Bystander effects induced by proton irradiated human chondrosarcoma cells

Mihaela Tudor[1,2], Mihaela Temelie[1], Roxana Cristina Popescu[1,3], Anna Rzjanina[4], Gennady Mytsin[4], François Chevalier[5,6] and 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 Politehnica University Of Bucharest (UPB) Department Of Science And Engineering Of Oxide Materials And Nanomaterials

4 Laboratory Of Nuclear Problems, Joint Institute For Nuclear Research

5 UMR6252 CIMAP, CEA - CNRS - ENSICAEN - Université De Caen Normandie, Caen, France

6 LARIA, Ircm, François Jacob Institute Of Biology, Drf-cea, Caen, France

Introduction

In radiobiology, bystander effects describe biological changes observed in cells which have not been directly irradiated.

Radiation-induced bystander effect (RIBE) depends on cell type, irradiation quality and dose.

RIBE includes genotoxic effects (DNA damage, chromosomal aberrations), cell death (apoptosis, necrosis) or adaptive response.

Proton therapy (hadron therapy) presents better specificity and lower toxicity for the surrounding normal tissue compared to conventional treatment and is standard care for some radioresistant tumors such as chondrosarcoma.

Chondrosarcoma is a chemo-radioresistant malignant bone tumor with high mortality, frequent metastasis and recurrence rate. In recent years, the treatment for chondrosarcoma focused on charged particle therapies such as hadron therapy and carbon ion therapy.

Research purpose

This study focuses on analysing cellular effects induced in bystander cells by 2D and 3D chondrosarcoma cells irradiated with protons of high and low energy versus X-rays.

Objectives:

- Investigating bystander effects in chondrocyte and endothelial cells;
- Characterizing the effects induced by proton irradiation versus X-ray;
- Assessing the effects induced by 2D and 3D models through cell survival and DNA damage assays.

Materials and methods

Cell lines:

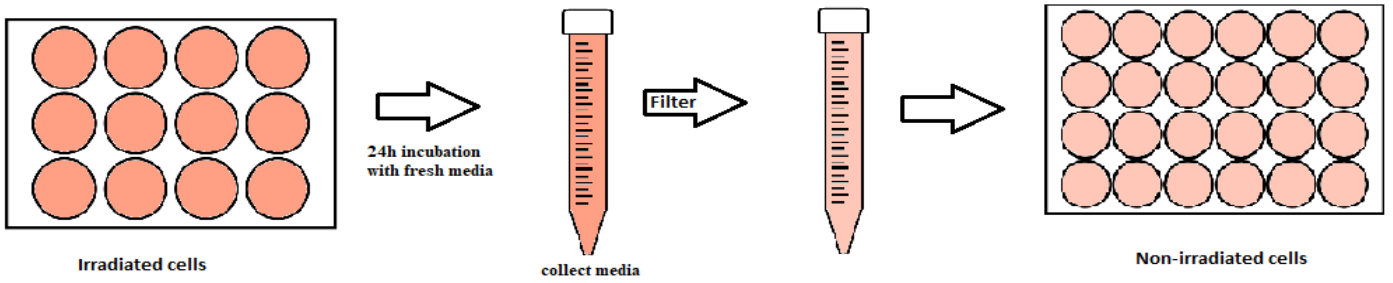
- Irradiated cells - chondrosarcoma cell line (2D and 3D): SW 1353;
- Bystander cells (2D):
  - Chondrocyte cell line: T/C-28a2;
  - Endothelial cell line: EA.hy926 .

Irradiation types:

- Proton: low energy (TR19 Cyclotron of IFIN-HH[1]), high energy (Phasotron of LNP from JINR[2]);
- X-ray (XSTRAHL XRC 160 machine of IFIN-HH[3]).

Materials and methods

Bystander effect induced by media transfer:



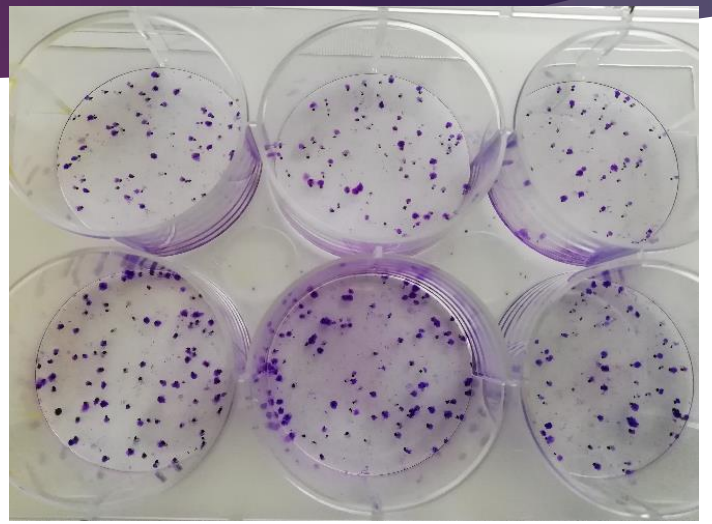
Experimental end-points:

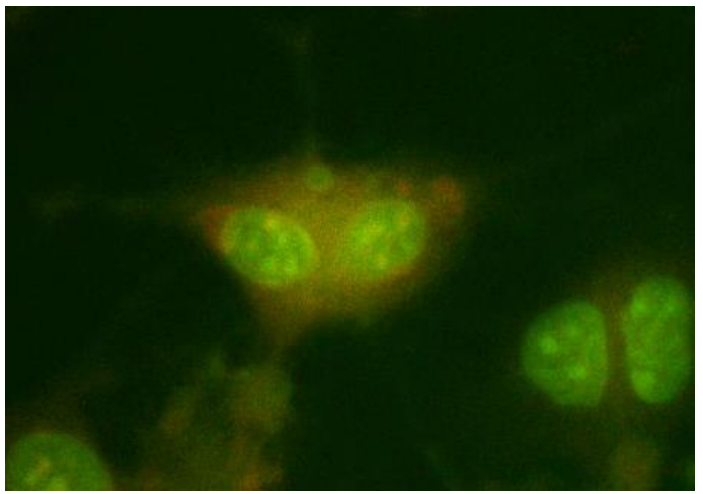
- Survival measurement - Clonogenic assay
- DNA damages measurement – Cytokinesis block micronucleus assay

Materials and methods

- Clonogenic assay = *in vitro* cell survival assay that evaluates a single cells' ability to grow into a colony.
  - Harvest, homogenize and count cells;
  - Plate 800 cells/well in a 6 well plate;
  - Incubate for 10-14 days, stain with a crystal violet solution and count colonies with at least 50 cells.

- The cytokinesis block micronucleus assay = a method that measures the DNA damage at chromosome level.
  - Cells were nurtured on 10mm cover-slides in a 24 well plate, after media transfer add the cytochalasin B solution;
  - After 24 h, samples were fixed in a 1:9 acetic acid – methanol solution and stained with acridine orange and were observed under a fluorescent microscope.

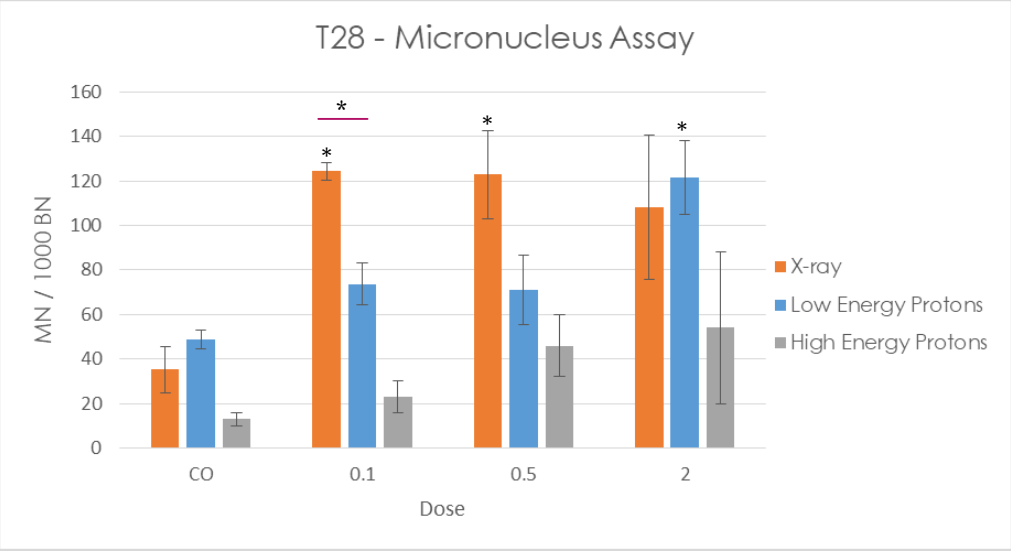


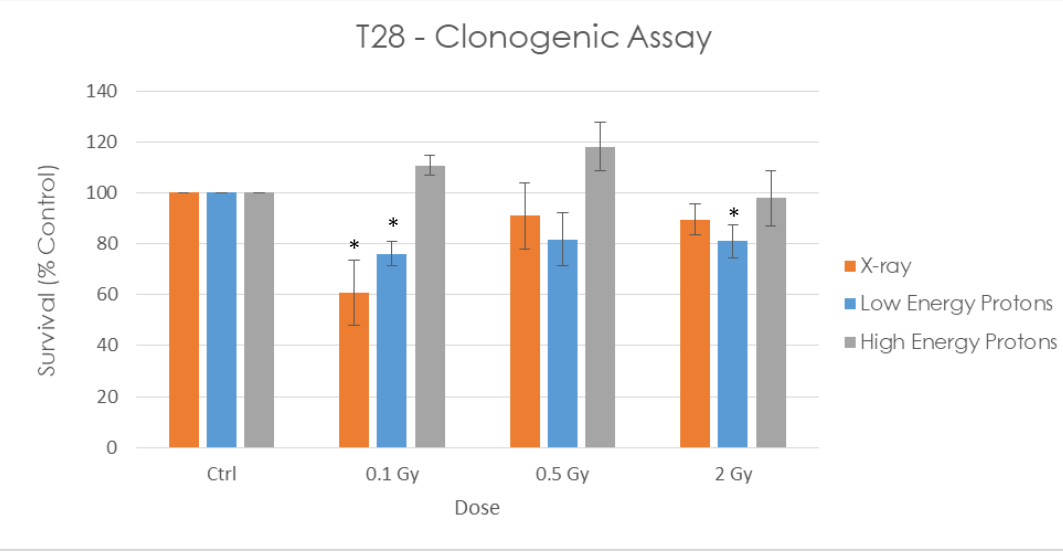


Results - Chondrocytes

Increased number of MN for X-ray at all doses, the highest values were obtained at 0.1 Gy and 0.5 Gy.

Cell survival decreased for all doses, with a pronounced effect at 0.1 Gy for X-ray and Low Energy Protons in contrast with High Energy Protons that produced no change in bystander cell survival.

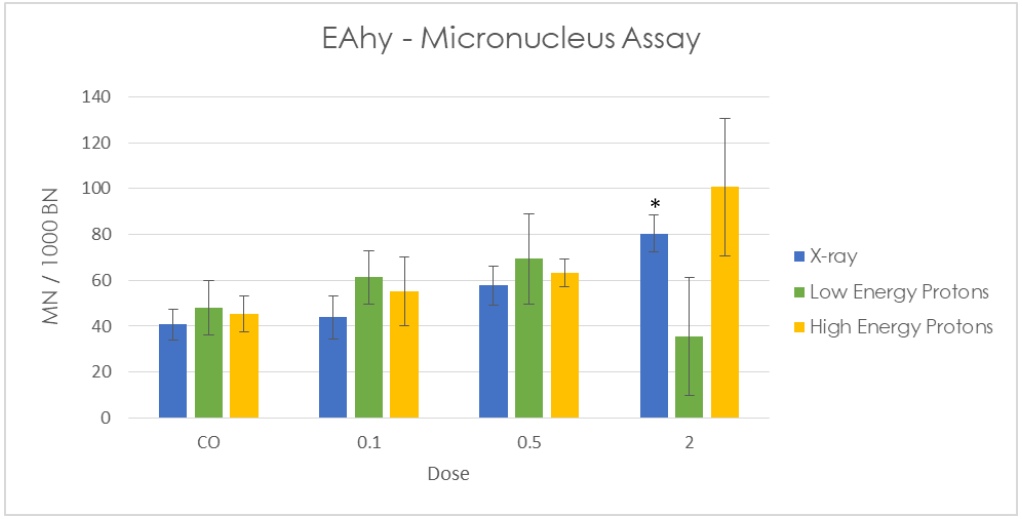


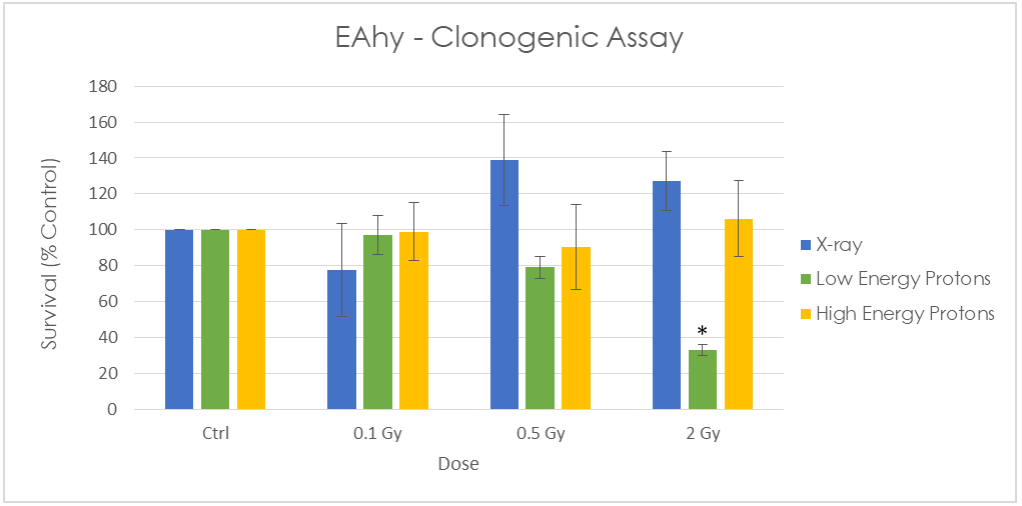


Results – Endothelial cells

Micronuclei number increased at 2 Gy for X-ray and High Energy Protons;

Low Energy Protons induced a dose dependent cell survival decrease in contrast to X-ray.

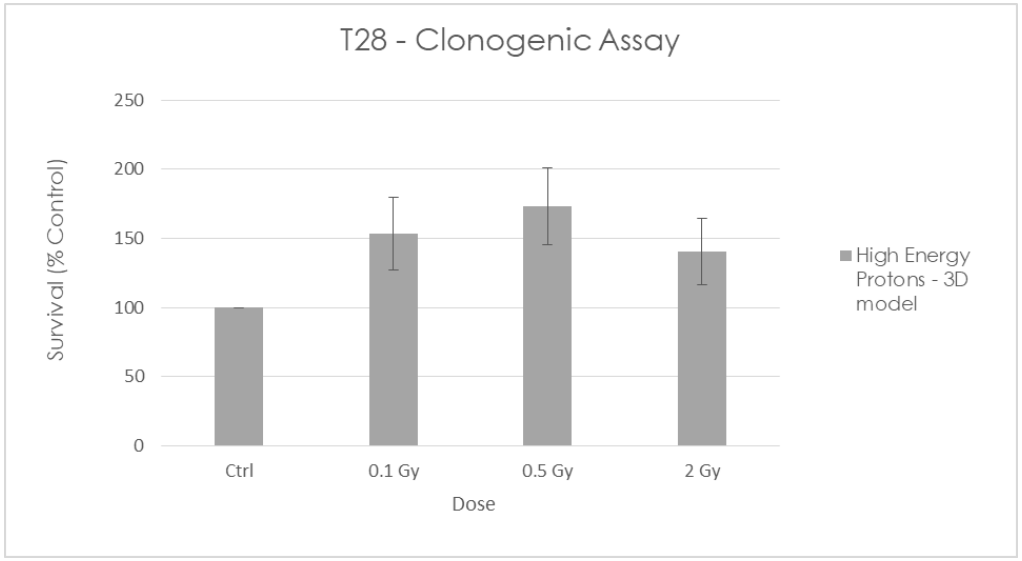


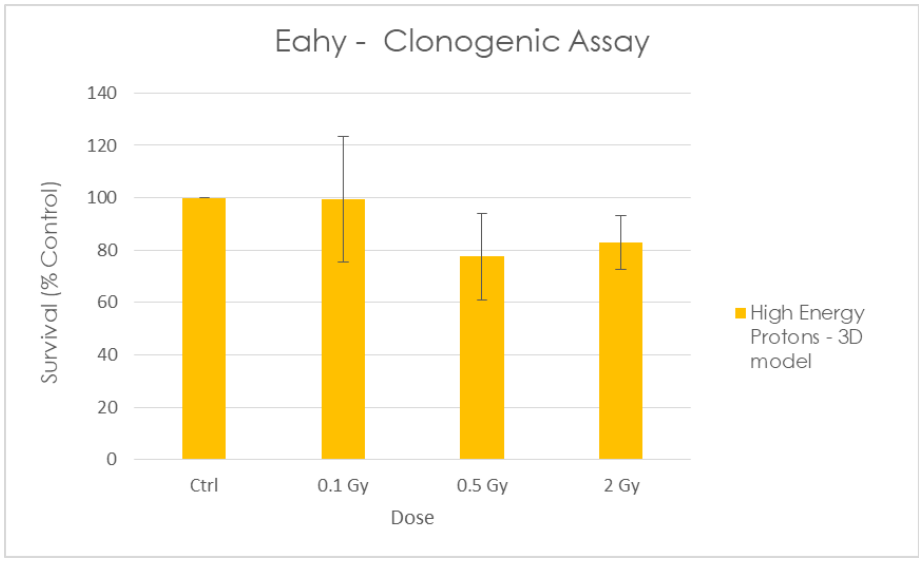


Results – 3D spheroid bystander

Preliminary results obtained after transferring media from our spheroid chondrosarcoma model:

High Energy Proton irradiation did not modify survival for chondrocytes or endothelial cells.





Conclusions

Irradiated chondrosarcoma cells have the ability to release stress factors inducing bystander responses in the non-irradiated chondrocytes/endothelial cells.

X-ray induced a more prominent bystander effect than proton irradiation in chondrocytes.

In contrast, endothelial cells show a growth in bystander response for proton irradiation.

High energy protons generated a diminished bystander effect in chondrocytes while at the same time reducing cell proliferation in endothelial cells. This findings might support the idea that high energy proton irradiation shields surrounding healthy tissue and delays angiogenesis.

Further *in vitro* studies are necessary to understand the biological mechanisms involved in the bystander effects of protons.

Future development

- Evaluation of intercellular signaling modulation in relationship to cellular architecture and radiation type;
- Identifying bystander effectors through metabolomics analysis;
- Additional endpoint investigations for the bystander effect induced by irradiated 3D spheroids.

Thank you for your attention!

We acknowledge the Romanian Ministry of Research and Innovation:
PN 19060203/2019, 36 PFE/2018, 18PCCDI/2018, 64PCCDI/2018.