Genetics and Molecular Biology, 29, 3, 558-561 (2006)

www.sbg.org.br

Short Communication

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

Flávio Monteiro Ayres[1,2,3], Aparecido Divino da Cruz[1,2], Patricia Steele[1] and Barry W. Glickman[1]

[1]*Centre for Environmental Health, Department of Biology, University of Victoria, Victoria, BC, Canada.*
[2]*Núcleo de Pesquisas Replicon, Departamento de Biologia, Universidade Católica de Goiás, Goiânia, GO, Brazil.*
[3]*Unidade Universitária de Ciências Exatas e Tecnológicas, Universidade Estadual de Goiás, Anápolis, GO, Brazil.*

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 1.6×10^{-6}. The groups exposed to 0.2, 0.5 and 1.0 Gy had *hprt* MFs of 2.3, 3.3 and 2.2×10^{-6}, respectively. The spontaneous MSI frequency per allele in non-selected cells was 5.4×10^{-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 $< 12 \times 10^{-3}$, 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.

Send correspondence to Aparecido Divino da Cruz. Núcleo de Pesquisas Replicon, Departamento de Biologia, Universidade Católica de Goiás, Av. Universitária 1069, Caixa Postal 86, 74605-010 Goiânia, GO, Brazil. E-mail: acruz@ucg.br.

***hprt* cloning assay.** Three groups of TK6 cells (1.6×10^6 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 $9.7 \pm 0.74\%$ Gy/min, using a ^{137}Cs γ-ray source (Gammacell 1000, Nordion International Inc., Ontario, Canada), and then incubated for three days at 37 °C and 5% CO_2 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,

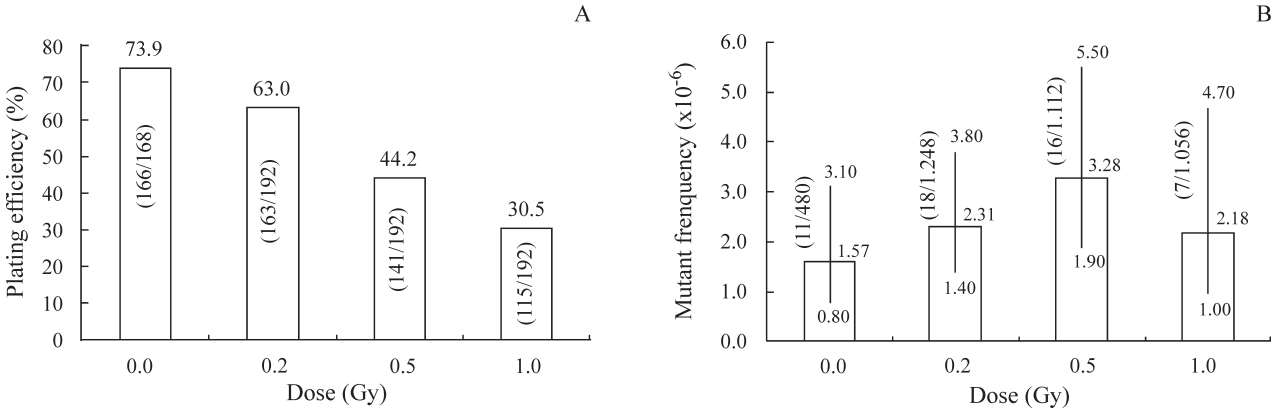

Figure 1 - Dose response of TK6 cells to ionizing radiation for (A) plating efficiencies and (B) mutant frequencies, using Poisson distribution, as described by Albertini *et al*. (2000). Error bars represent the standard deviation of each mutant frequency. Positive clones per well plated are shown in brackets. $\chi^2 = 22.92$ and 48.94 for the plating efficiencies of the groups exposed to 0.5 and 1.0 Gy, respectively. $\chi^2 = 110.96$, 296.33, and 31.96 for the mutant frequencies of the groups exposed to 0.2, 0.5, and 1.0 Gy, respectively. p = 0.001.

primers 100 pmol/μL, dNTP 25 mM (v/v), 2.5 U/μL Taq polymerase, and 100 ng of DNA preparation. The touchdown 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, *ANK1*, 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 1.57×10^{-6}, whereas the frequencies for the groups exposed to 0.2, 0.5 and 1.0 Gy were 2.31, 3.28, and 2.18×10^{-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 by other authors, who reported spontaneous mutation frequencies ranging from 1.3 to 3.8×10^{-6} (Giver *et al*., 1993; Nelson *et al*., 1994; Sussman *et al*., 1999; Tomita-Mitchel *et al*, 2000). The *hprt* mutant frequencies for the groups exposed to 0.2 and 0.5 Gy were closer to the mutant frequencies of TK6 cells exposed to 0.3 and 0.5 Gy described elsewhere (Grosovsky and Little, 1985). The previously reported *hprt* mutant fraction for lymphoblastoid cells exposed *in vitro* to 1.0 Gy is $17.1 \pm 7.8 \times 10^{-6}$ (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 $10.1 \pm 0.4 \times 10^{-6}$ 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 (5.4×10^{-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^R clones, MSI frequencies were estimated to be lower than 8.6, 4.7, 7.7 and 12×10^{-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.

	Unexposed group		Exposed groups (*hprt* mutant)		
	Plating efficiency	*hprt* mutant	0.2 Gy	0.5 Gy	1.0 Gy
Frequency	73.9%	1.57×10^{-6}	2.31×10^{-6}	3.28×10^{-6}	2.18×10^{-6}
N. of positive clones / N. of wells plated	166/168	11/480	18/1,248	16/1,112	7/1,056
N. of alleles studied[1]	1,112	116	212	130	82
N. of MSI/No. of alleles studied	6/1,112	0/116	0/212	0/130	0/82
MSI frequency	5.4×10^{-3}	$< 8.6 \times 10^{-3}$	$< 4.7 \times 10^{-3}$	$< 7.7 \times 10^{-3}$	$< 12 \times 10^{-3}$

[1]Microsatellite analysis was randomly performed among the positive clones, where each clone was considered as having two alleles.

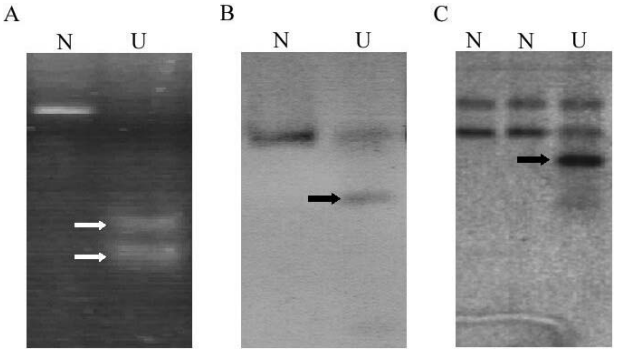

Figure 2 - MSI in TK6 cell clone PCR products for loci (a) *nm 23-H1,* (B) D8S135, (C) *p53* were separated in 7% polyacrylamide. Arrows indicate the new allele. N = Normal; U = Unstable.

microsatellite instability frequency higher than 30%, using a panel of microsatellite markers (Ayres *et al*., 2004). Moreover, *hprt* mutant selection is a rare event measured per ~10^{-6} assayed cells, in which it is assumed that only one mutant clone gives raise 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 x 10^{-3} found in this study is closer to the data reported for peripheral T lymphocytes, which vary from 2.9 to 5 x 10^{-3} (Shibata *et al*., 1994; Hackman *et al*., 1995). However, our spontaneous MSI frequency was higher than 8 x 10^{-4}, as reported by Li and colleagues (1994) for unexposed TK6 cells. Additionally, Davies and colleague (1999) found a spontaneous MSI frequency of 1 x 10^{-4} in *hprt* non-selected clones and of 3 x 10^{-3} in 6-TG^R 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 Grosovksy (2000) reported one event of MSI induced by ionizing radiation at 59 *tk* mutants (6 x 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^R 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.

References

Albertini RJ, Anderson D, Douglas GR, Hagmar L, Hemminki K, Merlo F, Natarajan AT, Norppa H, Shuker DEG, Tice R, Walters MD and Aitio A (2000) IPCS guidelines for the monitoring of genotoxic effects of carcinogens in humans. Mut Res 463:111-72.

Aquilina G and Bignami M (2001) Mismatch repair in correction of replication errors and processing of DNA damage. J Cel Physiol 187:145-54.

Ayres FM, Momotuk E, Bastos CC and da Cruz AD (2004) Detection of mutator phenotype in Brazilian patients with acute and chronic myeloid leukemia. Genet Mol Biol 27:483-8.

Berry SE, Davis TW, Schupp JE, Hwang HS, de Wind N and Kinsella TJ (2000) Selective radiosensitization of drug-resistant MutS homologue-2 (MSH2) mismatch repair-deficient cells by halogenated thymidine (dThd) analogues: Msh2 mediates dThd analogue DNA levels and the differential cytotoxicity and cell cycle effects of the dThd analogues and 6-thioguanine. Cancer Res 60:5773-80.

Davies MJ, Turner JG, Vives-Bauza C and Rumsby PC (1999) Investigation of mutant frequency at the *HPRT* locus and changes in microsatellite sequences in healthy young adults. Mutat Res 431:317-23.

Giver CR and Grosovsky AJ (2000) Radiation specific patterns of loss of heterozygosity on chromosome 17q. Mutat Res 450:201-9.

Giver CR, Nelson SL and Grosovsky AJ (1993) Spectrum of spontaneous *HPRT* mutations in TK6 human lymphoblasts. Environ Mol Mutagen 22:138-46.

Grosovsky AJ and Little JB (1985) Evidence for linear response for the induction of mutations in human cells by X-ray exposures below 10 rads. Proc Natl Acad Sci USA 82:2092-5.

Grosovsky AJ, Parks KK, Giver CR and Nelson SL (1996) Clonal analysis of delayed karyotypic abnormalities and gene mutations in radiation-induced genetic instability. Mol Cell Biol 16:6252-62.

Hackman P, Gabbani G, Osterholm A-M, Hellegren D and Lambert B (1995) Spontaneous length variation in microsatellite DNA from human T-cell clones. Genes Chromosomes Cancer 14:215-19.

Kleczkowska HE, Marra G, Lettieri T and Jiricny J (2001) hMSH3 and hMSH6 interact with PCNA and colocalize with it to replication foci. Genes Dev 15:724-36.

Li C-Y, Yandell DW and Little JB (1994) Elevated frequency of microsatellite mutation in TK6 human lymphoblast clones selected for mutations at the thymidine kinase locus. Mol Cell Biol 14:4373-9.

Nelson SL, Giver CR and Grosovsky AJ (1994) Spectrum of X-ray-induced mutation in the human *hprt* gene. Carcinogenesis 15:495-502.

Pavanello S and Clorofero E (2000) Biological indicators of genotoxic risk and metabolic polymorphism. Mutat Res 463:285-308.

Phillips EN, Gebow D and Liber LH (1997) Spectra of X-ray and spontaneous intragenic *HPRT* mutations in closely related human cells differentially expressing the *p53* tumor suppressor gene. Radiat Res 147:138-47.

Phillips EN, Xia F, Kelsey KT and Liber HL (1995) Spectra of spontaneous and X-ray-induced mutations at the *hprt* locus in related human lymphoblast cell lines that express wild-type or mutant p53. Radiat Res 143:255-62.

Shibata D, Peinaldo MA, Ionov Y, Malkhosyan S and Perucho M (1994). Genomic instability in repeated sequences is an

early somatic event in colorectal tumorigenesis that persists after transformation. Nat Genet 6:273-81.

Skopek TR, Liber HL, Penman BW and Thilly WG (1978) Isolation of a human lymphoblastoid line heterozygous at the thymidine kinase locus: Possibility for a rapid human cell mutation assay. Biochem Biophys Res Commun 84:411-6.

Sussman HE, Olivero OA, Meng Q, Pietras SM, Poirierr MC, O'Neil P, Finette BA, Bauer MJ and Walker VE (1999) Genotoxicity of 3'-azido-3'deoxythymidine in the human lymphoblastoid cell line, TK6: Relationships between DNA incorporation, mutant frequency, and spectrum of deletion mutations in *HPRT*. Mutat Res 429:249-59.

Tomita-Mitchel A, Kat AG, Marcelino LA, Li-Sucholeiki X-C, Goodluck-Griffith J and Thilly WG (2000) Mismatch repair deficient human cells: Spontaneous and MNNG-induced mutational spectra in the *HPRT* gene. Mutat Res 450:125-38.

Associate Editor: Catarina S. Takahashi