Time course of radiation induced chromosome aberrations in mouse bone marrow cells*

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The analysis of chromosome aberrations (CA) in cells at the first post-irradiation mitosis is an established technique to estimate the absorbed dose and to assess the radiation risk. Within the GREWIS consortium we will use this method to examine the effects of either α-particles, Fe-ions or X-rays on murine bone marrow cells (BMC). Since BMC are an asynchronously growing cell population and the number and the types of primary aberrations depend on the cell cycle stage at exposure, CA have to be scored at multiple fixation times to obtain a reliable estimate of the damage produced within the initial cell population.

In preparatory experiments BMC were isolated from C57BL/6 wild type mice as described elsewhere [1] and cultured for 2 hours in medium containing EdU (0,1 μM) and colcemid (0,025 μg/ml). After 2 hours chromosome spreads were prepared. Analysis of the sample showed that 26% of the interphase cells incorporated EdU, i.e. are in S-phase; the mitotic index was 3%. Interestingly, one third of the metaphases was EdU-positive pointing to a subpopulation of BMC with a short G2-phase ($<$ 2h) (Fig. 1).

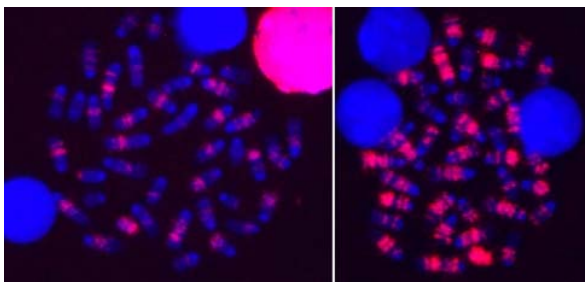

Figure 1: Chromosome spreads of BMC labelled for 2h with EdU. The labelling pattern shows that cells in late (left) or middle S-phase (right) reach mitosis within this time interval.

Subsequently, BMC were exposed *ex vivo* to 1 Gy X-rays (135kV, and 33,7mA). For chromosome analysis cells were harvested between 4h and 24h post-irradiation to cover the first post-irradiation cell cycle and stained with Giemsa. At 4h after exposure the mitotic index of BMC was too low for aberration scoring ($<$0.5%) indicating that radiation delays the progression of cells to mitosis. At the later times the mitotic index recovered and 100 metaphases were analysed per time-point. As shown in Fig. 2A a low yield of CA was found at 8h after exposure. The number of CA increased with time and reached a maximum between 16 and 20h. The aberration yield declined at 24h due to the dilution with undamaged or less damaged cells in second cycle metaphases (Fig. 2A). Chromatid-type aberrations dominated the aberration spectrum up to 20h post-exposure, while at 24h mainly chromosome-type aberrations were found (Fig. 2B). Altogether, the specific changes in the time-course of CA and the aberration spectrum demonstrate that most BMC reach the first mitosis after an exposure to 1 Gy X-rays within 24h. In subsequent studies we will examine, whether a later time-point is necessary for studies applying higher doses.

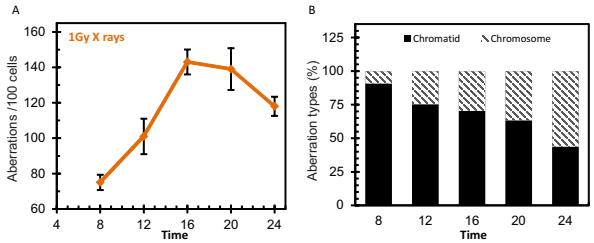

Figure 2: Aberration yield (A) and percentage of chromatid- and chromosome-type aberrations (B) measured in murine BMC at multiple harvesting times after exposure to 1Gy X-rays.

Recently we extended these studies to a human TNF-alpha transgenic mouse model [2]. The animals develop spontaneously arthritis and will be used within the consortium to study the health effects of radon in comparison to X-rays. As described above, BMC were isolated, exposed *ex vivo* to X-rays and CA were measured at several sampling times. The time course of CA in BMC of TNF-alpha mice was similar to that of wild type mice, with a maximum between 16 and 20h. Yet, the aberration yields were slightly higher in BMC from TNF-alpha mice. Currently, the experiments are repeated and mFISH analysis is performed to consolidate these results.

References

[1] N. Paz et al, GSI Scientific Report 2012 (2013)

[2] B. Frey et al, Autoimmunity, 42(4), May 2009, p. 346-8.

* Work supported by BMBF, grant 02NUK017A and 02NUK017G.

 doi:10.15120/GR-2014-1-BIOPHYSICS-22