Biological Science

27B/2009G640

Cell-killing effect for targeted cell nuclear irradiation with monochromatic X-ray microbeams

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Introduction

Radiobiological effects induced by direct irradiated cells is the basis for the current system for risk estimation from radiation and the risk of radiation-induced cancer after high and moderate doses are relatively known, based on the data from detailed epidemiological studies of the Japanese atomic bomb survivors in Hiroshima and Nagasaki [1]. However, it recently has been challenged by so called epigenetics-like effects, such as genomic instability, adaptive response and bystander effect, and such radiation-induced epigenetics-like effects may have important implications for risk evaluation of low doses.

In this study we focused on low-dose radiobiological effects using monochromatic X-ray microbeams. We have been studying the cellular responses in normal human fibroblasts irradiate with X-ray microbeams. The last year we reported the preliminary result in cell-killing effect induced by the targeted irradiation with cell nucleus. This year we clarified the bystander cellular effect induced by targeted cell nucleus irradiations.

Materials and Methods

The irradiation system for the targeted cell nucleus of normal human fibroblasts was reported in Photon Factory Activity Report 2009 [2]. Briefly, approximately 300 exponentially growing normal human fibroblasts were inoculated into the microbeam dish, which was stretching a 2.5μm-thick Mylar film over the bottom of the hole for X-ray window, 2 days before microbeam irradiation and irradiated with monochromatic X-ray microbeams (5.35keV) generated with the X-ray microbeam cell irradiation system at BL27B2. X-ray-microbeam (10μm x 10μm size) irradiation was carried out using the targeted nuclear irradiation system and each cell nucleus stained by Hoechst 33342 was captured by the computerized cell irradiation system [2], and irradiated 40R with each cell nucleus in either 100% cells or randomly selected 10% cells. The cell-killing effect was measured with a colony-forming assay as the reproductive cell death. After irradiations, cells were trypsinized, counted of the cell numbers and plated onto 100mm plastic dishes (BD Falcon 353003) to make 60 to 70 colonies per dish. The colonies were fixed and stained with 20% methanol and 0.2% crystal violet after a 14-day incubation period. Any colony consisting of more than 50 cells was scored as a surviving clone.

Results and Discussions

The percent of cell survival to targeted cell nuclei irradiated with 40R were to be (52±3.4)% for 100% cell nuclei and (79±3.8)% for randomly selected 10% cell nuclei. Furthermore, the percent of cell survival to randomly selected 10% cell nuclei increased to (99±3.3)% when using a specific inhibitor of gap-junction mediated cell-cell communication (Fig.1). We can calculate the cell survival to 10% cell nuclei irradiations to be 95%, assuming no bystander effect, based on the data of 100% cell nuclei irradiations. However, the result clearly shows that cell survival to 10% cell nuclei is significantly low beyond our calculation. There is clear evidence that bystander cell-killing effect is observed in the targeted cell nucleus irradiation and gap-junction mediated cell-cell communication plays an important role in inducing bystander effect.

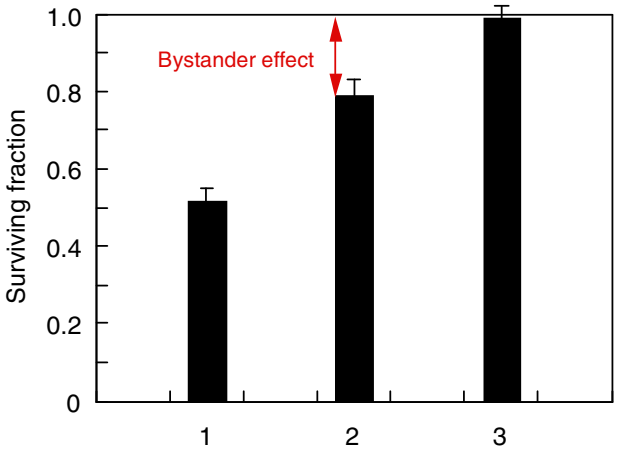

Fig.1 Cell-killing effect for the targeted cell nucleus irradiation with monochromatic X-ray microbeams (40R). 1; 100% cell nuclei, 2; randomly selected 10% cell nuclei, 3; randomly selected 10% cell nuclei with a specific inhibitor of gap-junction.

References

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