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Cytogenetic analysis of mouse bone marrow cells after radiation exposure *

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Chromosome analysis is an established technique to estimate the absorbed dose and to assess radiation risk. Most studies rely on damage produced in peripheral blood lymphocytes that represent mature cells. Yet, little information is available on the damage produced in their progenies that are found in the bone marrow. Studies in mice have shown that genetic background and dose play a role in the immediate induction of aberrations in bone marrow cells and in maintaining genetic stability [1-3].

To contribute to this issue we examine the quantity and the quality of chromosomal damage induced in mouse bone marrow cells by X-rays, α-particles or heavier ions. First, we optimized the isolation and in vitro cultivation of bone marrow cells for chromosome analysis (Fig. 1). Bones (femurs and tibias of hind legs) from C57BL/6 and rhodopsin (*Rho*) mutant mice were kindly provided by Prof. Layer (TU-Darmstadt). Both femurs and tibias were dissected and bone marrow cells were isolated by repeated flushing with culture medium. From each bone marrow specimen one culture was set-up in 4ml medium, respectively.

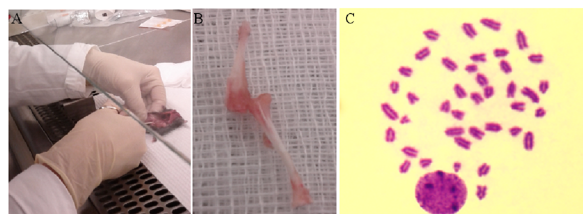

Figure 1: Isolation of bone marrow cells for chromosome analysis. (A) Preparation of bones, (B) Isolated femur and tibia, (C) Giemsa stained chromosome sample of bone marrow cells.

Cells were exposed in a flask to 1Gy X-rays (135kV, and 33,7mA) and chromosomes were prepared directly after exposure as well as 2, 6, 8, 24h post-irradiation. Briefly, colcemid (0.25µg/ml) was added to the culture medium 1h prior harvest to accumulate metaphase cells. Hypotonic treatment (0,075 M KCl) was performed at room temperature for 10 min. Then, cells were fixed with methanol and acetic acid (3:1) and dropped onto wet slides. Samples were stained with 3% Giemsa (Fig. 1C) and the mitotic index was determined. At 2h and 6h post-irradiation the mitotic index was <0.5%, while at 8h and 24h about 1.4 and 1.6% reached mitosis. Based on these measurements samples collected at 8h and 24h were selected for aberration scoring.

In *Rho* mutant mice the proportion of aberrant cells was 45% at 8h and increased to 68% at 24h post-irradiation (Fig. 2A). At both time points the most frequent aberrations were chromatid-type breaks accounting for 88% and 81% of all aberrations. On the other hand, 40% of the bone marrow cells of C57BL/6 mice were aberrant at 8 and 24h, respectively. At 8h the majority of aberrations (i.e. 87%) were chromatid-type breaks as observed in the mutant strain, but at 24 h this proportion decreased to 44% and chromosome-type aberrations such as chromosome-type breaks and dicentrics dominate. For a more detailed karyotype analysis the multicolour fluorescence in situ hybridisation technique (mFISH) is currently being used. An example is shown in Fig. 2B

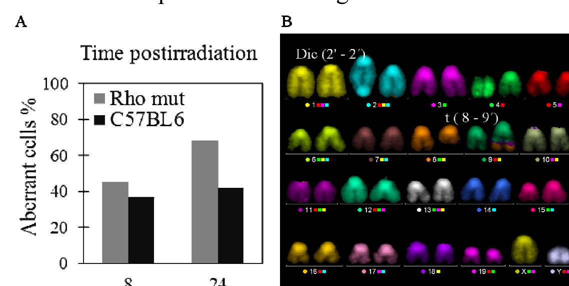

Figure 2: Cytogenetic analysis: (A) Percentage of aberrant cells measured at 8 and 24h post-irradiation following Giemsa staining (B) C57BL/6 bone marrow metaphase cell harvested 24h after exposure to 1Gy X-rays visualized with mFISH. The cell has 41 chromosomes and a dicentric chromosome (dic 2'-2') and a translocation (t (8-9') are indicated by an arrow.

The observed differences in the aberration spectrum at 24h post-irradiation point to a shorter cell cycle time of C57BL/6 bone marrow cells compared to the rho mutant cells, because aberration types reflect the duplication status of the chromosomes at the time of exposure. The appearance of only chromosome-type aberrations indicates that the cell was exposed in G0 or G1. Chromatid-type aberrations are formed when the affected chromosome region is already split or duplicated, i.e. during S or G2 phase. Yet, it cannot be excluded that a subset of C57BL/6 bone marrow cells has already undergone cell division. To account for the cell cycle effects, we are currently establishing a technique that allows both aberration analysis and identification of cells that were in S-phase at the time of exposure.

References

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