mBAND analysis of early and late damages in the chromosome of human lymphocytes after exposures to gamma rays and Fe ions

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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. Chromosome aberrations were analyzed in cells collected at first mitosis and at several time intervals during the culture period post irradiation. With gamma irradiation, about half of the damages observed at first mitosis remained after 7 day- and 14 day- culture, suggesting the transmissibility of damages to the surviving progeny. At the doses that produced similar frequencies of gamma-induced chromosome aberrations as observed at first mitosis, a significantly lower yield of aberrations remained at the same population doublings after Fe ion exposure. At these equitoxic doses, more complex type aberrations were observed for Fe ions, indicating that Fe ion-induced initial chromosome damages are more severe and may lead to cell death. Detailed analysis of breaks participating in total chromosome exchanges within the first cell cycle post irradiation revealed a common hotspot located in the 3p21 region, which is a known fragile site corresponding to the band 6 in the mBand analysis. The breakpoint distribution in chromosomes collected at 7 days, but not at 14 days, post irradiation appeared similar to the distribution in cells collected within the first cell cycle post irradiation. The breakpoint distribution for human lymphocytes after radiation exposure was different from the previously published distribution for human mammary epithelial cells, indicating that interphase chromatin folding structures play a role in the distribution of radiation-induced breaks.

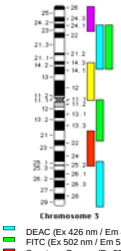
MATERIALS AND METHODS

Peripheral whole blood was collected from a healthy donor in vacutainer cell tubes containing sodium heparin. Mononuclear cells were immediately separated by centrifugation, washed twice with PBS, counted and resuspended in RPMI1640/2mM Glutamine/10%FBS. Cells were exposed *in vitro* to γ-ray doses of 2Gy or 4Gy using a ^{137}Cs-source at a dose rate of 0.5 Gy/min. 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) and100 IU/ml IL2 (Invitrogen) for long term culture. Chromosomes were collected at 48h which represented the first mitosis, and 7days and 14days 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).

RESULTS

1. Multicolor banding *in situ* hybridization (mBAND)

Fig. 1. XCyte 3-labeling scheme



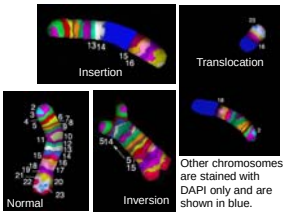
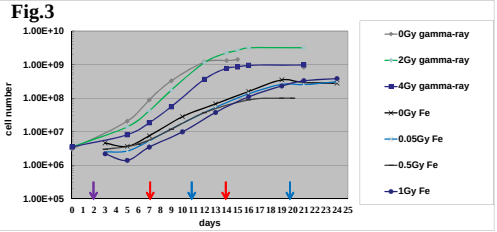


Fig. 2. Examples of chromosome 3 of human lymphocytes painted with mBAND after 4 Gy γ-ray exposure.

2. Growth curve of PBMC

Fig.3



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

3. Damages in Chromosome 3

Fig.4. Frequency distribution of cells containing aberrant chromosome 3

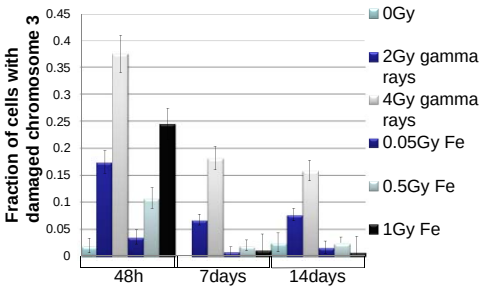


Fig.5. Frequency distribution of breaks in chromosome 3 at 48h after irradiation

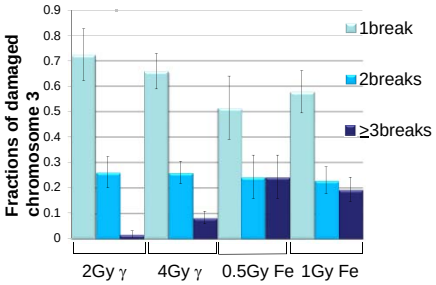


Fig. 6. Frequency distribution of chromosome break ends involved in inter- vs. intrachromosomal exchanges at 48h after irradiation

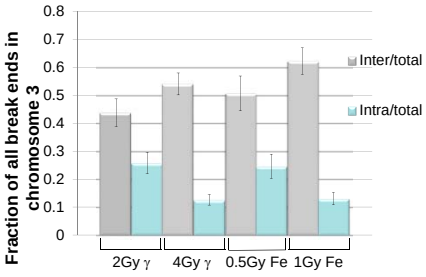
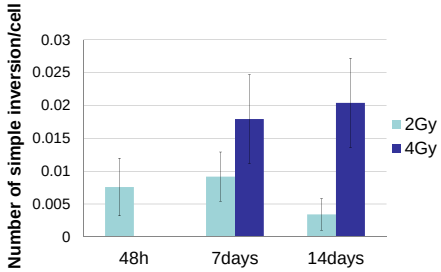
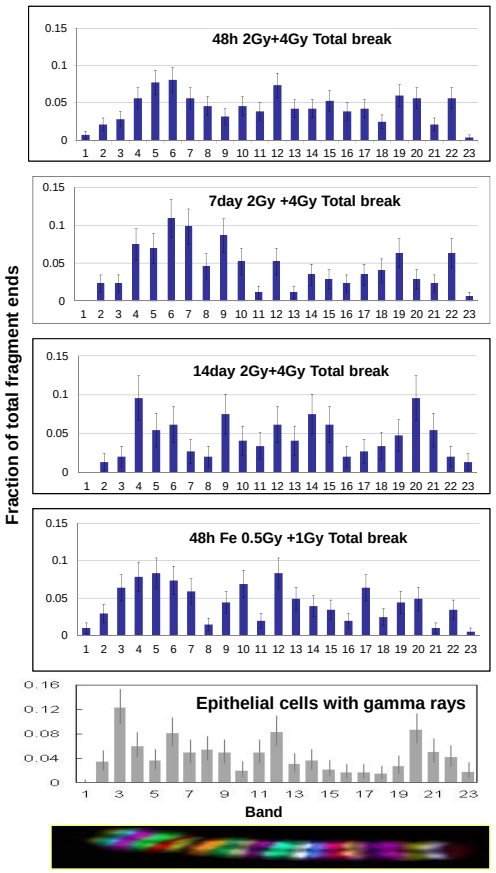


Fig.7. Frequency of simple inversions in chromosome 3 induced by gamma-rays



4. Breakpoint distribution in each banded chromosome 3

Fig. 8. Distribution for total chromosome ends



CONCLUSIONS

About half of the cells initially damaged in chromosome 3 induced by gamma rays within the first mitosis remained in the cells cultured up to 3 weeks post-irradiation, while a significantly lower fraction of the damaged cells remained after Fe ion exposures (Fig. 4).

Comparison of the number of breaks indicates that Fe ions produced 3 or more breaks in about 20 % of the damaged chromosome 3, whereas a lower fraction of cells with 3 or more breaks in chromosome 3 were found after γ ray exposures. Unlike γ rays, increasing doses of Fe ions did not produce more breaks in a damaged chromosome 3 (Fig. 5).

Increasing doses of exposure resulted in less fraction of chromosome break ends involved in intra-chromosomal exchange induced by either γ rays or Fe ions in the first mitosis (Fig.6).

Interestingly, simple inversions in chromosome 3 were found in only the 7 and 14 day samples after 4 Gy gamma irradiation. It is not clear whether cells containing simple inversions had already progressed through the first cell division at 48 hr, or the simple inversions were induced after the first cell division. (Fig.7).

The breakpoint distribution in chromosomes collected at 7 days, but not at 14 days, post irradiation appeared similar to the distribution in cells collected within the first cell cycle post irradiation. Further investigations are needed to determine whether some of the aberrations were formed after 7 days of cell culture (Fig.8).
The distribution of total breaks for human lymphocytes after radiation exposure was different from the previously published distribution for human mammary epithelial cells, indicating that interphase chromatin folding structures play a role in the distribution of radiation-induced breaks

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