Tissue-specific Deletion and Discontinuous Loss of Heterozygosity Are Signatures for the Mutagenic Effects of Ionizing Radiation in Solid Tissues

Olga N. Ponomareva, Jennifer A. Rose, Michael Lasarev, Janet Rasey, and Mitchell S. Turker

Center for Research on Occupational and Environmental Toxicology [O. N. P., J. A. R., M. L., M. S. T.] and Department of Molecular and Medical Genetics [M. S. T.], Oregon Health Sciences University, Portland, Oregon 97201 and Department of Radiation Oncology, University of Washington, Seattle, Washington 98195 [J. R.]

ABSTRACT

The mouse Aprt locus on chromosome 8 was used as the selectable target for the study of spontaneous and ionizing radiation-induced mutations in kidney epithelia and ear fibroblasts. Fifty-two Aprt heterozygous mice were exposed to 7.5 Gy of $^{137}$Cs-$\gamma$ radiation on their right sides, and Aprt-deficient clones were isolated from enzymatically digested tissues at times ranging from 1 day to 14 months after irradiation. A statistically significant increase in the mutant frequencies for the irradiated tissues was observed when compared with the spontaneous mutant frequencies for the nonirradiated tissues. A molecular analysis of spontaneous mutations observed for the nonirradiated tissues revealed tissue-specific differences; apparent chromosome loss was common in kidney mutants but infrequent in the ear mutants, whereas apparent deletions were common in the ear mutants but not detected in the kidney mutants. For the irradiated kidneys, apparent deletions were observed commonly demonstrating that these events are markers for ionizing radiation mutagenesis in this tissue. All of the loss of heterozygosity (LOH) tracts observed in the spontaneous mutants were continuous, but discontinuous LOH patterns were observed in 6–8% of ionizing radiation-induced ear and kidney cell mutants. Work with kidney-derived cell lines showed that discontinuous LOH is a novel signature for delayed ionizing radiation mutagenesis. Considered together, these results suggest that ionizing radiation-induced mutations in vivo can result from both direct and delayed mutagenic effects.

INTRODUCTION

Mutations observed in cancer cells can result from aberrant events that occur in normal cells as a result of exposure to endogenous or exogenous genotoxins and/or as a result of functional loss of DNA repair pathways (1–6). To better understand mutagenesis that results from these different conditions, systems have been developed that allow the detection and characterization of mutations that occur in vivo. These systems include bacterial transgenes in the mouse (7), and in vivo allow the detection and characterization of mutations that occur from these different conditions, systems have been developed that repair pathways (1–6). To better understand mutagenesis that results from direct and delayed mutagenic effects.

MATERIALS AND METHODS

Mouse Strains. The Aprt knockout allele (20) had been backcrossed more than eight times into the C57BL/6 and DBA/2 backgrounds when the experiments were initiated. F1 hybrid mice heterozygous for Aprt were obtained in most cases by breeding C57BL/6 males bearing the Aprt knock out allele with wild-type DBA/2 females. In a limited number of cases, DBA/2 males bearing the Aprt knock out allele were bred with wild-type C57BL/6 females. Hemielposure of Mice to $^{137}$Cs-$\gamma$ Irradiation. Each mouse was protected on its left side with a 4.5-cm lead shield and exposed to 7.5 Gy of $^{137}$Cs-$\gamma$ radiation at a dose rate of 1.50 Gy/min in a J. L. Shepherd model 81–14A $^{137}$Cs Irradiator. Initial tests, in which thermal luminescence dosimeter tags were placed at the exposed and protected tissues sites of sacrificed animals to determine the total doses that were received, demonstrated that the tissues on the left side received exposures <0.15 Gy at this dose and dose rate (data not shown). The irradiated mice were returned to their cages, and no special treatment was required until the time of sacrifice. Most mice (86%) were between 2 and 6 months of age at the time of exposure; the remaining animals were 7–8 months of age.

Isolation of Aprt-deficient Cells from Ear and Kidney Tissue. The methods used for the enzymatic digestion of ear and kidney tissues, and selection of Aprt-deficient primary clones are as described previously (8, 16, 21). Briefly, minced ear flaps were digested with a collagenase/dispase solution, and minced kidneys were digested with a collagenase solution. The cell suspensions were then plated into 100-mm dishes (five per ear and 10 per kidney) in the presence of 80 $\mu$g/ml 2′-6′-diaminopurine, which is used to select for Aprt-deficient cells.

LOH Analysis for Polymorphic Markers on Chromosome 8. DNA preparations from mutant cells were isolated from two different sources. The first was from clonally derived mutants cells that were expanded in culture until the cells were confluent in T25 cm$^2$ flasks. DNA was isolated from these cells using a conventional “salting out” method. Because a significant fraction of primary clones do not survive this expansion process, which is independent of the selection process (8), we also used an approach by which the DNA was isolated directly from the Aprt-deficient primary clones without expansion. A nitrosourea induced point mutations at both Aprt and Tk in splenetic T cells (11, 13), but X-rays were reported to be ineffective at increasing Aprt mutant frequencies in these cells (13).

Ionizing radiation induces mutational events via two broadly defined pathways (see Ref. 17 and references therein). One pathway results from direct damage caused by the radiation exposure. The second pathway results from indirect consequences of exposure and includes a delayed effect characterized by evolving chromosomal aberrations and a delayed increased in mutant frequencies, although no specific mutational event or pattern has been described. The current study was designed to determine whether ionizing radiation exposure could increase the frequency of mutations affecting the endogenous Aprt locus in the cells of two solid tissues, kidney and ear, and, if so, to search for mutational events that are “signatures” for exposure. The results identified apparent interstitial deletional events and a novel mutational pattern, termed discontinuous LOH (18, 19), as signatures for ionizing radiation exposure in the kidney and in both tissues, respectively. Work with immortalized kidney cells suggests that discontinuous LOH may result from the delayed mutagenic effect caused by ionizing radiation.
times ranging from 1 day (0 months) to 14 months before being sacrificed for mutant frequency determinations. The mice ranged in age from 2–20 months at the time of sacrifice. A cloning efficiency analysis to determine the number of clonable units recovered per organ (i.e., the number of cells that will form colonies when plated in culture) demonstrated that cell viability was reduced in the irradiated tissues. The cloning efficiencies for the irradiated tissues did not recover to levels observed for the nonexposed tissues even when the animals were sacrificed many months after exposure. The average reduction in cloning efficiency for the irradiated ears was 35% (95% CI of 23–45%; \( P < 0.001 \)) and for the irradiated kidneys was 47% (95% CI of 36–55%; \( P < 0.001 \)). The median cloning efficiencies (in clonable units per organ) for the irradiated versus nonirradiated kidneys were 8,000 and 16,670, respectively, and for the irradiated versus nonirradiated ears were 19,600 and 34,468, respectively.

**Mutant Frequencies Are Increased in Irradiated Tissues.** Significant variability for mutant frequencies for the nonirradiated (left side) and irradiated (right side) tissues was observed with frequencies ranging from \( 1.0 \times 10^{-5} \) to as high as \( 1.2 \times 10^{-3} \) (Fig. 1). Using Kendall’s \( \tau \) rank correlation (26), no association was found for the spontaneous mutant frequencies of nonirradiated kidneys and ears from hemi-irradiated animals, and 12 additional nonirradiated animals (Ref. 15; \( P \) ranges, 0.16–0.47). These results demonstrate that elevated mutant frequencies in one tissue do not correlate with elevated mutant frequencies in other tissues of a given animal. An increase in spontaneous mutant frequencies (i.e., from left tissues) as a function of age was observed for the ear (\( P = 0.0006 \); data not shown). The \( P \) for a rise in spontaneous kidney mutant frequencies as a function of age was suggestive (\( P = 0.09 \)).

A comparison of the mutant frequencies for the irradiated tissues versus the nonirradiated tissues demonstrated significant increases for both the kidney and the ear (representative examples shown in Fig. 1). For the kidney, the average increase was 4.5-fold (95% CI, 3.2–6.6; \( P < 0.001 \)) and for the ear the average increase was 2.9-fold (95% CI, 1.9–4.2; \( P < 0.001 \)). A more detailed analysis, which incorporates the lag time after irradiation, demonstrated that the increase in mutant frequencies for the irradiated ears relative to the nonirradiated ears did not appear until 2 months after irradiation. The mutant frequencies increased for ~8 months relative to the nonirradiated ears (95% CI, 5.5–10.3 months) and then began to decrease (\( P = 0.027 \); data not shown). A similar curve could not be discerned for the kidney tissue (\( P = 0.11 \); data not shown). In this case, an elevated mutant frequency was observed even at the earliest time point (1 day after irradiation), and there was no significant change as a function of time after irradiation (data not shown).

**Tissue-specific Differences in the Spectrum of Spontaneous Mutational Events.** DNA preparations from 50 Aprt-deficient ear mutants isolated from 21 nonirradiated ears and 35 Aprt-deficient kidney mutants isolated from 19 nonirradiated kidneys were analyzed for LOH at as many as 13 polymorphic loci on mouse chromosome 8. The LOH analysis allowed each mutational event to be placed into one of four broad mutational categories: (a) intragenic event (i.e., bp substitution, frameshifts, gene silencing, and so on; Fig. 2A); (b) chromosome loss (e.g., Fig. 2B); (c) mitotic recombination (e.g., Fig. 2C); and (d) interstitial deletion (Fig. 2, D–F). One observation common to all of the spontaneous mutations examined is that when LOH was observed for two or more polymorphic loci the LOH tracts were continuous.

For a statistical analysis, the spontaneous mutations for each tissue were grouped in two ways. One was to count all of the identical appearing mutations from a given tissue as one event, and the second was to include every mutational event in the analysis regardless of tissue origin (Table 1). A comparison of the spontaneous mutational
spectra for the ear and kidney tissues revealed two significant differences. The first was that apparent chromosome loss was common in the kidney mutants (41%; 43%) but infrequent in the ear mutants (6%; 6%; \(P = 0.002\) and 0.001, respectively). The second was that apparent interstitial deletion events were observed in ear mutants (25%; 18%; Fig. 2D) but not in the kidney mutants (\(P = 0.015\) and 0.005, respectively). Spontaneous mitotic recombinational and intragenic events occurred at similar percentages in both the ear and kidney mutants (Table 1). \(\text{Aprt}\) alleles containing spontaneous intragenic events from one kidney and four ear mutants were sequenced, and two point mutations were identified (C insertion and G \(\rightarrow\) A bp substitution). No mutations were observed in the other three DNA preparations consistent with epigenetic inactivation, which is a relatively common mechanism for inactivation of mouse \(\text{Aprt in vivo}\) (12, 16).

**Ionizing Radiation Induces Apparent Deletions in the Kidney.** DNA preparations from a total of 100 ear mutants and 78 kidney mutants obtained from irradiated (right side) tissues were analyzed for LOH events (Table 1). The ear mutants were isolated from 23 different animals, and the kidney mutants were isolated from 25 different animals. A sequence analysis of 2 kidney and 4 ear mutants from irradiated tissues that fell into the intragenic mutational category failed to identify specific mutational changes, again consistent with epigenetic inactivation.

A comparison of the mutational spectra for the irradiated tissues with those obtained from the nonirradiated tissues demonstrated that apparent deletions were common (30%; 27%) in the \(\text{Aprt}\) mutants isolated from the irradiated kidneys (Fig. 2F). In contrast, none were observed in mutants obtained from the nonirradiated kidneys (\(P = 0.0002\) and 0.0005, respectively). The deletional events were identified in mutants isolated from animals that were sacrificed from 0 to 9 months after ionizing radiation exposure. This observation suggests that the damage causing this type of mutational events occurs very early after irradiation and persists in the exposed cells, consistent with a direct mutagenic effect. Whereas the percentage of deletion mutations increased for the irradiated ear (24%; 29%; Fig. 2E) when compared with the nonirradiated ear (18%; 25%), this difference was not statistically significant.

**Ionizing Radiation Induces Discontinuous LOH in Both Tissues.** A second difference between the irradiated and nonirradiated tissues involved a mutational pattern that was observed previously after exposure of a kidney cell line to hydrogen peroxide. This pattern, termed discontinuous LOH (18), is characterized by LOH events on chromosome 8 that are apparently unlinked to the mutational events that cause loss of \(\text{Aprt}\) expression. No examples of discontinuous LOH were observed in mutants isolated from nonirradiated ear and kidney tissues. In contrast, five independent examples were observed in kidney mutants (Fig. 3A) and five independent examples (six total) were observed in ear mutants (Fig. 3B) isolated from the irradiated tissues. The earliest time point after irradiation that yielded a mutant exhibiting discontinuous LOH was 4 months; the remaining examples of discontinuous LOH were observed in mutants isolated from animals sacrificed \(\geq\) 8 months after irradiation suggesting a delayed effect in vivo. Because of the relatively few discontinuous LOH events, a statistical analysis for this LOH pattern failed to reach statistical significance when the irradiated ears and kidneys were compared independently with the nonirradiated ears and kidneys, respectively. Statistical significance was observed (\(P = 0.023\)) when the data from these two tissues were pooled.

**High Mutant Frequencies in Vivo Are Not Attributable Solely to the Presence of sib Cells.** It has been suggested that the presence of mutant sib cells is the most likely explanation for elevated spontaneous \(\text{Aprt}-\)mutant frequencies in a given tissue (12). To address this issue we examined all of the cases in which at least four mutational events were determined from a given organ (Table 2). Four suitable cases were found for mutants isolated from nonirradiated tissues, and in only one case (left ear of animal 351) was it possible to place many spontaneous mutants in one category (intragenic events). An additional four mutations were identified that represent at least three independent mutational events, for a minimum total of four independent events from this ear. Most of the mutations observed for the

![Fig. 2. LOH patterns reveal apparent deletional events occur spontaneously in ear tissues, and in irradiated ear and kidney tissues. LOH patterns for polymorphic chromosome 8 loci representing intragenic events including epigenetic silencing (A), chromosome loss (B), an example of mitotic recombination (C), and apparent deletions identified in mutants isolated from the left (nonexposed) ears (D), right (irradiated) ears (E), and right (irradiated) kidneys (F). Numbers underneath the deletion patterns represent total number of mutants for each pattern. A lack of a circle indicates that information was not obtained for that locus. Numbers on left side of figure represent polymorphic loci used for the LOH analysis.](image-url)

### Table 1. Classification of mutational events from irradiated and nonexposed tissues

<table>
<thead>
<tr>
<th>Event</th>
<th>IR ear</th>
<th>% Non-IR ear</th>
<th>% IR kidney</th>
<th>% Non-IR kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td>Delet.</td>
<td>17:24</td>
<td>29:24</td>
<td>8:9</td>
<td>25:18</td>
</tr>
<tr>
<td>MR</td>
<td>20:24</td>
<td>34:24</td>
<td>12:18</td>
<td>37:36</td>
</tr>
<tr>
<td>IE</td>
<td>8:30</td>
<td>14:30</td>
<td>10:20</td>
<td>31:40</td>
</tr>
<tr>
<td>CL</td>
<td>9:16</td>
<td>15:16</td>
<td>2:3</td>
<td>6:6</td>
</tr>
<tr>
<td>DLOH</td>
<td>9:16</td>
<td>15:16</td>
<td>2:3</td>
<td>6:6</td>
</tr>
<tr>
<td>Totals</td>
<td>59:100</td>
<td>32:50</td>
<td>32:50</td>
<td>64:78</td>
</tr>
</tbody>
</table>

\(\text{IR, irradiated; Delet., deletional event; DLOH, discontinuous LOH pattern; MR, mitotic recombination; IE, intragenic events including epigenetic silencing and bp changes; CL, chromosome loss.}\)

* Includes tissues from both sides of some nonirradiated animals.
* For each tissue the number on the left represents the minimal number of independent mutational events and the number on the right represents the total number of mutational events that were identified. See “Results” section “Tissue-specific Differences in the Spectrum of Spontaneous Mutations” for more details.
* IR, irradiated; Delet., deletional event; DLOH, discontinuous LOH pattern; MR, mitotic recombination; IE, intragenic events including epigenetic silencing and bp changes; CL, chromosome loss.
remaining three cases were also found to represent independent events, suggesting that the presence of mutant sib cells is not the sole explanation for elevated spontaneous mutant frequencies.

An analysis of 14 suitable cases for irradiated tissues demonstrated that mutant cells from an irradiated tissue source could exhibit a variety of mutational events (Table 2). Independent mutational events made up the majority of mutational events identified for all but 2 of the 14 cases (irradiated ears for animals 328 and 687). This analysis for the irradiated tissues provides additional evidence for the induction of mutational events by exposure to ionizing radiation in vivo.

**DISCUSSION**

The current study examined mutant frequencies and mutational spectra for spontaneous and ionizing radiation-induced mutations in kidney epithelia and ear fibroblasts. Analysis of mutations in the nonirradiated sides of the animals demonstrated animal to animal variation for spontaneous mutant frequencies similar to that observed in previous work by two other groups examining Aprt mutant frequencies in the mouse (12, 27) and in previous work by us (15). Interindividual variation has also been noted in humans for HPRT (10, 28) and GPA (29, 30) mutant frequencies. Whereas some of this variation may be explained by the presence of mutant sib cells (12), an analysis of spontaneous mutants from four organs (Table 2) suggests that high mutant frequencies can also result from elevated numbers of independent mutational events.

The most striking observation in regard to spontaneous mutations was that apparent loss of chromosome 8 was common in the kidney cells but occurred infrequently in the ear cells. In contrast, apparent interstitial deletional events occurred spontaneously in the ear cells, but no spontaneous examples were observed in the kidney mutants (Fig. 3C). No examples of discontinuous LOH were observed in 27 spontaneous mutants of the K435 cell kidney cell line (data not shown).

### Table 2: Mutation distributions<sup>a</sup> for organs with high mutant frequencies

<table>
<thead>
<tr>
<th>Tag</th>
<th>Organ</th>
<th>Mut. freq.&lt;sup&gt;b&lt;/sup&gt;</th>
<th>IE</th>
<th>MR</th>
<th>Delet.</th>
<th>CL</th>
<th>DLOH</th>
</tr>
</thead>
<tbody>
<tr>
<td>351</td>
<td>Left ear</td>
<td>$3.1 \times 10^{-3}$</td>
<td>6 (1)</td>
<td>2 (1)</td>
<td>1 (1)</td>
<td>1 (1)</td>
<td></td>
</tr>
<tr>
<td>554</td>
<td>Left kidney</td>
<td>$3.4 \times 10^{-3}$</td>
<td>1 (1)</td>
<td>2 (2)</td>
<td>2 (1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NTA</td>
<td>Left ear</td>
<td>$5.9 \times 10^{-4}$</td>
<td>2 (2)</td>
<td>1 (1)</td>
<td>2 (1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NTB</td>
<td>Left ear</td>
<td>$1.4 \times 10^{-4}$</td>
<td>1 (1)</td>
<td>1 (1)</td>
<td>2 (1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>177</td>
<td>IR kidney</td>
<td>$1.3 \times 10^{-3}$</td>
<td>8 (1)</td>
<td>3 (2)</td>
<td>4 (1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>179</td>
<td>IR ear</td>
<td>$1.1 \times 10^{-3}$</td>
<td>1 (1)</td>
<td>3 (2)</td>
<td>1 (1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>266</td>
<td>IR ear</td>
<td>$5.5 \times 10^{-4}$</td>
<td>2 (1)</td>
<td>1 (1)</td>
<td>4 (2)</td>
<td>5 (1)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>301</td>
<td>IR ear</td>
<td>$1.6 \times 10^{-3}$</td>
<td>9 (1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>328</td>
<td>IR ear</td>
<td>$3.1 \times 10^{-3}$</td>
<td>3 (1)</td>
<td>2 (2)</td>
<td></td>
<td></td>
<td>1 (1)</td>
</tr>
<tr>
<td>334</td>
<td>IR ear</td>
<td>$4.5 \times 10^{-4}$</td>
<td>1 (1)</td>
<td></td>
<td>3 (1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>554</td>
<td>IR kidney</td>
<td>$1.2 \times 10^{-3}$</td>
<td>2 (2)</td>
<td>5 (3)</td>
<td>2 (1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>556</td>
<td>IR ear</td>
<td>$2.5 \times 10^{-4}$</td>
<td>3 (1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>556</td>
<td>IR kidney</td>
<td>$7.4 \times 10^{-4}$</td>
<td>1 (1)</td>
<td>2 (2)</td>
<td>1 (1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>687</td>
<td>IR ear</td>
<td>$1.0 \times 10^{-4}$</td>
<td>5 (1)</td>
<td>3 (1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NTA</td>
<td>IR ear</td>
<td>$1.0 \times 10^{-3}$</td>
<td>2 (1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NTA</td>
<td>IR kidney</td>
<td>$3.3 \times 10^{-3}$</td>
<td>3 (3)</td>
<td>2 (2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NTA</td>
<td>IR ear</td>
<td>$3.1 \times 10^{-4}$</td>
<td>1 (1)</td>
<td>2 (2)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Number represent total number of mutants that fall into each mutational class; number in parentheses represents the minimum number of independent mutants.

<sup>b</sup> Animals are identified by tag numbers placed in ears when they were genotyped. Animals NTA and NTB lost their tags.

<sup>c</sup> IR ear and kidney are right-side irradiated organs; left-side ear and kidney were not exposed to ionizing radiation.

<sup>d</sup> Mutat. freq., mutation frequency; IR, irradiated; Delet., deletion event; DLOH, discontinuous LOH pattern; MR, mitotic recombination; IE, intragenic events including epigenetic silencing and base-pair change; CL, chromosome loss.
(Table 1). No significant difference in the spontaneous mutational spectra was observed in a study by others when mutations in Apert-deficient ear fibroblasts and T cells were compared (14). Moreover, no examples of spontaneous deletional events were observed in the ear fibroblast mutants (12, 14), which is in contrast to our observation that such events occurred spontaneously in 18–25% of the ear mutants examined. This contrasting result suggests that strain differences could play a role in spontaneous mutagenesis, because we used C57BL/6 X DBA/2 hybrids, whereas 129/Sv X C3H/HeJ hybrids were used in the other studies. One potential explanation for the strain difference is the extent of genetic variation of the different strains used to make the hybrids. Genetic variation between the C57BL/6 and DBA/2 strains is approximately twice that for the 129/Sv and C3H/HeJ strains, including for chromosome 8 (31). Moreover, it has been shown recently that the level of homology between chromosome homologues can have significant impact on recombinational events (32).

Interstitial deletions have been reported to be a signature mutation for ionizing radiation mutagenesis in cultured cells (33–35) including our work with mouse Apert (36). In the present study, apparent deletional events were induced in kidney cells in vivo by exposure to 7.5 Gy of 137Cs-γ irradiation. Because the cells of this tissue have a very low spontaneous background for deletional events, these events provide a signature mutation for ionizing radiation mutagenesis in the kidney. However, the relatively high spontaneous occurrence of deletional mutations in ear tissue (18–25%) makes continued study of the induction of interstitial deletional events difficult for this cell type, at least when the B6D2 hybrid strain is used.

Discontinuous LOH patterns are common in human cancers (18) and may reflect a form of genomic instability. These mutational patterns were observed in mutants isolated from both the irradiated ears and kidneys, in all but one case 8 months or more after irradiation. Discontinuous LOH was not observed in mutants isolated from the nonexposed tissues. The apparent lag in the formation of the discontinuous LOH pattern suggests that these mutations are the result of delayed genomic instability. Consistent with this possibility is the observation of discontinuous LOH as a signature for delayed instability in two kidney cell lines.

Several caveats should be noted in regard to the tentative conclusion that discontinuous LOH in vivo results from delayed genomic instability induced by ionizing radiation. One is that the growth properties of the kidney and ear cells after radiation can be divided into at least three categories, dying cells (which are irrelevant for our assay and for cancer), cells that divide shortly after exposure to radiation, and cells that undergo prolonged quiescence before undergoing cell division. It is formally possible that direct damage from the radiation exposure, coupled with delayed cell division after a prolonged quiescence, could set the stage for discontinuous LOH in the ear and kidney cells. A second caveat is that the cell culture work, which supplied supporting evidence for the hypothesis that discontinuous LOH is an in vivo marker for delayed mutagenesis, required the use of immortalized cells. Cellular immortalization can cause various types of genomic instability (37), although it is noted that we have not observed spontaneous examples of discontinuous LOH in kidney cell lines. Finally, complex mutations that are similar in molecular appearance to the discontinuous LOH patterns have been reported as a direct effect of ionizing radiation exposure for a human chromosome 11 homologue in a hamster cell background (38). However, these complex mutations did not involve LOH, because only one human chromosome 11 homologue is present in the hamster cell, although recent work with two chromosome 11 homologues suggest that complex cytogenetic aberrations can occur as a consequence (39). Additional work will be necessary to determine whether such aberrations are present in Apert mutant cells exhibiting discontinuous LOH to better understand how this mutational pattern is triggered by ionizing radiation exposure.

ACKNOWLEDGMENTS

We thank Blythe Gage, Alice Osis, and Sandra Krussel for large amounts of technical assistance in determining mutant frequencies, Lay Chin and Linda Wiens for mouse irradiation, and Jeff Schwartz, Peter Stambrook, and Leona Samson for helpful suggestions. We also thank Peter Stambrook and Jay Tischfield for sharing the Apert knockout mouse strain with us.

REFERENCES


Tissue-specific Deletion and Discontinuous Loss of Heterozygosity Are Signatures for the Mutagenic Effects of Ionizing Radiation in Solid Tissues
