normalized to control</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 h*</td>
<td>0.5 h*</td>
<td>2 h*</td>
</tr>
<tr>
<td>Control (fresh medium)</td>
<td>100.00 ± 4.3</td>
<td>100.00 ± 0</td>
</tr>
<tr>
<td>Conditional medium</td>
<td>88.77 ± 8.8</td>
<td>89.52 ± 1.8</td>
</tr>
<tr>
<td>Medium from 5 Gy irradiated cells</td>
<td>92.76 ± 6.8</td>
<td>97.54 ± 0.9</td>
</tr>
<tr>
<td>5 Gy irradiated medium without cells</td>
<td>95.64 ± 8.2</td>
<td>105.16 ± 1.5</td>
</tr>
</tbody>
</table>

*Confluent RKO36 cells were irradiated at 5 Gy and incubated for 0.5 or 2 h before medium transfer.

### Table 3. Death-inducing effect in RKO36 cells as measured by cell killing and induced genomic instability as measured by mixed GFP\textsuperscript{+/−} colonies

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Surviving fraction ± SD</th>
<th>% GFP\textsuperscript{+/−} colonies ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh medium</td>
<td>0.29 ± 0.044</td>
<td>0.056 ± 0.096</td>
</tr>
<tr>
<td>Medium from RKO36</td>
<td>0.283 ± 0.019</td>
<td>0.636 ± 0.599</td>
</tr>
<tr>
<td>B2</td>
<td>0.333 ± 0.094</td>
<td>2.095 ± 0.841</td>
</tr>
<tr>
<td>C5</td>
<td>0.306 ± 0.031</td>
<td>3.504 ± 2.270*</td>
</tr>
<tr>
<td>C17</td>
<td>0.315 ± 0.007</td>
<td>1.012 ± 0.442</td>
</tr>
<tr>
<td>C18</td>
<td>0.328 ± 0.014</td>
<td>0.586 ± 0.034</td>
</tr>
<tr>
<td>C32</td>
<td>0.300 ± 0.031</td>
<td>2.690 ± 0.737</td>
</tr>
<tr>
<td>C41</td>
<td>0.298 ± 0.025</td>
<td>1.834 ± 0.721</td>
</tr>
<tr>
<td>C89</td>
<td>0.322 ± 0.016</td>
<td>1.862 ± 0.654</td>
</tr>
<tr>
<td>GM10115</td>
<td>0.300 ± 0.042</td>
<td>0.233 ± 0.328</td>
</tr>
<tr>
<td>LS12</td>
<td>0.270 ± 0.085</td>
<td>2.622 ± 3.200</td>
</tr>
<tr>
<td>Fe-10-3</td>
<td>0.228 ± 0.002</td>
<td>1.574 ± 0.393</td>
</tr>
</tbody>
</table>

*$P < 0.05$, one-way ANOVA.
medium from several other unstable clones (B2, C17, C18, C32, C41, C89, and unstable GM10015 clones LS12 and Fe-10-3) had only modest effects. These results suggest that chromosomally unstable cells can produce genome-distabilizing factors, but these are not necessarily cytotoxic.

Discussion

Our studies on adaptive responses, bystander effect, and death-inducing effect in RKO36 cells measured by cell killing all yielded negative results. However, when we used a unique GFP-based assay to measure induced genomic instability, we observed a protective adaptive response after exposure of cells to low doses of radiation. We also found that medium from some chromosomally unstable RKO36 clones could induce genomic instability in untreated RKO36 cells, but no bystander effect was observed after transfer of medium from irradiated cells.

The adaptive response refers to the phenomenon by which cells irradiated with a sublethal dose of ionizing radiation (an adaptive dose of a few centigrays) become less susceptible to subsequent exposure to high doses of radiation (a challenge dose of several grays). The adaptive response was first described as a reduction in chromosomal aberration frequency in stimulated human lymphocytes (20). Subsequent adaptive responses include reduction of cell killing (32), micronuclei formation and sister chromatid exchange (33), mutation (34), and transformation (35) in pretreated cells exposed to a high dose of ionizing radiation. An adaptive response has also been described after clinical, environmental, and occupational exposure to radiation (36, 37). The mechanism for the observed adaptive response is thought to be that low radiation doses enhance DNA repair ability and antioxidiant activity, resulting in more proficient cellular responses to the subsequent challenge (33, 38).

Reports of the adaptive response to radiation are conflicting because adaptive responses are not consistently observed in all cell systems or in all humans investigated in vitro or in vivo (22, 39, 40). The variation among different laboratories could be related to a number of factors including cell type, cell culture conditions, cell cycle effects, types of radiation used, doses and dose rates, as well as time interval between irradiations (30, 41).

In our experiments, varying the cell culture conditions (serum) or the time interval between adapting and challenge did not influence the lack of an adaptive response for cell survival. However, we did observe a small adaptive response for genomic instability, suggesting that a low adapting dose can protect against delayed homologous recombination and mutation/deletion.

Radiation-induced bystander effects are considered a competing phenomenon with respect to an adaptive response (42, 43). Bystander effect describes the nontargeted effect observed in cells that were not irradiated per se but were either in contact with irradiated cells or received soluble signals from irradiated cells. These cells exhibit phenotypes typically associated with direct radiation exposure including reduced survival (28), increased sister chromatid exchange (44), increased micronuclei formation, modification of gene expression (45), up-regulation of oxidative metabolism, gene mutation, oncogenic transformation, and chromosomal instability (10, 11).

The precise mechanisms underlying signaling for these bystander effects remain poorly understood. A number of studies suggest that the damage signal(s) are either transmitted from irradiated cells to bystander cells via gap junction–mediated intercellular communication (45), nitric oxide (46), or by the release of cytokines or other soluble extracellular factors from irradiated cells (47). Bystander effects seen in growth medium transfer experiments seem to be strongly dependent on cell type, genotype, and density at the time of irradiation (27, 47, 48). We tested a variety of conditions including different doses, dose rates, cell densities, and time interval between irradiation and medium transfer, but none resulted in a bystander effect as measured by cell killing.

Nagar et al. (15, 16, 29) showed that filtered medium from chromosomally unstable GM10115 clones was cytotoxic to parental GM10115 cells. This phenomenon is separate from a bystander effect and was called death-inducing effect. Recent studies indicate that one or more factors secreted by unstable cells induce DNA breakage, micronuclei formation, apoptosis, and genomic instability in rare GM10115 cells surviving death-inducing effect (29). We report here that medium from the unstable clone Fe-10-3 only slightly reduced plating efficiency in RKO36 cells whereas exposure to medium from LS12 or seven chromosomally unstable RKO clones resulted in no reduction in plating efficiency. These data show that RKO cells do not produce a cytotoxic death–inducing effect factor and/or do not respond to such a factor. Interestingly, however, as we observed for the adaptive response, medium from chromosomally unstable RKO36 clones was able to elicit a small increase in genomic instability as measured by an increase in GFP+/- colonies. This is an interesting observation because we have previously found that GFP+/- colony induction and radiation-induced chromosomal instability are separate delayed effects of radiation exposure (23). Genomic instability can be considered the increased rate of accumulation of genetic alterations following radiation insult. If mixed GFP+/- colonies are clonally expanded, they continue to display mixed GFP+ and GFP- cells, although, over time, we have observed that the number of GFP+ cells declines probably due to expression of the GFP protein and subsequent proliferative disadvantage.

The goal of extrapolating data from in vitro and in vivo model systems to humans is to predict cellular responses and as indication for carcinogenic risk. Current models for extrapolation rely on assumed low dose linearity (6). However, adaptive responses, bystander effects, and radiation-induced genomic instability all affect this assumption. Extrapolation for cancer data from animal models to humans incorporates any role for bystander effect and radiation-induced genomic instability. However, they cannot incorporate any potential advantage presented by an adaptive response. Using our GFP-based plasmid assay for delayed radiation effects, we show for the first time an adaptive response that functions to minimize genome instability. Radiation-induced genomic instability shares many common features with the instability associated with carcinogenesis (49). Whereas it remains imperative to better understand the relationship between delayed radiation effects and carcinogenesis, our data indicate potential beneficial effects associated with exposure to low doses of radiation that might influence the risks occurring in cells subsequently challenged with high doses of radiation.

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