## The Effect of Ionizing Radiation on MCF-7 Breast Cancer Cell Line

### Agnė Bartnykaitė<sup>1</sup>, Rasa Ugenskienė<sup>1</sup>, Arturas Inčiūra<sup>2</sup>, Elona Juozaitytė<sup>2</sup> <sup>1</sup>Oncology Research Laboratory, Oncology Institute, Lithuanian University of Health Sciences, Kaunas, Lithuania; <sup>2</sup>Oncology Institute, Lithuanian University of Health Sciences, Kaunas, Lithuania

#### Objective

Breast cancer is one of the most common cancers worldwide. Radiotherapy is widely applied for breast cancer treatment, however, cancer cells resistance to ionizing radiation (IR) causes problems for the best treatment outcomes. The evidence suggests that some chemical substances in combination with IR could generate synergistic effect and increase death of cancerous cells. Mefenamic acid (MEF) is one of non-steroidal anti-inflammatory drugs. Literature sources present the data of MEF potential to improve the radio-sensitization of cancer cells. However, this type of analysis has never been done on breast cancer cells. Lithuanian University of Health Sciences participates in Horizon 2020 project "INfraStructure in Proton International Research" (INSPIRE) in the Framework Programme for Research and Innovation. In INSPIRE project we aimed to analyze the molecular basis of breast cancer cell resistance to ionizing radiation. Consequently, the aim of this study was to determine the radiation-induced effects on MCF-7 breast cancer cells in vitro and to analyze the effect of different MEF concentrations on breast cancer cell proliferation.

#### Results

Colony formation assay demonstrated that MCF-7 viability decreased with increasing doses of IR (Fig. 2). Results of apoptosis revealed that MCF-7 cell line exhibited a significant number of apoptotic cells at 24 hours after the highest doses of IR (8 and 10 Gy). Changes of cell cycle distribution following IR did not show significant results. Nevertheless, MCF-7 cell number increased in the G0/G1 phases following the exposure to all IR doses. In the following steps we aimed to analyze the effect of MEF on MCF-7 cells proliferation. During the experiments we observed that MEF was not completely dissolved and the selected concentrations produced needle-like crystals which could change biological activity of MEF pharmaceutical compound. Even though we performed proliferation analysis, however, we tend to think that MTT assay provided inaccurate results as they were very diverse in replicate samples. In the subsequence steps we aimed to dissolve MEF in the different solutions (for example, PBS pH 7.4), though, this resulted in even worse solubility. Our data suggests that due to its poor solubility MEF is not suitable for further research in our setting.

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#### Methods

Human breast cancer cell line MCF-7 was used for the study. Cells were exposed to 2, 4, 6, 8, 10 Gy of IR from Clinac 2100C/D linear accelerator. Radiation-induced effect was assessed using colony formation, apoptosis and cell-cycle assays.

To determine the effect of MEF, MCF-7 cell proliferation was estimated using MTT assay. The powders of MEF were dissolved in dimethyl sulfoxide (DMSO) to achieve a concentration of 178 mM of stock solution. The stock solution was frozen at -20 °C in small quantities to prevent freeze-thaw cycles. Working solutions were prepared before each experiment. Final DMSO concentration was 0.28 % in all the samples. Exponentially growing cells were plated into 96-wells plates for 24 hours and then treated with indicated concentrations (0, 10, 25, 50, 100, 250, 500 µM) of MEF (Fig. 1). Then cells were grown at 37 °C in a 5 % CO<sub>2</sub>-enriched humidified air atmosphere for 24, 48 and 72 hours.





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Figure 1. Inverted microscope images of mefenamic acid (MEF) crystals for different concentrations of solution (a) 178 mM stock solution, (b) 5500 µM working solution, (c) 0 µM solution, (d) 10 µM solution, (e) 25 µM solution, (f) 50 µM solution, (g) 100 µM solution, (h) 250 µM solution, (i) 500 µM solution



Figure 2. MCF-7 breast cancer cell survival following the exposure to ionizing radiation. The results are mean  $\pm$  standard deviation

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#### Conclusions

Our results are in agreement with the literature and suggest that MCF-7 cells are not highly resistant to IR. MCF-7 cell line could be use as control for comparison to other cell lines response to IR analysis. Furthermore, the study demonstrated that MEF tends to produce crystals which could exist in various polymorphic forms. Therefore, this drug will not be considered for further radiosensitizing analysis in our study.

#### Key words

breast cancer, MCF-7, ionizing radiation, mefenamic acid