Time-dependent Gene Expression Analysis in Human Peripheral Blood Lymphocytes for Biodosimetric Applications after Low and High Dose Gamma-Irradiation

Katja Knops[1], Sonja Boldt[2], Olaf Wolkenhauer[2], Ralf Kriehuber[1]

[1]Department of Safety and Radiation Protection, Forschungszentrum Jülich, D-52425 Jülich, Germany, k.knops@fz-juelich.de
[2]Department of Computer Science, Systems Biology and Bioinformatics Group, University of Rostock, D-18051 Rostock, Germany, boldt@informatik.uni-rostock.de

Introduction: In case of a large-scale radiation accident with involvement of individuals without physical dosimeters it is important to identify individuals who have received a moderate to high radiation dose to ensure proper medical care. As current methods are time-consuming, a fast and reliable method based on gene expression alterations is developed.
Methods: Human blood of 3 male and 3 female healthy donors, belonging to 3 different age classes, was irradiated *ex vivo* with 0, 0.02, 0.1, 0.5, 1, 2 and 4 Gy (γ-rays, Cs-137). Peripheral blood lymphocytes (PBL) were isolated and cultured for 6, 24 and 48 h in the medium- and high dose range (0.5 – 4 Gy) and for 24 and 48 h after low dose irradiation (0.02 and 0.1 Gy). Subsequently RNA and proteins were isolated and RNA was applied for processing whole human genome microarrays (Agilent) to analyze expression profiles. In the medium- and high dose range the most robust altered genes were selected for further qRT-PCR and protein expression analysis. To examine the radiation-specificity of the candidate genes, PBL were exposed to the DNA-damaging agents Paracetamol (25 and 200 µg/ml) and Mitomycin C (0.1 and 0.4 µg/ml) for 6, 24 and 48 h and gene expression was accordingly analyzed.
Results: By a p-value and fold-change driven gene selection 9 genes were identified in the low dose range and 16 genes in the medium- and high dose range allowing a radiation dose prediction accuracy of 96% independently on the time-point post irradiation up to 48 h. For 6 predictive genes in the medium- and high dose range and for two genes in the low dose range the observed radiation-induced gene expression profiles were confirmed and validated by qRT-PCR measurements in pooled and non-pooled samples. Additionally, qRT-PCR analysis revealed that the radiation dose predictive genes are highly radiation-specific when compared to exposure with Paracetamol or Mitomycin C. Protein expression analysis showed only for two genes a weak correlation between gene and protein expression after irradiation.
Conclusion: In vitro gene expression analysis in human PBL based on whole human DNA-microarray data allowed identifying a rather small set of radiation dose predictive and radiation-specific genes with a high potential for biodosimetric applications *in vivo* after low-, medium and high dose exposure.

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