

Mechanisms of mutagenesis in human cells exposed to 55 MeV protons

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Abstract

Protons represent the major type of charged particle radiation in spaceflight environments. The purpose of this study was to assess mutations arising in human lymphoid cells exposed to protons. Mutations were quantitated at the thymidine kinase (*TK1*) locus in cell lines derived from the same donor: TK6 cells (wt *TP53*) and WTK1 cells (mutant *TP53*). WTK1 cells were much more susceptible to mutagenesis following proton exposure than TK6 cells. Intragenic deletions were observed among early-arising *TK1* mutants in TK6 cells, but not in WTK1 cells where all of the mutants arose by LOH. Deletion was the predominant mode of LOH in TK6 cells, while allelic recombination was the major mode of LOH in WTK1 cells. Deletions were of variable lengths, from < 1 cM to 64 cM, while mutations that arose by allelic recombination often extended to the telomere. In summary, proton exposures elicited many types of mutations at an autosomal locus in human cells. Most involved large scale loss of genetic information, either through deletion or by recombination.

KEYWORDS: Protons, mutation, *TK1* locus, *TP53*.

1. Introduction

Human spaceflight involves exposure to complex radiation fields. In low earth orbit, the inclination affects time spent in the trapped radiation belts while altitude influences the amount of atmospheric shielding. The galactic cosmic radiation contributes to human exposure in low earth orbit and in interplanetary space. Periodic solar particle events contribute to human exposure particularly during extravehicular activities. Each environment includes a panoply of charged particles, but protons predominate.

Protons are sparsely ionizing unless they are near the end of their path. Very low energy protons have unusual biological effects, exceeding those produced by higher Z ions with similar LETs [1-3]. Low energy protons are a minor part of the initial proton energy spectrum in space.

In the present study, the mutagenic effectiveness of 55 MeV protons was measured. Two lymphoblastoid cell lines were employed that were derived from the same donor. Mutations were measured at the autosomal, heterozygous *TK1* locus. The active *TK1* allele is located on the same copy of chromosome 17q in TK6 and WTK1 cells [4-5]. TK6 cells express only wild-type *TP53*, while WTK1 cells express homozygous mutant *TP53* [6-8]. These cell lines are differentially mutable following radiation exposures [9-11].

2. Materials and methods

2.1. Cell lines

TK6 and WTK1 cells were grown at 37°C in a 5% CO₂ atmosphere in RPMI-1640 medium supplemented with 10% heat-inactivated horse serum.

2.2. Irradiations

Cells were exposed to 55 MeV protons in suspension at the 88" cyclotron at LBNL. The LET was measured throughout the thickness of a flask using a series of silicon detectors and averaged 1.46 keV/μm.

2.3. *TK1* mutation assays

Cells were pre-treated with CHAT according to standard methods [12]. Following irradiation, cells were kept in standard growth medium for three days to allow for phenotypic expression. TK6 cells were seeded into 96-well microtiter dishes at 4 × 10⁴ cells/well in the presence of 2.0 μg/ml of trifluorothymidine (TFT). An aliquot of each culture was seeded at 1 cell/well without TFT to determine the plating efficiency. WTK1 cells were seeded into TFT medium at 200-1000 cells/well. Early-arising *TK1* mutants were scored after 11 days. Dishes were re-fed with TFT. Late-arising mutants were scored after 18 days. *TK1* mutation frequencies were calculated according to standard methods [12].

2.4. Characterization of *TK1* mutants

Individual mutants were collected after exposure to 190.5 cGy of protons. Each DNA sample was cut with *SacI* and subjected to electrophoresis and Southern blotting using the *TK1* cDNA probe [13]. Gene dosage analysis was performed by re-probing each blot with the cDNA for *BCL-2*, located on chromosome 18 [14]. A ratio was established between the 8.4 kb band revealed by the *TK1* probe

in the control heterozygote and the corresponding 8.4 kb band revealed by the *BCL-2* probe. This established the intensity ratio for a single copy of the silent *TK1* allele. Similar intensity ratios were established for each mutant. Mutation tract lengths were analyzed using 14 markers linked to *TK1* [15].

3. Results

3.1. Mutation induction at the *TK1* locus

WTK1 cells were nearly 100-fold more susceptible to *TK1* mutation than TK6 cells (induction for TK6 cells $2.1 \pm 0.11 \times 10^{-7}/\text{cGy}$, $r^2 = 0.95$; induction for WTK1 cells $184 \pm 30 \times 10^{-7}/\text{cGy}$, $r^2 = 0.78$).

3.2. *TK1* mutation spectra

The early-arising mutants in TK6 cells included small mutations, partial gene deletions or rearrangements, and mutants that lost heterozygosity (LOH). Similar mutants in WTK1 cells all showed LOH (χ^2 , 1 d.f. = 47.03, $p < 0.001$). Only 1/25 early-arising LOH mutants in TK6 cells arose via allelic recombination (gene dosage for the silent *TK1* allele $> 1.8x$ control), while 44/60 early-arising mutants of WTK1 arose by allelic recombination (χ^2 , 1 d.f. = 34.04, $p < 0.001$). For late-arising mutants of TK6 cells, 60/71 arose by LOH, while 60/60 late-arising mutants of WTK1 cells arose by LOH (χ^2 , 1 d.f. = 10.15, $p < 0.002$). Recombination was less common in TK6-derived mutants than in WTK1 derived mutants (χ^2 , 1 d.f. = 15.68, $p < 0.001$).

Deletion tracts were < 20 cM in early-arising TK6 mutants, but were larger in WTK1 cells (χ^2 , 1 d.f. = 8.475, $p < 0.025$). Deletions were longer amongst late-arising clones and frequently extended over at least 29 cM. Longer deletion tracts occurred in WTK1 cells (χ^2 , 1 d.f. = 8.945, $p < 0.005$). Recombination tracts generally extended to the most telomeric marker (> 21 cM). Five of 90 mutants that arose via recombination had interstitial LOH tracts indicating double exchanges. In summary, proton exposures elicited many types of mutations in human cells. Most involved large scale loss of genetic information. The increase in mutant frequency in the *TP53* mutant WTK1 cells was associated with large LOH tracts and a higher proportion of recombination-mediated mutations.

4. Discussion

Large scale genetic loss is a common feature of ionizing radiation-induced mutations. Protons are very effective at producing extensive deletion mutations. Protons elicit allelic recombination that can initiate at any point centromeric to the *TK1* locus (data not shown). Mitotic recombination occurs *in*

vivo [16, 17] and the recombination events are typically single exchanges. Ionizing radiation can initiate large LOH tracts by deletional and recombinational mechanisms [18]. The high susceptibility to proton-induced *TK1* mutation and allelic recombination in WTK1 cells confirms earlier work with X-rays and alpha particles [9-11] and supports the hypothesis that apoptosis, which occurs rapidly in TK6 cells but more slowly in WTK1 cells, may help cleanse the genome of cells that are prone to recombination [6, 19].

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