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X-ray scattered radiation induced ROS generation and cell viability change related to absorbed dose

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Introduction (1)

- Radiotherapy is one of the conventional cancer treatment therapies applied in oncology clinics. Ionising radiation effects on cancer cells and healthy tissues has to be thoroughly researched, for appropriate tumor treatment.

- The poorly predictable side scattering of ionizing radiation during the application of complex and multi-field radiotherapy techniques (IMRT, VMAT and SRS) gives rise to a significantly higher complexity of low dose effect to cells.

Introduction (2)

- On top of that, it is already known that a nearby significant apoptotic cell signaling can change the behavior of unaffected cells. The relevance of such ionizing radiation treatment phenomenon is in place, since at least part of the affected cells are killed through the pathway of apoptosis.

- Therefore, there is a need for broad range of in vitro studies concerning application of low doses of ionizing radiation to cells.

- Aim of this study: apply our designed protocol to study side scattered and low dose effects on cells.

- Materials and methods:
  - Chinese hamster ovary cell line.
  - Clonogenic assay.
  - 24 and 96 well plates.
  - H2DCF-DA dye for ROS evaluation.

Irradiation procedure

- Varian Clinac DMX
- 6 MV energy photons
- Field size 4x4cm
- 3 Gy/min dose rate
- 8 Gy dose

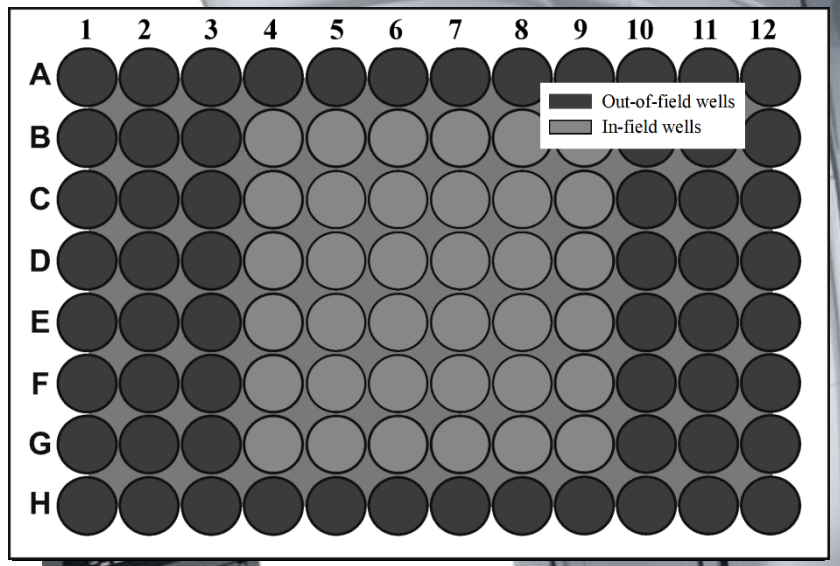

Fig. 3 . 96 well plate with marked in-field wells and out-of-field wells.

- 24 and 96 well plate (86x128 mm)

placed inside 86x128 mm hole in 30x30x11 cm PMMA plastic

- 100% isodose at the base of the plate. 4 cm of build-up plastic below and above plate.

Fig. 1 Linear accelerator used for irradiation.

Cell plating

- Trypsinization:
  - Growing medium is removed
  - 1 ml PBS to wash cells
  - 2 ml 0.05% trypsin/EDTA solution for 3 minutes
  - 1 ml of growth medium containing FBS
- For irradiation:
  - 500 000 cells/well (for 24 well plate)
  - 50 000 cells/well (for 96 well plate)
- For clonogenic cell viability assay:
  - 400 cells/dish

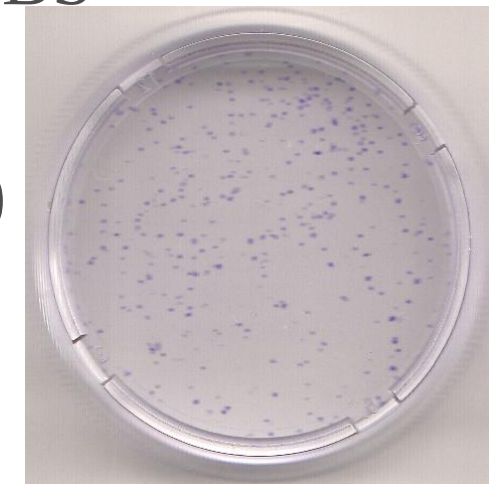

Fig. 4 Stained cell colonies using clonogenic assay

H2DCF-DA dye

- Our recent study showed that ROS generation during and after irradiation correlates to cell DNA damage and cell death.
- 50 μM/ml of H2DCF-DA for 60 min. prior to the irradiation and then distributed in each well. After irradiation 50 μl of 96 % ethanol was put on cell suspension disperse DCF dye.

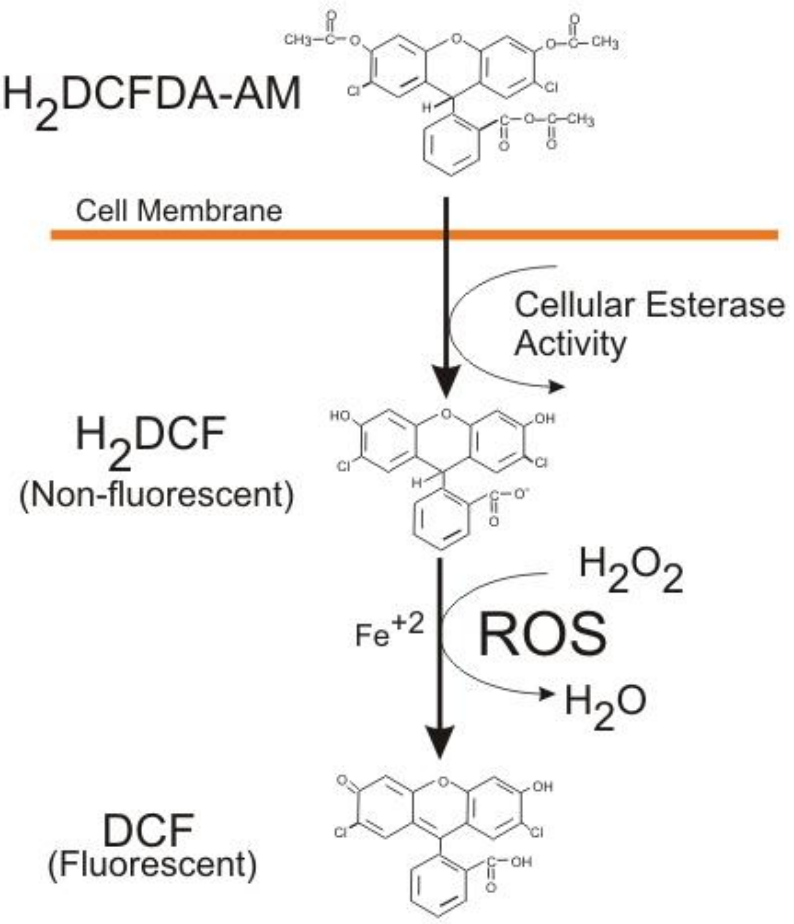

Fig. 5 Formation of fluorescent compound DCF by ROS[1].

[1] Held, Paul, and Kheng Newick. "Using BioTeks Synergy™ HT Reader to Measure Reactive Oxygen Species (ROS) Generation in Stimulated Cells." *BioTechniques*, vol. 46, no. 1, 2009, pp. 61–62.

Clonogenic assay.

- For cell viability assay 24 well plate was used.
- Cell suspension with growth medium at concentration of 10^6 cells/ml was prepared and 500 µl of suspension placed in 4 center wells (in-field) and 4 corner wells of plate (out-of-field).
- 3 h after irradiation the growth medium of each well was removed, centrifuged and placed onto Petri-dishes with 400 cells per dish. After 7 days colonies were counted.

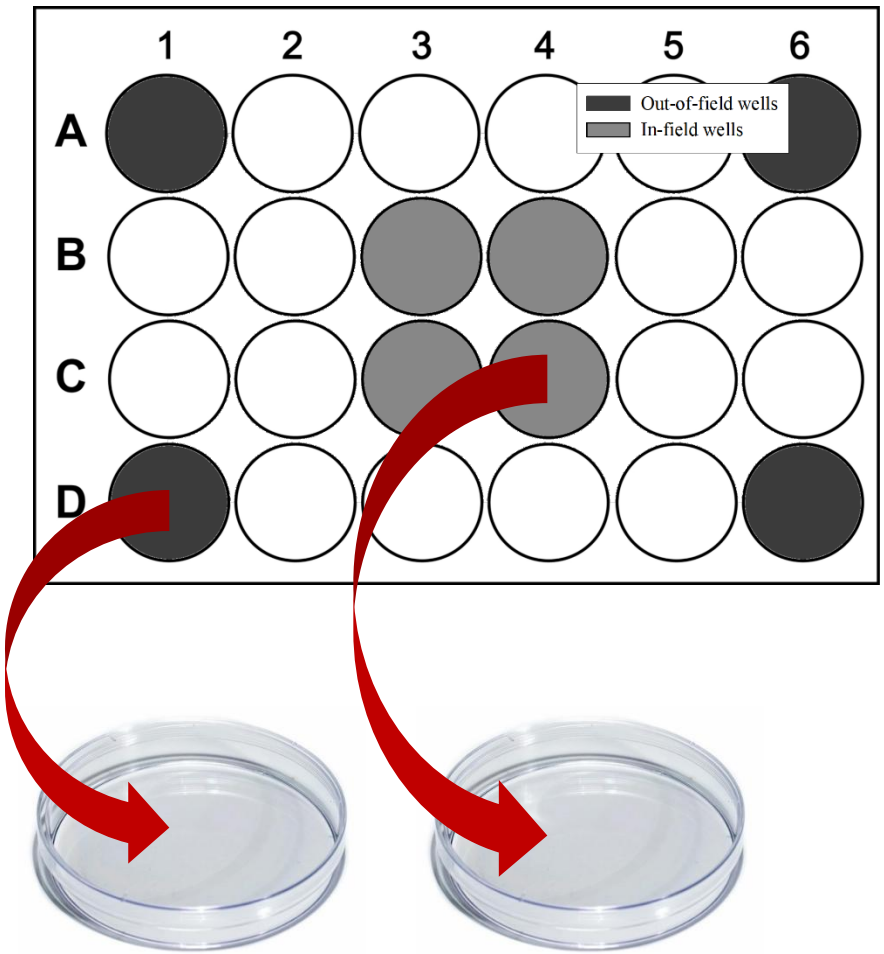

Fig. 6 Assessment of irradiated growth medium effect on cells viability

Results (1)

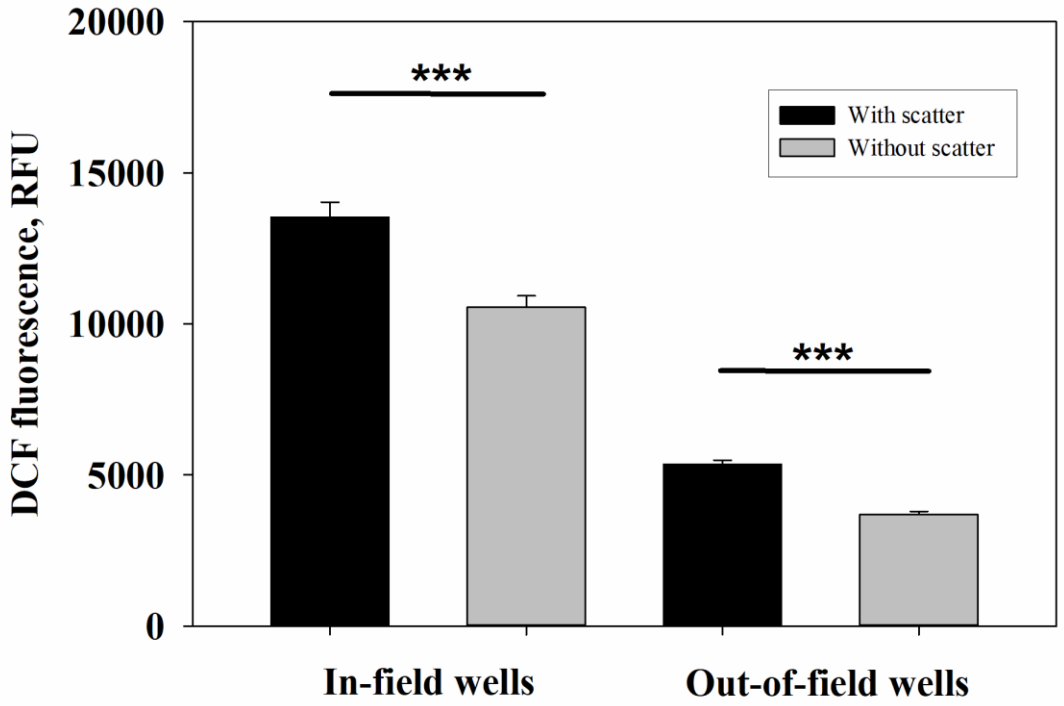

Fig. 7 Comparison of average values of DCF fluorescence between in-field wells and out-of-field wells with and without scatter material between wells. The increase of generated ROS is 28.18 % for in-field wells and by 45.07 % for out-of-field wells. *** signifies $p < 0.001$.

Results (2)

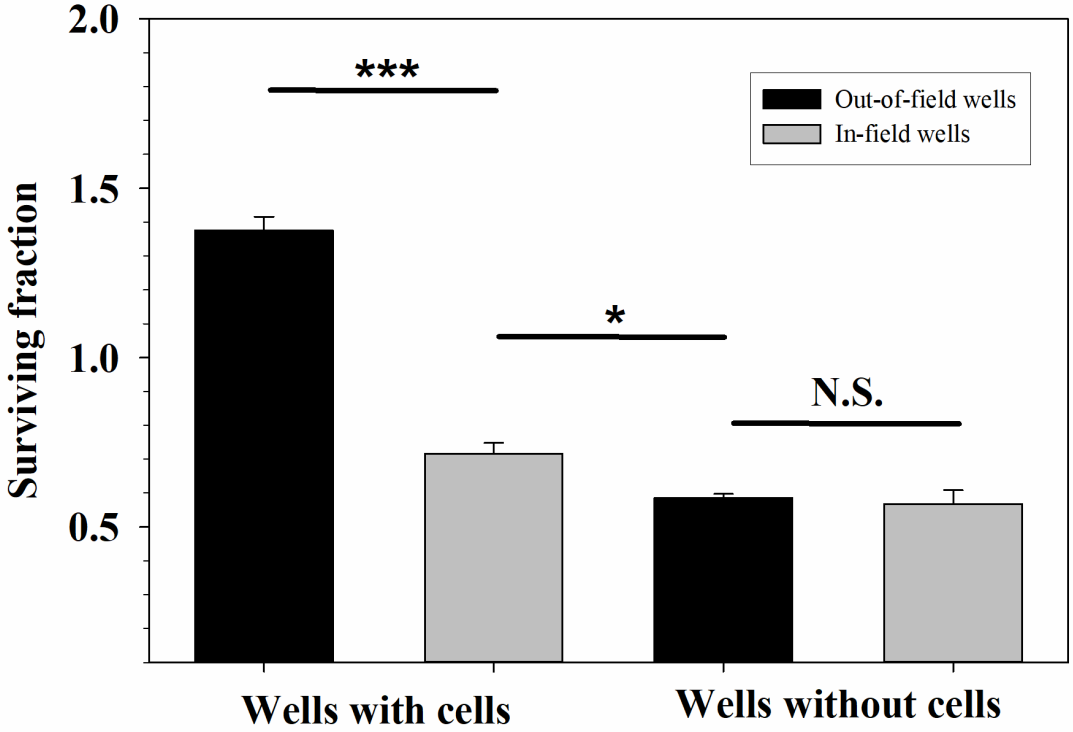

Fig. 8 Comparison of surviving fraction of cells after irradiation between in-field wells and out-of-field wells with and without cells. Results are normalized to 400 colonies. N.S. signifies p values > 0.05, * signifies p values < 0.05, *** signifies p < 0.001;

Conclusions

- Radiation induced side scattering effects affect in-field and out-of-field located cells, thus altering ROS generation. Since cell death after irradiation is a result of DNA damage generated radiation induced ROS, evaluation of ROS generation is crucial for the evaluation of out of field located cells viability. Significant radiation induced out-of-field ROS generation was observed performing our investigations which was contributing to the viability changes of the affected cells.

Discussion

- The usage of 96 well plate in irradiation experiments allows to "turn on" or "turn off" side scattering effects since one can fill the air gaps between wells in the plate and investigate direct effect on generation of ROS. This lead to conclusion, that side scattering effect must be considered when evaluating cells behavior after irradiation.
- Bystander effect was not observed with our setup but we revealed positive cell growth effect on cells affected by side scattered radiation.

Thank you for attention!