Painting analysis of chromosome aberrations induced by energetic heavy ions in human cells

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The Space Radiation Environment

Representation of the major sources of ionizing radiation of importance to manned missions in low-Earth orbit. Note the spatial distribution of the trapped radiation belts.

- **SOLAR PARTICLE EVENT** (Protons to Iron Nuclei)
- **INNER RADIATION BELT** (Protons)
- **OUTER RADIATION BELT** (Electrons)
- **GALACTIC COSMIC RADIATION (GCR)** (Protons to Iron Nuclei)
- **SOUTH ATLANTIC ANOMALY** (Protons)
Figure D.1. Abundances (a) and Energy Spectra (b) of GCR

Relative Abundance

Atomic Number (Z)

Differential Flux (m^2·sterad·sec·MeV/nucleon)^{-1}

Kinetic Energy (MeV/Nucleon)

(a) (b)
DSB induction

High-LET

Low-LET
Radiation-induced chromosome aberrations in lymphocytes in vitro

Why do we study chromosomes?

Chromosome aberrations in astronauts’ lymphocytes are analyzed to determine the biological dose received from long-term space missions.

### Biodosimetry results

<table>
<thead>
<tr>
<th>Subject</th>
<th>TLD reading (cGy)</th>
<th>Biological dose measured using values for translocation (cSv)</th>
<th>Average RBE*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.06</td>
<td>16</td>
<td>4.2</td>
</tr>
<tr>
<td>2</td>
<td>3.78</td>
<td>18</td>
<td>3.8</td>
</tr>
<tr>
<td>3</td>
<td>5.68</td>
<td>20</td>
<td>2.8</td>
</tr>
<tr>
<td>4</td>
<td>4.16</td>
<td>23</td>
<td>4.4</td>
</tr>
<tr>
<td>5</td>
<td>4.16</td>
<td>14</td>
<td>2.7</td>
</tr>
<tr>
<td>6</td>
<td>4.16</td>
<td>12</td>
<td>2.3</td>
</tr>
</tbody>
</table>

*25% correction for high-LET radiation in TLD measurement is included.

Mission duration: 3-5 months
Altitude: 190 NM
Inclination: 51.6 degree
Objectives

• Are there bio-signatures for space radiation exposure?
• Are chromosome aberrations associated with radiation risks?
Chromosome staining/painting techniques

- Giemsa
- FISH
- mFISH
- mBAND
Chromosome aberration

Stable

Unstable

Intra-chromosome exchange

Inter-chromosome exchange
Telomere Analysis

Human lymphocytes exposed to 2 Gy gamma rays. Chromosomes #2 and #4 were painted.

False incomplete exchange
Most of the incomplete exchanges analyzed with FISH are actually complete.
Human fibroblast cells exposed to radiation of different qualities

- The fraction of unrejoined chromosome breaks are higher for high LET
- Unrejoined breaks and incomplete chromosomal exchanges are possible biosignatures of high-LET radiation

High-LET radiation induces more unrejoined DNA double strand breaks

Desai, Davis, O’Neill, Durante, Cucinotta and Wu, Rad. Res. 2005
Complex aberrations -- mFISH analysis

mFISH showed a higher fraction of complex and incomplete exchanges for high-LET
Interphase vs. metaphase: Issues of biosignature
(F ratio: Ratio of dicentrics to centric rings)

Centromere probes were used.

<table>
<thead>
<tr>
<th>Radiation</th>
<th>Dose (Gy)</th>
<th>Harvest method</th>
<th>F ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\gamma$ ray</td>
<td>2</td>
<td>PCC</td>
<td>15.3±6.3</td>
</tr>
<tr>
<td>$\gamma$ ray</td>
<td>2</td>
<td>Meta</td>
<td>12.5±5.9</td>
</tr>
<tr>
<td>$\gamma$ ray</td>
<td>5</td>
<td>PCC</td>
<td>8.2±2.0</td>
</tr>
<tr>
<td>$\gamma$ ray</td>
<td>5</td>
<td>Meta</td>
<td>9.1±2.5</td>
</tr>
<tr>
<td>1 GeV/u Fe</td>
<td>3</td>
<td>PCC</td>
<td>5.2±0.9</td>
</tr>
<tr>
<td>1 GeV/u Fe</td>
<td>3</td>
<td>Meta</td>
<td>9.1±2.2</td>
</tr>
</tbody>
</table>

Wu, George, Kawata, Willingham and Cucinotta, Rad. Res. 2001
mBAND analysis

Normal

Inversion
Inter- vs. intra chromosome exchanges (mBAND)

Graph showing aberrations in chromosome 3/cell versus dose (Gy) for different radiation sources, including Fe (600 MeV/n) and 137 Cs γ-ray.
Most inversions were involved with other inter- and/or intra-chromosome rearrangements
Do spaceflight factors alter the cellular response to radiation exposure?

Hammond et al. Nature Medicine 1999
Chromosome aberration frequencies in pre- and post-flight astronaut lymphocytes irradiated in vitro with low-LET radiation (Wu et al. Phys. Med. 2001)

Nission: STS-103

Duration: 8 days

Blood draw schedule:
- 10 days before launch, JSC, kept at 4 C for 1 day before exposure
- 0 days after landing, KSC, kept at 4 C and received next day. Kept at 4 C before exposure
- 14 days after landing, KSC, kept at 4 C for 1 day before exposure

Irradiation: Whole blood was irradiated to gamma rays

Procedure: Whole blood was stimulated to grow with PHA in growth medium and chromosomes were collected following standard procedures.

Chromosome analysis: Chromosomes #1 and #5 were painted.
Do spaceflight factors alter the cellular response to radiation exposure?

Wu, George, Willingham and Cucinotta, Physica Medica 2001

Summary

• FISH, mFISH, mBAND, telomere and centromere probes have been used to study chromosome aberrations induced in human cells exposed to low- and high-LET radiation in vitro

• High-LET induced damages are mostly a single track effect

• Unrejoined chromosome breaks (incomplete exchanges) and complex type aberrations were higher for high-LET

• Biosignatures may depend on the method the samples are collected

• Recent mBAND analysis has revealed more information about the nature of intra-chromosome exchanges

• Whether space flight/microgravity affects radiation-induced chromosome aberration frequencies is still an open question.