Cytogenetic signature of heavy charged particles: impact of LET and track structure

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Heavy ion irradiation: characteristic features

Cancer therapy with heavy ions

- Advantages: inverted dose-depth profile, greater biological effectiveness
- ~3000 patients in Japan and Germany
- Questions: late effects
Dose-distribution in nanometer scale

\[
\text{LET} = \frac{dE}{dx}
\]

Linear energy transfer (LET)

(M. Krämer)

13 keV/\mu m

170 keV/\mu m

720 keV/\mu m
Heavy ion irradiation: characteristic features

- Quantity: >300 times higher dose
- Quality: particles
- Dose contribution of Fe-ions
- ~500% uncertainties in health risk for long-term space missions

Radiation exposure in Space

\[ \text{Dose} \propto Z_{\text{eff}}^2 \]

Energy spectrum of ions in galactic cosmic rays

Applied radiations
- X-rays (LET value: 2 keV/\(\mu\)m)
- 990 MeV/u Fe-ions (155 keV/\(\mu\)m)
- 100 MeV/u C-ions (28.9 keV/\(\mu\)m)
- 11.6 MeV/u C-ions (175 keV/\(\mu\)m)
DNA DSB induction after heavy ion irradiation

Cell
Chromosome
Nucleus
(10 \( \mu m / 1 \mu m \) DNA)

DNA
nm scale

Inhomogeneous dose distribution

Primary lesions on the DNA molecule
DNA hierarchical structure: from a polymer thread to spatial organisation of chromosomes
Average dose: 2 Gy, Yield: 200 events/Gy/Cell
LET (linear energy transfer): 16, 120, 720 keV/µm
(200, 15, 1 MeV/u C-ions)

(M. Scholz)
Chromosome aberrations

- Originate from DNA damage (effect of induced DSBs processing)
- Important **biomarker** of cancer risk
- Used as "biological dosimeter"
- Model system: human peripheral blood lymphocytes
- Standard protocol: cell collection at a single time point (48 h)
- Open questions:
  - Cell cycle delay
  - Apoptosis
  - Inter/intra-donor variability
  - Radiation quality

(Ritter, Lee, Nasonova)
Standard experimental design: synchronous cells irradiated in $G_0$

Metaphase analysis (M)

Irradiated cells are stimulated to enter the cell cycle

G$_2$/M checkpoint

Intra-S-phase checkpoint

G$_1$/S checkpoint

lymphocytes

$G_0$

+ PHA
+ BrdU

GSI
Time-course of aberrations in M-cells

X-rays               100 MeV/u C       990 MeV/u Fe      177 MeV/u Fe        4.1 MeV/u Cr
LET:  2 keV/µm        29 keV/µm       155 keV/µm        335 keV/µm         3160 keV/µm

Only first cycle metaphases were scored.
RBE as a function of LET and time!

Analysis of damage at one sampling time will unavoidably result in an under- or overestimation of damage
Analysis of biological data and models involved

• The way out…

To interpret time-dependent yield of aberrations and their distribution—collection of cells over the complete time course of the first mitosis along with a mathematical analysis = integration of data (Scholz et al. 1998, Nasonova et al. 2001, Gudowska et al., 2005)
Random sums of random elements and compound Poisson distribution of lesions.

\[ x(t) = \sum_{j=1}^{n(t)} X_j \]

\[ n(t) = \min\left\{ n : \sum_{j=1}^{n} T_j > t \right\} \]

\[ G_{x(t)}(s) = G_{n(t)}[G_X(s)] \]

\[ G_X(s) = \exp\left( \frac{X}{X_i} \right) \left\{ \exp\left( X_i \left[ e^{-s} - 1 \right] - 1 \right) \right\} \]

\[ \lambda = \langle n \rangle \]

\[ \mu = \langle X_i \rangle \]

\[ (x+1)P(x) = \langle x \rangle \sum_{k=0}^{x} C_k P(x-k) \]

\[ C_k = \frac{\mu^k e^{-\mu}}{k!} \]

\[ P(0) = \exp\left[ \frac{\lambda}{\mu} (e^{-\mu} - 1) \right] \]

In brief: induction can be modelled as a CTRW (renewal process)…

\[ \langle x \rangle = \lambda \mu \]

• Generally: counting Poisson process is time-dependent (cell-cycle delay of damaged cells)

• Intensity rate of secondaries \( X_j \) depends on local, spatial distribution of imparted energy

• The average number of aberrations scored in a population becomes time-dependent
Random sums of random elements and compound Poisson distribution of lesions.

Simple approximation reads…

\[
P(x) = \frac{e^{-\lambda}}{x!} \sum_{n=0}^{\infty} \frac{n^x}{n!} (e^{-\mu} \lambda)^n
\]

1. The number of particle traversals per cell nucleus \( n \), \( \lambda = \langle n \rangle \)
2. The average number of aberrations induced by a particle hit \( \mu \)

175 keV/\( \mu m \) C-ions
2 Gy (7.13x10^6 /cm^2), 48 h
0.89 aberrations per cell
1.78 particle hits/nucleus
Frequency distribution of aberrations per cell

155 keV/µm Fe-ions, 2.3 Gy (9x10⁶ /cm²)

No hit: 11 %, 1 hit: 24 %, 2 hits: 27 %, 3 hits: 20 %, more: 18%

dimension of the cell nucleus
Frequency distribution of aberrations per cell: effect of LET

175 keV/µm C-ions, 2 Gy
(7.13x10^6 /cm^2)

29 keV/µm C-ions, 2 Gy
(43.1x10^6 /cm^2)
Frequency distribution of aberrations per cell: similar LET but different energy and track structure

175 keV/µm C-ions, 2 Gy
(7.13x10⁶ /cm²)
Energy 11.6 MeV/n

155 keV/µm Fe-ions, 2.3 Gy
(9x10⁶ /cm²)
Energy 1 GeV/n
Back to fundamentals:

Physical characteristics of ion beams

Simulation of $\delta$-electron emission by 1 MeV/u protons and C-ions.

11.4 MeV/n C-ions ($R_{\text{max}}=2.3 \mu m$)

Local dose deposited in particle tracks.

(M. Krämer, GSI)
Back to fundamentals: Physical characteristics of ion beams

Fe 990 MeV/n

C 15 MeV/n

Local dose deposited in particle tracks.
Total amount of aberrations/ aberrant cells (integration analysis)

\[ N_k = \prod_{i=1}^{k} N_0 (1 + M_{i}) \]

\[ M_{i}^* = M_{i} \frac{N_k}{N_0} = M_{i} \prod_{i=1}^{k} (1 + M_{i}) \]

- \( A_{\text{tot}} \) total no. of aberrations
- \( a_i \) number of aberrations found at a time \( i \)
- \( M_{i}^* \) corrected mitotic index at a time \( i \)
- \( \Phi_{\text{tot}} \) total fraction of aberrant cells

Reconstructed growth of population with respect to the starting number of cells

\[ A_{\text{tot}} = \sum_{i} a_i M_{i}^* \]

\[ \Phi_{\text{tot}} = \sum_{i} f_i M_{i}^* \]
Cell kinetics in mitosis and cycle delay: lymphocytes
Fe 990 MeV/u, 2.23 Gy

Flux of aberrant cells (cells/h x 10^-3)

Modal values are shifted towards later times

Delay in mitosis can be correlated with a number of aberrations carried by a cell

Gudowska-Nowak et. al.
Detection of cell-cycle delay: time variation of parameter $\lambda$

Time-variation of “hits” (parameter $\lambda$) reflects delay in mitosis of damaged cells
Cell kinetics in mitosis: integrated data for fraction of cells which completed mitosis up to 84h

Survival fractions in first mitosis

Note „efficiency” in the response for different LET values
The microscopic structure of energy deposition of heavy ions influences the production of aberrations.

<table>
<thead>
<tr>
<th><strong>Low LET (2-30 keV/mm)</strong></th>
<th><strong>High LET (150-3000 keV/mm)</strong></th>
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</thead>
<tbody>
<tr>
<td>Homogeneous dose deposition</td>
<td>Inhomogeneous dose deposition</td>
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<tr>
<td>Aberrations are <em>Poisson</em> distributed</td>
<td>Aberrations are distributed according to the <em>compound-Poisson</em> statistics</td>
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<tr>
<td>Aberration yield does not change with time</td>
<td>Correlation between the number of particle hits and delay</td>
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<tr>
<td>Standard metaphase method (48 h) is sufficient</td>
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<td><em>M</em>- (or G2-assay) at several times should be assessed</td>
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SUMMARY

- Data clearly demonstrate that there is a selective delay of heavily damaged cells.

- Cell cycle delays have to be taken into account for a realistic estimate of the cytogenetic effects of heavy ions.

- Data measured at only time-point in metaphase or G2-phase cells insufficient: may mask real efficiency of particles.

? Use of aberrations for risk assessment of high LET particles
2 Gy C-ions versus 2 Gy X-rays

Complex aberrations

Time-course of aberrations
detected by M-FISH
Higher biological effectiveness

Nanometer scale:

complex damage due to localized energy deposition
Microscopic dose distribution of X-rays or ions

90 MeV/u C-ions
29 keV/µm
2 Gy, 43*10^6 /cm²

990 MeV/u Fe-ions
155 keV/µm
2.3 Gy, 9*10^6 /cm²

Particle fluence of 4*10^6 ions/cm²
corresponds to 1 particle hit in average per nucleus
(No hit: 37 %, 1 hit: 37 %, 2 hits: 18 %,
more than 3 hits: 8 %)

177 MeV/u Fe-ions
335 keV/µm
2 Gy, 3.7*10^6 /cm²

4.1 MeV/u Cr-ions
3160 keV/µm
20.3 Gy, 4*10^6 /cm²

X-rays
2 keV/µm
2 Gy
Features of the statistics: overdispersion

comparison of frequency patterns

\[ G_{N+P}(Z) = G_P(Z)G_N(Z) = \exp[\lambda(e^{\mu(Z-1)} - 1) + a(Z - 1)] \]

\[ Z = \exp(isx) \]

\[ G = \langle \exp(isx) \rangle \]

\[ \sigma^2 = \frac{\lambda \mu^2 + \lambda \mu + a}{\lambda \mu + a} = 1 + \frac{\lambda \mu^2}{\lambda \mu + a} \]
Correlation between damage and delay times

Ar ions

X-rays

Gudowska-Nowak et al.
IJRB (2005)
Direct correlation between the average delay time in entering 1st mitosis and the average number of aberrations carried by a cell.

Gudowska-Nowak et al. IJRB (2005)
Mitotic delay as a function of dose

Lymphocytes of the same donor were exposed to either X-rays or Fe ions.
Dose-distribution in micrometer scale

(M. Scholz)

(G. Taucher-Scholz, B. Jacob)