BL-27B/2019G651

Radio-adaptive response induced by the targeted cytoplasmic irradiation in normal human fibroblasts with X-ray microbeams

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1 Introduction

It should be a powerful tool to use a microbeam-irradiation system in the research field of radiation science for understanding fundamental radio-biological responses induced by low-dose- or low-fluence radiations. Especially, a targeted irradiation to either cell nucleus or cytoplasm enables us to identify biological cellular responses, such as bystander effects, genomic instability and radio-adaptive response, more in detail, and the study of such cellular responses for low linear energy transfer (LET) radiation can surely provide the critical information for evaluating radiation risk such a low-dose (rate) exposure as the accident of Fukushima Daiichi Nuclear Power Plants caused by the Great East Japan Earthquake at 2011.

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. The most part of biological studies for such cellular responses induced in cells irradiated with microbeam radiations have been carried out using high-LET-particle radiations and so far only limited studies are available to examine biological effects induced by low-LET electromagnetic radiations, such as X or gamma rays.

We already reported that the cellular bystander effect, such as chromosomal damage, in normal human fibroblasts via gap-junction mediated cell-to-cell communication was not induced in cells immediately after random irradiations with both cell nucleus and cytoplasm of the X-ray microbeams [1]. On the other hand, the bystander cell-killing effect was induced in normal human fibroblasts when cells were irradiated with targeted cell nucleus alone [2]. 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-to-cell communication [3, 4]. 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, National Institutes for Quantum and Radiological Science and Technology. 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 micrbeams and lower-LET X-ray micrbeams.
(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 even if low-LET X-ray micrbeams were used.
(4) The cell-killing effect for 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 cells is 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 (Radio-adaptive response)."

We have trying to verify the hypothesis using the X-ray micrbeams produce by synchrotron radiations in this study. The last year in order to make clear the radio-adaptive response induced in each cell by intracellular response, targeted cytoplasm of 100% cells in the microbeam-irradiation dish were irradiated with X rays (10R=0.092Gy) beforehand and then targeted cell nuclei of 100% cells were irradiated with X rays (0.092Gy). Cell-killing effect, which was detected with a colony-forming assay as a reproductive cell death, was shown in Fig.1. The surviving fraction for the targeted cell nucleus irradiation was around 0.80 and no cell-killing effect was induced by the targeted cytoplasm irradiation. On the other hand, the surviving fraction 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 cell-killing effect was drastically recovered by the pre-irradiated cytoplasm of the low-dose-X rays. There is clear evidence that the radio-adaptive response should occur in each cell pre-irradiated to cytoplasm with the low-dose X rays. The

data clearly showed the radio-adaptive response induced in intracellular response.

This year we have been trying to make clear whether the radio-adaptive response was induced in intercellular response or not.

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 microbeam 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 [5]. 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. 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). In order to examine the radio-adaptive response induced in intercellular response, targeted cytoplasm randomly selected 10% of all cells in the microbeam-irradiation dish were irradiated with X rays (0.092Gy) beforehand and then targeted cell nuclei of 100% cells were irradiated with X rays (0.092Gy) at the 180min interval within which the cells were kept in a CO_2 incubator at 37°C after the first cytoplasmic irradiation. Cell-killing effect was measured with a colony-forming assay. 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 preliminary data was shown in Fig.1 that the cell-killing effect for randomly selected 10% of targeted cytoplasmic irradiation (0.092Gy) ---> (180min interval) ---> 100% of targeted nuclear irradiation (0.092Gy) clearly indicated to be going up as well as 100% of the pre-irradiated cytoplasm. The result suggests that the unirradiated cell to its cytoplasm is capable of inducing radio-adaptive response against following irradiation with cell nucleus. One of the possible mechanisms is to be communication and transmission of unknown signals from the cells irradiated with cytoplasm and inducing radio-adaptive response, suggesting intercellular response. In the next step, we must make clear what kinds of factor(s) are activated by a low-dose irradiation in cytoplasm. 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.

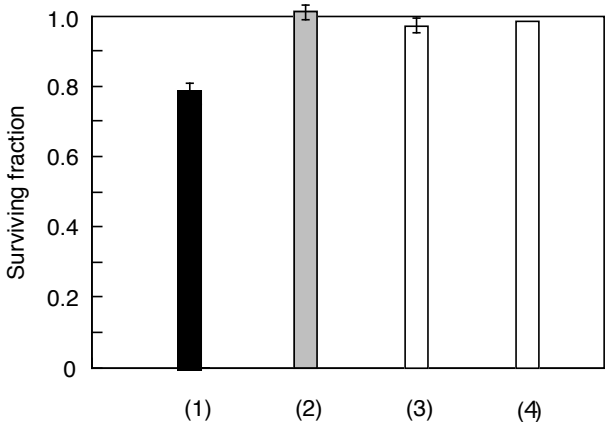

Fig.1: Cell-killing effect of normal human fibroblasts irradiated with either targeted nuclear or cytoplasmic irradiation of monochromatic X-ray microbeams. (1) 100% of targeted nuclear irradiation (10R=0.092Gy) alone, (2) 100% of targeted cytoplasmic irradiation (0.092Gy) alone, (3) 100% of targeted cytoplasmic irradiation (0.092Gy) ---> (180min interval) ---> 100% of targeted nuclear irradiation (0.092Gy), (4) Randomly selected 10% of targeted cytoplasmic irradiation (0.092Gy) ---> (180min interval) ---> 100% of targeted nuclear irradiation (0.092Gy). The data of (1), (2) and (3) showed the average and the standard error of 6 independent experiments.

References

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