INVESTIGATION OF RADIATION INDUCED MITOTIC ARREST IN IRRADIATED CELLS

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INTRODUCTION

Radiotherapy is a conventional localized cancer treatment method using ionizing radiation. Interaction of high energy photons with biological tissue results in production of the reactive oxygen species (ROS), that in turn may cause significant cellular damage. Depending on the X-ray energy delivered to the irradiated cells four scenarios are possible: 1) No significant changes in cell viability at low irradiation doses; 2) Significant damages in irradiated cells caused by radiation induced ROS that may lead to temporary mitotic arrest at the G1/S, S or G2/M stages of the cell cycle at high doses; 3) Initiation of the apoptotic cell death due to severe ROS damage; 4) Necrosis of cells because of colossal unrepairable damage done by radiation generated ROS.

It is known that the application of 2 Gy per fraction for cancer treatment is sufficient enough for effective balancing between tumor destruction and possible low health tissue complications. Due to the fact that 2 Gy irradiation most likely is responsible for the apoptotic cancer cell death, significantly less inflammation and other systemic response is introduced, as compared to necrotic cell death. Also other cellular reactions to irradiation are possible, e.g. radiation induced continuous proliferation of tumor cells. Moreover, cells that survive significant DNA damage and proliferate can induce secondary, non-metastatic, tumor.

In the performed research radiation induced ROS generation, cellular DNA damage and exposed cell viability were investigated, and irradiation dose related complex cellular response was discussed. We also demonstrated cellular response to different irradiation doses in form of ROS production and mitotic arrest. Performed analysis of the results led to suggestion of a irradiation dose threshold between 2 Gy and 4 Gy, which differentiates between the stages of cell response to irradiation. Presence of a threshold was approved by the results of DNA damage evaluation, assessment of post irradiative ROS production, relative mitotic arrest in cells and cell viability investigations.

MATERIALS AND METHODS

In order to perform complex numerical evaluation of radiation impact on the processes that contribute to the damage of DNA, cells were irradiated by 6 MeV X-ray photons to different doses (2-10 Gy). The increase of ROS generated inside the cell and in the medium, cell DNA damage, cell viability and relative mitotic arrest was evaluated after irradiation of cells.

Chinese hamster ovary cells (CHO-K_1) were cultured at 37°C in water-saturated air containing 5% CO2. The cells were routinely passaged in growth medium supplemented with 10 % of fetal calf serum. The cell doubling time was approximately 12 h.

Irradiation of cells was performed in linear accelerator Varian Clinac DMX using 6 MeV X-ray photons. The treatment dose delivery rate to the target of 3 Gy/min was applied. The irradiation dose varied between 0.5 and 10.0 Gy. Irradiation was performed in broad beam geometry using 10x10 cm^2 irradiation field. Petri dish (ϕ35 x 10 mm) with cells was placed in a special cavity located at 6 cm depth of a (30 x 30 x 11) cm^3 PMMA phantom. Dose simulation was performed using Varian Aria AAA algorithm keeping 100% isodose at the depth of 6 cm.

Clonogenic cell assay was used to assess radiation induced effects in irradiated cells. Cell colonies were manually counted using ImageJ software plug-in Colony Counter. Colony residence area was assessed using ImageJ software plug-in Analyze Particles, which enabled analysis of the scanned image of Petri dish with cells detecting separate cell colonies and calculating the average area of colonies within the dish.

RESULTS AND DISCUSSION

Generation of ROS in CHO cell cultures (10^6 cells/ml with added 50 µM/ml DCFH-DA dye for the evaluation) irradiated to the doses from the interval of 1 Gy to 8 Gy were investigated 1 hour after irradiation. The results of dose dependent DCF fluorescence intensity changes in irradiated cells are provided in Fig.1.

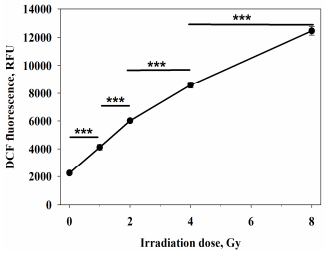

Fig 1. Dose dependent generation of ROS observed 1h after the irradiation. Results are presented as the mean values ± S.E.M. Triple asterisks (***) indicate $p < 0.001$.

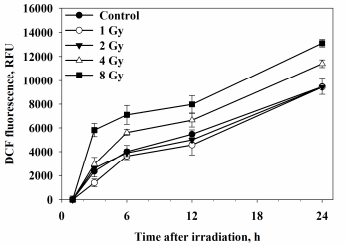

Fig 2. Additional ROS production dynamics within 24h. Results are presented as the mean values ± S.E.M

DCF fluorescence intensity measured 1 h after irradiation was identified as a primary result of radiation induced ROS. However, in order to assess the real dynamics of ROS generation in irradiated cells, further DCF fluorescence intensity measurements were performed 3, 6, 12 and 24 h after irradiation (Fig.2). Since the evaluated DCF fluorescence intensity for all irradiation doses were almost linear, it was possible to subtract corresponding fluorescence values measured 1 h after the cell irradiation from the values obtained performing later measurements. This allowed the follow up and comparison of ROS generation dynamics in differently irradiated cells within 24 hours. It was found that the DCF fluorescence intensity was non-uniformly increasing with the increasing time after the exposure of cells. The statistically significant DCF fluorescence intensity growth was observed in the cells irradiated to > 2 Gy doses, indicating similar intensity growth rate for the cells irradiated to higher doses. According to (1, 2) observed buildup of post irradiative ROS inside cell suspension can be identified as the signals of apoptosis.

The assessment of cell viability was another step of the complex investigation of radiation induced biological effects in the cells. For this reason, Clonogenic assay was used to sort out the viable and dead cells after irradiation to different doses. Usually Clonogenic assay is performed by counting colonies of cells that are capable to proliferate after irradiation and comparing them to control cells. We have modified the clonogenic assay protocol and evaluated additionally the average area of the formed cell colonies using open source imaging software ImageJ in order to evaluate cell mitotic arrest. Taking into account that the colony size is dependent on cell division ability, comparison of colonies formed by control cells and those formed by irradiated cells enabled the evaluation of the delayed relative mitosis after irradiation. The results on irradiated cell survival are shown in Fig. 3. Notable loss of cell viability from 82±5% evaluated after irradiation to 2 Gy to 48±1.8% after irradiation to 4 Gy was observed. The tendency of cell viability loss was followed by the increasing irradiation dose to 6, 8 and 10 Gy and resulted in cell viability of 24±3.4%, 12±0.7%, 9±0.7% respectively. Decreasing tendency of irradiation formed areas occupied by cell colonies with the increasing irradiation dose was also observed, however at the doses > 6Gy no significant changes in formed cell colony area were registered.

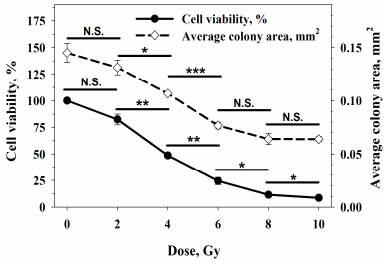

Fig 3. Cell survival dependence on delivered dose and corresponding average cell colony area after irradiation. Results are presented as the mean values ± S.E.M. in the graph. A single asterisk (*) indicates $p < 0.05$; double asterisks (**) indicate $p < 0.01$; triple asterisks (***) indicate $p < 0.001$; N.S. - no significance.

CONCLUSIONS

Based on performed analysis time dependent dose threshold of 2-4 Gy for for maximal delayed mitosis effect in irradiated cells has been set. Presence of such a threshold was approved by the results on DNA damage evaluation, post irradiative ROS production, cell viability and partly by data on relative mitotic arrest in irradiated cells indicating that application of dose threshold based irradiation model can contribute to the improvement of fractionated radiotherapy treatment efficiency.

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