ENSAR2

Midterm Meeting of Networking Activity 5: MediNet

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Vinča Institute of Nuclear sciences, University of Belgrade

Hadrons on Malignant Cells: Recent Activities within Collaboration between LNS-INFN and Vinca Institute of Nuclear Sciences

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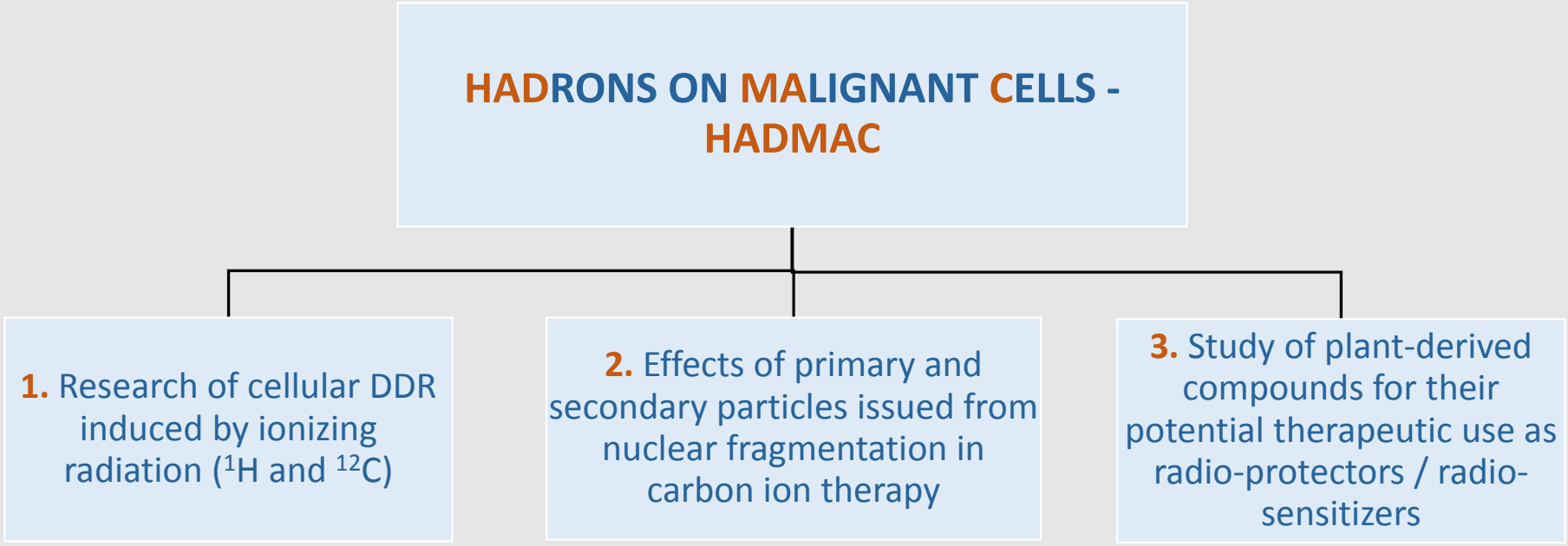

HADRONS ON MALIGNANT CELLS - HADMAC
1. Research of cellular DDR induced by ionizing radiation (^{1}H and ^{12}C)
2. Effects of primary and secondary particles issued from nuclear fragmentation in carbon ion therapy
3. Study of plant-derived compounds for their potential therapeutic use as radio-protectors / radio-sensitizers

Background:

1.

- ✓ Effects of low and high LET (linear energy transfer) radiations, i.e. γ – rays, protons or carbon ions, on different human cancer cells.

- ✓ The accent is given on the cellular DNA damage response (DDR) that can be induced by various stress signals, which is in this study ionizing radiation, i.e., ^{1}H and ^{12}C . Ionizing radiation acts on cellular DNA, either directly by inflicting DNA damages or indirectly by affecting DNA metabolism. DNA double-strand breaks represent critical lesions which can lead to genomic instability and cell death. They trigger a number of protective mechanisms such as up regulation of repair pathways, cell cycle arrest and sometimes programmed cell death.

2.

- ✓ In the fragmentation tail of ^{12}C ions, that is crucial for therapy and treatment planning systems, one can differ the contribution and the efficiency of each secondary ion specie, both experimentally and numerically.
- ✓ Numerical simulations with the GEANT4 code can offer this information by means of calculating each particle flunce, LET and dose.
- ✓ Radiobiological and immunocytochemical studies that are based on evaluation of damages induced in DNA, can provide necessary data to improve knowledge about the effects of primary and secondary particles in ^{12}C ion therapy. Some of these secondary ions are interesting for hadrontherapy also because there is a growing attention for their use as primary particles.
- ✓ The start of radiobiological analysis of secondary particles will be with ^{4}He, ^{6}Li and ^{11}B.

3.

- ✓ Radiation therapy has certain limitations including non-specific toxicity towards normal cells, thus reducing the efficacy of treatment.

- ✓ The exposure to ionizing radiation also creates intracellular reactive species, which consecutively lead to DNA strand breaks and conformational alterations of biomolecules. There are certain compounds that could be effectively used to scavenge free radicals and thus protect normal cells from radiation induced injury. Therefore, screening and testing of natural compounds as well as synthetic products in order to find effective radio-protectors able to inhibit radiation damage not only during radiotherapy of cancer patients, but also to healthy individuals undergoing occupational and accidental exposure to radiation is permanently in focus.

- ✓ Within HADMAC experiment the prospective application of different plants that are known to have anti-inflammatory, antioxidant and immunomodulatory compounds as well as plant-derived polyphenols with radio-sensitizing properties is analyzed.

Pomegranate *(Punica granatum L.)*

It is fruit-bearing shrub which originates from Middle East and India and is known as medicinal plant since ancient times. Historically, pomegranate has well documented appreciation in many cultures as a symbol of longevity and health.

All parts of the tree, including peel, have particular application especially in traditional medicinal systems (Chinese, Ayurvedic, and Unani).

In ethno medicine it is used as a remedy for various pathological conditions such as diabetes, dysentery, malaria, dental plaque and intestinal infections.

Some of the modern uses of pomegranate include treatment of **acquired immune deficiency syndrome (AIDS)**, **cancer**, **cardiovascular condition**, in addition to the use in **oral hygiene** and **cosmetics**.

Over the last few decades, accumulating scientific data reveal that **antioxidant**, **anti-inflammatory** and **anti-cancer activity of pomegranate** can be largely **attributed to its high content of polyphenols**.

Thus, pomegranate extracts could find clinical applications in many diseases where chronic inflammation is believed to play an essential etiology, such as cancer.

Both edible parts as well as nonedible peel are rich sources of bioactive ingredients.

Antioxidant activity of pomegranate juice is three times higher compared with green tea and red wine.

Punicalagin is ellagitannin unique to pomegranate and it is a major contributor to antioxidant activity of pomegranate juice.

Pomegranate peel (PP) is significant **natural source of phenolics** such as **ellagitannins**, **proanthocyanidins**, and **flavonoids**. Among typical compounds, ellagic and gallic acids, punicalagin and punicalin are reported.

Experimental conditions

- 4 human cancer cell lines:

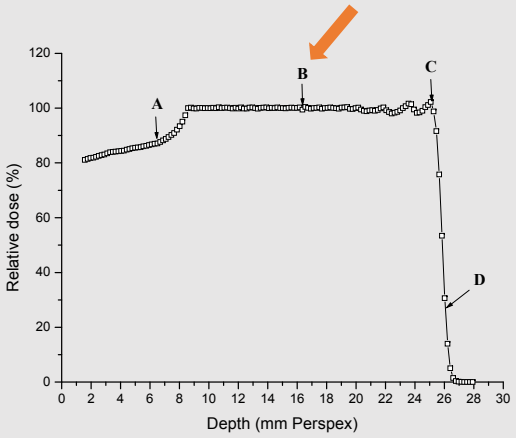
  HTB140 melanoma,

  CRL-5876 lung carcinoma,

  HTB177 lung carcinoma,

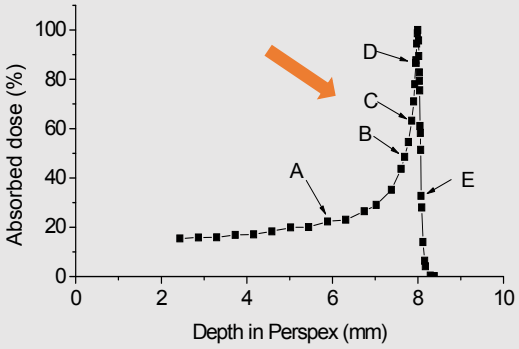
  MCF-7 breast adenocarcinoma ⟸

  MRC5 normal human lung fibroblasts

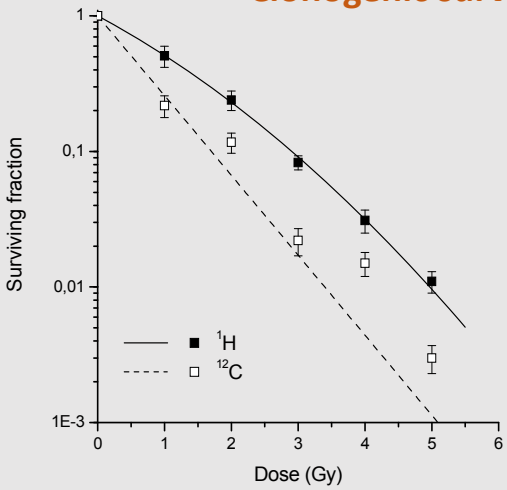


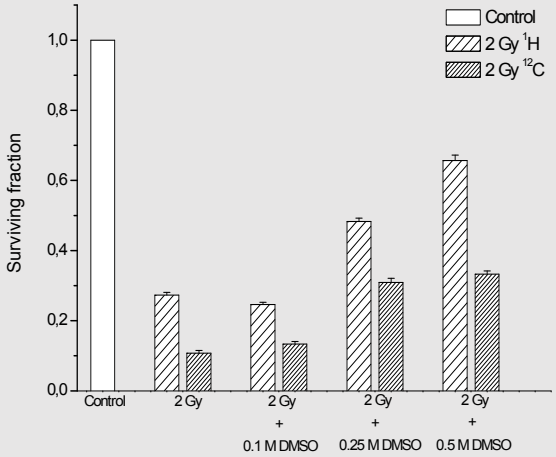
- Irradiation parameters for 62 MeV/u ^{1}H and ^{12}C

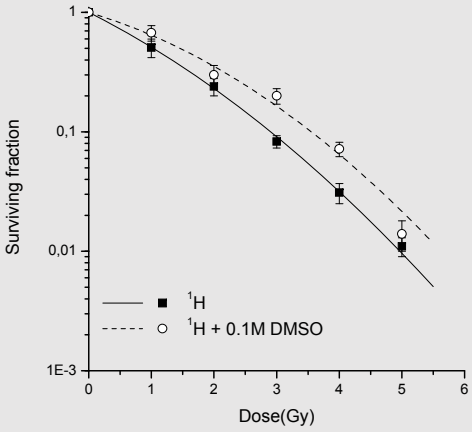
Irrad. type	Relative Dose (%)	E (MeV)	LET (keV/µm)
$^{1}H_{SOBP}$	100.0±1.6	34.9±2.2	4.7±0.2
$^{12}C_{SOBP}$	100.0±1.8	121.1±2.6	202.0±3.6

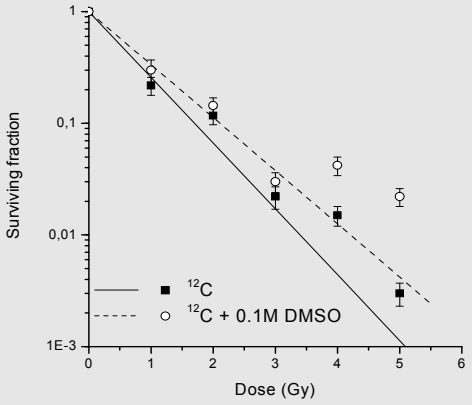


Clonogenic survival of MCF-7 cells



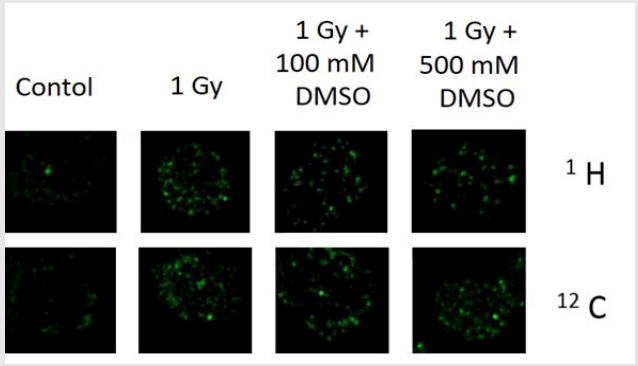




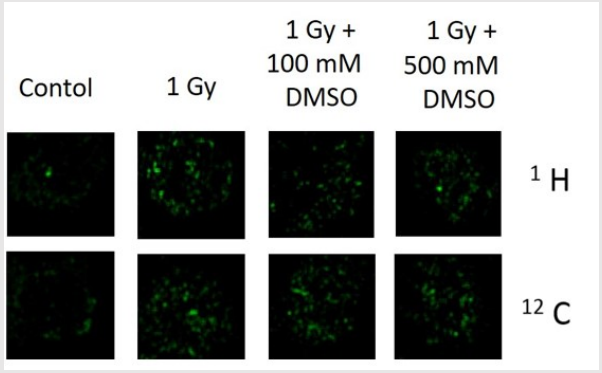


- Irradiations with either ^{1}H or ^{12}C strongly inactivate MCF-7 cells, with the SF2 values being 0.24 and 0.07, respectively.
- Radical scavenging potential of DMSO was tested for doses of 0.1, 0.25 and 0.5 M. The cells were treated with DMSO 1 h before irradiations. The concentration of 0.1M was chosen for clonogenic survival and immunochemical analyses.
- ^{1}H irradiations combined with 0.1M DMSO increasecell survival, thus changing SF2 values from 0.24 to 0.35 and D10 from 2.9 Gy to 3.54 Gy.
- ^{12}C irradiations in combination with 0.1M DMSO also increase cell survival, with SF2 values expanding from 0.07 to 0.11 and D10 from 1.7 Gy to 2.1 Gy.

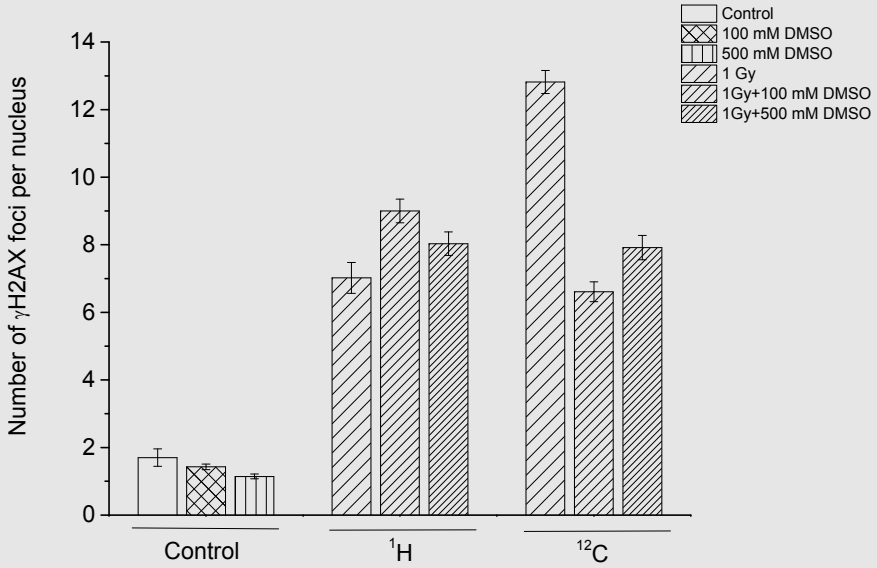
ICC analysis of γH2AX foci in MCF-7 cells pre-treated with 100 mM and 500 mM DMSO 1 h before irradiation with ^{1}H or ^{12}C ion beam.

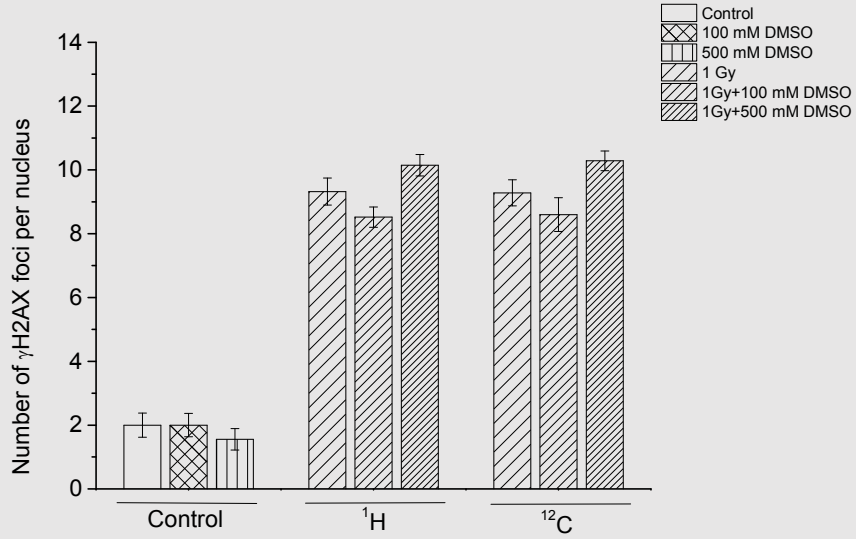


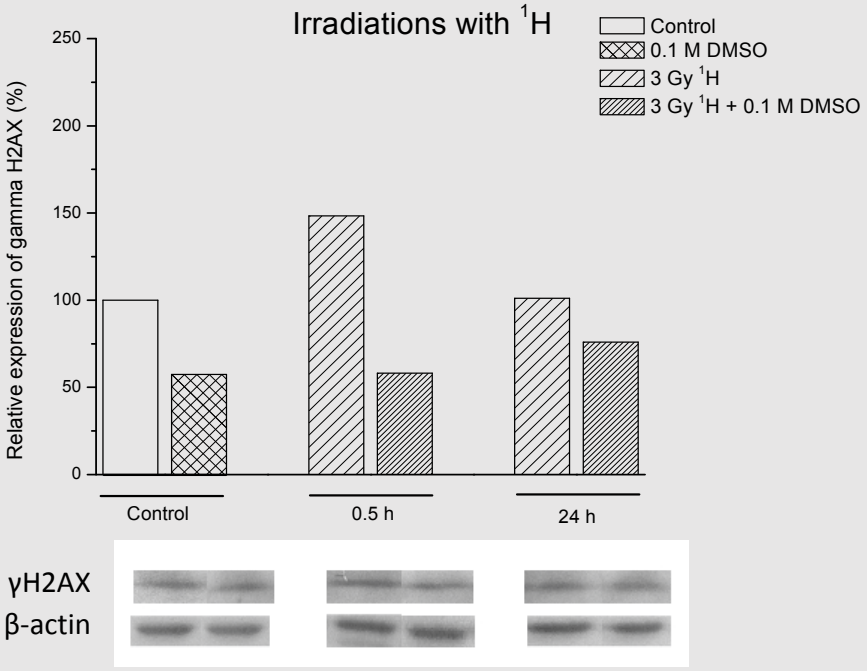
MCF-7 cells 0.5 h after irradiation

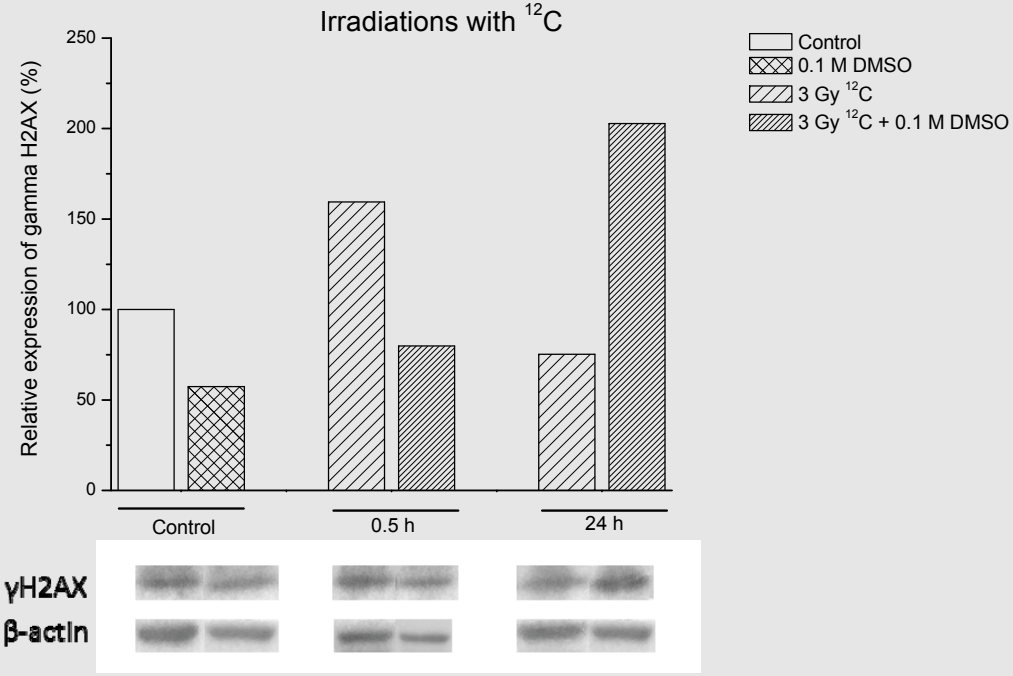


MCF-7 cells 24 h from irradiation





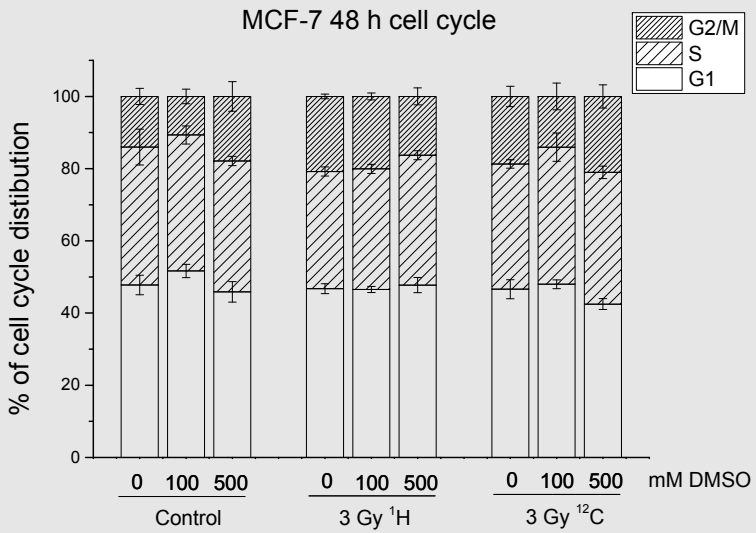


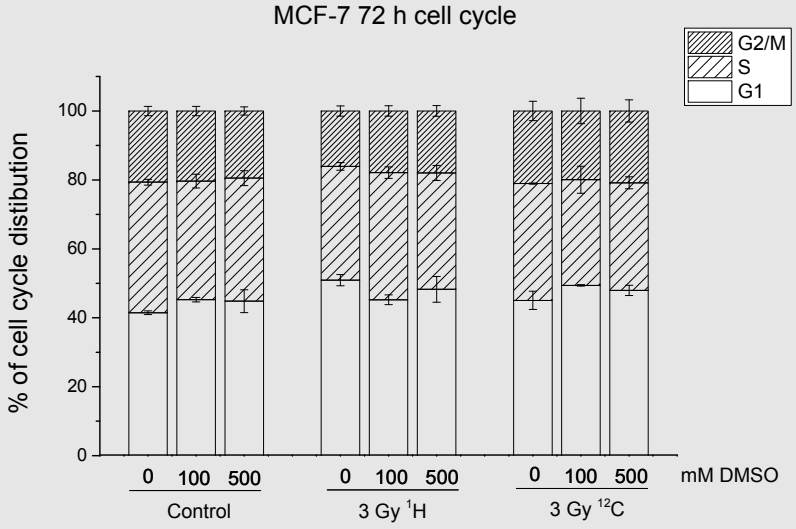


Elevated **expression of γH2AX protein** is observed in MCF-7 cells 0.5 h after irradiation with either ^{1}H or ^{12}C, while pre-treatment with 0.1 M DMSO causes the drop of γH2AX expression comparing to the control.

Further incubation up to 24 h of ^{1}H irradiated cells that were pretreated with 0.1 M DMSO decreases the level of γH2AX, while irradiation itself shows no difference comparing to the control. Irradiations with ^{12}C ions reduces the level of γH2AX, while the level of this protein is almost two times higher in samples pretreated with 0.1 M DMSO (this needs to be checked).

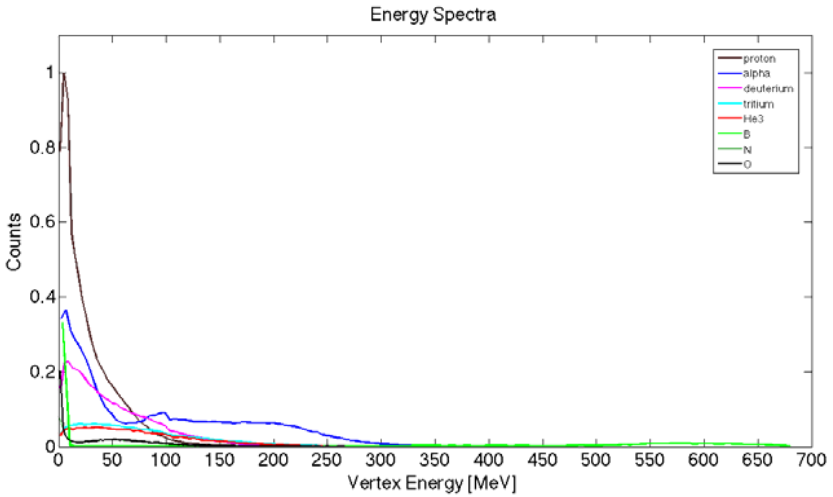
Cell cycle analysis of MCF-7 cells pre-treated with 100 and 500 mM DMSO 1 h before irradiation with ^{1}H or ^{12}C ion beam

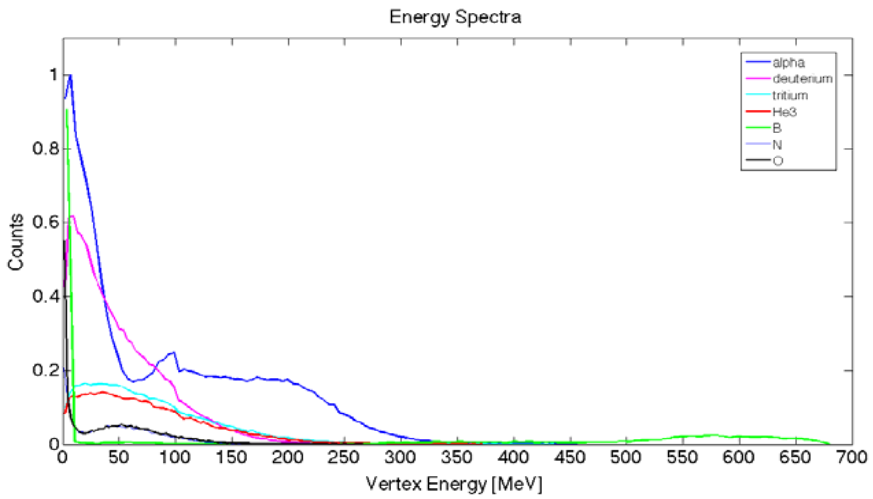




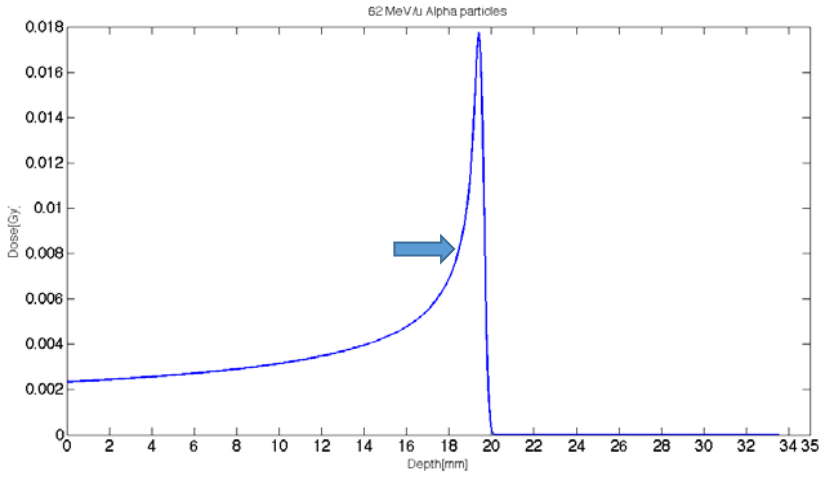
GEANT4 simulations

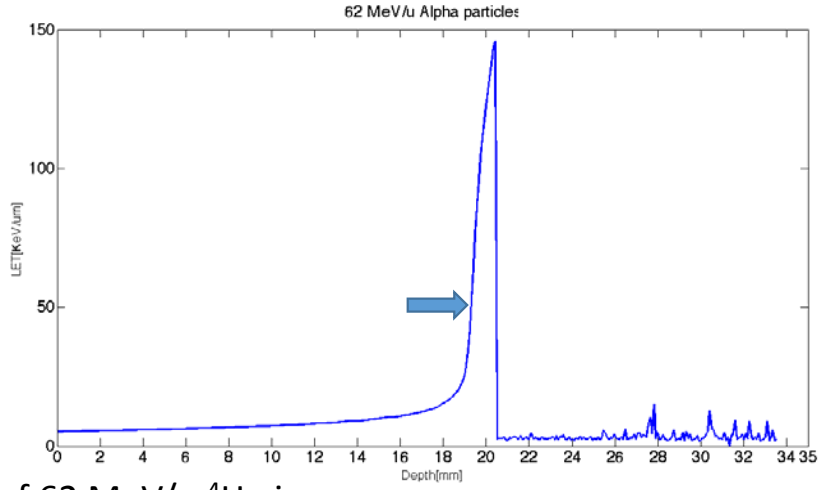
2.





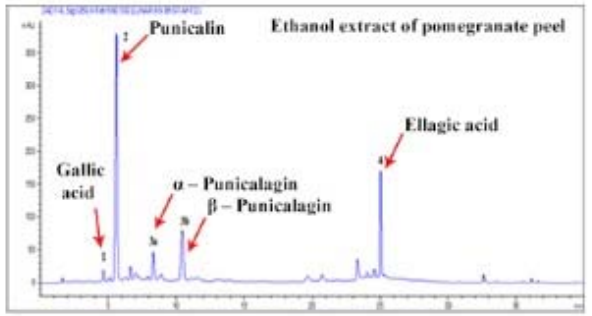
Energy spectra of secondary particles issued from fragmentation of 62 MeV/u ^{12}C ions.





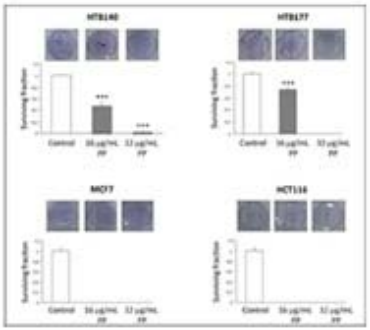
Dose and LET depth distributions of 62 MeV/u ^{4}He ions

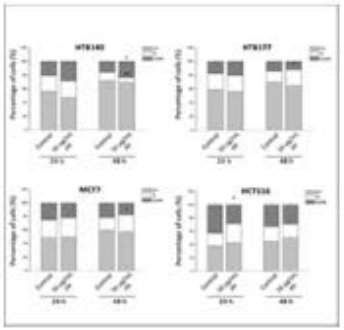
3.

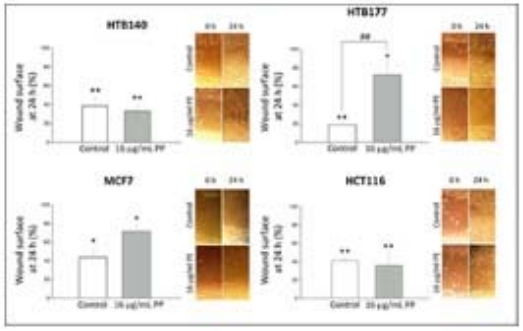


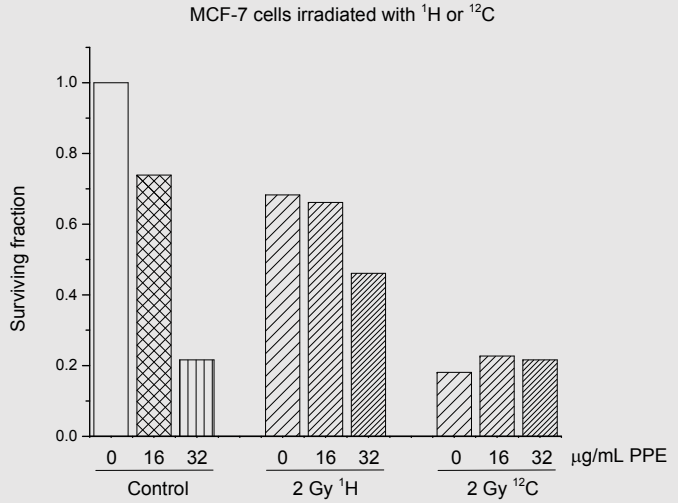
HTB140, HTB177, MCF7, HCT116 cancer cells

SI (selectivity index)		
	24 h	48 h
HTB140	1,42 ± 0,02	1,06 ± 0,01
HTB177	3,02 ± 0,06	1,6 ± 0,01
MCF7	4,28 ± 0,26	6,05 ± 0,32
HCT116	2,23 ± 0,09	4,71 ± 0,15
MRC-5	1	1

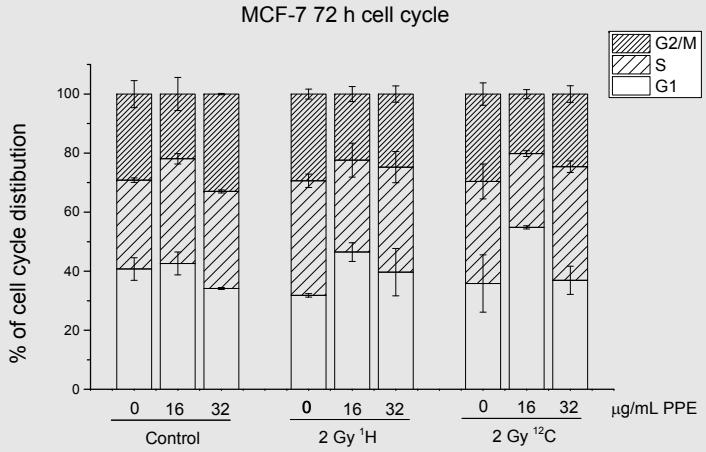






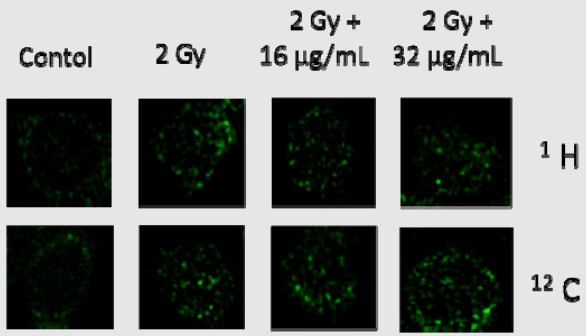


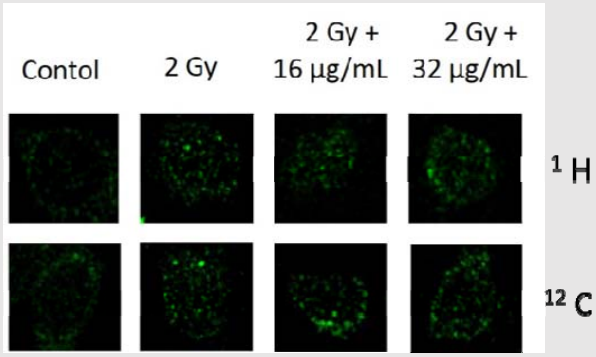
Clonogenic survival at 2 Gy of MCF-7 cells pre-treated with 16 and 32 µg/mL pomegranate peel extract (PPE), 6h before exposure to ^{1}H or ^{12}C ion beam. Analysis was performed 7 days after irradiation.



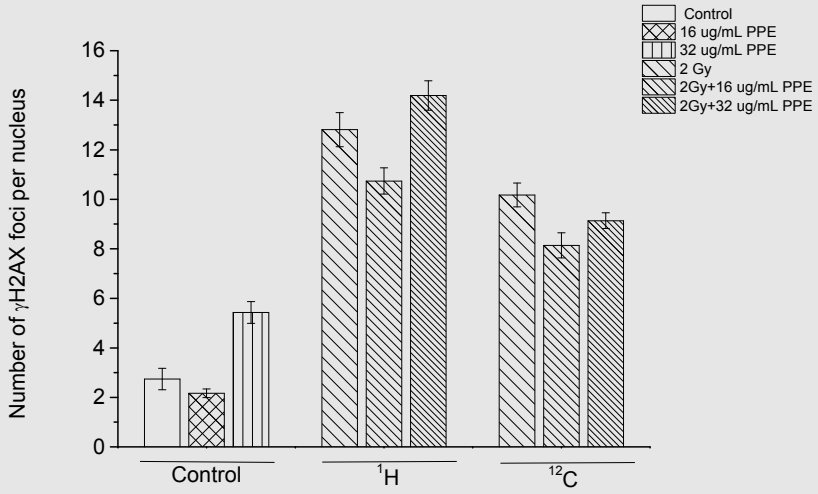
Cell cycle analysis of MCF-7 cells pre-treated with 16 and 32 µg/mL pomegranate peel extract (PPE), 6h before exposure to ^{1}H or ^{12}C ion beam. Analysis was performed 72 h after irradiation.

ICC analysis of γH2AX foci in MCF-7 cells pre-treated with 16 and 32 µg/mL pomegranate peel extract (PPE) 6h before exposure to ^{1}H or ^{12}C ion beam

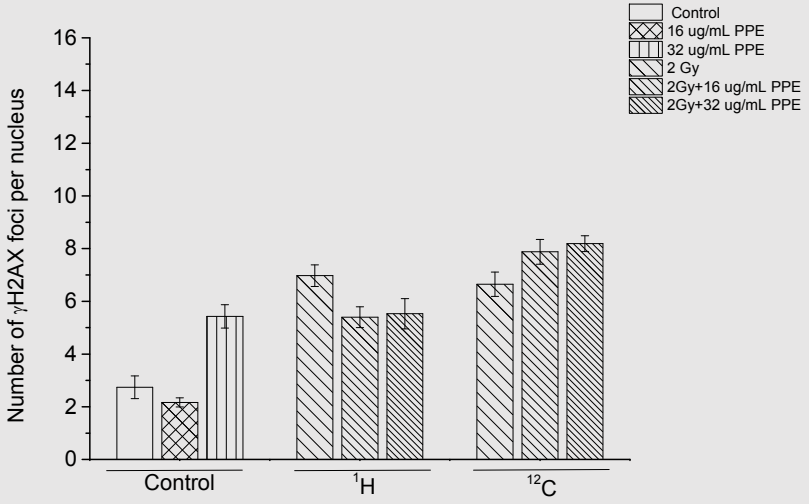




MCF-7 cells 0.5 h from irradiation



MCF-7 cells 24 h from irradiation



Conclusions

1.

- ✓ Irradiations with either ^{1}H or ^{12}C ions strongly inactivate MCF-7 cells, with ^{12}C ions being more efficient.
- ✓ Cell cycle analysis of MCF-7 cells pre-treated with 100 and 500 mM DMSO 1 h before irradiation with ^{1}H or ^{12}C ion beam revealed G1/S block at 48 and 72h post-irradiation.
- ✓ Number of γ-H2AX foci reached maximum 0.5 h after irradiation, particularly with ^{12}C , and decreased with the prolonged incubation up to 24 h.

2.

- ✓ Irradiations with secondary particle issued from ^{12}C fragmentation will be done with 62 MeV/u ^{4}He ions at LET energy of ~ 50 keV/µm, which corresponds to irradiation position of ~ 40 % dose within the proximal part of the pristine Bragg peak.

3.

- ✓ Clonogenic survival at 2 Gy as well as immunecytochemical analysis of MCF-7 cells pre-treated with 16 and 32 µg/mL pomegranate peel extract (PPE), 6h before exposure to ^{1}H or ^{12}C ion beam, showed strong cell inactivation, particularly with ^{12}C ions.
- ✓ Scavenging properties of PPE (16 and 32 µg/mL) on MCF-7 cells were revealed.

Selected publications:

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