Time-dependent gene expression analysis for biodosimetric applications in low and high irradiated human PBL

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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.

Conclusion

***In vitro* gene expression analysis in human PBL based on whole human microarray data allowed identifying a rather small set of radiation dose predictive and radiation-specific genes with high potential for biodosimetric applications *in vivo* after low-, medium- and high dose exposure.**

Chip-based gene selection

Ex vivo irradiation

High dose: 0.5, 1, 2, 4 Gy

Low dose: 0.02, 0.1Gy

Human blood of 6 healthy donors (3 male and 3 female; belonging to 3 different age classes)

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Isolation of lymphocytes

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High dose: Isolation of RNA 6, 24 and 48 h after irradiation

Low dose: Isolation of RNA 24 and 48 h after irradiation

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Pooling of RNA of the 6 donors from the same run, irradiated with the same dose and isolated at the same time point after irradiation

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Measuring of gene expression changes after irradiation using whole human genome microarrays (Agilent)

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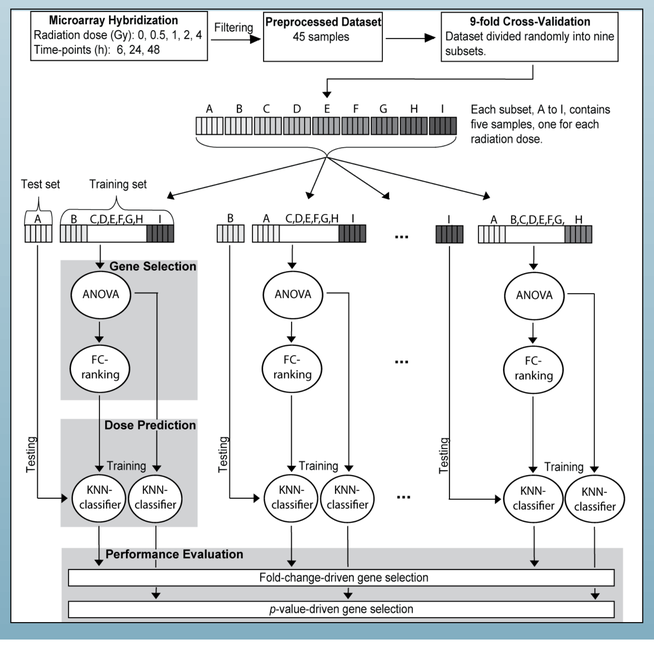
Analysis of microarrays to identify radiation responsive genes by a *p*-value and fold-change driven gene selection technique, based on the frequency of selections from cross-validations



Microarray-based gene lists for *in vitro* dose prediction

High dose prediction		Low dose prediction
TNFSF4	*RP4-42C19.3*	*FDXR*
FDXR	*ISG20L*	*PFKFB3*
SPATA18	*LOC283454*	*THC253753*
DOK7	*TCL1A*	*MKL2*
PHLDA3	*TNC2651023*	*C10orf39*
VWCE	*CNTNAP2*	*A_32_P138939*
LGR6	*C8orf38*	*FLJ35379*
PRICKLE	*E2F7*	*BU561469*
		Y6G5C
Prediction accuracy: 95.7%		Prediction accuracy: 95.6%

Tab. 1: List of 16 genes suitable for radiation dose prediction in the high dose range (0.5 Gy – 4 Gy) up to 48 h after irradiation. The red coloured genes were applied for further qRT-PCR and Western Blot analysis.
Tab. 2: List of 9 genes suitable for radiation dose prediction in the low dose range (0.02 Gy – 0.1 Gy) up to 48 h after irradiation. The red coloured genes were applied for further qRT-PCR analysis (Knops *et al*., accepted) .

Validation of microarray results using qRT-PCR

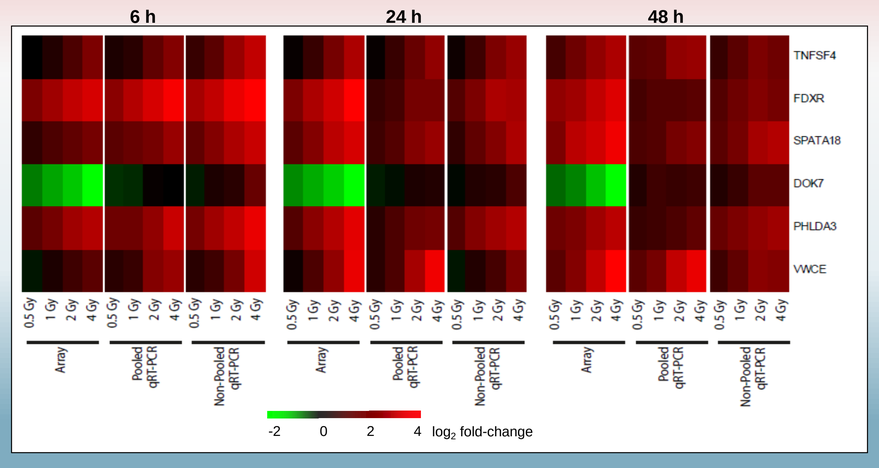


Fig. 1: Gene expression profiles of biomarker genes measured by microarrays and qRT-PCR after high dose irradiation. For the majority of genes very similar expression profiles were detected in qRT-PCR and microarray gene expression analysis (Boldt *et al.*, 2012).

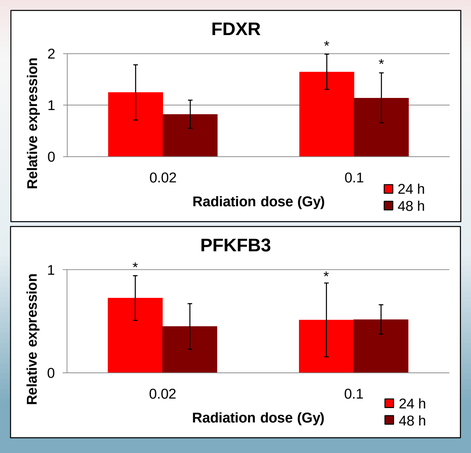


Fig. 2: Gene expression profiles after low dose irradiation measured by qRT-PCR. *FDXR* and *PFKFB3* featured expression alterations after irradiation.

Protein expression analysis after irradiation

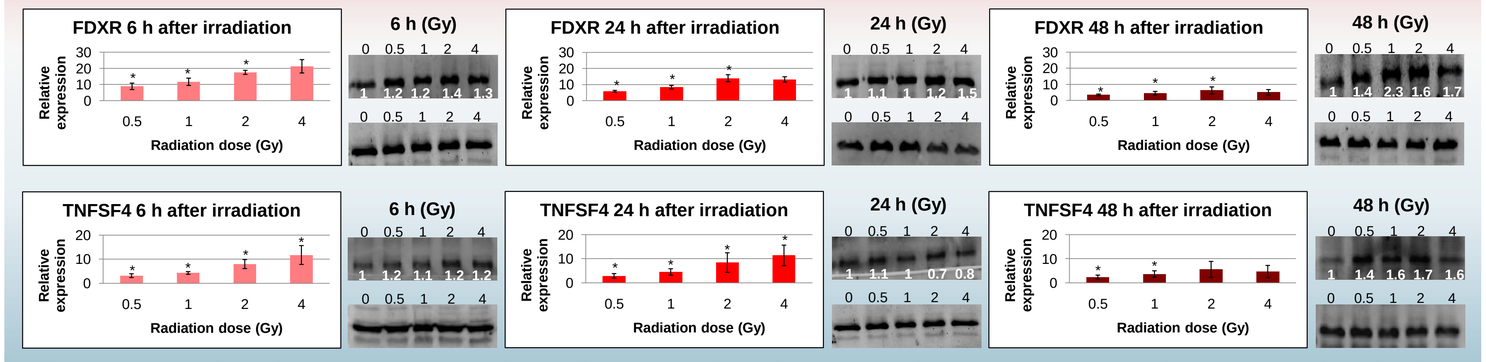


Fig. 3: Comparison between gene and protein expression of FDXR and TNFSF4 measured at 6, 24 and 48 h after high dose irradiation (0.5 Gy – 4 Gy) by qRT-PCR and Western blots. The gene expression of *FDXR* and *TNFSF4* increased with rising dose especially 6 h after irradiation, whereas the protein expression increased slightly.

Gene expression profiles after 4-Acetamidophenol and Mitomycin C treatment

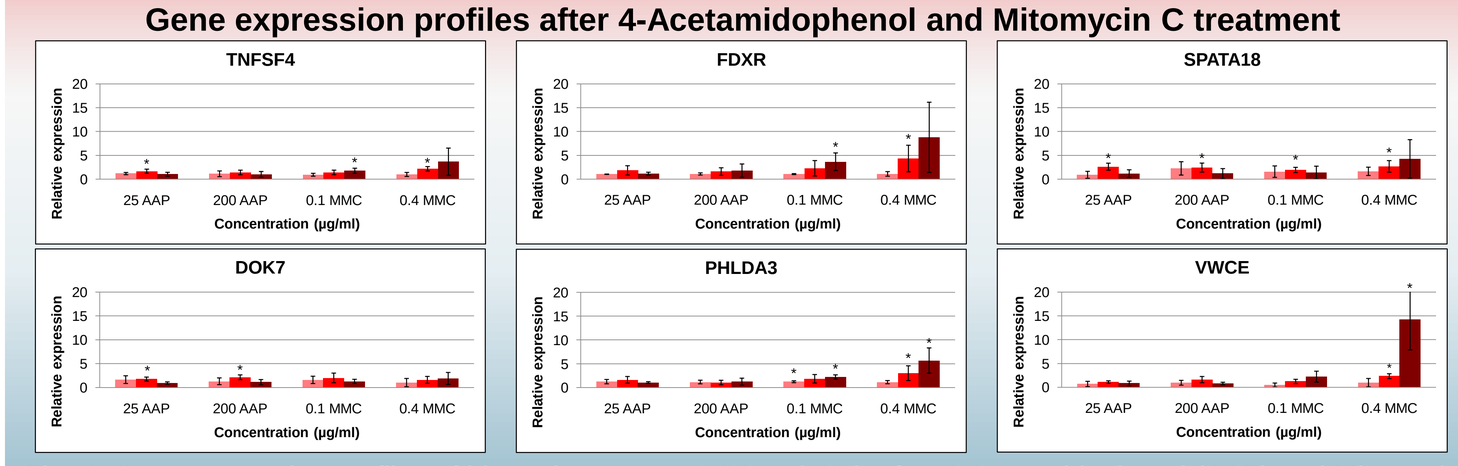


Fig. 4: Gene expression profiles of biomarker genes 6, 24 and 48 h after 4-Acetamidophenol (AAP) and Mitomycin C (MMC) treatment. To examine radiation-specific gene inductions lymphocytes were incubated with the DNA-damaging agents AAP and MMC. Only 0.4 µg/ml MMC treatment yielded considerable expression alterations.

6 h after irradiation 24 h after irradiation 48 h after irradiation; * p < 0.05; qRT-PCR control (0 Gy) = 1

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