Involvement of Oxidative Metabolism and Membrane Signaling in Bystander Response: A Microbeam Study.

M. Hanot[a], J. Hoarau[a], M. Carriere[a], J. Angulo[b] and H. Khodja[a]

[a] *CEA Saclay, Pierre Süe Laboratory, CEA-CNRS UMR9956, 91191 Gif syr Yvette, France;* [b] *CEA Fontenay aux Roses, Laboratory of genetics of radiosensitivity, 92260 Fontenay aux Roses, France*

maite.hanot@cea.fr

Charged particles microbeam facilities are a unique tool that allows targeting of single cells and analysis of the induced damage on a cell-by-cell basis. The ability of these tools to discriminate between hit and non-hit cells, and to deliver a precise dose is of particular interest in the investigation of various phenomena related to low dose radiation responses and more particularly in bystander effects.

Since spring 2004, the choice was made to develop a device specifically designed and dedicated to radiobiology studies, with for imperative to adapt the device to the ideal conditions for cell culture. Today the single-cell microbeam system is operational: it can deliver 10 individual alpha particles (3 MeV) to selected cell nuclei in a precisely known proportion of cells in a population.

The investigated endpoint in radiobiology studies is the identification of signaling pathways implicated in the appearance of non targeted effects. Here, the induction of bystander effect was studied by assessing DNA double-strand breaks (DSB) formation with the γH2AX and 53BP1 foci formation assay. Then, a comparative kinetics was established between these foci formation assay and reactive oxygen species (ROS) generation. This kinetics was accomplished in a range of time going from 30 minutes till 24 hours post-irradiation. By this way, we showed the presence of ROS in the hit cells as well as in the bystander cells. This was noticed till 2 hours post irradiation and correlated in observation of foci in both populations of cells.

Moreover, alpha particles induced generation of ROS in the hit cells by a mitochondria-dependent way. This ROS generation was partly involved in a process of radio-induced apoptosis (24h post-irradiation) in the hit cells. However, no apoptosis was detected in bystander cells. On the other hand, the cell membrane seemed to play a decisive role in the signaling leading to the generation of ROS in the hit and bystander cells. Indeed, the use of filipine, which destabilized the rafts of cholesterol in the membrane, annihilated the ROS generation in these two populations of cells. We thus support the hypothesis that ROS, generated by mitochondria as well as cell membrane, belongs to signaling pathways directly involved in bystander effect.

Article published by EDP Sciences and available at http://www.radioprotection.org or http://dx.doi.org/10.1051/radiopro:2008548