Processing of radiation-induced DNA double-strand breaks (DSBs) differs in human hematopoietic stem and progenitor cells versus mature lymphocytes

D. Kraft[1,2], M. Volcic[3], A. Groo[1], A. Stahl[3], D. Salles[3], H. Bönig[2], M. Durante[1], G. Taucher-Scholz[1], C. Fournier[1] and L. Wiesmüller[3]

[1]GSI, Darmstadt, Germany; [2]Institute for Transfusion Medicine and Immunohaematology, Frankfurt, Germany; [3]Department of Obstetrics and Gynecology, University of Ulm, Germany

Introduction

Stem and progenitor cells of the hematopoietic system (HSPC) are the origin of radiation-induced leukemogenesis [1]. Ionizing radiation generates DNA double-strand breaks (DSBs) and error-prone DSB repair activities are thought to be responsible for generating chromosomal rearrangements that can lead to hematopoietic malignancies [2]. Regarding repair of radiation-induced DSBs and its accuracy in HSPC, data are scarce, in part contradictory and were obtained almost exclusively in murine model systems [e.g. 2]. Having identified differences in DSB repair pathway usage in HSPC versus mature peripheral blood lymphocytes (PBLs) (see report 2011), we exposed cells to different radiation qualities (X rays, heavy Titanium/Calcium ions), which may induce different types of chromosomal damage and thus different DSB repair mechanisms [3,4], and monitored processing of radiation-induced DNA lesions in HSPC versus PBLs.

Materials and Methods

Human CD34$^+$ HSPC and PBLs were isolated from healthy donors. While HSPC were pooled from 4-9 donors and cultivated for 72h as described [4] PBLs were individually cultivated in RPMI 1640 medium supplemented with 20% FCS, 3mM Glutamine and 3% phytohemagglutinin (PHA). Cell cultures were irradiated with 2 Gy of X rays or particle irradation (Titanium, Calcium, 1GeV/u) and further cultivated for the indicated times. After cytospin harvest cells were fixed, extracted, and immunolabeled as in [5]. Fluorescence micrographs were collected on an Olympus BX51 epifluorescence microscope equipped with AnalySIS software including mFIP module (3.2, Soft Imaging System) for image acquisition and automated identification. Antibodies used were: 53BP1 rabbit (Novus), phospho-RPA (p-RPA) mAb clone S33 (Bethyl), Rad51 rabbit H-92 (sc-8349, Santa Cruz), and Alexa Fluor® 555 conjugated secondary antibodies (Invitrogen).

Results

Recently, we observed that HSPC and PBLs differ in the usage of DSB repair pathways and fidelity of the same, even though the cell cycle distribution under the assay conditions following *ex vivo* cultivation for 72h was comparable (see report 2011 and data not shown). Next, we dissected DSB processing in response to treatment with ionizing radiation by quantitative immunofluorescence microscopy of discrete nuclear signals (foci) indicative for the accumulation and/or removal of DNA lesions (53BP1), single-stranded DNA (p-RPA) and the assembly/disassembly of the machinery for homologous recombination (Rad51), as this repair activity had differed up to 16-fold between the two cell types. Exposure to 2Gy of X rays caused a sharp increase of 53BP1, p-RPA and Rad51 foci in HSPC. Strikingly, PBLs displayed 2-3fold lower foci numbers per nuclear area 1h (53BP1, p-RPA) and 2h (Rad51) after irradiation. Interestingly, 53BP1 foci numbers were equivalent when irradiating cells with Titanium or Calcium ions (not shown) suggesting that with complex chromosomal damage differences in damage processing between HSPC and PBLs disappear.

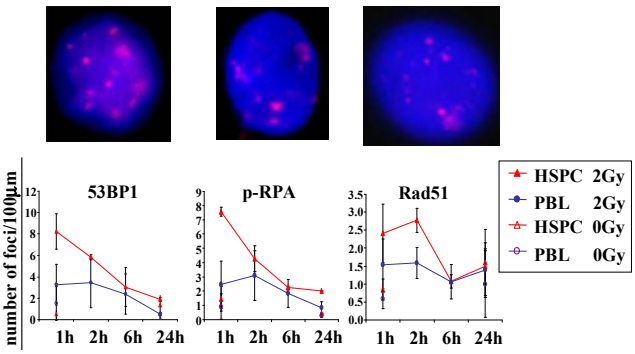

Figure 1: Analysis of DNA damage processing in human HSPC versus PBLs *ex vivo*. HSPC and PBLs were cultivated for 72h followed by exposure to 2Gy X rays and further cultivation for the indicated times. HSPC and PBLs were processed for immunofluorescence analysis and 53BP1, p-RPA, and Rad51 foci quantified (representative images for HSPCs). Mean values and SDs from 50-100 nuclei for N=2-3 HSPC cultures and N=6-9 PBL donors are shown graphically.

References

[1] Rithidech et al (2007), Radiat Environ Biophys, 46(2):137-145
[2] Mohrin et al (2010), Cell Stem Cell, 7(2):174-185
[3] Durante et al (2001), Radiat Res, 156(5 Pt 2):662–667
[4] Becker et al (2009), Int J Radiat Biol, 85(11):1051-1059
[5] Volcic et al. (2012), Nucleic Acids Res, 40(1):181-195

* Work financed by DLR/BMWi contract No. 50 WB 1225.