NF-κB-dependent DNA damage-signaling differentially regulates DNA double-strand break repair mechanisms in immature and mature human hematopoietic cells

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Introduction

Hematopoietic stem and progenitor cells (HSPC) are the presumed target for leukemic transformation, which can be induced by ionizing radiation due to failed repair of DNA double-strand breaks (DSBs) and generation of chromosomal rearrangements. As little is known about the repair of radiation-induced DSBs and its accuracy in HSPC, we explored the activity, quality, and molecular components of DSB repair in human HSPC as compared with mature peripheral blood lymphocytes (PBL).

Materials and Methods

The enrichment of HSPCs from CD34+ cell population and PBL from the peripheral blood of healthy donors as well as cultivation were performed as described [1,2]. The usage of different pathways of DSB repair was measured using a plasmid based EGFP-reporter system [3]. To measure repair following inhibition of NF-κB signaling additionally IκBα-SR expression plasmid was added to the DNA mixture. In the NF-κB reporter assay a reporter plasmid for NF-κB-dependent GFP production was included. The statistical significance of differences was determined using nonparametric Mann-Whitney test for unpaired samples (**P*<0.05, ***P*<0.01, ****P*<0.001) with the software GraphPad Prism 5.01. Columns show mean values; bars, SEM.

Results

The measurements of the reconstitution of EGF wildtype gene of the reporter plasmid revealed that HSPC were severely compromised in the usage of Non-homologous end joining (NHEJ) and HR+SSA (Fig. 1), but not in microhomology mediated end joinig (not shown). This result indicates a differential repair pathway usage between HSPC and PBL, although formation and removal of γH2AX foci (not shown) and induction and rejoining of chromosomal breaks [4] is similar for both cell types. Functional screening analysis revealed that DNA repair kinases (ATM/ATR/DNA-PK), p53 signaling and chromatin remodeling do not play a major role in the pathway usage. However, overexpression of the NF-kB inhibiting protein IkBα reduces HR pathway usage in PBL significantly, but not in HSPC (Fig. 2a), pointing to NF-kB signaling as the molecular component underlying the observed differences in pathway usage. This is consistent with the higher transcriptional activity of NF-kB for PBL compared to HSPC observed in a NF-kB reporter assay (Fig. 2b). The low NF-kB activity in HSPC is also related to a concomitant accumulation of 53BP1 foci after irradiation [5] that in turn limits the expression of DSB repair genes and bears a risk for inaccurate repair.

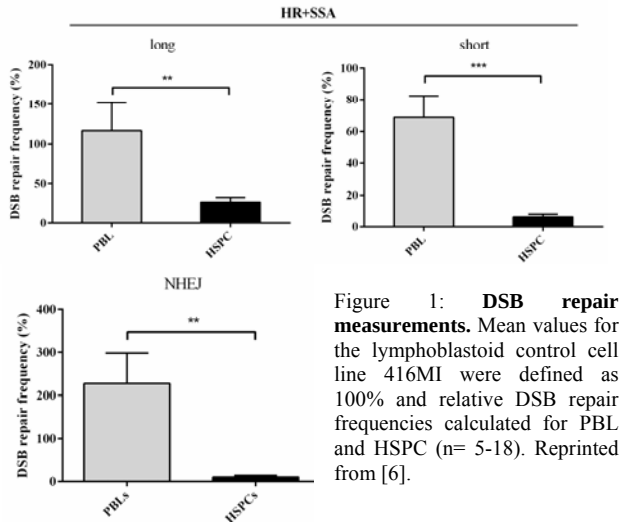

Figure 1: **DSB repair measurements.** Mean values for the lymphoblastoid control cell line 416MI were defined as 100% and relative DSB repair frequencies calculated for PBL and HSPC (n= 5-18). Reprinted from [6].

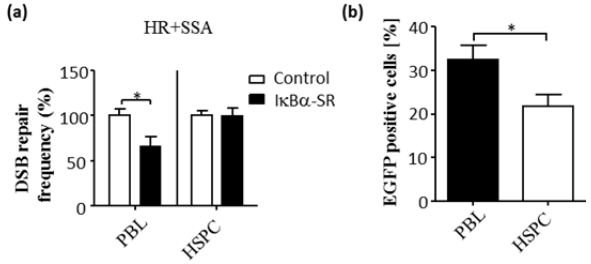

Figure 2: **(a) DSB repair measurements following inhibition of NF-κB signaling.** Mean values for samples nucleofected with control plasmid were set to 100% for each cell type and each experimental day (n=6-12). (b) **NF-κB reporter assay (**n=6). Reprinted from [6].

References

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