

Cell-killing effect by the targeted cytoplasmic irradiation in normal human fibroblasts with monochromatic X-ray microbeams (2)

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1 Introduction

The biological research using a microbeam can provide us very important implications in the research field of radiation science. Especially, a targeted irradiation to either cell nucleus or cytoplasm using a microbeam enable us to understand biological cellular responses, such as bystander effects, genomic instability and radio-adaptive response, induced by low-dose or low-fluence irradiations more in detail. The study of such biological responses for low linear energy transfer (LET) radiation can surely provide the important information for evaluating risk such a low-dose (rate) exposure as the accident of Fukushima Daiichi Nuclear Power Plants. However, most studies for such biological effects induced in cells irradiated with a microbeam have been carried out using high-LET-particle radiations and so far only limited data is available to understand biological effects induced by low-LET electromagnetic radiations, such as X or gamma rays. It should be a powerful source for the microbeam of low-LET electromagnetic radiations to use the synchrotron radiations.

We have been studying cellular responses induced by the targeted irradiations using the cell-irradiation system of X-ray microbeams at BL27B [1]. Our final goal is to identify the molecular mechanism(s) of radiation induced cellular responses, and then to clarify radiation risk concerning the issue of the accident of Fukushima Daiichi Nuclear Power Plants.

We already reported that the cellular bystander effect via gap-junction mediated cell-cell communication was not induced in cells immediately after random irradiations with both cell nucleus and cytoplasm of the X-ray microbeams [2]. However, the bystander cell-killing effect was induced in cells irradiated with just cell nucleus when using the system of the targeted cell nuclear irradiation [1]. And also the higher frequency of gene mutation at the hypoxanthine-guanine phosphoribosyl transferase (*HPRT*) locus was induced in the progeny of the following 20-cell generations from the X-ray-microbeam irradiated cells than that in the progeny of non-irradiated control cells and it was reduced to the non-irradiated control level when treating with a specific inhibitor of gap-junction mediated cell-cell communication [3]. Our study suggests that it should be very important to investigate low-LET-radiation induced bystander cellular effects irradiated with targeted cell nucleus or cytoplasm using the X-ray microbeams as the

accident of Fukushima Daiichi Nuclear Power Plants in order to evaluate the risk of cancer by low-dose exposure.

This year, we examined cell-killing effect of either targeted cell-nuclear or cytoplasmic irradiations expanding the last year's experiment.

2 Experiment

Normal human skin fibroblasts distributed by the RIKEN BioResource Center Cell Bank (Cell name : NB1RGB, Cell No. : RCB0222) were used in this study. Approximately 1,000 exponentially growing cells were inoculated into the center of each microbeam dish, which was stretching a 2.5 μ m-thick Mylar film over the bottom of the hole for X-ray window, one day before irradiations. Each cell nucleus stained by Hoechst 33342 was captured by the computerized cell irradiation system. Targeted cytoplasmic irradiations with the monochromatic X-ray microbeams (5.35keV) to NB1RGB cells were carried out using the cell-irradiation system according to the last year's report. Briefly, we made the microbeam covering the areas of 30 μ m x 30 μ m in which the center of the microbeams the gold-made mask that was 22 micrometer in diameter and 20 micrometer in height on a thin SiN film was set in order to shield the nucleus (Fig.1, Fig.2).

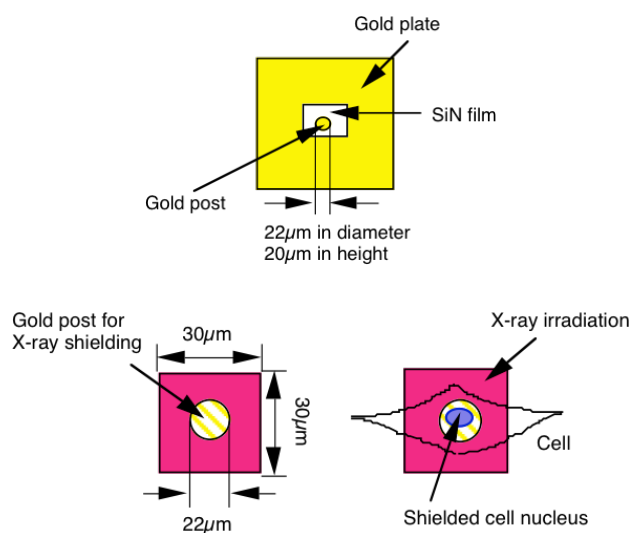


Fig.1: The method for the targeted cytoplasmic irradiation using X-ray microbeams. This method regarding the targeted cytoplasmic irradiations to the normal human fibroblasts were explained in the last year's report.

This year, we tried to examine the radio-adaptive response induced in the cells pre-irradiated cytoplasm with low-LET electromagnetic radiations. The cytoplasm of all cells captured by the computerized irradiation system was irradiated with 10R first, and then the cell nucleus of all cells captured was irradiated with 10R at the 3hr interval, within which the cells were kept in a CO₂ incubator at 37°C after the first cytoplasmic irradiation, using the X-ray microbeams collimating the beam size of 10μm x 10μm. Cell-killing effect was measured with a colony-forming assay as a reproductive cell death. Immediately after irradiation, cells were trypsinized and a defined number of cells plated onto 100mm plastic dish to make 60-70 colonies per dish. The colonies fixed and stained with 20% methanol and 0.2% crystal violet for 16-day incubation. The colonies consisting of more than 50 cells were scored as a survivor.

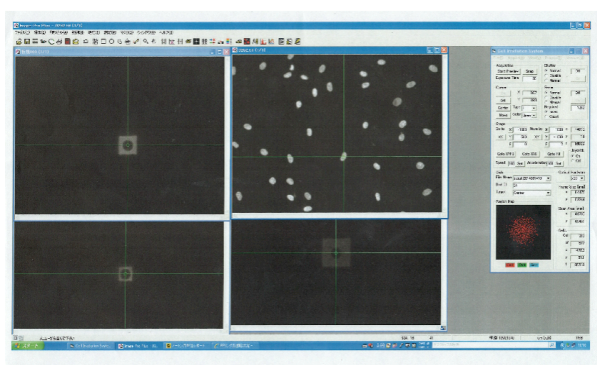


Fig.2: The computerized targeted cytoplasmic irradiation system of X-ray microbeams. The upper photo in the left side shows 60-square-micrometer microbeam and the lower photo in the left side shows 30-square-micrometer microbeam with the gold post at the center of the microbeams. This figure regarding the targeted cytoplasmic irradiations to the normal human fibroblasts was taken from the last year's report.

3 Results and Discussion

The preliminary result was shown in Fig.3. The surviving fraction for the targeted cell nucleus irradiation was around 0.79 and no cell-killing effect was induced by the targeted cytoplasm irradiation. The surviving fraction in the cells irradiated with the targeted cell nuclei when irradiated the cytoplasm beforehand was increased at 0.96. The obtained results clearly suggest that the radio-adaptive response should occur in the cells pre-irradiated to cytoplasm with the X-ray microbeams.

In this experiment we chose 3hr as the interval between the first cytoplasmic irradiation and the second nuclear irradiation. The result indicates that the cellular response led to the radio-adaptive response will be completed within 3hr after the first cytoplasmic irradiation, but it is still unclear whether the "3h interval" is maximum efficient for inducing the radio-adaptive response or not. We will continue to study biological responses induced by the targeted cytoplasmic irradiation to make clear the

conditions for inducing the radio-adaptive response, such as interval times or irradiation doses of the first irradiation.

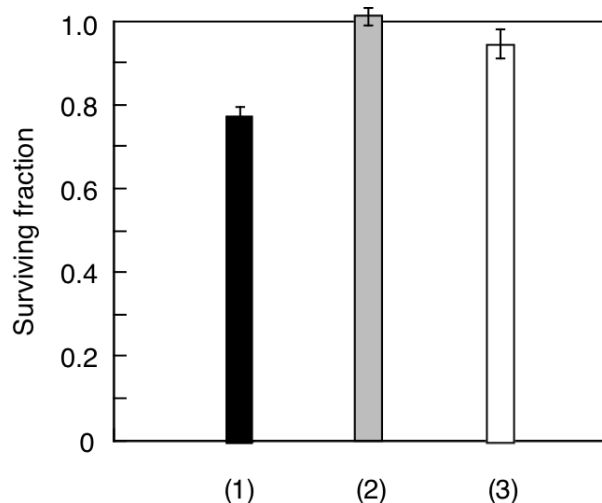


Fig.3: Cell-killing effect of normal human fibroblasts irradiated with either targeted nuclear or cytoplasmic irradiation of monochromatic X-ray microbeams (5.35keV). (1); targeted nuclear irradiation (10R) alone, (2); targeted cytoplasmic irradiation (10R) alone, (3); targeted cytoplasmic irradiation (10R) → (3hr interval) → targeted nuclear irradiation (10R). The data show the average and the standard error of 3 independent experiments.

References

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