NINTH INTERNATIONAL
CONFERENCE ON RADIATION
IN VARIOUS FIELDS OF RESEARCH
June 14-18, 2021 | Hunguest Hotel Sun Resort | Herceg Novi | Montenegro

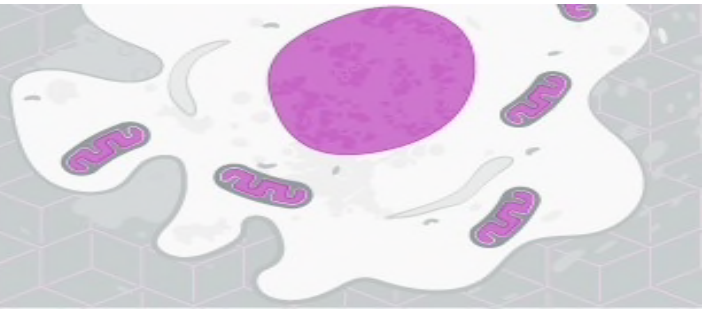
Nanoparticle radiosensitization experiments at IFIN-HH, Romania

Roxana Cristina Popescu, Mihaela Tudor, Diana Savu

National Institute for R&D in Physics and Nuclear Engineering "Horia Hulubei"

RAD2021

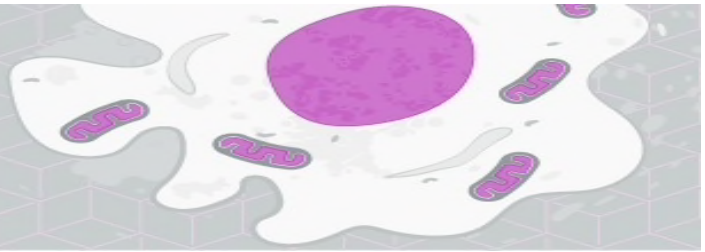
Abstract



- Translational cancer radiotherapy aims to deliver a high dose at the tumor site, to inhibit the development of cancer cells, while, in the meantime, trying to protect the healthy cells. In order to reach this goal and to minimize the adverse systemic effects, targeted nanoparticle therapies have been proposed as a solution.
- We have employed different types of radiation therapy in combination with iron oxide nanoparticles (IONP) to be used as radiosensitizers.
- Due to the secondary reactive species production following the interaction with radiation, intermediate Z IONP determined the alteration of the tumor cells metabolic function, DNA damage, protein expression, division or even induction of cell death.
- We have proved that the sensitizing response is influenced by the nanoparticles targeting ability and intracellular localization, as well as radiation type and properties, like energy, dose and flow.

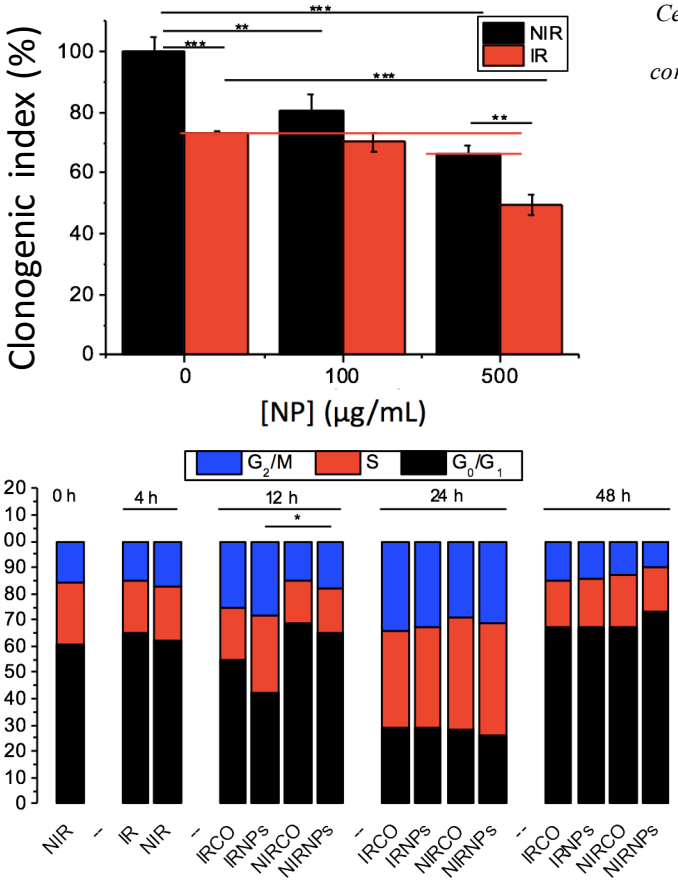
- **Acknowledgements:** This work was supported by Romanian Ministry of Research National grants no. PN 19060203, 543PED.

Introduction

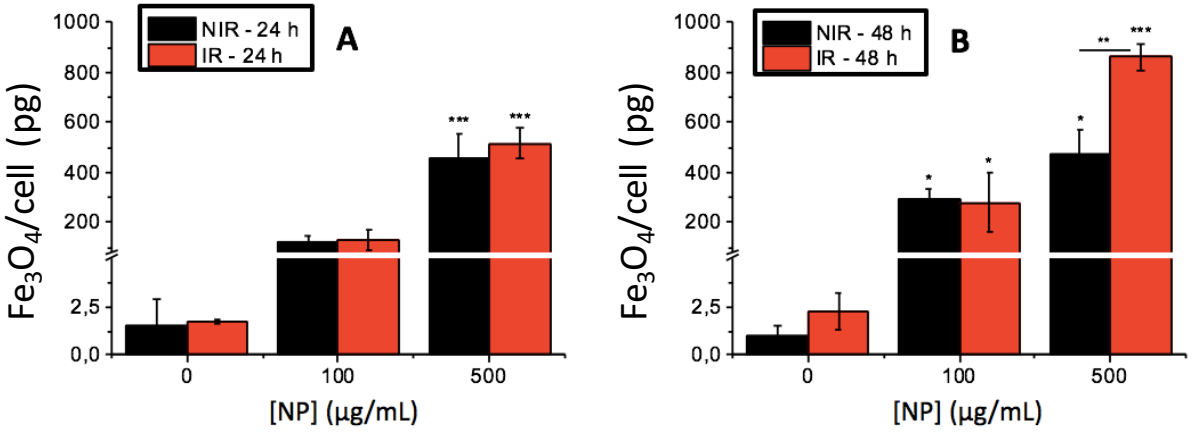


- **Nanotechnology** → potential improvement of cancer treatment efficiency ← ability to target the tumor area;
- Cancer therapy using **radiation** → high dose at the tumor site → inhibit the growth of tumor cells → protect the surrounding healthy cells;
- **Radiosensitizers based on high Z elements** → absorb incident radiation → deliver secondary radiation → enhances the production of reactive oxygen species, interaction and intercalation into the DNA.
- The **biological effects** of nanoparticles radiosensitization imply the alteration of cellular signaling pathways involved in:
  - oxidative stress;
  - cell cycle disruption;
  - DNA repair inhibition;
- **Shortcomings** to clinical translation
  - non-specific targeting;
  - inability of internalization and interaction with the cell compartments;
  - inability of nanoparticles to be intracellulary retained.

Treatment with radiation followed by NPs



*Cell survival of MG-63 previously exposed to 0Gy, 1Gy X-Rays, 500 µg/mL, 100 µg/mL Fe_3O_4@DOX or combined treatment, during 48h; data is shown as mean ±SEM; *P<0,05 **P<0,01 and ***P<0,001.*



*Quantity (pg) of internalized Fe_3O_4 in MG-63 osteosarcoma cells exposed to different concentrations of Fe_3O_4@DOX during 24, respectively 48h; a group of cells as previously exposed to 1Gy; data is shown as mean ±SEM; *P<0,05 **P<0,01 and ***P<0,001.*

*Cell cycle distribution for MG-63 cells exposed to radiation treatment (0, 1 Gy) and NPs (0, 500 µg/mL Fe_3O_4@DOX) at different time intervals; *P<0,05.*

[Int. J. Molec. Sci., 2020, 21(19), 7220;]
Acknowledgement: Prof. M.R. Veldwijk, Univ. Heidelberg

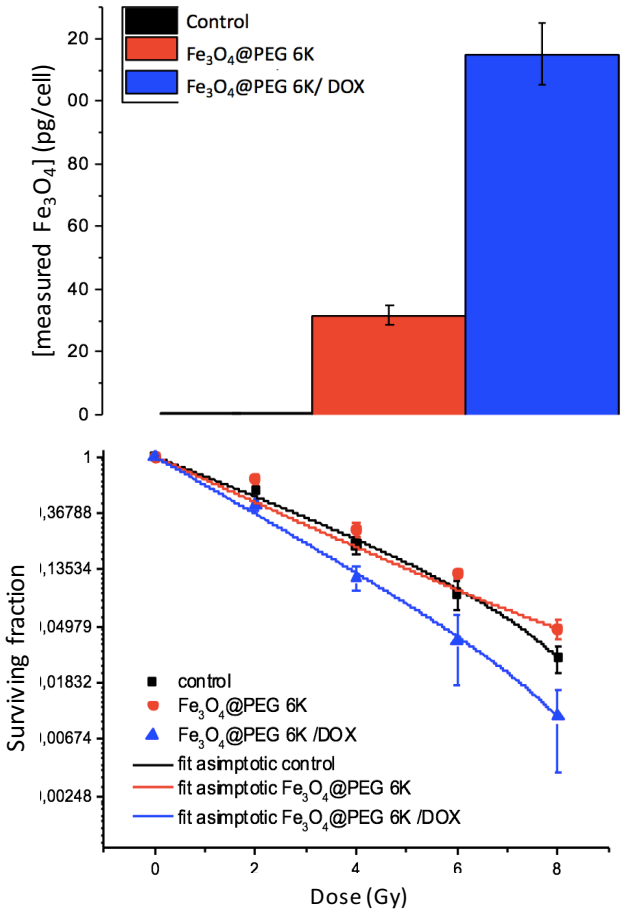
Treatment with radiation followed by NPs

- The potential cytotoxic effects of radiation treatment followed by exposure to Fe_3O_4@DOX in MG-63 human osteosarcoma cells. MG-63 cells at confluence were irradiated with 1 Gy (150 KeV), allowed to attach during 4h and then exposed to different concentrations of Fe_3O_4@DOX in the range of 0-500 μg/mL.

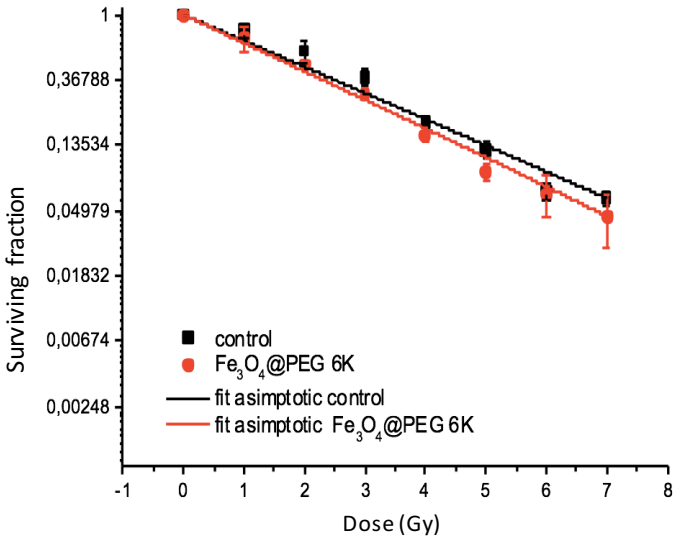
- Combined treatment → increase of the cytotoxic effects compared to NP alone.
- Temporary cell cycle arrest in G_2/M ←cells previously exposed to 1Gy X-Rays ←enhanced internalization of Fe_3O_4@DOX.
- 1Gy + 500 μg/mL NP → early G_2/M at 12h from irradiation, maximum at 24h after treatment.

[Int. J. Molec. Sci., 2020, 21(19), 7220;]

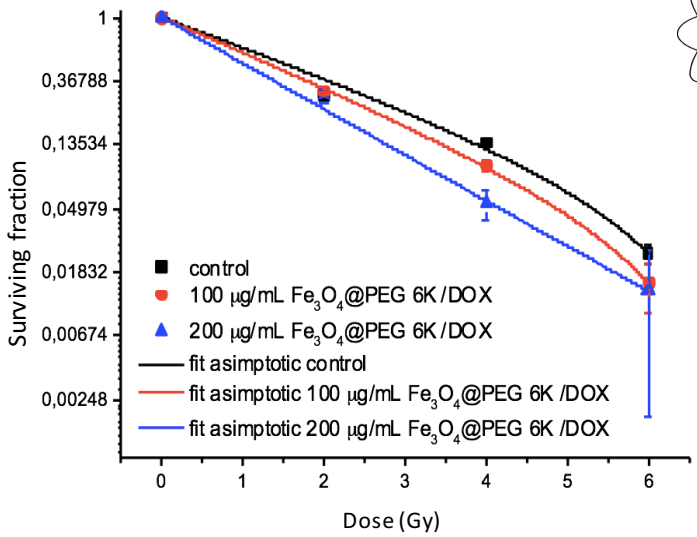
Treatment with NPs followed by radiation



Quantity of internalized Fe_3O_4 in HeLa cells incubated with 0, respectively with 100 µg/mL Fe_3O_4@PEG 6K (/DOX) during 16h, at 24h from NP removal; data is shown as mean±SEM (n=3);



Clonogenic survival rate of HeLa cells exposed to 100 µg/mL Fe_3O_4@PEG 6K during 16h and followed by 50kV X-Ray exposure. Data is shown as mean ±STDEV (n=3);

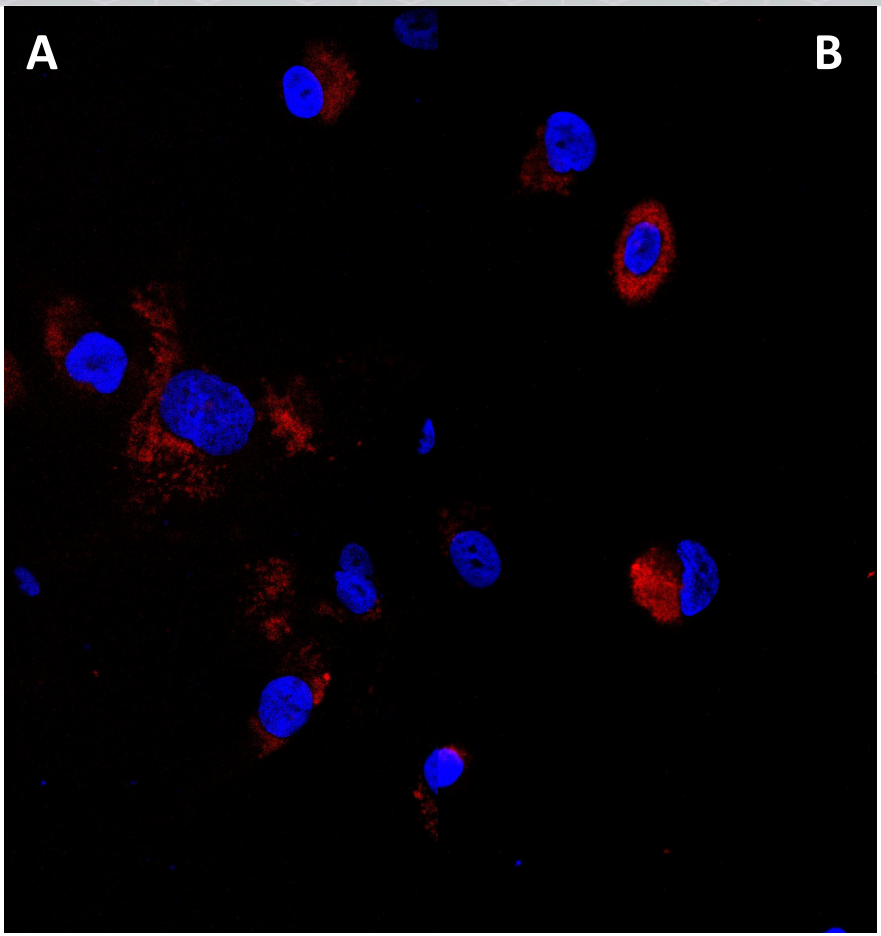


Clonogenic survival of HeLa cells exposed to 0, 100, respectively 200 µg/ mL Fe_3O_4@PEG 6K/DOX during 16h and followed by 150 kV X-Ray at different doses; data is shown as mean ± STDEV (n=3);

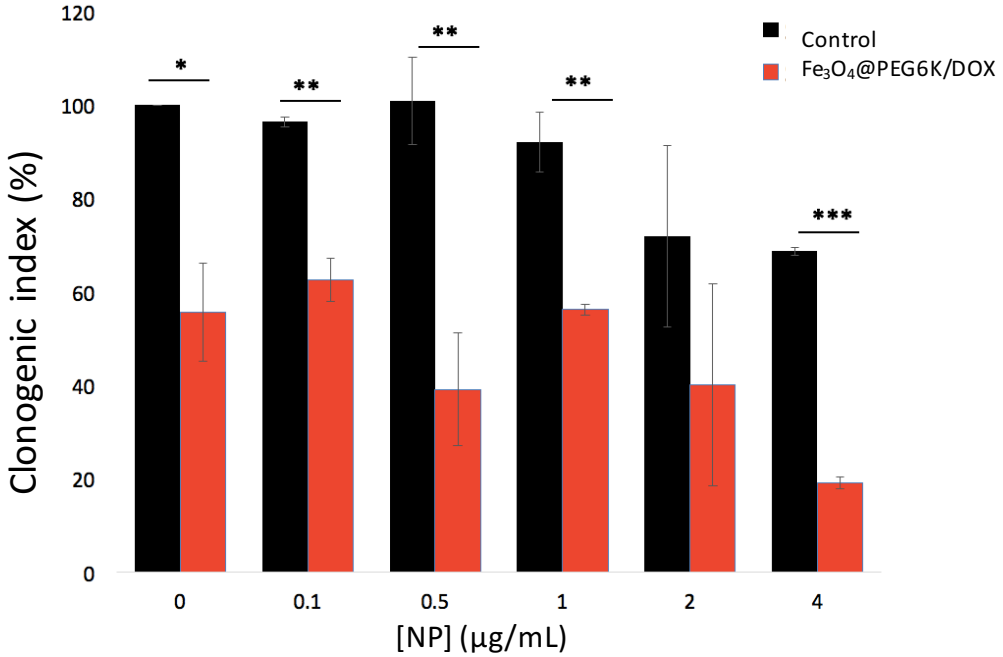
Clonogenic survival rate of HeLa cells exposed to 100 µg/mL Fe_3O_4@PEG 6K(/DOX) during 16h and followed by 6MV X-Ray exposure. Data is shown as mean ±STDEV (n=3);

Acknowledgement: Prof. M.R. Veldwijk, Univ. Heidelberg [Sci. Rep., 2020, 10, 10530]

Treatment with NPs followed by radiation



Internalization of Fe_3O_4@PEG 6K/DOX in SW1353 cells incubated with with 200 µg/mL Fe_3O_4@PEG 6K (/DOX) during 16h, at 24h from NP removal;



*Cell survival of SW1353 cells exposed to 200 µg/mL Fe_3O_4@PEG 6K/DOX during 16h and followed by 155 MeV proton exposure. Data is shown as mean ±STDEV (n=3); *P<0,05 **P<0,01 and ***P<0,001*

Acknowledgement: Dr. A. Rzjanina; Dr. G. Mytsin, JINR Dubna

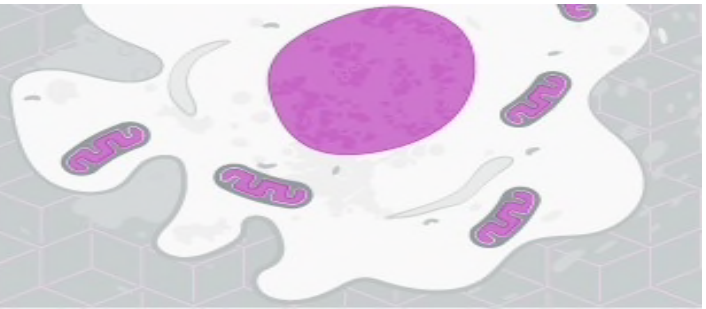
JOINT INSTITUTE FOR NUCLEAR RESEARCH
IFIN-HH

Treatment with NPs followed by radiation

- In order to evaluate the radiosensitization potential of Fe_3O_4@PEG 6K(/DOX) nanoparticles, human cervical adenocarcinoma HeLa and human chondrosarcoma SW1353 were incubated with NP during 16h and then irradiated. X-Rays with different energies were used: high energy (6MV), as well as medium (150 kV) and low energies (50 kV), respectively protons (155 MeV).
- 100 µg/mL DOX- free Fe_3O_4@PEG 6K + 6MV X-Ray → no statistically significant effect compared to radiation alone in HeLa.
- 100 µg/mL DOX- free Fe_3O_4@PEG 6K + 50 kV X-Ray → radiosensiziting effect $DMF_{SF\ 0.1}$= 1,13 ±0,05 in HeLa.
- 100 µg/mL Fe_3O_4@PEG 6K/DOX + 6 MV X-Ray → radiosensitizing effect $DMF_{SF\ 0.1}$= 1,3 ±0,1 in HeLa.
- 100 µg/mL Fe_3O_4@PEG 6K/DOX + 150 kV X-Ray → radiosensitizing effect $DMF_{SF=0,1}$=1,29±0,04 in HeLa.
- 200 µg/mL Fe_3O_4@PEG 6K/DOX + 150 kV X-Ray → radiosensitizing effect $DMF_{SF=0,1}$=1,55±0,12 in HeLa.
- 200 µg/mL Fe_3O_4@PEG 6K/DOX + 155 MeV protons → increased cytotoxicity in SW1353.

Acknowledgement: Prof. M.R. Veldwijk, Univ. Heidelberg
Dr. A. Rzjanina; Dr. G. Mytsin, JINR Dubna
[Sci. Rep., 2020, 10, 10530]

Conclusions



- The implication of magnetite nano-systems in the dual treatment of cancer involving radiotherapy and chemotherapy was evaluated through the monitorization of cytotoxic effects by applying the ionizing radiation treatment before or following the incubation of cells with nanoparticles.
- In the first case, was investigated the correlation between the increase of Fe_3O_4@DOX NP internalization following the irradiation of the cells with the cell cycle progression, as well as the characterization of the cytotoxicity. A higher significant percent of magnetite nanoparticles was measured in cells that were irradiated previous to NP exposure, phenomena associated with a more rapid entrance of the cells in the G_2/M phase of the cell cycle.
- The incubation of the tumor cells with Fe_3O_4@PEG 6K (/DOX) nanoparticles followed by irradiation at different energies and doses proved a radio-sensitization effect dependent on the concentration of the nanoparticles, concentration of DOX, but also the energy and dose of ionizing radiation. The obtained results proved that DOX free Fe_3O_4@PEG 6K determined a decrease of cell survival in HeLa cells following the radiation with low energy X-Rays (50 kV), but not following radiation with high energy (6MV). Through DOX encapsulation, a radio-sensitizing effect was obtained on HeLa cells at both high (6MV) and medium (150 kV) energies. Irradiation with 155 MeV protons showed a significantly increased cytotoxicity against chondrosarcoma cells for dual treatment.
- Results proved the efficiency of functionalized magnetite nanoparticles in the controlled delivery of anti-tumor substances and the chemical and/or radiological sensitization of human tumor cells. These observations confirm the potential use of the resulted nano-systems as potential candidates in the chemo- and radio-therapy mediated by nanoparticles.