The radiation-induced G2 cell cycle delay is comparable in human hematopoietic stem- and progenitor cells and mature lymphocytes*

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Introduction

Hematopoietic stem and progenitor cells (HSPC) constantly renew all mature blood cells e.g. peripheral blood lymphocytes (PBLs). Ionizing radiation generates DNA double-strand breaks (DSBs). Error-prone DSB repair activities can lead to chromosomal rearrangements which are considered to contribute to leukemogenesis [2]. Thus, the quality of DNA repair is essential. Because several mechanisms exist to repair DNA damage, we first measured the usage of distinct repair pathways after the induction of DSBs [3] and found differences in DNA repair pathway usage between HSPC and PBLs [4]. The choice of DNA repair mechanism is dependent on several factors, e.g. the cell cycle phase. When damage is introduced the cell cycle can be arrested at specific cell cycle checkpoints for repair. In this process many sensor and effector molecules are involved. Thus, we investigated in both cell types the cell cycle distribution upon stimulation and X-irradiation and a protein involved in the induction of cell cycle arrests.

Materials and Methods

CD34+ HSPC and PBLs were isolated from peripheral blood of healthy donors as described in [5]. G0-phase cells were cultured for 72h in expansion media supplemented with cytokines (HSPC) or PHA (PBLs) [5] to allow cell cycle entry. Asynchronous cells were exposed to 2Gy of X-rays (16mA, 250kV). At defined time points post irradiation cell cycle distribution was determined by DAPI staining and flow cytometry and protein amounts were determined by Western Blot analysis. Immunodetection was performed using the polyclonal anti-phospho-Chk2 respectively anti-Chk2 (both Cell Signalling) and anti-GAPDH (Abcam) antibody (loading control).

Results and Discussion

Figure 1 shows the cell cycle entry of stimulated PBLs resulting in an asynchronous cell cycle distribution after 72h. This is similar to HSPC (not shown). After irradiation with 2Gy of X-rays, PBLs undergo a transient G2 cell cycle arrest again similar to HSPC (not shown). All cells entered the cell cycle again after 48h post irradiation.

As the cell cycle analysis revealed a G2/M cell cycle delay we investigated the G2/M checkpoint protein Chk2. Figure 2 shows that the phosphorylation of Chk2 detected

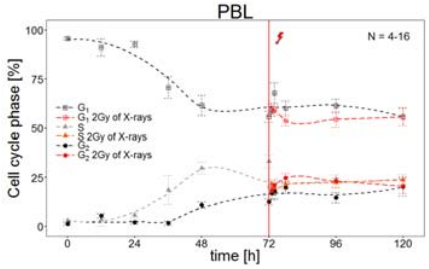

Figure 1: Cell cycle distribution in % for PBLs (n = 4–16) over a time period of 120h with stimulation for 72h before irradiation with 2Gy of X-rays (shown in red). Data are shown as mean ± SEM.

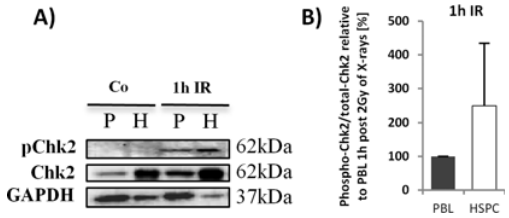

Figure 2: A) Immunodetection of phospho-Chk2, total Chk2 and GAPDH in PBLs and HSPC before (Co) and post irradiation (IR, 1h) with 2Gy X-rays. B) Phospho-Chk2 relative to total Chk2 normalised to PBL 1h post-IR (mean ± SD from 2 experiments)

1h after irradiation correlates with the observed G2 cell cycle delay for HSPC and PBLs (Fig.2, A). The relative phosphorylation of Chk2 to the total Chk2 amount revealed no significant difference in the phosphorylation activity in HSPC and PBLs (Fig.2, B).

Cell cycle distribution, radiation induced delay and Chk2 signalling are similar in PBLs and HSPC and thus cannot explain the observed differences in DNA repair pathway usage.

References

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