Low doses of gamma ionizing radiation increase hprt mutant frequencies of TK6 cells without triggering the mutator phenotype pathway

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Abstract

The TK6 lymphoblastoid cell line is known to be mismatch repair (MMR) and p53 proficient. Deficiency in MMR results in a mutator phenotype characterized by microsatellite instability (MSI) and increased hprt mutant frequency (MF). Increased hprt MF is also a biomarker of effect for exposure to ionizing radiation. In order to test if a mutator phenotype could be induced by low doses of gamma ionizing radiation, an hprt cloning assay and a MSI investigation were performed after radiation exposure. The spontaneous MF was 1.6 x 10^{-6}. The groups exposed to 0.2, 0.5 and 1.0 Gy had hprt MFs of 2.3, 3.3 and 2.2 x 10^{-6}, respectively. The spontaneous MSI frequency per allele in non-selected cells was 5.4 x 10^{-3}, as evidenced at the loci D11S35, nm23-H1, D8S135 and p53. MSI frequencies in the groups exposed to 0.2, 0.5 and 1.0 Gy were found to be < 4.7, < 7.7 and < 12 x 10^{-3}, respectively. The frequencies of hprt mutants and MSI found in this study suggest that low doses of ionizing radiation increase hprt mutant frequency without triggering the mutator phenotype pathway.

Key words: mutator phenotype, hprt, microsatellite instability, TK6 cells, ionizing radiation.

Received: September 28, 2004; Accepted: January 6, 2006.

TK6 is a lymphoblastoid cell line heterozygous at the thymidine kinase locus (Skopek et al., 1978) that has been characterized by the karyotype 47,XY,+13,14q+,21p+ (Grosovsky et al., 1996). The cells harbor wild-type p53 (Philips et al., 1997) and are mismatch repair (MMR) proficient (Tomita-Mitchel et al., 2000; Kleczkowska et al., 2001). The MMR system recognizes base mismatches during DNA replication and eventually trigger to apoptosis (Berry et al., 2000). MMR deficiency results in mutator phenotype associated with microsatellite instability and elevated mutation rates at the hprt locus (Aquilina and Bignami, 2001). Increased hprt mutant frequency is also a biomarker of effect (Pavanello and Clorofero, 2000) for potential low-dose ionizing radiation exposure (Albertini et al., 2000).

In this study, we used the hprt cloning assay and microsatellite analysis to investigate if low doses of gamma ionizing radiation can induce a persistent genetic instability through the mutator phenotype pathway in TK6 hprt mutant clones.

hprt cloning assay. Three groups of TK6 cells (1.6 x 10^6 cells/mL) were exposed in vitro to the doses of 0.2, 0.5, and 1.0 Gy of gamma ionizing radiation, respectively. Cells were irradiated at a dose-rate of 9.7 ± 0.74% Gy/min, using a 137Cs γ-ray source (Gammacell 1000, Nordion International Inc., Ontario, Canada), and then incubated for three days at 37 °C and 5% CO2 before the hprt cloning assay. For hprt cloning assay, 6 cells/well in the unexposed group and 3 cells/well in the exposed groups were plated into 96-well microtitre plates in non 6-TG-containing medium for determining plating efficiency, and at 10^4 cells/well in 2.0 μg/mL 6-TG for hprt mutant selection. RPMI 1640 medium was supplemented with 6 mg/mL penicillin, 10 mg/mL streptomycin, 1% fungizone and 10% bovine serum. After 14 days, clones were scored and expanded individually to 6-well plates in 2 mL of growth medium/well. Wells with approximately 10^6 cells/mL were harvested for microsatellite analysis.

Microsatellite analysis. PCR was performed in 10 mM Tris, 50 mM KCl, 1.5 mM MgCl2, TMCA 0.1 mM,
primers 100 pmol/μL, dNTP 25 mM (v/v), 2.5 U/μL Taq polymerase, and 100 ng of DNA preparation. The touch-down thermal protocol was 94 °C for 1 min, 65-55 °C (decreasing 1 °C/cycle for 10 cycles, followed by 20 cycles at 55 °C) for 1 min, 72 °C for 1 min, and 5 min extension at 72 °C. The CA repeats markers analyzed were D6S105, ANKI, D8S135, D11S35, nm23-H1 and p53. PCR products were visualized using a 7% polyacrylamide gel stained with ethidium bromide.

Statistical analysis. Chi-square analysis was performed to conclude if there were a difference between two plating efficiencies or between two mutant frequencies.

The spontaneous hprt mutant frequency found in this study was 1.57 x 10^{-6}, whereas the frequencies for the groups exposed to 0.2, 0.5 and 1.0 Gy were 2.31, 3.28, and 2.18 x 10^{-6}, respectively. The plating efficiencies and mutant frequencies for all groups are shown in Figure 1. Our spontaneous hprt mutant frequency was similar to that described elsewhere (Grosovsky and Little, 1985). The previously reported hprt mutant fraction for lymphoblastoid cells exposed in vitro to 1.0 Gy is 17.1 ± 7.8 x 10^{-6} (Phillips et al., 1995). Such a high value may be due to the selection of mutants using p53 non-proficient TK6 cells (WTK1 cell line) and further to the lower concentration of 6-TG (0.5 μg/mL) medium. For higher doses, Nelson and colleagues (1994) found a mutant fraction of 10.1 ± 0.4 x 10^{-6} for TK6 cells exposed in vitro to 2.0 Gy.

The unexposed group and the one not selected by 6-TG showed six events of MSI in 1,112 alleles investigated (5.4 x 10^{-3} MSI/allele), as described in Table 1. All the microsatellite instabilities observed were found to present a decrease of the wild-type allele size. Loci D11S35 (not shown) and nm23-H1 (Figure 2.A) exhibited two MSI per locus in different clones. Loci D8S135 (Figure 2.B) and p53 (Figure 2.C) exhibited one MSI per locus. However, because no MSI was detected in 6-TG^6 clones, MSI frequencies were estimated to be lower than 8.6, 4.7, 7.7 and 12 x 10^{-3} for the unexposed cells and for the cells exposed to 0.2, 0.5 and 1.0 Gy, respectively. It is worth noting that MMR deficiency is likely to be identified by a

### Table 1 - Frequency of MSI at 6 microsatellite loci in TK6 cell clones exposed and unexposed to ionizing radiation.

<table>
<thead>
<tr>
<th></th>
<th>Unexposed group</th>
<th>Exposed groups (hprt mutant)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Plating efficiency</td>
<td>hprt mutant</td>
</tr>
<tr>
<td>Frequency</td>
<td>73.9%</td>
<td>1.57 x 10^{-6}</td>
</tr>
<tr>
<td>N. of positive clones</td>
<td>166/168</td>
<td>18/1,248</td>
</tr>
<tr>
<td>N. of alleles studied</td>
<td>1,112</td>
<td>212</td>
</tr>
<tr>
<td>N. of MSI/No. of alleles studied</td>
<td>6/1,112</td>
<td>0/212</td>
</tr>
<tr>
<td>MSI frequency</td>
<td>5.4 x 10^{-3}</td>
<td>&lt; 8.6 x 10^{-3}</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>0.2 Gy</th>
<th>0.5 Gy</th>
<th>1.0 Gy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency</td>
<td>2.31 x 10^{-6}</td>
<td>3.28 x 10^{-6}</td>
<td>2.18 x 10^{-6}</td>
</tr>
<tr>
<td>N. of positives</td>
<td>18/1,248</td>
<td>16/1,112</td>
<td>7/1,056</td>
</tr>
<tr>
<td>N. of alleles</td>
<td>212</td>
<td>130</td>
<td>82</td>
</tr>
<tr>
<td>N. of MSI/No. of alleles</td>
<td>0/212</td>
<td>0/130</td>
<td>0/82</td>
</tr>
<tr>
<td>MSI frequency</td>
<td>&lt; 4.7 x 10^{-3}</td>
<td>&lt; 7.7 x 10^{-3}</td>
<td>&lt; 12 x 10^{-3}</td>
</tr>
</tbody>
</table>

1Microsatellite analysis was randomly performed among the positive clones, where each clone was considered as having two alleles.
MSI frequency was higher than $8 \times 10^{-4}$, as reported by Li et al. (1994; Hackman et al. 1995). However, our spontaneous MSI frequency of $5.4 \times 10^{-3}$ found in this study is closer to the data reported for peripheral T lymphocytes, which vary from $2.9 \times 10^{-3}$ (Shibata et al., 1994; Hackman et al., 1995). This suggests that only one mutant clone gives rise to each positive colony (Albertini et al., 2000). Thus, although the exact frequencies of microsatellite instability and mutator phenotype in hprt mutants induced by ionizing radiation remain to be determined, these results indicate the presence of an efficient MMR system in our mutant clones.

The spontaneous MSI frequency of $5.4 \times 10^{-3}$ found in this study is closer to the data reported for unexposed TK6 cells. Additionally, Davies and colleagues (1999) found a spontaneous MSI frequency of $1 \times 10^{-4}$ in hprt non-selected clones and of $3 \times 10^{-3}$ in 6-TG-resistant T-lymphocytes, results that are in disagreement with the data from this study. The last mentioned authors attributed the low rate of MSI for non-selected clones to the large number of alleles investigated. Giver and Grososky (2000) reported one event of MSI induced by ionizing radiation at 59 tk mutants ($6 \times 10^{-4}$ MSI/allele).

In conclusion, considering together the hprt mutant frequency for all the groups and the absence of MSI in the 6-TG resistant clones, our data suggest that ionizing radiation increases hprt mutant frequency without triggering the mutator phenotype pathway.

Acknowledgments

A Strategic Research Grant from the National Science and Engineering Research Council of Canada supported this work.

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Associate Editor: Catarina S. Takahashi