Cytokine secretion profiles and signaling pathways analysis of endothelial cells exposed to high doses of ionizing radiation

G. Tarlet[1*], V. Buard[1], B. Micheau[1], M. Benderitter[1], A. Francois[1], F. Milliat[1] and O. Guipaud[1*]

[1] Institut de Radioprotection et de Sûreté Nucléaire, Laboratoire de Radiopathologie et de Thérapie Expérimentale
IRSN/PRP-HOM/SRBE/LRTE, BP 17 – 92262 Fontenay-aux-Roses Cedex, France.
[*] Contact: georges.tarlet@irsn.fr, olivier.guipaud@irsn.fr

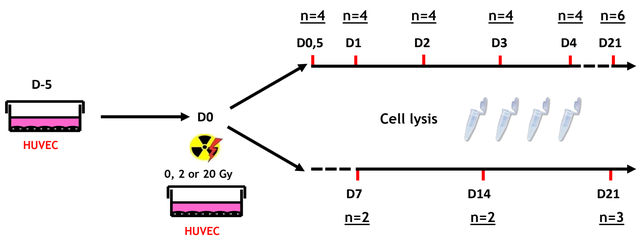
Introduction

Normal tissue damage after radiation therapy is characterized by a chronic altered phenotype of endothelium. The knowledge of molecular mechanisms involved in endothelium dysfunction following high doses of radiation exposure is needed to identify therapeutic targets and to develop strategies to prevent and/or reduce effects of irradiation. To clarify the molecular mechanisms involved in the response to irradiation, we examined irradiation-responsive proteins in cultured primary Human Umbilical Vein Endothelial Cells (HUVEC) using the Luminex technology.

Methodology

In vitro irradiation of HUVEC and protein extracts :

Cultured primary HUVEC (Human Umbilical Vein Endothelial Cells) were exposed to 0, 2 and 20 Gy using Co^{60} source. After different times of ionizing radiation exposure (D0,5, D1, D2, D3, D4, D7, D14 and D21), cells were harvested and lysed in an appropriate buffer compatible either for cytokine or for phosphoprotein assays. Protein extracts were dosed prior to multiplex bead-based assays using Bio-Plex system.

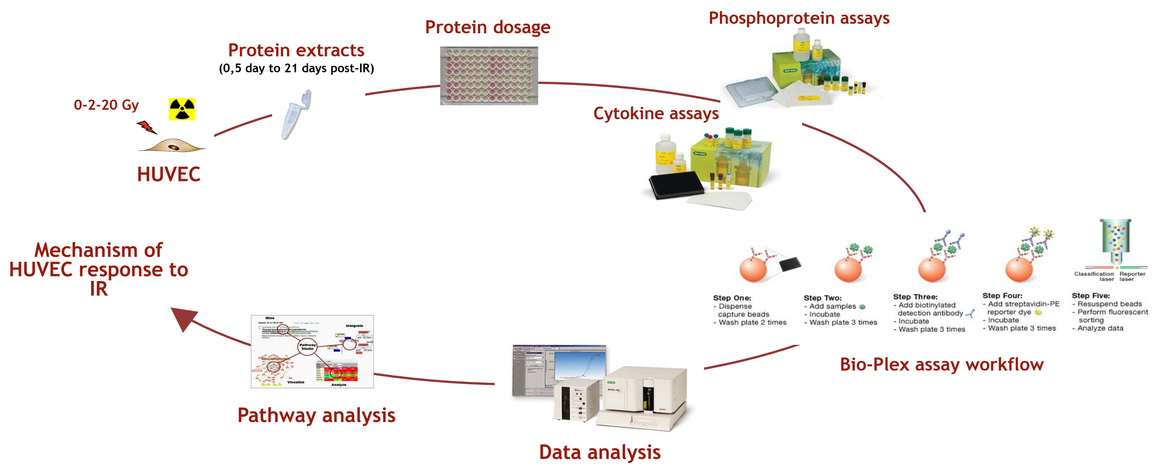


* Akt (Akt signaling), IκB-α (Immunity, cell inflammation), p53 (Cell cycle, checkpoint control), ERK1/2, JNK, p38 MAPK (MAP kinase signaling).

** Hu IL1β, Hu IL2, Hu IL4, Hu IL5, Hu IL6, Hu IL7, Hu IL8, Hu IL10, Hu IL12, Hu IL13, Hu IL17, Hu G-CSF, Hu GM-CSF, Hu IFNγ, Hu MCP-1, Hu MIP-1β, Hu TNFα.

Experimental strategy :

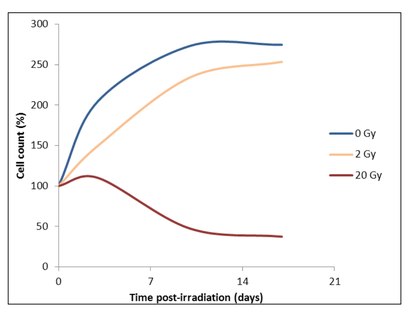
To investigate the protein secretion and signaling pathways, the Bio-Plex assay system is used to detect, in lysates of cultured primary HUVEC assayed 1, 2 and 3 weeks after ionizing radiation exposure, the secretion of 17 cytokines (interleukines, chemokines, growth factors) and levels of 5 intracellular phosphoproteins with their respective total forms.



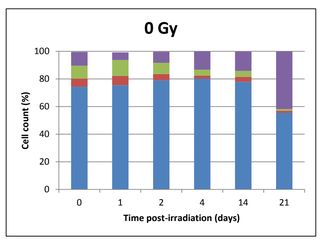
Results

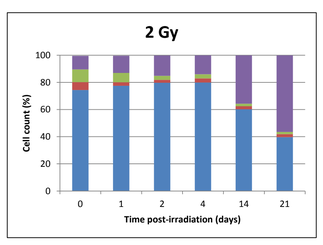
Characterization of the HUVEC model of irradiation

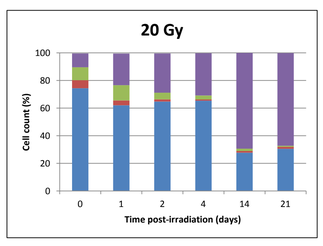
Cell growth



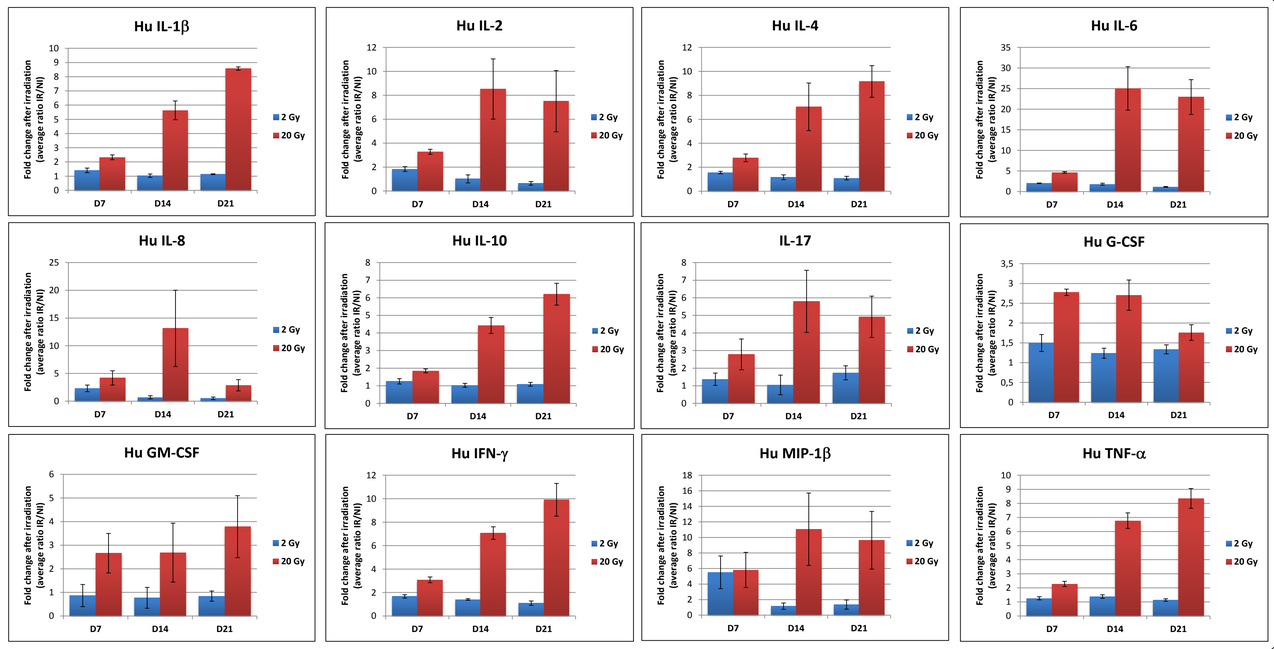
Flow cytometry(IP)



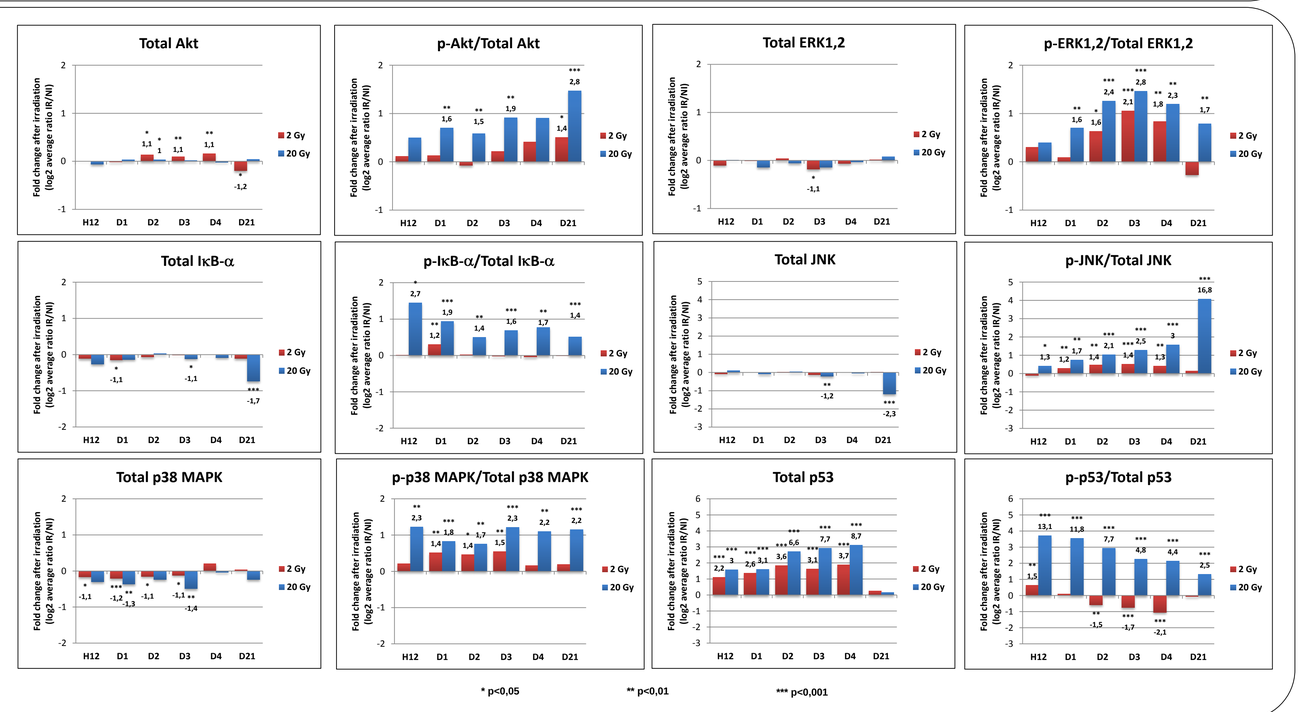




Cytokine secretions



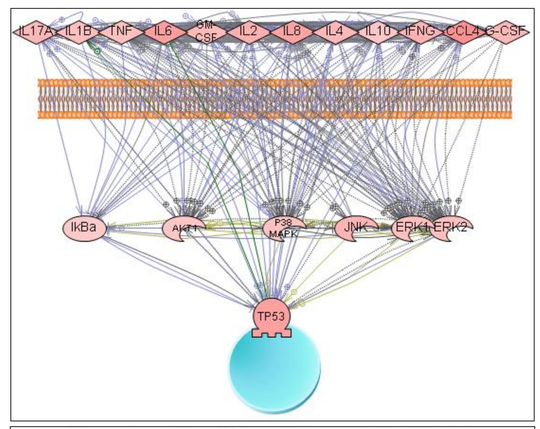
Protein phosphorylations



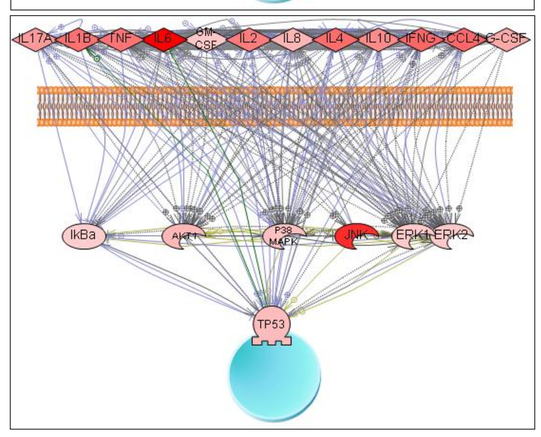
* p<0,05 ** p<0,01 *** p<0,001

Biological pathways analysis : Network visualized using Pathway Studio® software

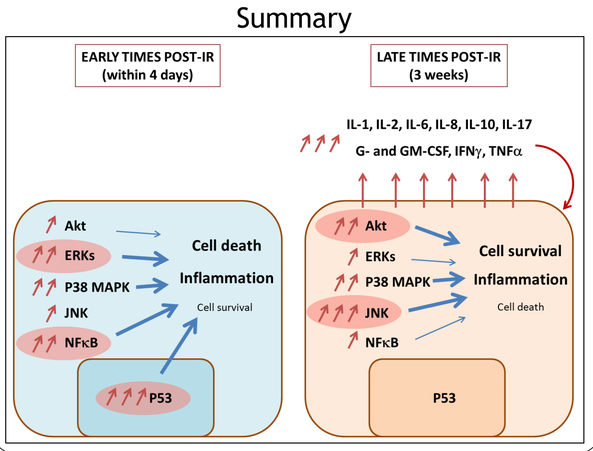
Day 4/7
O vs 20 Gy
Single dose



Day 21
O vs 20 Gy
Single dose



Summary



Conclusions

The molecular profile of endothelial cells exposed to high doses of ionizing radiations is quickly modified. Kinetic analyses show that the HUVECs acquired a chronic pathological phenotype 3 weeks post-irradiation. These results clearly indicate that the survival fraction of irradiated HUVEC always display a modified phenotype in long-term cultured cells after ionizing radiation exposure. This phenotype could reflect the continuous expression of stress and inflammatory signals in the survival fraction of irradiated HUVEC.

Financial supports

EDF
Électricité de France