

Molecular mechanisms of response of human peripheral blood mononuclear cells to ionizing radiation

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Introduction: The aim of this study was to compare reaction of quiescent and proliferating PHA (mitogenic lectin phytohemagglutinin)-stimulated human peripheral blood mononuclear cells (PBMCs) to γ -radiation (IR) and analyze changes of proteins related to repair of DNA damage and apoptosis, such as γ H2A.X, p53 and its phosphorylations on serine 15 and 392, and p21.

Materials and methods: PBMC were acquired from venous blood of normal donors by centrifugation over Histopaque. The lymphocytes were cultured in IMDM medium supplemented with 20% fetal calf serum and antibiotics. For mitogenic stimulation of lymphocytes was added 10 μ g/ml PHA. The viability of the PBMC has been identified by flow cytometry. Western blotting has been used for p53 and its phosphorylation form, p21 and H2AX detection. T-lymphocytes and PBMC were irradiated and lysed. To enhance detection sensitivity the Total p53 and PathScan p53ser15 Sandwich ELISA Kits were used.

Results: PBMCs were stimulated to enter the cell cycle by treatment with the PHA. After 72 h long stimulation by PHA 96 % of CD3+cells were CD25+ and 12 % entered cell cycle (S+ and G2 phase). PHA-stimulation itself causes increase in γ H2A.X, p53, and p21, but not phosphorylation of p53. After the irradiation of these stimulated PBMCs we detected increase in p53 and its phosphorylations on serine 15 and 392, and further increase in p21 from 4 h after the irradiation. Also level of γ H2A.X increased significantly. Increase of p53 phosphorylation on serine 15 is dose-dependent 4 h after the irradiation in the whole studied dose range (0.5-7.5 Gy) in stimulated PBMCs. We compared p53, p53ser15 and ser392, p21 and H2AX between grouply of phytohemagglutinin-stimulated and non-stimulated PBMC 4 hours after 0,5-7,5Gy as well as 1-72 hours after 4Gy irradiation.. We were unable to detect p53 accumulation or phosphorylation in response unstimulated PBMC to gamma radiation induced DNA damage. We induced cell cycling by phytohemagglutinin in the T-cell fraction of PMBC preparations. Althoughly total p53 protein expression was unchanged after 4 Gy irradiation, we found p53ser392 time-dependent expression (1-72 hours, 4 Gy) and p53ser15 dose-dependent expression in range of 0,5-7,5 Gy 4 hours after irradiation in phytohemagglutinin-stimulated PBMC. Neither total p53 protein nor p53ser392 was not altered by irradiation in non-stimulated PBMC.

Conclusions: We suggested that p53 protein accumulation is a common mechanism for induction apoptosis of irradiated cells. We suggest that this pathway is activated in proliferating lymphocytes, unlike quiescent lymphocytes.