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Genomic instability in human lymphoid cells exposed to 1 GeV/amu Fe ions

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Abstract

The goal of this study was to assess whether charged particle radiations of importance to spaceflight elicit genomic instability in human TK6 lymphoblasts. The incidence of genomic instability in TK6 cells was assessed ~21 days after exposure to 2, 4, or 6 Fe ions (1 GeV/amu, LET= 146 keV/µm). Three indices of instability were used: intraclonal karyotypic heterogeneity, mutation rate analysis at the thymidine kinase (*TK1*) locus, and re-cloning efficiency. Fifteen of sixty clones demonstrated karyotypic heterogeneity. Five clones had multiple indicators of karyotypic change. One clone was markedly hypomutable and polyploid. Six clones were hypomutable, while 21 clones were mutators. Of these, seven were karyotypically unstable. Six clones had low re-cloning efficiencies, one of which was a mutator. All had normal karyotypes. In summary, many clones that survived exposure to a low fluence of Fe ions manifested one or more forms of genomic instability that may hasten the development of neoplasia through deletion or by recombination.

KEYWORDS: Genomic instability, Fe ions, human cells.

1. Introduction

Ionizing radiation is a human carcinogen [1]. Carcinogenesis is a multi-stage process that involves multiple genetic changes. Persons with a mutator phenotype are predisposed to cancer [2-3]. Attempts have been made to ascertain whether densely ionizing radiations induce a persistent, transmissible genomic instability in the progeny of irradiated cells [4]. Genomic instability has been defined in many different ways. The purpose of this investigation was to assess whether low fluence exposures to Fe ions induce genomic instability in human TK6 lymphoblasts, as was shown for exposure to X-rays [5].

Fe ions represent the most abundant high Z particle in the galactic cosmic radiation [6]. Nevertheless, individual cells will be traversed by either one or no Fe ions during an extended mission in extramagnetospheric space [7]. In the present study, the incidence of genomic instability was assessed 25-30 generations after low fluence exposures wherein a majority of the cells were traversed by at least one Fe ion. Instability was assessed in three ways: by analysis of G-banded karyotypes, by measuring the mutation rate at the *TK1* locus, and by measuring the re-cloning efficiency of single cells.

2. Materials and methods

2.1. *Cell lines*

TK6 cells were grown at 37° C in a 5% CO_2 atmosphere in RPMI-1640 medium supplemented with 10% heat-inactivated horse serum.

2.2. *Irradiations and post-irradiation growth*

Cells were exposed to 0-6 Fe ions (1 GeV/amu) per cell in suspension at the AGS at Brookhaven National Laboratory. The LET was measured using a series of silicon detectors and averaged 146 keV/µm. The corresponding doses were 0- 189 cGy. Replicate cultures were exposed and split out immediately to ensure the independence of surviving clones. Cells were seeded into 96-well microtiter dishes at extremely low densities (0.1-4 cells/ well) to ensure the monoclonal origin of each colony. Five independent clones were selected from untreated cultures. Twenty independent clones were picked from cultures exposed to 2, 4, or 6 Fe ions respectively (60 irradiated clones). Each clone was grown for approximately 21 days post-irradiation prior to characterization.

2.4. *Karyotypic analysis*

Metaphase preparation was performed according to standard procedures [5]. For each clone, a minimum of 10 G-banded metaphase spreads was examined.

2.5. *Mutation rate determinations at the TK1 locus*

Mutation rates were determined by inoculating 16 flasks with 10^3 cells and allowing expansion to approximately 10^6 cells per culture. Each culture was seeded in its entirety into 96-well dishes containing 2 µg/ml trifluorothymidine. Mutation rates were determined after 14 or 21 days of growth using the P(0) method [5].

2.6. *Re-cloning efficiency*

TK6 cells were seeded in 96-well dishes at 1 cell per well. The cloning efficiency was determined after 14 days of growth [9].

3. Results

The analysis of karyotypic heterogeneity has been completed in 60 clones of Fe-exposed TK6 cells and 5 parallel control clones. The analysis of *TK1* mutation rates has been done in all 65 clones and three bulk populations of TK6 cells, and re-cloning efficiencies have been measured in all 65 clones and three bulk populations of TK6 cells.

The normal karyotype of TK6 cells is extremely stable. The karyotype remains 47, XY, 13+, 14q+, 21p+. This karyotype has remained the same since the original report [8]. Karyotypic heterogeneity in this study was defined as at least two sub-populations of cells among ten G-banded metaphase spreads from a given clone, at least one of which manifests a structural abnormality. Fourteen of sixty irradiated clones demonstrated karyotypic heterogeneity 25-30 population doublings post-irradiation, and six of these clones had multiple indicators of karyotypic change. This includes clones with three or more sub-populations, with at least two different structural abnormalities, or a clone with at least two sub-populations that includes at least one multiply rearranged chromosome (e.g. a derivative or marker chromosome). One clone demonstrated simple karyotypic heterogeneity and polyploidy.

TK1 mutation rates in the Fe-irradiated TK6 clones were checked 25-30 population doublings post-irradiation. The mutation rates were assessed for both 'normal-growth' (NG) and 'slow-growth' (SG) mutants. The *TK1* mutation rate among five unirradiated TK6 clones was combined with mutation rate determinations on three bulk populations of TK6 cells to serve as a baseline. The mean baseline mutation rate for NG *TK1* mutants was 4.3×10^{-7}/cell/generation (range ± 2 standard deviations = 0-9 x 10^{-7}/cell/generation), while the mean baseline mutation rate for SG *TK1* mutants was 1.03×10^{-6}/cell/generation (range ± 2 standard deviations = 0.57-1.48 × 10^{-6}/ cell/generation). A clone was defined as a mutator if it had a mutation rate > 2 S.D. above the mean for the controls (either NG or SG). One clone was markedly hypomutable. Chromosomal analysis showed that this clone was polyploid. Six others were hypomutable. In contrast, twenty one clones were classified as mutators. Of these, fourteen had stable karyotypes, five met the definition of karyotypic heterogeneity and two had multiple indicators of karyotypic change. Thus, there was some association between karyotypic change and the mutator phenotype.

The cloning efficiency of irradiated and non-irradiated cells was assessed 25-30 generations post-irradiation. The mean re-cloning efficiency for 5 unirradiated clones and three bulk populations of TK6 cells was 0.658 (range ± 2 S. D. = 0.444-0.782). Six of sixty irradiated clones had a re-cloning efficiency that was > 2 S.D. below the mean of the controls. One of these clones was a high mutator, but all had normal karyotypes. In this study, re-cloning efficiency did not seem to be a good predictor of other instability phenotypes.

4. Discussion

The process of carcinogenesis requires multiple genetic alterations to occur in an individual cell. These multiple alterations often include acquisition of a growth advantage, an escape from apoptosis, and the accumulation of chromosome changes. Radiation-induced genomic instability may account for the high frequencies of initiated cells both *in vitro* [10] and *in vivo* [11]. Differences in the sensitivities of mouse strains to radiation-induced mammary carcinogenesis have been associated with their susceptibility to karyotypic instability [12], and differences have been reported in the susceptibility of cells from different human donors to radiation-induced genomic instability [13-14].

Our results are in partial agreement with those obtained in human-hamster hybrid cells where X-rays induce chromosomal instability in a dose-dependent manner but were not effective at producing a mutator phenotype [15-16]. In contrast, our results agree with studies done in CHO cells that show induction of chromosomal instability and a mutator phenotype that reflects an increase in spontaneous mutagenesis [17]. In summary, low fluence exposures to Fe ions were potent inducers of genomic instability in human cells.

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REFERENCES

[1] Little MP, Muirhead CR. Curvature in the cancer mortality dose response in Japanese atomic bomb survivors: absence of evidence of threshold. Int J Radiat Biol 1998: 74; 471-480.

[2] Loeb LA. Mutator phenotype may be required for multistage carcinogenesis. Cancer Res 1991: 51; 3075-3079.

[3] Modrich, P. Mismatch repair, genetic stability, and cancer. Science 1994: 266; 792-801.

[4] Blakely EA, Kronenberg, A. Heavy-ion radiobiology: new approaches to delineate mechanisms underlying enhanced biological effectiveness. Radiat Res 1998: 150; S126-145.

[5] Grosovsky AJ, Parks K, Giver CR, Nelson SL. Clonal analysis of delayed karyotypic abnormalities and gene

mutations in radiation-induced genetic instability. Mol Cell Biol. 1996: 16; 6252-6262.

[6] NCRP (National Council on Radiation Protection and Measurements). NCRP Report no. 98. Bethesda, MD 1993.

[7] Curtis SB, Letaw JR. Galactic cosmic rays and cell hit frequencies outside the magnetosphere. Adv Space Res 1989: 9(10); 293-298.

[8] Yandell DW, Little JB. Chromosome 14 marker appearance in a human B lymphoblastoid cell line of nonmalignant origin. Cancer Genet and Cytogen 1986: 20; 231-239.

[9] Furth EE, Thilly WG, Penman BW, Liber HL, Rand WM. Quantitative assay for mutation in diploid human lymphoblasts using microtiter plates. Anal Biochem 1981: 110; 1-8.

[10] Kennedy AR, Cairns J, Little JB. Timing of the steps in transformation of C3H 10T 1/2 cells by X-irradiation. Nature (London) 1984: 307; 85-86.

[11] Kamiya K, Yasukawa-Barnes J, Mitchen JM, Gould MN, Clifton KH. Evidence that carcinogenesis involves an imbalance between epigenetic high-frequency initiation and suppression of promotion. Proc Natl Aca. Sci USA 1995: 92; 1332-1336.

[12] Ponnaiya B, Cornforth MN, Ullrich RL. Radiation-induced chromosomal instability in BALB/C and C57BL/6 mice: the difference is as clear as black and white. Radiat Res 1997: 147; 121-125.

[13] Kadhim MA, Lorimore SA, Hepburn MD, Goodhead DT, Buckle VJ, Wright EG. Alpha-particle-induced chromosomal instability in human bone marrow cells. Lancet 1994: 344; 987-988.

[14] Sabatier L, Lebeau J, Dutrillaux B. Radiation-induced carcinogenesis: individual sensitivity and genomic instability. Radiat Environ Biophys 1995: 34; 229-32.

[15] Limoli, CL, Corcoran JJ, Milligan JR, Ward JF, Morgan WF. Critical target and dose and dose-rate responses for the induction of chromosomal instability by ionizing radiation. Radiat Res 1999: 151; 677-685.

[16] Limoli, CL, Kaplan MI, Corcoran J, Meyers M, Boothman DA. Morgan WF. Chromosomal instablity and its relationship to other end points of genomic instability. Cancer Res 997; 57; 5557-5563.

[17] Little JB, Nagasawa H, Pfenning T, Vetrovs H. Radiation-induced genomic instability: delayed mutagenic and cytogenetic effects of X rays and alpha particles. Radiat Res 1997: 148; 299-307.