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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 research using a microbeam can provide us very important information in the research field of radiation science and it should be essential for us to understand radio-biological effects, such as bystander effect, genomic instability and radio-adaptive response, induced by low-dose or low-fluence irradiations. The study of such biological effects for low linear energy transfer (LET) radiation, such as X rays or gamma rays, can surely provide the important implications for evaluating risk such a low-dose (rate) exposure as the accident of Fukushima Daiichi Nuclear Power Plants.

We have been studying cellular responses induced by the low-density irradiations using the cell-irradiation system of X-ray microbeams at BL27B [1] in order to clarify radiation risk concerning the issue of the accident of Fukushima Daiichi Nuclear Power Plants and already reported that the cellular bystander effect via gap-junction mediated cell-cell communication was not induced in cells immediately after irradiation of the X-ray microbeams [2]. However, 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. Furthermore, the higher mutation frequency at 20-cell generations after irradiation was reduced to the non-irradiated control level when treating with a specific inhibitor of gap-junction mediated cell-cell communication [3]. We conclude that genomic instability detected with gene mutation occurs in future generations by the bystander effect via gap-junction mediated cell-cell communication, although no bystander cellular effects were observed in cells immediately after irradiation of the X-ray microbeams. Our study suggests that it should be very important for us to investigate biological late effects induced by low-LET electromagnetic radiations as the accident of Fukushima Daiichi Nuclear Power Plants in order to evaluate the risk of cancer by low-dose exposure.

Most studies for radiation-induced bystander effects using a microbeam, however, have been performed using high-LET heavy ions and only limited studies are available to understand cellular responses using low-LET electromagnetic radiations. In this study we have been studying low-LET-radiation induced bystander cellular effects irradiated with targeted cell nucleus or cytoplasm using the X-ray microbeams. This year, we examined cell-killing effect of either targeted cell-nuclear or cytoplasmic irradiations using the method described in the last year's reported.

2 Experiment

Normal human skin fibroblasts distributed by the RIKEN BioResource Center Cell Bank (Cell name : NB1RGB, Cell No. : RCB0222) were used in this research project. Approximately 1,000 exponentially growing cells were inoculated into the center of each microbeam dish, which was stretching a 2.5μm-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 the normal human fibroblasts were carried out using the cell-irradiation system according to the last year's report. Briefly, we made the microbeam covering the areas of 30μm x 30μm 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).

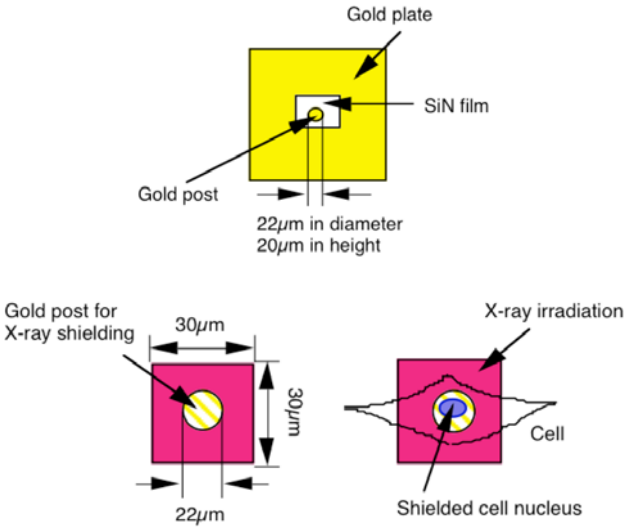

Fig.1: The method for the targeted cytoplasmic irradiation using X-ray microbeams. This method regarding the targeted cytoplasmic irradiations to the normal human fibroblasts were explained in the last year's report.

The cytoplasm of all cells captured by the computerized irradiation system was irradiated with either 10R or 40R. Also the targeted nuclear irradiation of all cells was performed either 10R or 40R of the X-ray microbeams collimating the beam size of 10μm x 10μm using the same computerized cell-irradiation system. Cell-killing effect was measured with a colony-forming assay as a reproductive dell 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 14 days after incubation. The colonies consisting of more than 50 cells were scored as a survivor.

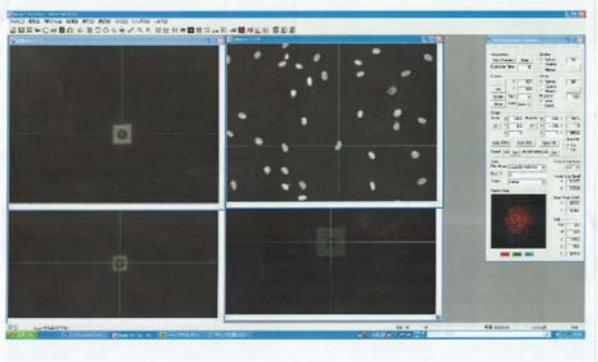

Fig.2: The computerized targeted cytoplasmic irradiation system of X-ray microbeams. The upper photo in the left side shows 60-square-micrometer microbeam and the lower photo in the left side shows 30-square-micrometer microbeam with the gold post at the center of the microbeams. This figure regarding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

3 Results and Discussion

The obtained results were shown in Fig.3. The surviving fractions for the targeted cytoplasmic irradiations were around 1.0 for both 10R and 40R. The data indicated that no cell-killing effect was induced by the cytoplasmic irradiations with the 5.35keV monochromatic X-ray microbeam ranging from 10R to 40R. On the other hand, the surviving fractions for the targeted cell-nuclear irradiation were 0.52 for 40R and 0.79 for 10R. Although we got the data with 2 different exposure doses, the cell-killing effect in cells irradiated with the targeted cell-nuclear irradiation was a dose-dependent manner. The results clearly showed that cell-killing effect occurred in just only cells deposited X-ray energy in cell nuclear.

Now we just start to examine a radio-adaptive response in the cell-killing effect by the targeted cell-nuclear irradiation when the cells were treated with the pre-irradiation to the targeted cytoplasm of the X rays. We will check the surviving fraction obtained by the targeted cell-nuclear irradiation whether it will be suppressed or not by the pre-irradiation with the X-ray microbeams to cytoplasm.

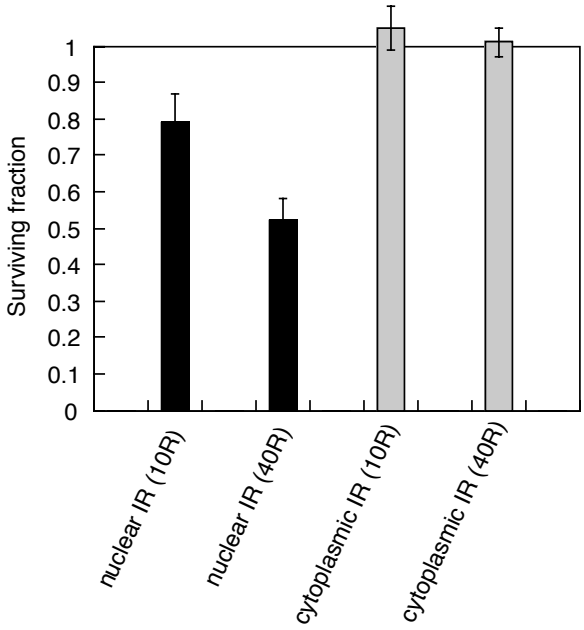

Fig.3: Cell-killing effect of normal human fibroblasts irradiated with either targeted nuclear or cytoplasmic irradiation (IR) of monochromatic X-ray microbeams (5.35keV). The data show the average and the standard error of 5 independent experiments.

References

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