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Original research article

Investigation of the bystander effect in CHO-K1 cells

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ABSTRACT

Aim: Investigation of the bystander effect in Chinese Hamster Ovary cells (CHO-K1) co-cultured with cells irradiated in the dose range of 0.1–4 Gy of high LET ^{12}C ions and X-rays.
Background: The radiobiological effects of charged heavy particles on a cellular or molecular level are of fundamental importance in the field of biomedical applications, especially in hadron therapy and space radiation biology.
Materials and methods: A heavy ion ^{12}C beam from the Heavy Ion Laboratory of the University of Warsaw (HIL) was used to irradiate CHO-K1 cells. Cells were seeded in Petri dishes specially designed for irradiation purposes. Immediately after irradiation, cells were transferred into transwell culture insert dishes to enable co-culture of irradiated and non-irradiated cells. Cells from the membrane and well shared the medium but could not touch each other. To study bystander effects, a clonogenic survival assay was performed.
Results: The survival fraction of cells co-cultured with cells irradiated with ^{12}C ions and X-rays was not reduced.
Conclusions: The bystander effect was not observed in these studies.

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1. Background

Knowledge of the radiobiological effects of heavy ions at the cellular and molecular level is of fundamental importance in the field of radiation therapy. It has been accepted for a long time that the damaging effects of ionizing radiation are the results of the direct ionization of cell nuclei. However, in addition to effects in cells directly targeted with heavy ions, there is an evidence of non-targeted biological effects in cells that have not been directly irradiated. The bystander effect of heavy ions manifests itself as the loss of clonogenic potential, alterations in gene expression profiles, and the elevated frequency of micronuclei, which arise in non-irradiated cells having received signals from irradiated cells.[1] The phenomenon of radiation-induced bystander response was first described by Nagasawa and Little in 1992,[2] when increased frequencies of sister chromatid exchanges (SCEs) were observed in about 30% of the cells exposed to α-particle by which <1% of the nuclei was traversed by a single α-particle track.

The effect induced by irradiated cells and their progeny on neighboring non-irradiated cells was studied. CHO-K1 cells were irradiated with ^{12}C ions and X-rays with three different doses: 0.1 Gy, 1 Gy, 4 Gy. Investigation of the bystander effect was enabled by co-culture of irradiated and non-irradiated cells in special transwell dishes. Clonogenic survival assay was used in these studies.

2. Materials and methods

2.1. Cell line and culture conditions

Chinese Hamster Ovary cells (CHO-K1) were exposed to two kinds of ionizing radiation – high LET ^{12}C ions and X-rays. The cell line is characterized by genetic stability, the ability to form colonies and a relatively rapid growth rate, with a cell cycle of 12–14 h. The cells were cultured in 5A McCoy (Gibco, USA) medium, containing 10% fetal bovine serum (FBS) (Gibco, USA), 1% penicillin and streptomycin (Gibco, USA) and incubated in a humidified atmosphere at 37 °C with 5% CO_2.

2.2. Irradiation facility

The experimental set-up has been described previously[3] and therefore only a short review is presented. An experimental set-up with a horizontal heavy ion beam designed for radiobiological research at the Heavy Ion Laboratory of the University of Warsaw (HIL) was used. It provides the possibility to irradiate biological samples at room temperature and atmospheric pressure by various ions at high LET. A view of the facility is shown in Fig. 1.

At the entrance the beam is collimated by a 2 mm aperture. To achieve a homogeneous radiation field over the area of $1 \times 1\,cm^2$ of the exit window (made from havar with thickness of $2.3\,mg/cm^2$), the ion beam was passively spread out by a scattering gold foil (Goodfellow Cambridge Limited, Huntington, UK) with thickness of $13\,mg/cm^2$. The on-line ion beam monitoring is ensured by a silicon detector placed at 20°.

2.3. Irradiation

One day before irradiation cells were seeded on the Petri dish made with mylar bottom with thickness of 6 μm. To obtain homogeneous irradiation of all CHO-K1 cells, biological samples were fastened to a movable, specially designed sample holder mounted on an x–y–z stepping motor with remote control, set at a distance of 1.2 cm from the exit window. At this system the time dependence of the beam intensity during the sample irradiation as well as the energy spectrum of the scattered beam were registered. The signals from the monitor detector were counted in a fast programmable scaler.

When the number of registered particles reached the preset value (defining the dose), a start signal was created and the target changed its position according to a planned route. The data were visualized on-line at the PC monitor by a graphical interface. Communication between the PC and the electronics was via a CAMAC crate controller.

Primary energy of the carbon ions was 48 MeV. Scattering gold foil and havar exit window degraded the ions energy to 27 MeV. Air layer and mylar bottom of the Petri dish again reduced energy of the ions and in the result carbon ions with 17 MeV energy hit the cells. Linear energy transfer (LET), namely the average amount of energy deposited

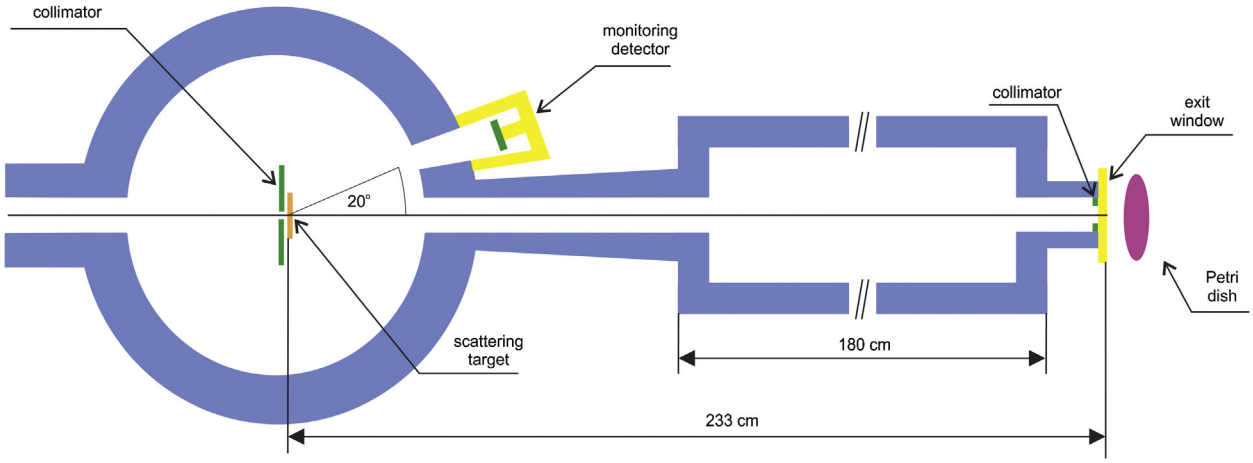

Fig. 1 – Schematic view of the set-up for radiobiological studies with the horizontal beam line.