

Gene expression based biodosimetry in lymphocyte subsets

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Abstract

Objectives: The triage of victims to radiation accidents is a great challenge for the health care system. So far, there is a clear lack of fast methods to quickly confirm an exposure to ionizing radiation. RT-QPCR based measurement of radiosensitive genes in human lymphocytes or peripheral mononuclear cells (PMNC) is a method that complies best to these requirements. However, interindividual differences in gene expression levels have limited the use of this method as a biodosimetric tool so far.

In addition, the different subsets of lymphocytes differ in their cellular response to ionizing radiation. This suggests a differential regulation of genes involved e.g. in DNA repair and apoptosis in lymphocyte subtypes.

As T-lymphocytes represent the major part of the mononuclear cells we investigated the transcriptional regulation of 6 well known radiosensitive genes (GADD45, BAX, CDKN1A, DDB2, PCNA and ATF3) in PMNC, CD4+ and CD8+ T-lymphocyte subtypes of 5 healthy donors.

Method: Peripheral blood lymphocytes (PBL) were isolated from the peripheral venous blood using immunodensity centrifugation with a human lymphocyte enrichment cocktail (RosetteSep[®], StemCell Tech.). PBL were then exposed to different doses of ionizing radiation (X-rays, 240 kV) and cultured with RPMI 1640 and 10 % autologous serum for 24 and 48 h. The lymphocyte subsets (CD4+ and CD8+) were isolated using the EasySep[®] immunomagnetic separation kit (StemCell Tech.). After RNA isolation and cDNA synthesis RT-QPCR was performed for all 6 genes. 18 S rRNA was used as an endogenous control.

Preliminary results: All 6 genes are expressed differentially in PBLs and CD4+ T-lymphocytes. Using the absolute gene expression level of 4 genes in CD4+ lymphocytes (GADD 45, DDB2, BAX and PCNA) we were able to completely differentiate between the sham irradiated control and the specimens exposed to 0,5 Gy. These results were stable up to 48 h after exposure. GADD 45 and PCNA showed a linear quadratic dose response relationship. ($r^2 = 0,82$ (GADD45) and $r^2 = 0,85$ (PCNA)).

BAX showed greatest induction of all genes ($8,8 \pm 1,5$ at 1 Gy 24 h after exposure).

Conclusion: The genes were also expressed differentially in PBLs, however at a lower level, implying that the gene expression analysis of radiosensitive genes in lymphocyte subtypes (e.g. CD4+) is a promising tool, especially for the confirmation of a low dose exposure.