DNA damage and cell killing in alpha particle irradiated and in medium mediated bystander AG1522 primary human

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In the framework of the NOTE Integrated Project (FP6-36465, Euratom), we used the alpha particle irradiator set up at the Istituto Superiore di Sanità to investigate DNA damage and cell killing in irradiated and bystander AG1522 primary human fibroblasts. In particular, we studied the timing of medium-mediated bystander effects in order to get insight on the mechanisms involved. To this purpose, both medium transfer and co-culture approaches were used. The experiments were carefully designed in order to produce data useful for modeling. For this reason a great importance was given to standardization of methodologies, and experimental variables such as cell growth phase, cell density, medium thickness/volume, were evaluated and optimized. Cells were seeded in stainless steel Petri dishes with Mylar bottom, especially designed for alpha particle irradiation. They were realized for housing permeable membrane insert(s) and exactly reproduce the geometry of commercial Cell Culture Insert Companion Plates. The alpha particle irradiator was positioned into a CO2 incubator in order to perform irradiation and co-culture incubation in physiological conditions. The data so far obtained show that 30 min of co-culture with 0.5 Gy irradiated cells are sufficient to start a significant induction of DNA damage in bystander cells. This damage increases after 2 h of co-culture to a level twice that of the sham irradiated cells (the percentage of cells showing γ-H2AX foci rising from $\sim$12% to $\sim$25% in bystander cells compared to $\sim$96% in 0.5 Gy irradiated cells). Moreover, the damage induced after 1 h co-culture is further increased by 1 h incubation in the presence of conditioned medium only. Cell killing data, obtained using the medium transfer approach, show a surviving fraction of 0.8 in bystander cells incubated with medium from cells irradiated with 0.1 or 0.5 Gy and kept for 1h at 37°C. It is interesting that the same surviving fraction is obtained in 0.1 Gy irradiated cells, while it drops to 0.2 after the dose of 0.5 Gy. The overall results we obtained for bystander induced effects at molecular and cellular level indicate that crucial signalling from irradiated to bystander cells occurs at relatively short time (0.5-1 h) after irradiation, giving constrains about the possible signalling factors involved.

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