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SENSITIVITY OF LUNG CARCINOMA CELLS TO γ- RAYS AND ERLOTINIB

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Abstract
In order to increase radio-sensitivity of human lung adenocarcinoma NCI-H1568 cells, targeted therapy drug, erlotinib was used. The impact of radiation and erlotinib on cell behaviour was analyzed using three biological endpoints. Irradiations with γ-rays resulted in reduction of cell survival, viability and proliferation. Erlotinib significantly inhibited cell growth and proliferation capacity. Combined treatments with radiation and erlotinib showed high level of reduction of cell viability and proliferation. Preliminary data encourage further investigations of mechanisms underlying the radiation responses enhanced by erlotinib.

Introduction
Non-small cell lung cancer (NSCLC) represents over 80% of all lung cancers. Radiotherapy and chemotherapy, alone or in combination are standard treatment strategies for this disease. The epidermal growth factor receptor (EGFR) is a member of the human epidermal growth factor (HER) family of receptors that is aberrantly expressed in a broad range of cancers including NSCLC. Ligand binding induces activation of tyrosine kinase (TK) domain of EGFR which leads to the activation of important signaling network involved in tumor cell proliferation and survival [1]. For this reason, blocking this signaling pathway by molecular targeting of EGFR is an attractive therapeutic strategy. Most targeted therapies include anti-EGFR monoclonal antibodies and small-molecule tyrosine kinase inhibitors (TKIs) [2]. Erlotinib (Tarceva) is quinazoline small-molecule inhibitor of HER1/EGFR tyrosine kinase approved by the United States Food and Drug Administration for the treatment of advanced NSCLC and pancreatic cancer [3]. There is evidence that radiation can activate EGFR signaling leading to accelerated proliferation or repopulation of tumour cells [4]. Considering the new insights into the role of EGFR in DNA repair through interaction with DNA-dependent protein kinase (DNA-PK) there is a considerable interest in using EGFR inhibitors for sensitizing tumours to radiotherapy in cancer patients [5].

Trying to improve the anti-tumour activity of γ-rays by erlotinib, combined effects of these agents on the human lung adenocarcinoma cell line NCI-H1568 were analyzed.

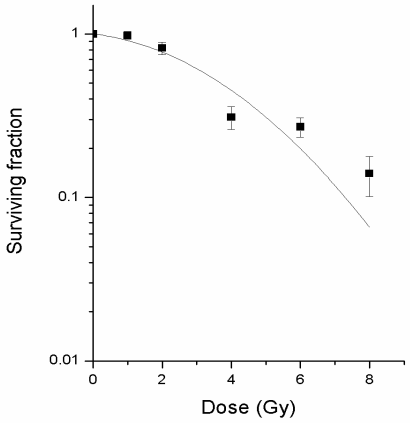

Figure 1. Dose-response curve of NCI-H1568 cells exposed to γ-rays, obtained by clonogenic assay.

Materials and methods

The NCI-H1568 cells were irradiated in air with ^{60}Co γ-rays at the Vinča Institute of Nuclear Sciences, Belgrade, Serbia. Cell survival was assessed 7 days after irradiation in the dose range from 1 to 8 Gy and at the dose rate of ~1 Gy/min. Viability and proliferation were measured 7 and 14 days post irradiation using SRB (MP Biomedicals, Inc) and BrdU (Roche Diagnostics GmbH, Mannheim, Germany) assays. The absorbance was measured at 450 and 550 nm for BrdU and SRB assays, respectively (Victor, Wallac, Turku, Finland). In combined treatments, following literature data, 5 μM erlotinib was added immediately after irradiation [6].

Results and Discussion

Dose-dependent inhibitory effects of γ-rays are justified by clonogenic assay. Surviving data were fitted to the linear-quadratic model and the best fit survival curve is given in Figure 1. The surviving fraction at 2 Gy (SF2) is 0.78. Obtained data indicated high level of radio-resistance of analyzed NSCLC cells, especially to lower doses of γ-rays (1 and 2 Gy), while the response of NCI-H1568 cells to higher doses was significant (Fig. 1). Treatment of irradiated NCI-H1568 cells with erlotinib caused very strong inhibition of cell survival and colony formation was not detected.

Based on the fact that analyzed cells are quite resistant to γ-rays, for further experiments dose range was extended with higher doses beyond the therapeutic values (12 and 16 Gy).

Dose-dependent cell viability obtained with SRB assay, 7 days after application of γ-rays, ranged from 31 to 99% (Fig. 2a). NCI-H1568 cells showed strong response to erlotinib alone and in combination with γ-rays, with viability less than 5% (Fig. 2a). Similar data were obtained 14 days after irradiation, where the values were between 16 and 90% while the number of viable cells after single and combined treatment with erlotinib was less than 10% (Fig.2b). For both incubation periods, good dose-dependent response to γ-rays was observed. According to the results obtained by BrdU assay, cell proliferation decreases after exposure to higher doses of γ-rays. Moreover, treatment with erlotinib alone and in combination provokes strong inactivation of proliferation, with values less than 8%, as compared to control (Fig. 2c). Proliferation of treated cells after 14 days of incubation is given in Fig. 2d. Obtained data showed slightly higher proliferation of cells, not exceeding 30%, therefore indicating that the cells recover after applied treatments.

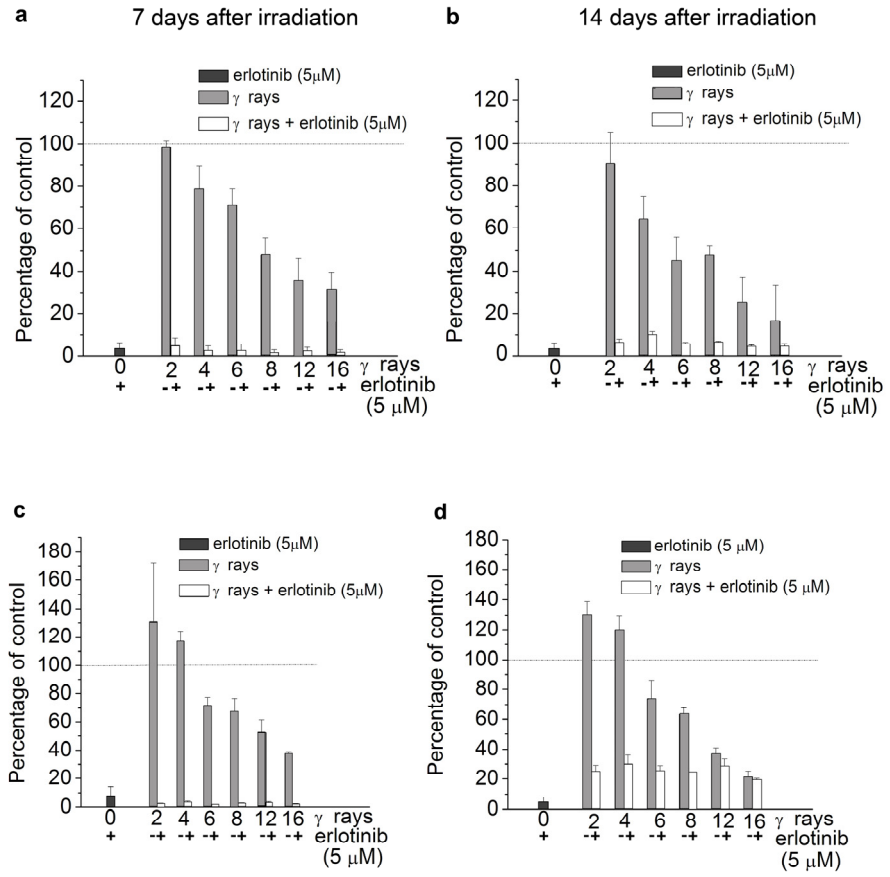

Figure 2. Effects of γ-rays and erlotinib on NCI-H1568 cells, 7 and 14 days after irradiation, estimated by SRB (a, b) and BrdU assays (c, d).

Conclusion

Presented results indicated that γ-rays inactivated NCI-H1568 cells in the dose-dependent way. Treatment with erlotinib highly sensitized the cells to γ-rays, thus making this agent valuable in cancer treatment when used in the synergy with radiation.

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