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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 study using a microbeam can provide us very important scientific evidence in the research field of radiation science and it should be essential to understand radio-biological responses induced by low-dose or low-fluence irradiations. 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. Furthermore, the study of such biological responses for low linear energy transfer (LET) radiation can surely provide the critical 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 examine biological effects induced by low-LET electromagnetic radiations, such as X or gamma rays.

Radiation-induced bystander effects are described as the ability of cells affected by irradiation to convey manifestations of damage to neighbor cells that are not directly irradiated. 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 [1]. However, the bystander cell-killing effect was induced in cells targeted irradiations with cell nucleus alone. 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 [2]. We also have been studying the radiation-quality dependent bystander cellular effects, such as cell-killing effect and gene mutation induced by high-LET heavy-ion microbeams at the Takasaki Ion Accelerator for Advanced Radiation Application, Takasaki Advanced Radiation Research Institute. Together with the data of X-ray microbeams in PF, so far we understand the cellular responses as follows:

(1) In the case of both irradiations with cell nucleus and cytoplasm at the same time, the bystander cellular effects were observed in the cells irradiated with medium-LET carbon-ion microbeams, but not higher-LET neon- or argon-ion microbeams and lower-LET X-ray microbeams.
(2) Secondary radiations with low-LET components were calculated to produce from higher-LET heavy-ion tracks in proportion to LET values by the Monte Carlo simulation. They irradiated the cells located in the neighborhood of the heavy-ion tracks.
(3) The bystander cellular effects were induced by the targeted cell-nucleus irradiations with low-LET X-ray microbeams.
(4) The biological effects of low-LET X rays were higher in the cells with the targeted cell-nucleus irradiations than those in the cells of both irradiations with cell nucleus and cytoplasm at the same time.

Thus we can set up a hypothesis from the above scientific evidence as follows:

"When the cytoplasm of targeted cells are irradiated with low-LET X rays, unknown cellular response(s) is induced in the cell and in consequence the cell becomes protective to radiation damage."

It should be a powerful source for the microbeam of low-LET electromagnetic radiations to use the synchrotron radiations. And we have trying to verify the hypothesis using the X-ray microbeams in this study.

2 Experiment

Normal human skin fibroblasts distributed by the RIKEN BioResource Center Cell Bank (Cell No.: RCB0222, Cell name : NB1RGB),were used in this study. Approximately 1,000 exponentially growing cells were inoculated into the center of each microbeam dish, which was stretching a 2.5μm-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 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 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 [3]. When cell

nucleus was irradiated, we used the X-ray microbeams collimating the beam size of 10μm x 10μm. The irradiation doses were selected to be 10R (0.092Gy) and 40R (0.37Gy).

This year, we examined the radio-adaptive response induced in the cells pre-irradiated cytoplasm with X-ray microbeams. The cytoplasm of all cells captured by the computerized irradiation system was irradiated with 10R first, and then the cell nucleus stained by Hoechst 33342 of all cells captured was irradiated with 10R at the 180min interval, within which the cells were kept in a CO_2 incubator at 37ºC after the first cytoplasmic irradiation. 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 16-day incubation. The colonies consisting of more than 50 cells were scored as a survivor.

3 Results and Discussion

The plating efficiencies (PE) of Hoechst + UV scanning, which was used for capturing the cell nucleus by the computerized cell irradiation system, targeted nuclear irradiation alone and targeted cytoplasmic irradiation alone were shown in Fig.1. Now the surviving fraction (SF), which was one of the indicators for a cell-killing effect, was calculated as the following formula;

SF = PE (irradiation) / PE (Hoechst + UV scanning)

The data clearly showed the cell-killing effect for the targeted nuclear irradiation alone increased with dose-dependent manner, but no cell-killing effect was observed in targeted cytoplasmic irradiation alone.

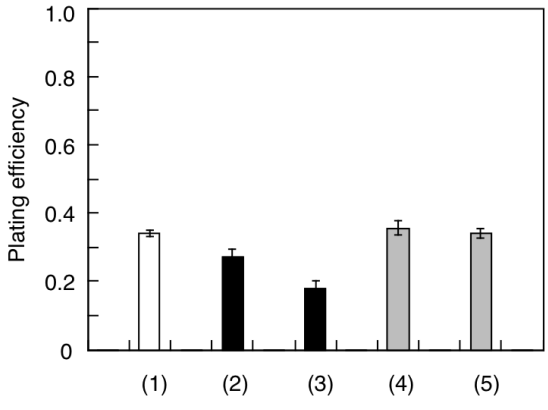

Fig.1: Plating efficiencies of normal human fibroblasts for each treatment; (1) Hoechst + UV scanning, (2) targeted nuclear irradiation (10R) alone, (3) targeted nuclear irradiation (40R) alone, (4) targeted cytoplasmic irradiation (10R) alone, (5) targeted cytoplasmic irradiation (40R) alone. The data showed the average and the standard error of 6 independent experiments.

In the case of the experiment to examine the radio-adaptive response, the cell samples were scanned under the condition of Hoechst + UV twice times putting 180-min incubation in a CO_2 incubator at 37ºC between two irradiations. The PE of Hoechst + UV scanning twice and targeted cytoplasmic and nuclear irradiations within 180-min interval were shown in Fig.2. The data suggested that these were almost the same, indicating the SF of the targeted cytoplasmic and nuclear irradiations within 180-min interval was around 1.0.

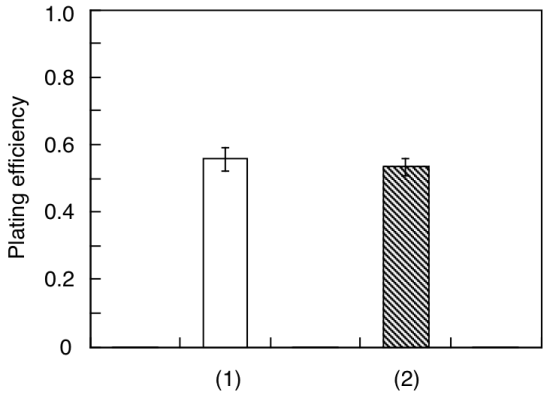

Fig.2: Plating efficiencies of normal human fibroblasts for each treatment; (1) Hoechst + UV scanning, (2) targeted cytoplasmic irradiation (10R) ---> (180min interval) ---> targeted nuclear irradiation (10R). The data showed the average and the standard error of 6 independent experiments.

To make clear the radio-adaptive response induced in the cells pre-irradiated cytoplasm, the SF data were plotted for targeted nuclear irradiation (10R) alone, targeted cytoplasmic irradiation (10R) alone and targeted cytoplasmic and nuclear irradiations (10R) within 180-min interval (Fig.3). The SF for the targeted cell nucleus irradiation was around 0.80 and no cell-killing effect was induced by the targeted cytoplasm irradiation (SF = 1.0). On the other hand, the SF in the cells irradiated with the targeted cell nuclei when the cells were irradiated the cytoplasm beforehand was increased at 0.97. The obtained data showed that the SF was drastically recovered by the pre-irradiated cytoplasm of the low-dose-X-ray irradiation, by which dose the SF was almost 1.0. There is clear evidence that the radio-adaptive response should occur in the cells pre-irradiated to cytoplasm with the low-dose irradiation of the X-ray microbeams. And we can conclude that our hypothesis is proven by this study.

In the first step, we can identify the radio-adaptive response of the cell-killing effect as the intracellular response. In the next step, we must make clear what kinds of factor(s) are activated by a low-dose irradiation in cytoplasm. In this study we chose 3hr as the interval between the first cytoplasmic and the second nuclear irradiations. 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. Also, we will try to examine the intercellular response i.e. participating bystander effect. We have a plan to examine the radio-adaptive response induced by the intercellular response focused on gap-junction mediated bystander effect in the next research project.

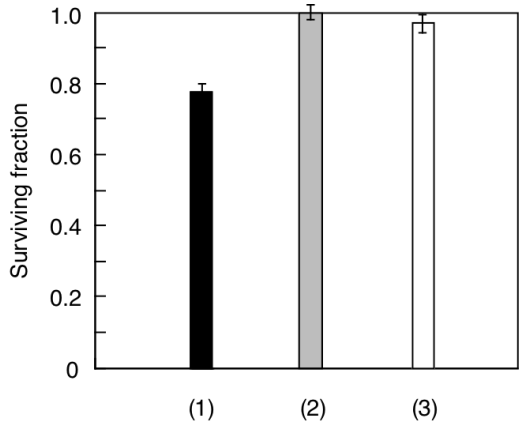

Fig.3: Cell-killing effect of normal human fibroblasts irradiated with either targeted nuclear or cytoplasmic irradiation of monochromatic X-ray microbeams. (1) targeted nuclear irradiation (10R) alone, (2) targeted cytoplasmic irradiation (10R) alone, (3) targeted cytoplasmic irradiation (10R) ---> (180min interval) ---> targeted nuclear irradiation (10R). The surviving fractions were calculated as PE (irradiation) / PE (Hoechst + UV scanning) and the data showed the average and the standard error of 6 independent experiments.

References

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