PROTON AND FE ION-INDUCED EARLY AND LATE CHROMOSOME ABERRATIONS IN DIFFERENT CELL TYPES

Presented By:
Rosalin Goss, Ph.D. candidate
Texas Southern University/NASA
Dr. Wilson/Dr. Wu

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Presentation Outline

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BACKGROUND

• Exposure to radiation induces different types of DNA damage.
• Increases mutation and chromosome aberration rates.
• Increases cellular transformation in vitro and in vivo.
• The susceptibility of cells to radiation depends on:

1. Genetic background
2. Growth condition of cells
3. Types of radiation
• Proton and Iron (Fe) are two of the most abundant types of charged particles in deep space.

• Both charged particles produce ionization along their path.

• Protons are called low-linear energy transfer because they are able to pass through matter without losing much energy.
• Stable type chromosome aberrations that survive multiple generations of cell division include translocation and inversions.

• An efficient method to detect an inversion is multi-color banding fluorescent in situ hybridization (mBAND) which allows identification of both inter- and intrachromosome aberrations simultaneously.

• Post irradiation, chromosome aberrations may also arise after multiple cell divisions as a result of genomic instability.

• To investigate the stable or late-arising chromosome aberrations induced after radiation exposure, we exposed human lymphocytes to gamma rays and Fe ions ex vivo, and cultured the cells for multiple generations.
Genomic instability: An increased tendency of the GENOME to acquire MUTATIONS when various processes involved in maintaining and replicating the genome are dysfunctional.

Some of these chromosomal changes were seen in all cells of a tumor but others were not, suggesting that tumor cells are the progeny of a genetically unstable single cell, which continues to acquire chromosomal abnormalities over time.
Two types of elements have a key role in instability leading to chromosome rearrangement:

- those that act in *trans* to prevent instability-
  - DNA repair
  - replication
  - DNA damage checkpoint factors

- those that act *in cis*-
  - chromosomal hotspots of instability such as fragile sites and highly transcribed DNA sequences

DNA DSB can give rise to chromosome instability
Mechanisms of double stranded break repair leading to different chromosome rearrangements

BIR; break-induced replication
SDSA; synthesis-dependent strand annealing

(*repeated fusion and breakage of chromosomes following the loss of a telomere)
Radiation-induced genomic instability

Untargeted radiation effects

observed in unirradiated cells and belong to two different but overlapping categories;

- **Delayed effects**
  arises in the descendants of the irradiated cells after many generations. Delayed phenotypes are not induced uniformly among the progeny of surviving cells.

- **Bystander effects**
  observed in cells that were close to the irradiated cells or that received damaging signals from irradiated cells (radiation-induced bystander effects).

  A) Gap junction intercellular communication
  Stimulating a damage-signaling pathway mediated by p53, P21/Waf1(CDKN1A), Up-regulation of Connexin 43 gap junctions.

  B) Secretion of soluble factors into the cell medium
  TGF-B or IL-8 etc. increase intracellular levels of ROS in unirradiated cells.
Objectives

(1) Determine the relationship between the quality and fluence of energetic charged particles and the induction of delayed damages in chromosomes, which is commonly referred to as chromosomal instability.

(2) Understand the relationship between chromosomal instability and other events at the cellular and molecular level that play a role in the maintenance of genome integrity.

Chromosome aberrations in long term human lymphocytes exposed in vitro to 4 Gy gamma rays
(Preliminary data)

we exposed peripheral blood mononuclear cells collected from healthy subjects to gamma rays, and cultured the cells for multiple generations.
MATERIALS AND METHODS

- Peripheral whole blood was collected from a healthy donor in vacutainer cell tubes containing sodium heparin.
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- Mononuclear cells were immediately separated by centrifugation, washed twice with PBS, counted and resuspended in RPMI1640/2mM Glutamine/10%FBS.
- Cells were exposed \textit{in vitro} to \(\gamma\)-ray doses of 2Gy or 4Gy using a \(^{137}\text{Cs}\)-source at a dose rate of 0.5 Gy/min.
MATERIALS AND METHODS cont’d

• For high LET radiation, the cells were exposed to Fe ions (600MeV/nucleon) at NASA Space Radiation Laboratory (NSRL) at Brookhaven National Laboratory.

• Immediately after irradiation. Cells were seeded at a density of 3 X 10^5 cells/ml in RPMI1640/2mM Glutamine/10%FBS containing 1% Phytohemagglutinin(PHA) and 100 IU/ml IL2 (Invitrogen) for long term culture.

• Chromosomes were collected at 48h which represented the first mitosis, and 7 days and 14 days after irradiation using a premature chromosome condensation (PCC) technique with Calyculin-A.

• Chromosome 3 was painted with the XCyte3 mBAND kit (MetaSystems) and intra- and inter-chromosomal aberrations were analyzed with the mBAND analysis system (MetaSystems).
Chromosomes were collected at 2, 7, 14 days after irradiation for gamma-ray samples, while 2, 11, 20 days for Fe ions samples at similar PDL.
Frequency distribution of early and late aberrations in chromosome 3 of human lymphocytes

Cells were exposed to gamma rays or 600 MeV/u Fe ions, and cultured for different time periods before harvested for chromosome analysis.

About half of the cells initially damaged in chromosome 3 by gamma rays remained after cultured for 14 days, while a significantly smaller fraction of the cells damaged with Fe ions remained for this culture period. The RBE value was high for initial chromosomal damages, but may be low for late damages.
Complex exchanges involving chromosome 3 of human lymphocytes induced by gamma rays or Fe ions

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CONCLUSION

• About half of the cells initially damaged in chromosome 3 by gamma rays remained after cultured for 14 days, while a significantly smaller fraction of the cells damaged with Fe ions remained for this culture period, resulting in a lower RBE value for late chromosome damages in comparison to the early damages.

• Further investigations are needed to determine whether some of the aberrations were formed during the culture period.

• The distribution of break ends participated in interchromosome exchanges for human lymphocytes after radiation exposure was different from the previously published distribution for human mammary epithelial cells, indicating that interphase chromatin folding may play a partial role in the distribution of radiation-induced breaks.