Distribution of chromosome breakpoints in human epithelial cells exposed to low- and high-LET radiation

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July 18, 2010
mBAND Analysis

XCyte 5-labeling scheme

- DEAC (Ex 426 nm / Em 480 nm)
- FITC (Ex 502 nm / Em 530 nm)
- Spectrum Orange (Ex 559 nm / Em 588 nm)
- Texas Red (Ex 595 nm / Em 615 nm)
- Cy5 (Ex 649 nm / Em 670 nm)
Example of chromosome 3 painted with mBAND

Interchromosomal exchange (Complex)

Deletion (Ring)
Intrachromosomal exchange (inversion)

Interchromosomal exchange

Normal

Example of chromosome 3 painted with mBAND
Irradiation

**High Dose Rate**
- $^{137}$Cs $\gamma$-ray: 2.0 Gy/min University of Texas, MD Anderson Cancer Center
- Fe ions: 0.5 Gy/min NASA Space Radiation Laboratory / BNL

**Low Dose Rate**
- $^{137}$Cs $\gamma$-ray: 1.7 cGy/h NASA/JSC
- Neutron: 2.5 cGy/h Los Alamos Nuclear Science Center (LANSCE) 30L

Neutron energy spectrum on the ISS, measured spectra on Mir and the normalized LANSCE energy spectra.

*The LANCE neutron energy spectrum is similar over a wide energy range to expected spectrum inside the International Space Station (ISS).*

Badhwar G.D. et al. (2000)
Induction of chromosome 3 aberration in human cells by neutrons, Fe-ions or γ-rays

Relative Biological Effectiveness (RBE)

Fe: 8.4
Neutron: 26.4
Frequency distributions of breaks/chromosome 3

- γ-rays (2 Gy/min)
- Fe-ions (0.5 Gy/min)
- Neutrons (2.5 cGy/h)
Induction of interchromosome exchanges (A) and intra-chromosome exchanges (B) in human chromosome 3 by neutrons, Fe-ions or γ-rays.

The dose responses for interchromosomal exchanges were linear in all four exposures. However, the dose response for intrachromosomal exchanges were none linear. Increasing dose of high dose rate exposure (Fe-ions or γ-rays) increase the fraction of cells with intrachromosome aberrations, whereas increasing dose of low dose rate exposure (neutrons or γ-rays) does not affect the fraction of cells with intrachromosome aberrations.
Interchromosome exchanges broken down as simple and complex types

- **Simple exchange**
- **Complex exchange**

### Neutrons (2.5 cGy/h)
- Fraction in damaged chromosome 3

### Fe-ions (0.5 Gy/min)
- Fraction in damaged chromosome 3

### γ-rays (2 Gy/min)
- Fraction in damaged chromosome 3

### γ-rays (1.7 cGy/h)
- Fraction in damaged chromosome 3

**Complex exchange:** Chromosome interexchanges involving at least 3 breaks in two or more chromosomes
Induction of inversion

![Graph showing induction of inversion](image)

**Y-rays** (1.7 cGy/h)  
**Y-rays** (2 Gy/min)  
**Fe-ions** (0.5 Gy/min)

Fraction of inversion in cell vs. dose (Gy)
Classification of inversion involved aberrations in chromosome 3

Inversion (simple)

Inter-exchange involved Inversion

Intra-exchange involved Inversion

Inter-exchange and Intra exchange involved Inversion

Fraction of total inversion

- Inv
- Inv + Intra
- Inv + Inter
- Inv + Inter + Intra

y-rays 4Gy  Fe  Neutron
Chromosome rearrangements in human cancer (Olopade et al.)
Distribution of total breaks

- **A**: Distribution of total breaks at 2 Gy and 4 Gy.
- **B**: Distribution of total breaks at 0.1 Gy and 0.2 Gy.
- **C**: Distribution of total breaks at 1 Gy and 2 Gy.
- **D**: Distribution of total breaks at 0.1 Gy and 0.2 Gy.
- **E**: Expected distribution of total breaks.

Bands: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23
Fragment ends participating in interchromosomal exchanges
Fragment ends participating in intrachromosomal exchanges

![Graphs showing fraction of total fragment ends across different bands.](image)
Interchromosome exchanges

- **Neutron**
- **Gamma low dose rate**
- **Fe**
- **Gamma high dose rate**

Frequency of break end vs Band
Comparison of inter-chromosomal breaks:

- Fe-N
- LG-N
- LG-Fe
- HG-N
- HG-Fe
- HG-LG

Comparison of intra-chromosomal breaks:

- Fe-N
- LG-N
- LG-Fe
- HG-N
- HG-Fe
- HG-LG

Statistical analysis
intrachromosomal exchange events between two fragment ends

Low-LET

High-LET
2-D representation of the locations of different segments of Chromosome 3 inside the cell nucleus.

Interchromosome exchange

Intrachromosome exchange

(A)

(B)

(C)
Comparison between the distributions of the genes and of the breakpoints
Conclusions

• Low- and high-LET radiations produced distinct breakpoint distributions.

• The difference of the breakpoint distributions between low- and high-LET only appeared in break ends involved in interchromosome exchanges.

• The breakpoint distributions for break ends participating in intrachromosome exchanges were similar.

• Gene-rich regions do not necessarily have more chromosome breaks.

• High-LET appeared to produce long live (data not shown) or longer live breaks that can migrate a longer distance before rejoining with other breaks.

• Domains occupied by different segments of the chromosomes may be responsible for the breakpoint distribution.
Acknowledgement

Prairie View A&M UNIVERSITY
   Dr. Prem B. SAGANTI
   Dr. Richard WILKINS
   Brad GERSEY

BNL/NSRL
   Dr. Adam Rusek
   NSRL physics dosimetry group

LANSCE
   Dr. Bruce E. TAKALA

Work supported by the NASA Space Radiation Health Program.

Thank you very much!