Direct and bystander effects of human chondrosarcoma cell line irradiated with protons

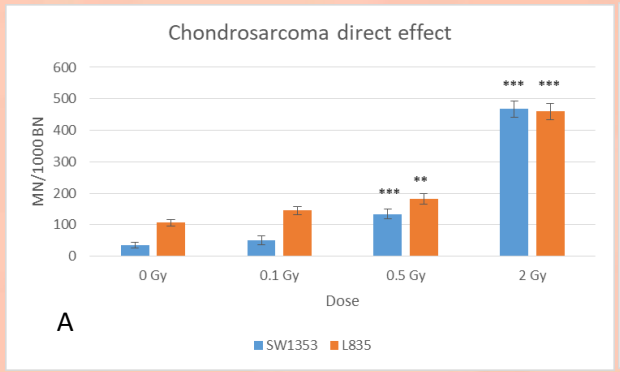
Mihaela Tudor[1,2], Roxana Popescu[1], Mihaela Temelie, François Chevalier[3,4], Diana Savu[1]

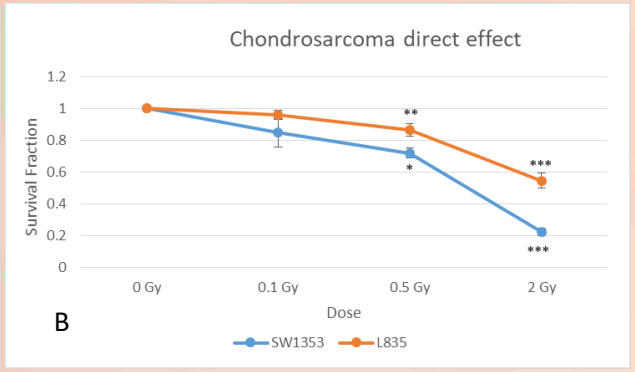
[1]Horia Hulubei National Institute for R&D in Physics and Nuclear Engineering (IFIN-HH); [2]University of Bucharest, Faculty of Biology; [3]UMR6252 CIMAP, CEA - CNRS - ENSICAEN - Université de Caen Normandie, Caen, France; [4]LARIA, iRCM, François Jacob Institute of Biology, DRF-CEA, Caen, France. Email: mihaela.tudor@nipne.ro

Ionizing radiation induces complex cellular effects in targeted cells as well as neighbouring "bystander" cells which have received signals produced by irradiated cells. Characterising both direct and non-targeted bystander effects might improve the overall outcome of radiation exposure which could have implications in radiotherapy and radioprotection.

In this study we focused on analysing direct and bystander cellular effects of proton irradiation of chondrosarcoma cells. Chondrosarcoma is resistant towards conventional chemo-/radiotherapy and can be un-operable. Charged particle radiotherapy is now a good alternative for chondrosarcoma. The advantages of this method include higher specificity (given by its specific Bragg pick) and increased biological efficacy (due to the high LET).

Two different chondrosarcoma cell lines (SW1353 and L835) were irradiated with low energy protons (10 MeV) at doses in the range 0.1-2 Gy. To study the bystander phenomena we used a medium transfer protocol. The bystander supernatant containing signals emitted by proton irradiated chondrosarcoma cells, was transferred to non-irradiated bystander normal chondrocyte (T/C-28a2) and endothelial (EA.hy926) cells. Cell survival and DNA damage were assessed in directly irradiated chondrosarcoma cells and in bystander chondrocytes and endothelial cells.

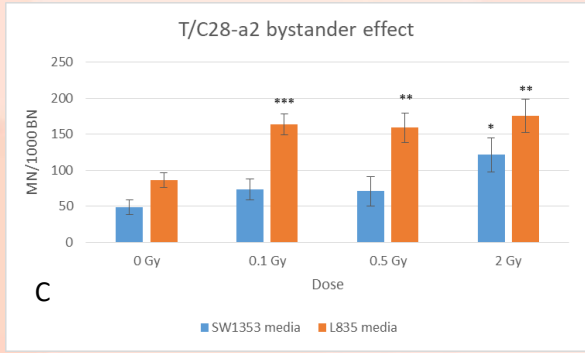


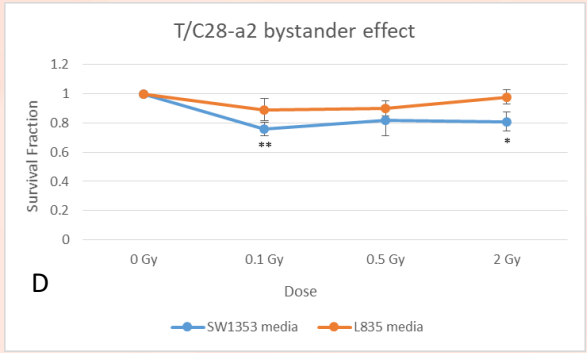


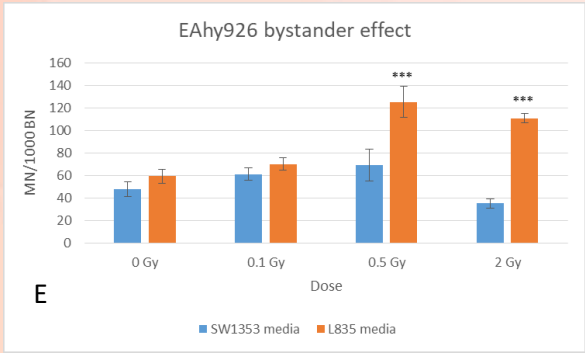
Proton irradiation induced a dose dependent direct effect in chondrosarcoma cells lines.
A. Irradiation induced a statistically significant genotoxic effect observed as a linear increase of the micronuclei number;
B. Cells exposed to proton irradiation presented a statistically significant a decrease in the long time survival rate over the 0.5 Gy dose.

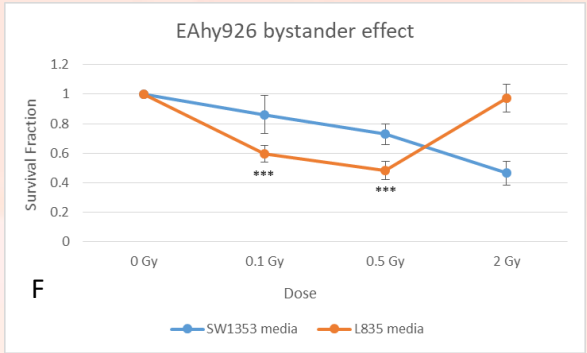
In conclusion, proton irradiation induces a dose-dependent effect in both chondrosarcoma cell line. Our results showed that proton-irradiated chondrosarcoma cells have the ability to release stress factors inducing bystander responses in the non-irradiated surrounding chondrocyte or endothelial cells.

Acknowledgements: This work was supported by Romanian Ministry of Research National grants no. PN 19060203, 543 PED/2019, 18 PCCDI/ 2018.









Bystander effect was induced in normal cell lines after media transfer from proton irradiated chondrosarcoma cells.
C. Condocyte cells presented an increased number of micronuclei after receiving media from irradiated L835 cells at all doses;
D. A decrease in survival rate for the dose of 0.1 Gy was observed in chondrocyte cells after receiving media from both chondrosarcoma cell lines;
E. Endothelial cells show a statistically significant increase in the micronuclei number at doses of 0.5 Gy and 2 Gy after receiving media from L835 cells;
F. . Clonogenic cell death measurements for cells exposed to media from L835 chondrosarcoma cells presented a statistically significant reduction in survival at doses of 0.1 Gy and 0.5 Gy.