Biological Science

27B/2007G702

Cell-killing effect in bystander cells induced by monochromatic X-ray microbeams

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Introduction

A central paradigm in radiation biology has been that only cells "hit" by a track of radiation would be affected to induce radiobiological consequences, and cells "not hit" should not be. This paradigm is the basis for the current system for risk estimation of radiobiological effects. However, it recently has been challenged by so called non-targeted effects, such as bystander effect, and such radiation-induced non-targeted effects may have important implications for risk evaluation of low dose / low dose rate radiations. Microbeams must be a powerful technique for radiobiological studies, especially non-targeted effects, however almost all of the studies were carried out using high-LET charged particle microbeams. In this study we have begun to investigate cellular bystander responses in normal human fibroblasts induced by low-LET X-ray microbeams.

Materials and Methods

Monochromatic X-ray microbeams (5.35keV) were generated with the cell irradiation system of X-ray microbeams at BL27B2 [1,2]. Approximately 6 x 10^5 exponentially growing normal human fibroblast cells were inoculated into each of microbeam dish, which was constructed with an acrylic resin ring of 36-mm diameter and attached 7.5μm-thick polyimide film on the bottom of the ring, 2 days before microbeam irradiation. One day before irradiation, half of the dishes were treated with a specific inhibitor of gap-junction mediated cell-cell communication (40μM of γ -isomer of hexachloro-cyclohexane). At the irradiation period, cultures were confluent. Cell cycle distribution of the confluent cultures was analyzed using a flow cytometory and around 95% of the cells were G_1- or G_0-phase (data not shown). X-ray-microbeam irradiation was carried out using the 284 (17x17-5)-cross-stripe method (Fig.1). Microbeams of 20μm x 20μm size were irradiated in each point with 0.4Gy.

Results and Discussions

Figure 2 showed cell-killing effect, which was detected with a colony-forming assay as reproductive cell death, in microbeam-irradiated dishes (IR) and microbeam-irradiated dishes with a specific inhibitor of gap-junction (L+IR). The percent cell survival in X-ray-microbeam irradiated dishes (a) was around 100%, while the survival in carbon-ion microbeam irradiated dishes (b) using the same experimental system was ranging from 83% to 94%. In our microbeam-irradiation method, we estimated that the percent of microbeam-direct hit cells was around 0.2% of all cells in the dish. The results showed that no cell-killing effect beyond expectation was observed in the X-ray-microbeam-irradiated cells, when compared to the carbon-ion-microbeam-irradiated cells. Our present results suggest that X-ray-microbeam-irradiated cells can't induce a bystander lethal response in neighbouring cells, which are not directly hit by X rays, under our experimental condition such as the confluent state of the normal human fibroblasts.

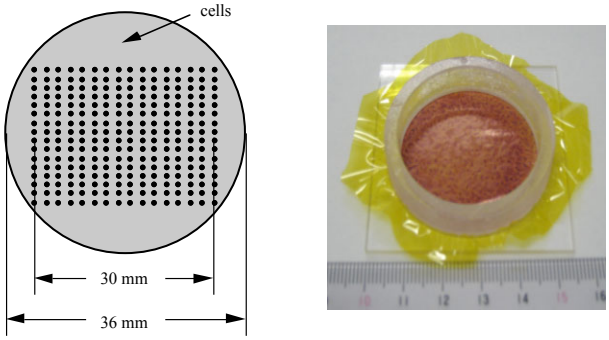

Fig.1 Microbeam-irradiation procedure of normal human fibroblasts using the 284 (17x17-5)-cross-stripe method.

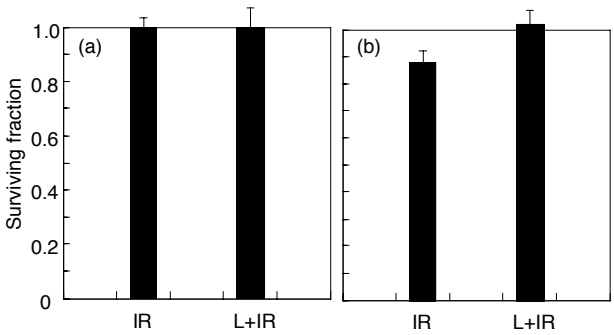

Fig.2 Cell-killing effect in microbeam-irradiated dishes (IR) and microbeam-irradiated dishes with a specific inhibitor of gap-junction (L+IR). (a); monochromatic X-ray microbeams. (b); carbon-ion microbeams.

References

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