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Biological effects of targeted cell nuclear irradiation with monochromatic X-ray microbeams

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Introduction

Microbeams applied to radiobiological studies must be a powerful technique, especially examining non-targeted effects, such as bystander effects, genomic instability and radioadaptive response. However almost studies were carried out using high-LET helium-ion microbeams and limited experiments were available for examining biological effects induced by microbeams of electromagnetic radiations. Furthermore, the study for investigating non-targeted effects in cells irradiated with targeted nuclear or cytoplasmic irradiations was limited. In this study we have begun to investigate non-targeted cellular biological effects in normal human fibroblasts induced by targeted nuclear or cytoplasmic irradiations of low-LET X-ray microbeams.

Materials and Methods

Monochromatic X-ray microbeams (5.35keV) were generated with the X-ray microbeam cell irradiation system at BL27B2 [1,2,3]. Approximately 300 exponentially growing normal human fibroblasts were inoculated into the centre of each 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 (Fig.1). At the irradiation, cultures were almost in confluent stage. X-ray-microbeam irradiation was carried out using the targeted nuclear irradiation system (Fig.2). Briefly, each cell nucleus stained by Hoechst 33342 was captured by the computerized cell irradiation system and irradiated 0.4Gy in each cell nucleus with X-ray microbeams of 10μm x 10μm size one by one. The cell-killing effect was measured with a colony-forming assay as the reproductive cell death. After irradiations, cells of all irradiated dishes 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

In the targeted nuclear irradiation system, the cell nuclei of normal human fibroblasts were successfully captured (See red dots in the right panel of Fig.2.) and irradiated 0.4Gy dose to the each captured cell nucleus. Although it was a preliminary data, the percent of cell survival irradiated with 0.4Gy to 100% cell nucleus was (50±14)%. Now we have begun to examine biological effects in cells irradiated with 100% cell nuclear and non-targeted effects using this irradiation system.

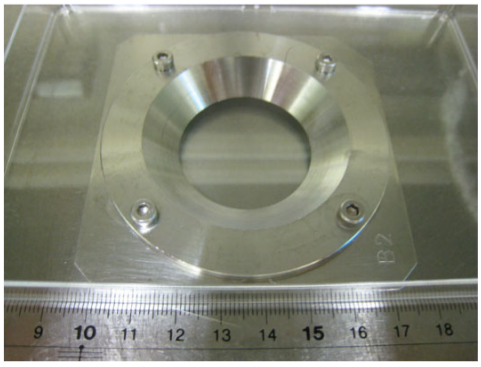

Fig.1 The microbeam irradiation dish used for the targeted cell nuclear irradiation.

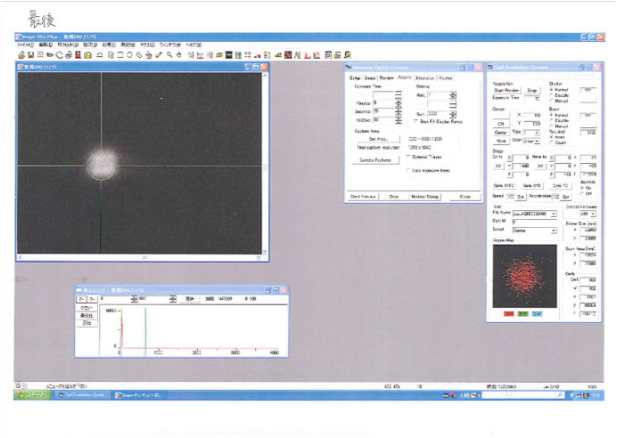

Fig.2 The computerized targeted nuclear irradiation system of X-ray microbeams. Near square beam on the left panel shows 10μm x 10μm X-ray microbeams.

References

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