



A PROTEOMIC APPROACH TO IDENTIFY PHASE-SHIFTS IN RESPONSES AT LOW DOSES AND DOSE RATES

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Introduction

The effects of low doses and dose rates of ionizing radiation on human cells are presently unclear. The risk projection models used to estimate risk at low doses and dose rates are based on data obtained at higher levels. But emerging evidence suggests that the biological responses to low- and high-doses may be significantly different which requires an accurate reflection of these processes.

Objectives

The objective of this study is to explore potential alterations in the profile of the proteome of primary human fibroblast cells (VH10) after low-dose gamma irradiation at different doses and dose rates and to identify single proteins involved in these biological responses by using 2D-electrophoresis (DIGE) and MALDI-TOF/MSMS.

Experimental Design

Variation in protein expression were measured by comparing non-irradiated and irradiated cells for different dose rates and irradiation conditions.

IR 1: Pre-irradiation plus challenging (acute) dose

Pre-irradiation dose: 40 mGy
dose rates 4.1 mGy/h, 50 mGy/h, 24000 mGy/h;

Challenging dose: 100 mGy, 2 hours post pre-irradiation

→ Investigation of adaptive response effects (IR1 – IR4&5)

IR 2: Pre-irradiation only

dose: 40 mGy
dose rate: 4.1 mGy/h, 50 mGy/h or 24000 mGy/h

→ Investigation of dose rate effects

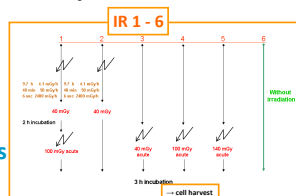
IR 3, 4 and 5: Challenging (acute) dose only

dose: 40 mGy (IR 3)
or 100 mGy (IR 4)
or 140 mGy (IR 5)

All challenging doses were applied with a dose rate of 24000 mGy/h

→ Investigation of low dose effects

IR 6: Control



Results

4.1 mGy/h

➢ Hardly any differentially regulated proteins found

50 mGy/h

➢ In total, **54 proteins** had statistically significantly altered regulation levels (1-ANOVA, $p \leq 0.05$); **17 proteins** were identified using MS

➢ The fold ratios between non-irradiated and irradiated samples varied in the majority of cases from **10-50%**

24000 mGy/h

➢ In total, **47 proteins** had statistically significantly altered regulation levels (1-ANOVA, $p \leq 0.05$); **14 proteins** were identified using MS

➢ The fold ratios between non-irradiated and irradiated samples varied in the majority of cases from **10-20%**

Differences in the cell response after exposure to 140 mGy in total were most pronounced comparing combined (adapting and challenging dose) versus acute irradiation (IR1 vs IR5).

Methods

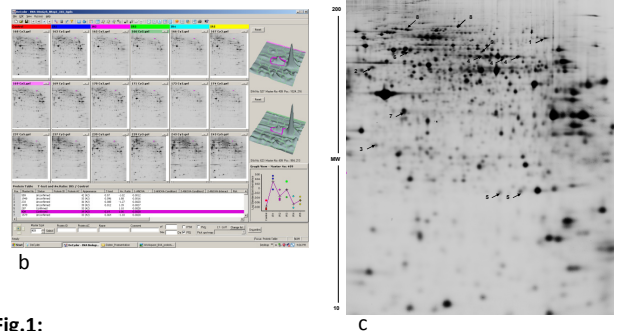
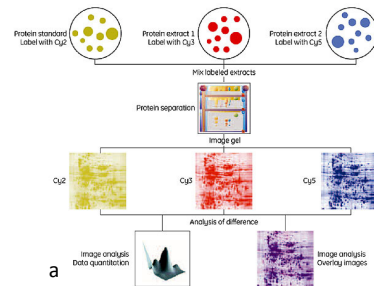


Fig.1:
a) 2D DIGE-technique and b) DeCyder® software (GE Healthcare)
c) 2D-gel (total cell extract)

Validation

Dose rate 50 mGy/h: Vimentin, an intermediate filament protein, is one of the proteins which was significantly altered. It was found to be expressed in several isoforms differing in molecular weight and isoelectric point.

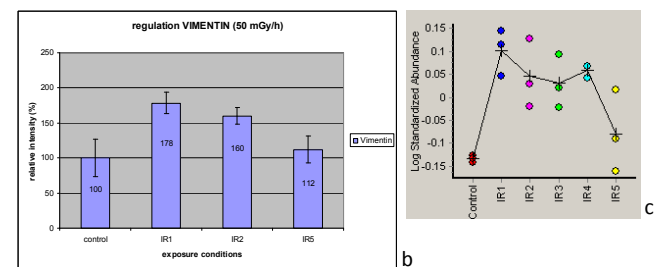
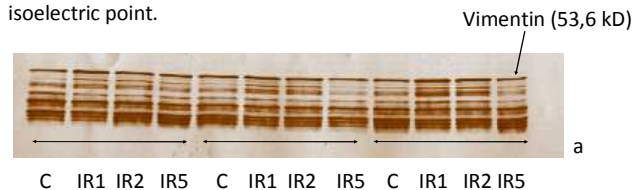


Fig.2:
a) Western blot of three independent biological samples exposed at 50 mGy/h (control, IR 1, 2, 5).
Western blot data (b) are in accordance with 2D results (c).
The Stain-Free technology without HKP from Bio-Rad was applied for data-normalization.