MOLECULAR NATURE OF SPONTANEOUS AND RADIATION-INDUCED 
MUTATIONS WITH ALLELIC LOSS IN HUMAN CELLS

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Abstract

An assay system was developed for dissecting the second step in loss of function mutations and for determining the potency of physical and chemical agents to produce such mutations. Results obtained suggest that somatic recombination and/or deletion occurs close to the border between the heterochromatic and euchromatic regions of chromosome 16q.

Constitutional loss or inactivation of one copy of a tumor suppressor gene, as exemplified by hereditary retinoblastoma, increases the propensity for malignancy by reducing the number of events required for the complete loss of the negative regulatory function. An immortalized B-lymphoblastoid cell line (LCL), WR10, derived from an obligate heterozygote of hereditary 2,8-dihydroxyadenine urolithiasis, adenine phosphoribosyltransferase (APRT) deficiency, has enabled us to develop an assay system for dissecting the second step in loss-of-function mutations, i.e. the forward mutation from $APRT^{+/-}$ to $APRT^{-/-}$ or $APRT^{0/-}$, and for determining the potency of physical and chemical agents to produce such mutations [1]. The non-functional $APRT$ allele on the chromosome 16 (16q24.3) in WR10 cells bears a nonsense mutation in the exon 3. WR10 was found to be heterozygous also for a SphI RFLP associated with the gene, which allowed the functional and the constitutionally non-functional allele to be distinguished by Southern blot analysis using an $APRT$ probe.

The base-line frequency of cells resistant to 100 μM of 2,6-diaminopurine (DAP) was found to be $1.1 \times 10^{-5}$ with a mutation rate of $1.65 \times 10^{-6}$ / cell / generation, which was 5-10 fold higher than the rates for 6-thioguanine resistant ($TG^r$) mutation measured with other LCLs from normal individuals. Recloned $DAP^r$ mutants in general grew as rapidly as wild-type WR10 cells (the population doubling time of approximately 36 h). Exposure of WR10 cells to γ-rays resulted in a dose-dependent increase of $DAP^r$ mutant fraction up to $2.5 \times 10^{-4}$ at 2 Gy, whereas induced mutant fraction was $4.7 \times 10^{-5}$ for $TG^r$ with the base-line frequency of $1 \times 10^{-6}$. Irradiation of WR10 cells with far-UV light from a 15-W germicidal lamp resulted in
dose-dependent increases both for DAP\textsuperscript{r} and TG\textsuperscript{r} mutations. However, the increase in the fraction of DAP\textsuperscript{r} mutant was only 2.2 fold at most and statistically significant (p< 0.05) above 17.6 J/m\textsuperscript{2} which allowed merely 15 % survival (Figure 1), whereas the doubling dose for TG\textsuperscript{r} mutations was approximately 2.2 J/m\textsuperscript{2}. Thus, in contrast with ionizing radiations, UV-light is not more effective in inducing mutations at the APRT locus than at the HPRT locus [2].

![Image](image1.png)

Figure 1. Cell survival and mutant fractions as a function of dose after irradiation with (A) \(\gamma\)-rays and (B) UV-light in WR10 cells. Open square, DAP\textsuperscript{r}; closed square, TG\textsuperscript{r}. Each point represents a single determination and bars represent 95% confidence intervals.

Table 1 The structural change of APRT gene in DAP-resistant mutants

<table>
<thead>
<tr>
<th>Treatment</th>
<th>number of mutant clones analyzed</th>
<th>number of clones with allelic loss</th>
</tr>
</thead>
<tbody>
<tr>
<td>None (spontaneous)</td>
<td>26</td>
<td>22 (85%)</td>
</tr>
<tr>
<td>(\gamma)-rays (2 Gy)</td>
<td>69</td>
<td>64 (93%)</td>
</tr>
<tr>
<td>UV-light (17.6 J/m\textsuperscript{2})</td>
<td>31</td>
<td>17 (55%)</td>
</tr>
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</table>
The *APRT* gene, consisting of 5 exons, spans 2.5 kb. A substantial proportion of the spontaneously-arising mutants (22/26, 85%) and virtually all of γ-ray-induced mutants (64/69, 93%) lost the functional allele, judged from loss of heterozygosity (LOH). On the contrary, only 25% of UV-induced mutants were considered to bear LOH (Table 1), when the *contribution of spontaneous mutants with allelic loss was taken into account*. Major DNA alterations are, therefore, unlikely to be involved in UV-induced mutations at the *APRT* locus, as previously demonstrated for the *HPRT* locus [3]. Dosage blotting revealed that about half of the spontaneously-arising and γ-ray-induced mutants with LOH showed a reduction to homozygosity of the mutant allele, implying that the mutated allele was duplicated due to mitotic recombination or gene conversion. Non-disjunction with reduplication of the mutant chromosome 16 was ruled out based on the retention of heterozygosity at 16p microsatellite loci (the D16S298 and the D16S292) in all of the mutants tested with LOH at the *APRT* locus. Approximately 70% of mutants both arising spontaneously and induced by ionizing radiation showed LOH at three proximal loci on the long arm, the D16S266 (16q23.3), the D16S265 (16q21) and the D16S308 (16q12.2). The distribution of the sites for somatic recombination or for deletion breakpoints in radiation-induced mutants seemed to be non-random and indistinguishable from that in spontaneously-arising mutants. These results suggest that somatic recombination and/or deletion occur preferentially close to the border between the heterochromatic and the euchromatic regions of the chromosome 16q, implicating an untargeted nature of these events incurred by ionizing radiation.

**References**

